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5MESMAP

The Fifth International Mediterranean Symposium on Medicinal and Aromatic Plants

April 24-26, 2019 / Cappadocia - TURKEY

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The Fifth International Mediterranean Symposium on Medicinal and Aromatic Plants

MESMAP – 5
PROCEEDINGS BOOK
ABSTRACTS & FULL PAPERS

April 24th – 26th, 2019

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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. MESMAP Symposiums provide a platform for herbal medicines, botany, plant biotechnology, ethnobotany, phytopharmacology, pharmacognosy, food, agriculture and forestry, plant biology, phytochemistry and aromatherapy.

That symposium was the fifth meeting series of MESMAP, and you can find abstracts of all the scientific works presented in MESMAP-5 in this PROCEEDINGS BOOK. We are proud to announce that selected full papers will be published in the contracted journals of the symposium after scientific evaluation. We are happy to invite MESMAP-5 participants to submit their full papers which were presented at the symposium ‘Molecules’, ‘Annals of Phytomedicine’, International Journal of Agriculture, Environment and Food Sciemces’, ‘Journal of Institute of Science and Technology-Iğdır University’, and ‘Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)’.

We would like to thank for their sincere supports of Turkish General Directorate of Forestry, TURKISH AIRLINES, Austrian Drug Screening Institite, Kilis 7 Aralık University, Khon Kaen University, Kumamoto University, Iğdır University, Rural Federal University of Rio de Janeiro (UFRRJ)-Brazil, AMAPMED, Association of Pharmaceutical Teachers of India, Cosmetic Producers and Researchers Associatons, Talya Herbal Compony, and MASMANA Olive Oils and all the other supporters.

Organizing Committee hope that MESMAP-5 Symposium participants would have an amazing experience and unforgettable memories to take back their homes, and would like to thanks for all MESMAP-5 participants for their valuable contributions. We would like to remind you that MESMAP Symposium series will be organized every year. Hope to meet you in the sixth meeting series of MESMAP-6 in 2020 spring.

Sincerely,
Symposium Chairman

Prof. Dr. Nazım ŞEKEROĞLU
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‘Selected full papers will be published in the official journals of MESMAP- 5 after the symposium’

Molecules (Publication fee will be covered by authors)	https://www.mdpi.com/journal/molecules
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PLENARY LECTURE

CHEMICAL DIVERSITY OF BIOACTIVE DITERPENOIDS OF ENDEMIC ANATOLIAN LAMIACEAE PLANTS

Gülaçtı Topçu*

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Mediterranean region, particularly Anatolia is one of the most rich regions for the Lamiaceae family plants in the world. Since over 30 years, ten genera belonging to Lamiaceae family have been studied by our group, particularly *Salvia*, *Sideritis*, *Teucrium*, *Ajuga*, *Nepeta*, and *Lavandula* species and in recent years *Micromeria*, *Origanum*, *Stachys* plants. In Turkey, the genus *Salvia* is represented by over 100 species while Anatolia is one of two gen centers of the *Sideritis* species with 78% endemism ratio, 39 being endemic among 45 species (53 taxa). Till today, over 50 *Salvia*, 15 *Sideritis*, 12 *Nepeta*, 10 *Teucrium*, 2 *Lavandula* species were investigated by our group for their biologically active compounds, particularly diterpenoids. Considering the studies on those mentioned Anatolian Lamiaceae plants *Salvia* species were found to be highly rich and divers in abietane diterpenoids while *Sideritis* in *ent*-kaurane and *Teucrium* species in neo-clerodane diterpenoids while the other genera species were not specifically rich in any type of diterpene skeleton [1]. *Nepeta* and *Origanum* and *Stachys* species are rich in phenolics and flavonoids rather than diterpenoids and other terpenoids.

Antioxidant, antiradical, antimicrobial including antiviral, and anticholinesterase, anti-inflammatory, and cytotoxic activities of a series diterpenoids from above Lamiaceae plants were investigated by *in vitro*, and anti-repellent/insecticidal activities *in vivo*. *Salvia* species investigated by our group afforded about 170 diterpenoids showed highest chemodiversity (abietanes, seco-abietanes, nor-abietanes, fully aromatic abietanes, labdanes, pimaranes)[2] with multitargeted activity, particularly high antioxidant, anticholinesterase and cytotoxic activity. *Sideritis* species were rich in *ent*-kaurane diterpenoids with strong anticholinesterase and antirepellent/insecticidal activities, on the other hand they were rich in glycosides of phenolics and flavonoids, *Teucrium* species also showed chemodiversity in their neo-clerodane diterpenoids, *Ajuga*, *Nepeta* and *Lavandula* species have not shown such distinct diterpenoids.

Structures of the isolated pure diterpenoids and other constituents were elucidated by 1D- and 2D-NMR and Mass and other spectroscopic methods. The extracts of the Lamiaceae plants from different genera with their pure diterpenoids were studied for mainly *in vitro* antioxidant, cytotoxic, antibacterial, antiviral and anti-cholinesterase activities [1-3], activity results obtained will be presented.

Keywords: Lamiaceae, chemodiversity, diterpenoids, biological activity.

References:

[1] Topcu G. and Gören A., (2007). Biological Activity of Diterpenoids Isolated from Anatolian Lamiaceae Plants, Records *of Natural Products*, 1, 1-16.
[2] Topçu G., Yücer R., Şenol H., (2017). "Bioactive Constituents of Anatolian *Salvia* Species" in Salvia Biotechnology, Georgiev V., Pavlov A., Eds., Springer International Publishing AG, part of Springer Nature pp.31-132.
[3] Topçu G., Kuşman T., (2014). Lamiaceae Family Plants as a Potential Anticholinesterase Source in the Treatment of Alzheimer's Disease, Bezmialem Science, 2, 1-25.

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PLENARY LECTURE

ELEPHANT TRUNK, BIRDS NEST, CAT WHISKERS AND FATİMA'S DAGGER: CAN NATURAL PRODUCTS FROM MALAYSIA TREAT DIABETES MELLITUS?

Mohammad Rais Mustafa

Department of Pharmacology, Faculty of Medicine and Centre for Natural Products Research and Drug Discovery (CENAR), University of Malaya, 50603, Kuala Lumpur, Malaysia.

Natural products are naturally derived metabolites and/or by-products from microorganisms, plants, or animals. These products have been exploited for human use for thousands of years continuously providing a broad and structurally diverse arsenal of pharmacologically active compounds that have been effectively utilised in the treatment of numerous diseases. More than 45 % of all marketed drugs in the world are natural products or their derivatives. Medicinal use of natural products in the treatment and prevention of diseases including diabetes has a long history compared to conventional medicine. Diabetes mellitus is one of the major public health concerns all over the world including Malaysia. Malaysia has the highest rate of diabetes in Asia and this prevalence is projected to rise to 21.6% by the year 2020. Most of diabetic patients are affected by type 2 diabetes with hyperglycemia, insulin resistance and insulin secretion defect. Despite the greater awareness and increasing number of drugs available, diabetes continues to be poorly treated with many patients succumbing to serious complications such as kidney failure, vision loss, heart diseases, and foot amputations. Natural products including herbal medicines are commonly used in Malaysia as a form of complementary and alternative therapy for the treatment of diabetes and its complications. This is attributed to Malaysia being one of the ten mega biodiversity countries in the world and having a mixed population of Malays, Chinese, Indians and indigenous tribes, each with its own ethnic health system. This talk will highlight local practices using natural products including elephant trunk (*Clinacanthus nutans*), birds nest (*Aerodramus fuciphagus*), cat whiskers (*Orthosiphon stamineus*) and Fatima's dagger (*Marantodes pumilum)* to treat diabetes and similar scientific efforts being undertaken by researchers from the Centre for Natural Products Research and Drug Discovery (CENAR) at the University of Malaya.

Keywords: Natural products, diabetes, *Clinacanthus nutans*, *Aerodramus fuciphagus*, *Orthosiphon stamineus, Marantodes pumilum*

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INVITED LECTURE

COMBINATION IS THE KEY – ANALYTICAL AND BIOLOGICAL INVESTIGATIONS IN PHYTOPHARMACY, PHYTOCOSMETICS AND PHYTONUTRITION

Thomas A.E. Jakschitz[1]; Günther K. Bonn[1,2]

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Phyto-sciences are playing an increasingly important role, not only because of scientific interest, but also because the strongly growing demand for nature-derived products in pharma, cosmetics and nutrition.

Compared with synthetic formulations, it is a big issue to generate effective and safe products as one has to deal with hundreds of different compounds in highly diverse concentrations. For identification of active compounds in these multi-component mixtures multiple different extracts have to be investigated by analytical technologies and biological assays to enable bioinformatic investigations with meaningful results. Once the active compounds are identified, the extraction procedure has to be optimized in terms of increasing the yield of active ingredients and eliminating the yield of disturbing/toxic compounds. The next hurdle is the production of sufficient quantities of plant material of appropriate quality. Especially in wild collections, the concentrations of the ingredients usually vary greatly, making the production of high-quality products significantly more difficult. The solution to this problem is controlled cultivation of optimized seedlings under optimized conditions like soil-composition, temperature and altitude.

This brief overview clearly figures out the importance of combined technologies at the very different development stages from an idea to an effective and safe product.

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INVITED LECTURE

SOME INVESTIGATIONS ON THE MEDICINAL PLANTS GROWING IN NORTHERN CYPRUS

Filiz Meriçli

Department of Phytotherapy, Faculty of Pharmacy, Near East University, Nicosia, Turkish Republic of Cyprus, filiz.mericli@neu.edu.tr

Objective / Purpose: Cyprus, the third largest island of the Mediterranean Sea; is located at the crossroads between Asia, Africa and Europe. More than 1800 plants are growing wildly in Northern Cyprus, and 22 of them are endemic plants. Folk remedies and medicinal plants of Northern Cyprus are investigated by the scientists working in Faculty of Pharmacy at Near East University. The purpose of these researchs is to evaluate the medicinal plants grown on Northern Cyprus in pharmaceutical sciences, to develop the medicinal products to be patented and to gain economic value.

Material and Methods: Fatty oils of seeds of *Prunus dulcis* and *Opuntia ficus indica* were collected from different place of Northern Cyprus; obtained using supercritical CO2 extraction method, and fatty acids were determined by GC-MS. Alkaloid content of *Colchicum pusillum* samples roots and flowers were determined by HPLC. Cladodes and fruits of *Opuntia ficus indica* were collected from Güzelyurt and phenolic compounds were analyzed by HPLC. Anticancer activities of these plants were investigated on human colon carcinoma or breast cancer cell lines. Volatile oil of *Crataegus azarolus* were investigated by GC-MS; phenolic compounds were determined by HPLC.

Results: Our results indicated that all of these plant materials can be evaluated in pharmaceutical sciences for human health; further investigations on developing the medicinal products are still going on.

Conclusion / Discussion: According to the results to develop the medicinal products to be patented has been stil going on.

Keywords: *Prunus dulcis*, *Opuntia ficus indica*, *Colchicum pusillum*, *Crataegus azarolus*, Northern Cyprus, anticancer activity.

References:

[1] Eda Becer, Duygu Yiğit Hanoğlu, Hilal Kabadayı et al, (2018). The effect of *Colchicum pusillum* in humon colon cancer cells via Wnt/β-catenin pathway, *Gene*, *686*, 213-219.

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INVITED LECTURE

EVIDENCE-BASED AFRICAN NATURAL MEDICINES - EXPLORING THE SYNERGY BETWEEN ANCIENT WISDOM AND MODERN SCIENCE

Alvaro Viljoen

Department of Pharmaceutical Sciences and SAMRC Herbal Drugs Research Unit, Tshwane University of Technology, Pretoria, 175 Nelson Mandela Drive, Private Bag X680, Pretoria, 0001, South Africa

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

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INVITED LECTURE

NEUROPROTECTIVE BEHAVIOR FOR A HEALTHY BRAIN

Monica Hancianu, Oana Cioanca, Ursula Stanescu

Department of Pharmacognosy, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania

Objective: Reactive oxygen species (ROS) and oxidative stress represent key factor for multiple health problems such as metabolic syndrome, neurodegeneration and cancer. The negative impact is expressed as lipid peroxidation, protein misfolding, DNA damage and mitochondrial dysfunction which lead to cell death. Neuroprotection is a major strategy for brain health and may include natural molecules with antioxidant potential. Often, neuronal alteration, subtle cognitive and behavioral changes occur long before the clinical stage of dementia symptoms. Newer trends in neuroscience imply that depression and dementia might have a common cause or they might be linked. It is thought that earlier-onset depressive symptoms are an indicator for higher risk of developing dementia later in life.

Material and Methods: Our study briefly presents the neuroprotective potential of several selective extracts and herbal compounds against ROS and on scopolamine-induced anxiety and depression animal models.

Results: Our results indicated that all of the extracts had a notable antidepressant and anxiolytic activity which lead to better memory and learning abilities for the tested rats. The intensity of the behavior changes and the antioxidant value depends on the concentration and the type of the substances included in the extracts.

Conclusion: All in all, the obtained results are suggesting the importance of herbs for neuroprotection; a moderate daily amount could possible help against oxidative damage. Diet comes most at hand when the most risk factors cannot be influenced (genes, age, sex), by adopting neuroprotective behaviors we can maintain the neuronal plasticity of our brains.

Keywords: Neuroprotection, depression, anxiety, oxidative stress

References:

[1] Singh-Manoux, A., Dugravot, A., Fournier, A., Abell, J., Ebmeier, K., Kivimaki, M., & Sabia, S. (2017). Trajectories of depressive symptoms before diagnosis of dementia: a 28-year follow-up study. *JAMA Psychiatry*, 74(7), 712-718.

MES

INVITED LECTURE

CAN WE PROTECT OUR BRAIN FROM NEUROTOXICITY BY NATURAL PRODUCTS?

Noureddine Djebli

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Neurotoxicity is the damage to the brain and/or the peripheral nervous system from toxic chemicals. These chemicals include insecticides, solvents, lead, mercury, cadmium, car exhaust, formaldehyde, chlorine, phenol and thousands of others. Our environment has become polluted with large doses of these. It is difficult for the individual to avoid exposure. Symptoms for the brain toxicity are short term memory loss, loss of circulation, imbalance, and flu-like symptoms.

Researchers believe that there may be a link between neurotoxicity and progressive neurodegenerative disorders such as dementia, Parkinson’s disease, amyotrophic lateral sclerosis, and multiple sclerosis. There’s no doubt that we are living in a sea of neurotoxins, and it’s not possible to avoid them all. But what you can do instead is reduce the total load — the total number of neurotoxic “burdens.” The attentions of researchers have been inclined towards the phytochemicals, many of which have minimal side effects. Phytochemicals are promising therapeutic agents because many phytochemicals have anti-inflammatory, antioxidative as well as anticholinesterase activities. Various drugs of either synthetic or natural origin applied in the treatment of brain disorders

The aim of this lectur is to evaluated the some of the medicinal plants and their active constituents that have been used in different methods induced neurotoxicity.

Keywords: Neurotoxic, heavy metals, neurodegeneratives diseases, medicinal plants, mice.

INVITED LECTURE

BIOLOGICAL POTENTIAL OF COMPOUNDS ISOLATED FROM ALGERIAN PLANTS

Zahia Kabouche

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Algeria is characterized by a rich flora. Caryophylaceae, Amaranthaceae, Fabaceae, Euphorbiaceae and Pinaceae have been the subject of our studies. Flavonoids and terpenoids were the principal secondary metabolites isolated from the selected species. Antioxidant, antimicrobial, anti-inflammatory, anti-tyrosinase and cytotoxic activities of the isolated compounds have been established.

Keywords: Caryophylaceae, Amaranthaceae, Fabaceae, Euphorbiaceae, Flavonoids, Terpenoids, Biological activities.

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INVITED LECTURE

SYNTHESIS OF PLANT BASED HYBRID NANOFLOWERS AND EXPLORATION IN NOVEL DRUG DISCOVERY

Ufuk Koca-Çalışkan

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Objective/Purpose: Organic–inorganic hybrid nanoflowers have received much attention due to their simple, fast and green synthesis, large surface-to-volume ratio, high efficiency, and enzyme stabilizing abilities [1-2]. Zare and co-workers reported protein-inorganic hybrid nanoflowers initially that was made of proteins (BSA, laccase etc.) and $Cu_3(PO_4)_2.3H_2O$ [1], further, different kinds of organic molecules (such as protein, DNA, alginate etc.,) and ions (Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, etc.,) containing hybrid nanoflowers have been subsequently reported. In this review study, synthesis of novel hybrid nanoflowers exploiting plant extracts and copper (II) ions in order apply as enzyme inhibitor agents were given.

Material and Methods: Medicinal plants (*Achillea wilhelmsii*, *Ajuga chamaepitys*, *Phlomis grandiflora*, *Rosa canina*, *Solanum melongena)* that are grown in Turkey, have traditional applications against different diseases were collected and extracted according to their ethnopharmacological usage. The dried plant extracts in different concentratios were used as an organic component to synthesis hybrid nanoflowers All the organic-inorganic hybrid structures were synthesized with the collaboration of Dr. Nalan Özdemir laboratory (Erciyes University Kayseri/Turkey). The morphologies of the synthesized nanoflowers were examined using Scanning Electron Microscopy (SEM), the chemical and crystal structures of them were characterized using Fourier Transform Infrared Spectroscopy (FTIR,) and X-Ray Diffraction (XRD) analysis.

Results: Synthesized nanoflowers were analyzed with different physicochemical techniques. According to analyzed SEM images the concentration of the best shaped nanoflowers were selected for further antioxidant and enzyme inhibitory analysis. The data demonstrated that some of the hybrid nanoflowers (*Achillea wilhelmsii*, *Rosa canina*) have better antioxidant activity than the plain extracts. Moreover, some of the hybrid nanoflowers (such as *Achillea wilhelmsii*, *Ajuga chamaepitys*, *Phlomis grandiflora*) demonstrated better alfa-glicosidase inhibition acitivity, whereas some nanoflowers (such as *Ajuga chamaepitys*, *Phlomis grandiflora* and *Rosa canina*) alfa-amilase anzyme inhibitory activity compared to plain extracts.

Conclusion/Discussion: The synthesis of hybrid nanoflowers containing plant extracts is an important factor considering its suitability for commercial applications in pharmacy, cosmetics and various applications in industry.

Keywords: Nano flowers, hybrid nanoflowers, plant extracts, enzyme inhibition,

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INVITED LECTURE

PROPAGATION, IMPROVEMENT, PRESERVATION AND ENHANCEMENT OF FOREST SPECIES IN TUNISIA

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Abstract

The Republic of Tunisia, located in northern Africa, faces various environmental challenges caused by anthropogenic practices such as overgrazing, deforestation, and desertification. The conversion of natural ecosystems is the major cause of plant biodiversity loss.

Because of this great environmental diversity, there are distinctive vegetation and various genetic resources in Tunisia. However, these genetic resources are endangered and the risk of degradation due to various socio-economic and industrial (deforestation, wood industries and paper, ...) as well as environmental and climatic stresses (drought, salinity, erosion, ...) and it is therefore essential, to preserve in order to exploit them effectively and sustainably. The conservation and exploitation of these resources must be done in a rational and long term. This research was conducted to investigate propagation, improvement and preservation of forest species and aromatic and medicinal plants. The objective of this work is to achieve:
- The conservation of forest species and medicinal plants by *in vitro* and *ex vitro* techniques. This is to prevent them of degradation and erosion of forest genetic resources but also to maintain the adaptive potential of species or populations in long term.
- The recovery of forest species and medicinal use and exploitation by their essential oil to identify the bioactive substances of agronomic interest (ie biological herbicides and pesticides) and pharmaceuticals (phytotherapy, synthesis of new antibiotics and anticancer drugs ...).

The results show that the techniques of *in vitro* culture improve the rate of propagation of threatened forest species and also allow their conservation and solve the problem of genetic conformity. Similarly, the use of essential oils and fixed natural resources could contribute economically to solve several problems
The results show that the techniques of in vitro culture significantly improve the rate of propagation of the forest species (*Arbustus*, *argania spinosa*, *cupresus*, *pinus*, …) and also allow their conservation and solve the problem of genetic conformity. Similarly, the use of essential oils and fixed natural resources could contribute economically to solve several problems.

Keywords: Propagation, improvement, preservation, enhancement, forest species, *in vitro* culture, essential oil, antibacterial activity.

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ORAL PRESENTATIONS

ORAL PRESENTATION

IN VITRO SALVIA GLUTINOSA BIOLOGICAL EFFECTS DEPEND ON EXTRACTION PROCEDURE AND CELL TYPE

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Objective/Purpose: Cytotoxicity, antibacterial and *in vitro* immune effects were investigated for aqueous (tea and cooked) and methanol extracts of *Salvia glutinosa* in three different *in vitro* cellular systems: fibroblast, tumor and stem cell lines, bacteria and mononuclear blood cells.

Material and Methods: The herb of *Salvia glutenosa* was subject to extraction with cold and hot water and methanol, respectively. The extracts were tested for cytotoxicity in HS fibroblasts cell line and B16F10 cancer cell line systems and palatal stem cells, in ATCC and clinical *E. coli* and *Pseudomonas* isolates and also in whole blood cultures from vaccinated bovine. The antioxidant capacity was determined by assessing free radical scavenging effect over 1,1-diphenyl l-2-picrylhydrazyl (DPPH) radical for all extracts in duplicate.

Results: The antioxidant activity was the highest in cooked extract (87.402%), while the metanolic extracts were less active (83.64%). The cytotoxicity was the lowest in the tea treated cells, with a VTI% of 101.853 at a dosage of 0.95 µl on HS cells and of 102.580 at a dosage of 7.5 µl on B16F10 cell line. No antibacterial effects were observed in any of the extracts against the used bacterial strains. While the metanolic sage extract was the most active in bovine blood cultures at the beginning of the experiment, the aqueous extracts were highly immune stimulating for the mononuclear blood cells 14 days post vaccination.

Conclusion / Discussion: The *in vitro* biological effects of *Salvia glutinosa* were dependant on the extraction procedure, radical scavenging activity and solvent, but also the cell type used, showing no cytotoxicity and antibacterial activities but with strong immune enhancing effect.

Keywords: *Salvia glutinosa* extracts, cell-lines, antioxidant, immune stimulating, antibacterial

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MESMAP-5 PROCEEDINGS BOOK

ORAL PRESENTATION

IN VITRO ANTIOXIDANT PROPERTIES OF EXTRACTS AND NANOPARTICLES OBTAINED FROM *EQUISETUM* SP.

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Objective: Vegetal extracts contain antioxidant compounds able to block reactive oxygen species, metals involved in oxidative processes or enzymes that catalyze oxidative processes. In our study we evaluated the antioxidant properties of extracts from *Equisetum species* and for silver nanoparticles obtained from these.

Material and Methods: We analysed ethanol and water extracts obtained from *Equisetum pratense*, *Equisetum sylvaticum* and *Equisetum telmateia*. *In vitro* antioxidant properties have been evaluated by lipoxygenase inhibition test, iron chelating test, hydroxyl scavenging and superoxide scavenging tests [2]. Silver nanoparticles (AgNPs) have been prepared using extracts and AgNO3 (1:10) [1]. The synthesis of AgNPs has been evaluated by UV-Vis spectrophotometry.

Results: Organic compounds especially polyphenols from extracts react with silver to form AgNPs that modify the wavelength of maximum absorption to 420 nm. Antioxidant properties of extracts and nanoparticles depend on the chemical composition of these. On lipoxyenase inhibition test ethanol extracts were more active than water extracts. The *Equisetum sylvaticum* ethanol extract was the most active (EC50 = 19.17±0.80 μg/mL). AgNPs are at least twice more active than extracts, so on lipoxygenase inhibition test for *E. sylvaticum*-AgNPs the value of EC50 decreased to 8.98±0.49 μg/mL.

Conclusion / Discussion: Polyphenols presented in water and methanol extracts react with sliver and form AgNPs that improves the biological properties of extracts. Excepting superoxide scavenging test, extracts from *E. sylvaticum* presented the most important antioxidant properties.

Keywords: antioxidant, *Equisetum species*, nanoparticles, silver

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ORAL PRESENTATION

ETHNO–BOTANICAL STUDIES OF PLANTS USED BY TRIBES IN MAHARASHTRA FOR TREATMENT OF KIDNEY STONES

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Objective/Purpose: A renal calculi or kidney stones are becoming one of the most prevalent life styles diseases globally. Treatment of kidney stones is expensive and there is also recurrence of the same. Kidney being a complex organ needs to be treated carefully otherwise it results in irreversible kidney damage. A survey in Raigad region of Maharashtra revealed that the tribal communities have been successfully using plants and their extracts for dissolution of the kidney stones. A survey was conducted to collect information on the plants being used by these tribes and the tribal medical Practitioners.

Material and Methods: A detailed survey was conducted in Raigad district of Maharashtra by meeting and observing the tribal community and local medical practitioners to learn about the plants and plant parts which were being used by them to treat patients with kidney stones. Patients who were treated and were relieved of kidney stones were also interviewed. A plant based system using cystoliths of Ficus *elastica* leaves and raphides of *Colocasia* petiole has been developed to demonstrate the efficacy of the plants used by the tribals and the local medical practitioners. Phytochemical analysis of the extracts of *Tectona grandis* fruits and *Bauhinia purpurea* leaves was done using standard methods.

Results: The tribal community in Raigad used several plants for successful treatment of kidney stones. In this research phytochemical analysis of extracts of fruits of *Tectona grandis* and *Bauhinia pupurea* leaves revealed that major components were flavonoids, saponins and phytosterols. These extract had effective lithotropic activity as revealed by the test developed in our laboratory.

Conclusion: Aqueous extracts of *Tectona grandis* fruits and *Bauhinia purpurea* leaves were effective in dissolving calcium oxalate and calcium carbonate crystals.

Keywords: Kidney stones, Lithotropic, *Tectona grandis* fruits, *Bauhinia purpurea* leaves.

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ORAL PRESENTATION

CONIFERS IN THE CONQUEST OF CANCER

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Objective / Purpose: Cancer is a global disease, causing millions of deaths every year. Along with the existing anticancer drugs, identification of new antiproliferative agents is necessary for patients in poor general condition who cannot tolerate conventional cytostatic drugs or in cases of resistance to the available chemotherapeutics. This study is aimed at identification of plant species, producing metabolites with high antiproliferative activities and with potential application as sources of lead compounds for drug discovery.

Material and Methods: *Juniperus sabina* var. *balkanensis* was delivered from its natural habitat in the Rhodope Mountains.[i] Other conifer representatives and *Buxus sempervirens* were from the Arboretum of the University of Forestry, Sofia, Bulgaria. *Sequoiadendron giganteum, Ruta graveolens, Betula pendula, Inula helenium* were collected from the Botanic garden area of the Bulgarian Academy of Sciences. *Rhodiola rosea* was of commercial origin. Antiproliferative activities of the extracts were evaluated by their half-maximum growth-inhibitory concentrations (IC_{50}), derived from the dose-response curves of the MTT-assay after treatment of NB4 cells with the corresponding plant extracts, as it was described previously.

Results: In the group of studied plant species, the best antiproliferative activities were determined after treatment of NB4 (acute promyelocytic leukemia) cells with the leaves extracts of *Juniperus sabina* var. *balkanensis* R. P. Adams & A. N. Tashev (IC_{50}=0.3±0.03 microgram/ml) and *Sequoiadendron giganteum* (Lindl.) J. Buchh. (IC_{50}=0.9±0.1 microgram/ml) in comparison with less active leaves extracts of other conifers *Taxus baccata* L., *Cupressus arizonica* Greene, *Chamaecyparis lawsoniana* Parl., *Chamaecyparis pisifera* (Siebold & Zucc.) Endl, and other medicinal plant species *Buxus sempervirens* L. (leaves), *Ruta graveolens* L. (leaves), *Inula helenium* L. (roots), *Betula pendula* Roth (cortex), *Rhodiola rosea* L. (rhizomes). Podophyllotoxin and other lignans were identified in the *J. sabina* var. *balkanensis* extract by UPLC/HRMS. Identification of *S. giganteum* metabolites is in progress. While *S. giganteum* (giant sequoia) belongs to the endangered plant species and its metabolites are useful as lead compounds for design of anticancer chemotypes, *J. sabina* varieties are easy for cultivation and feasible for industrial exploitation.

Conclusion / Discussion: The leaves extracts of *Juniperus sabina* var. *balkanensis* and *Sequoiadendron giganteum* were identified as new natural agents with superior anticancer activities in leukemia cells. Extension of their antiproliferative properties in other types of cancer cells is envisaged in the near future.

Keywords: Anticancer conifers, *Juniperus sabina* var. *balkanensis*, *Sequoiadendron giganteum*

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ORAL PRESENTATION

CURCUMIN, A POLYPHENOL DERIVED FROM *CURCUMA LONGA*: CLINICAL EVIDENCE AND MOLECULAR MECHANISMS IN PREVENTION AND MANAGEMENT OF CANCER

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Background and Purpose: Cancer is the second leading cause of death globally, and it has been a major health problem in the past decades. Several strategies are involved in treatment and prevention of cancer cases such as chemotherapy, immune therapy, radiation, hormone therapy, targeted therapy and herbal therapy. Among them, herbal therapy with medicinal and aromatic plants has been gained great importance in recent years. Medicinal plants and their bioactive compounds may offer clinically useful anticancer agents, due to their rich anticarcinogenic and chemoprotective potentials.

Curcumin, the rhizome of Curcuma longa, is widely used in therapeutic purposes because of its several biological properties such as anti-inflammatory, anticancer, antioxidant, and etc. From this point of view, this review is aimed to comprehensively highlight the effects of curcumin for the prevention and treatment of cancer. Methods: This report reviews the pharmacological and anticancer effects of curcumin with a focus on its molecular mechanisms in prevention and management of various cancer cases. For this purpose, electronic databases, including PubMed, Scopus, ScienceDirect, Cochrane library, Google Scholar and MedlinePlus, etc. were searched to summarize *in vitro*, *in vivo* and clinical studies on anticancer effects of *Curcuma longa* and curcumin, polyphenolic bioactive compound of the plant up-to-date.

Results and Conclusion: To sum up, curcumin, the active ingredient of turmeric and exerts remarkable therapeutic potential for the treatment of various types of cancers. Curcumin is strong anti-oxidant and anti-inflammatory properties and therefore it possesses anticancer potentials through various molecular mechanisms. Based on documented data this natural compound could be a key source of anticancer agent in modern anticancer therapy after clinical confirmation.

Key Words: *Curcuma longa*; curcumin; anti-cancer agents; molecular mechanism; natural products; medicinal plants.

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ORAL PRESENTATION

BLUE ESSENTIAL OIL OF CHAMOMILE VARIETY "*LIANKA*" WITH THE HIGHEST CONTENT OF /-/-α-BISABOLOL AND ITS IMPORTANCE TO NEW PHARMACEUTICAL FORMULATIONS

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Objective/Purpose: Based on the study of pharmacodynamics properties, the chamomile variety "*LIANKA*" was bred at the University of Presov, Slovakia, between the years 2008 – 2013. Currently, this variety obtains the Certificate by the Community Plant Variety Office (CPVO) in Angers, France (in 2018).

Material and Methods: 5 samples of German Chamomile, diploid variety "*LIANKA*", from the small-cultivation in Trebisov, the Eastern Slovakia (2018), with weight of 10 g was grounded in a blender. The essential oil was prepared by hydro-distillation in Clevenger-type apparatus according to the EU Pharm. The GC/MS analyze were carried out on a Varian 450-GC connected with a Varian 220-MS. Identification of components was made by comparison of their mass spectra with those stored in NIST 14. Kovat's samples (C-5 to C-22 alkane mixture) injected during sample analyze and Kovat's indices calculated from retention time using a third order polynomial. 40 reference authentic compounds (Extrasynthese, Merck, Sigma and Roth) were purchased. Many authenticated from MS data or retention index were compared by literature (Adams, 2007).

Results: The GC/MS results confirm earlier reports (Salamon et al., 2016) that major volatile constituents obtained from the chamomile anthodia were /-/-α-bisabolol and chamazulene. The selected variety, environmental conditions, agronomic management practice and effective distillation (Salamon, 2007) were obtained of substantial contents of /-/-α-bisabolol (69.87 ±2.1%) and chamazulene (10.69 ±0.5%) and the low contents of /-/-α bisabololoxides A and B (< 3 %). The blue essential oil content was ranged from 0.65 to 0.85 % in order to time of single flower harvests. /-/-α-bisabolol is the most important component of the blue chamomile essential oil, also known as levomenol, chemically is a natural monocyclic sesquiterpene alcohol. The chemist Sorm and his research team isolated and discovered the /-/-α-bisabolol in chamomile essential oil in 1951. The isopropylindene structure of natural /-/-α-bisabolol was proved through infrared (IR) and nuclear magnetic resonance spectra (Franke and Schilcher, 2005).

Conclusion/Discussion: Formulation several liquid and ointment preparations (Schilcher, 2004) were standardized to contain essential oil, high contents of /-/-α-bisabolol and chamazulene from flowers. These formulations are used on inflammations of the gums and oral cavity, rectum and urogenital region, and other mucous membranes. The formulations are also used for skin baths and enemas and as aides in curing bronchitis, stomach ulcers, digestive disorders, wounds, and dermatitis. The chamomile variety "*LIANKA*" and its large-scale cultivation, extraction and distillation is very forward raw-material to the pharmaceutical industry in the world.

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ORAL PRESENATION

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OILS OF BULBS AND BULBILS FROM *ALLIUM SATIVUM* L.

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Objective: This work aims to study in vitro, antioxidant, antibacterial, antifungal and cytotoxic activities of essential oils of bulbs and bulbils from garlic (*Allium sativum*).

Material and Methods: The chemical composition of these essential oils extracted by conventional hydrodistillation method was analysed using GC/MS. The antimicrobial activity was assessed against bacterial and fungal strains clinically significant and the antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Also, the in vitro cytotoxicity of the bulbs essential oil was evaluated on human cancer cell lines, the HeLa cell line derived from cervical carcinoma from a 31-year-old female [ATCC® CCL-2™, ECACC, Sigma Aldrich, Saint-Quentin Fallavier, France] and the MDA-MB-231 cell line derived from human breast adenocarcinoma [ATCC® HTB-26™, ECACC, Sigma Aldrich, Saint-Quentin Fallavier, France] by the UptiBlue Viable Cell Counting Assay.

Results: A total of twenty-three compounds were identified and the diallyl disulfide, methyl allyl trisulfide and diallyl trisulfide were found to be the predominant compounds for both essential oils. These oils showed respectively potential DPPH radical scavenging activity with IC_{50} (concentration providing 50% inhibition) values of 1.87 and 2.85 mg/ml for bulbs and bulbils. Similarly, antimicrobial activity showed moderate to potent broad spectrum activities. However, the strains *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Trichophyton violaceum and Epidermophyton flocosum* proved to be the most sensitive. Moreover, after 48 hours of incubation, the oil tested proved to be potent against both cell lines with a mortality of almost 100% at a concentration of 0.05%, the HeLa cell line appears more sensitive with a much lower viability at 50% for a concentration of 0.005%.

Conclusion: Based on these results, further experiments will be carried out on this golden vegetable in order to use it as an alternative product in food and pharmaceutical proposes.

Keywords: Garlic essential oil, GC-MS, Antioxidant activity, Antimicrobial activity, Cytotoxicity.

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ORAL PRESENATION

SEMI-SYNTHESIS OF BIOACTIVE GYPSOGENIN DERIVATIVES

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Objective / Purpose: Gypsophila species have been used for the traditional Chinese medicine. Gypsophila is well known to contain saponins. Saponins are glycosides which are found in many plants. These saponins have variety of biological activities including, antimicrobial, antifungal, antitumor, antioxidant, cytotoxic activity. In this study, gypsogenin aglycone which is a natural saponin was obtained from the roots of *Gypsophila arostii*. Gypsogenin and synthesised compounds were evaluated for activity. We synthesized some bioactive compouns.

Material and Method: We synthesized new gypsogenin derivatives (**1-9**). Purification has been carried out using chromatographic methods (Column Chromatography).

Results: Synthesized compounds (**1-9**) was determined by IR, UV, ^{1}H NMR, APT and LCMS analysis.

Conclusion / Discussion: Gypsogenin and synthesised compounds were evaluated for antimicrobial and cytotoxic activities.

Keywords: Gypsogenin, semi synthesis.

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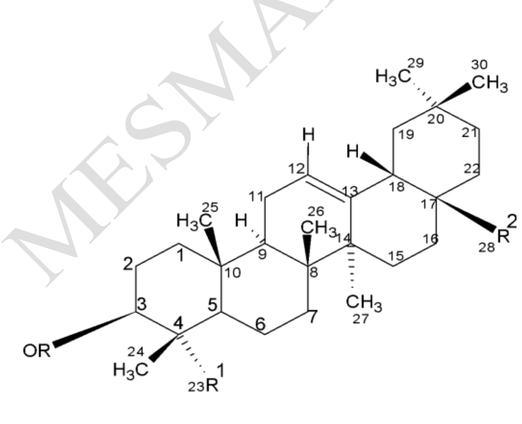

	R	R^1	R^2
Gypsogenin	-OH	-COH	-COOH
Compound 1	-OH	-CH=N-OH	-COOH
Compound 2	-OCO-CH_3	-CHO	-COOH
Compound 3	-OH	-CHO	-CO-OCH$_2$-Ph
Compound 4	-OCO-CH_3	-CH=N-OH	-COOH
Compound 5	-OCO-CH_3	-CH=N-NH-CS-NH_2	-COOH
Compound 6	-OH	-CH=N-OH	-CO-OCH$_2$-Ph
Compound 7	-OH	-CH=N-NH-CS-NH_2	-CO-OCH$_2$-Ph
Compound 8	-OCO-CH_3	-CHO	-CO-OCH$_2$-Ph
Compound 9	-OCO-CH_3	-CH=N-OH	-CO-OCH$_2$-Ph

ORAL PRESENTATION

INFLUENCE OF AN AQUEOUS MINT (*MENTHA PIPERITA*) EXTRACT ON HEALTH STATUS AND IMMUNE RESPONSE IN YOUNG CALVES

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Objective/Purpose: The susceptibility to diseases in calves is increased during the neonatal period, thus natural products, such as plant extracts with increased bioavailability and less side effects could help in boosting immunity. *Mentha piperita*, an ubiquitous plant, with considerable antimicrobial activity, could serve for this purpose.

Material and Methods: Three equal groups of calves (n=7), with homogenous age within the group (15-25 days), were subjected to plane milk (I) or milk plus 200 ml(II) or 400 ml (III) of mint tea treatment for 10 days. The clinical health status of animals was monitored daily and blood was sampled on heparin (50 IU) on days 0 and 10. Blast transformation of mononuclear cells was quantified by a micromethod. Cultural variants included treatment with alcoholic extracts of *M. pipperita, Echinacea angustifolia, E. purpurea, Calendula officinalis, Vaccinium myrtillus, Thymus vulgaris, Symphitum officinale* and propolis (1µl/well). The antioxidant activity of the aqueous extract of mint was determined by assessing free radical scavenging effect over DPPH (%) radical. Excel program was used to evaluate the statistical significance of the treatments' effects by group.

Results: All calves stayed clinically health during the entire experiment. The antioxidant activity of plants rendered from *E. purpurea* being the most to *C. officinalis* the least active antioxidant. The mint tea antioxidant activity was of 76.2%. The blast transformation indices were high in all groups and did not differ significantly, but with higher values for all plant extract treatments in the 200 ml tea treated group.

Conclusion / Discussion: Mint tea treatment somewhat increased the spontaneous blast transformation capacity of mononuclear cells but did not significantly influence the *in vitro* response to other plant extracts.

Keywords: calves, mint tea, antioxidant, alcoholic plant extracts, blast transformation

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ORAL PRESENTATION

INVESTIGATION OF THE CYTOTOXIC, ANTICANCER AND APOPTOTIC DNA FRAGMENTATION PROPERTIES OF *BONGARDIA CHRYSOGONUM* L. EXTRACTS ON BRAIN GLIOMA AND LUNG CARCINOMA CANCER CELLS

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Background and Objective: *Bongardia chrysogonum* L. is commonly known as ‘Uruf-el-Deek’ and locally known as ‘Catlak Otu’ in the Anatolia, where the tubers of this plant have been used for the treatment of prostate hypertrophy, hypercholesterolemia, hemorrhoids, diabetes, epilepsy and hematological malignancies in folk remedy. **Material and Methods:** The purpose of the current study was to investigate potential anticancer and antiproliferative activities of the tubers of *B. chrysogonum* L., extracted with water, ethanol and chloroform solvents, against human lung carcinoma (A549), non-small lung cancer (H1299), glioma (C6) cancer cells, and non-tumorous human umbilical vein endothelial cells (HUVECs). In this context, cell proliferation and cell viability of the cells treated with the plant extracts at various concentration ranged from 10, 25, 50, 100 to 200µg/mL were determined by MTT and trypan blue exclusion assays. The dose response curve was used to generate the IC_{50} (µg/mL) values for each cell line. ANOVA (one way) was used for evaluation statistical differences between the groups, and the experiments were carried out at least triplicate. Based on their cytotoxicity against A549, H1299 and C6 cancer cells, the concentrations of the extracts were selected for *in vitro* micronucleus (MN) assay. Apoptotic activity induced in the plant extracts treated and untreated cancer cell lines were analyzed for DNA fragmentation using the ‘Apoptotic DNA-Ladder Kit’ (QIAamp DNA mini kit, Qiagen), and the release of the enzyme lactate dehydrogenase (LDH) activity was also performed for detection of necrosis in the cultured cells. **Results:** The tuber extracts of *B. chrysogonum* L. exerted significant anticancer and antiproliferative activity towards all treated cancer cells with the IC_{50} values ranged from 8.25±0.01 to 42.05±0.03µg/mL. The methanolic-tuber extract seem to be possessed the strongest anticancer and antiproliferative activities on A549 cells (IC_{50}=8.25±0.01µg/mL at 50µg/mL concentration), whilst chloroform extract of the tuber was found to have the weakest ones on C6 cells. With regard to LDH activity, it was determined that the methanolic-tuber extract lead to higher leakage activity towards C6 cells as compared to that of lung cancer cell lines, in dosage and time dependently. In addition, the tuber extracts of the plant-induced apoptosis was determined in the cancer cells, and multinucleated cells treated with the extracts at different concentration were distinguished as apoptotic or necrotic cells under the microscope. **Conclusions:** As can be seen from the literature survey, the results presented herein could be considered as the first report on probable anticancer and antiproliferative effects of the tuber of *B. chrysogonum* L. However, the data obtained from the current research would be provided valuable information to discover plant-based chemotherapeutics for the cure of cancers, the extracts should be analyzed by using further *in vitro* and *in vivo* techniques for specify the bioactive phytochemicals responsible for this excellent anticancer potential.

Keywords: *Bongardia chrysogonum* L., anticancer, apoptosis, necrosis, DNA fragmentation, micronucleus

Acknowledgments: The author would like to thank to taxonomist Res. Assist. Fatih Yayla for his kind contribution of collecting and identifying the plant material, and Prof. Dr. Nazim Sekeroglu for his kind scientific contribution to the research.

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MESMAP-5 Final Proceedings Book

ORAL PRESENTATION

COMPOUNDS FROM AN AROMATIC ANNUAL PLANT GROWING IN ARID ZONE OF ALGERIA AND SCREENING OF ITS ANTIBACTERIAL ACTIVITY

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The Asteraceae *Matricaria pubescens* (Desf.) is a spontaneous species from arid regions of Algeria. To get its volatile fraction, the aerial parts of this plant were subjected to an hydrodistillation using a cleavenger apparatus. A weak yield (0.1%) was obtained. The sample was analyzed by GC-MS on a non polar capillary column. Among identified components in this volatile composition were; spathuleol (10.59%), decanoic acid (5.03%), trans isoelemicin (4.70%), cadinol (1.59%).

Beside, the purpose of the present study was to continue the exploration of the biological effects of *M. pubescens*. Indeed, the prepared essential oil and its fragrant water have been the subject of an antibacterial investigation. However, most of the microorganisms tested were resistant to the tested samples.

Keywords: GC-MS, volatile composition, bioactivity, spontaneous plant, arid zones.

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ORAL PRESENTATION

IMPACT OF DIFFERENT CUTTING STAGES ON THE CONTENT, CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITE OF ESSENTIAL OILS FROM *SALVIA OFFICINALIS* L.

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Objective: *Salvia officinalis* L. from Lamiaceae family is a worldwide cultivated aromatic herb [1]. Generally, this species has been used herbal tea for a wide range of disorders and illnesses [2]. Also, the essential oils of *S. officinalis* exhibit antioxidant, antimicrobial, antispasmodic, and stimulant properties [3]. The aim of the present study was to investigate the effect of different cutting stages on chemical composition and antibacterial activities of essential oils from *S. officinalis* L. cultivated in Turkey.

Material and Methods: Chemical composition of essential oils obtained by hydro-distillation from the dried aerial parts of *S. officinalis* cultivated in Bolu/Turkey ecological conditions harvested before flowering (BF), 50% of flowering (50%F) and full flowering (FF) stages were determined GC and GC/MS. The disc diffusion assay (Kirby-Bauer Method) was used to screen for antibiotic activity.

Results: The essential oil contents obtained were 0.30% in BF stage, 0.40% in 50%F, and 1.00% in FF stage. α-thujone (28.46%, 36.52% and 48.94%) were found to be the major component of the essential oils from BF, 50%F, and FF stages, respectively. The essential oils exhibited strong antibacterial activity against the microorganisms (*Salmonella typhimurium, Serratia marcescens, Proteus vulgaris, Enterobacter cloacae, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus, Staphylococus epidermidis* and *Escherichia coli*) examined.

Conclusion/Discussion: The highest amount of essential oil and α-thujone content were obtained from the plants which were harvested in FF stages. The contents, chemical compositions and antimicrobial activities of essential oils from *S. officinalis* varied according to cutting stages.

Keywords: Disc diffusion, essential oil, GC/MS analysis, *Salvia officinalis* L.

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ORAL PRESENTATION

MEDICINAL PLANTS AND PHYTOCHEMICALS: A SOURCE FOR GLUT4 STIMULATORS AND DIGESTIVE ENZYMES INHIBITORS

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Diabetes is one of the world's most widespread diseases, which is characterized by persistent hyperglycemia and related to metabolic disorders. It affects over 327 million people and causes about 300,000 deaths annually [1]. Despite great advances in the prevention and therapy, existing treatments for this disorder have serious side effects. Plants used in traditional medicine represent valuable sources in the search for new medicinal compounds. In traditional system of medicine, at least 1200 species of medicinal plants are used for their antidiabetic attributes. However, only a small proportion of such plants have been scientifically validated [2].

The objective of this study was to assess the *in vitro* inhibitory activity of selected local anti-diabetic medicinal plants on carbohydrate and lipid digestive enzymes; namely, α-glucosidase, pancreatic α-amylase, and pancreatic lipase. The inhibitory activities of ethanol: water (50%:50%) herb extracts were evaluated using the enzymatic colorimetric assays. At a concentration of 0.2 mg/ml, among six herbal extracts tested, only *Allium sativum* (bulb) showed an inhibitory activity against the intestinal sucrase (α-glucosidase). *Cinnamomon cassia* (bark) showed an inhibitory activity against lipase. *C. cassia* (bark) and *Olea europaea* (leaves) inhibited pancreatic α-amylase with IC_{50} values of 24±3.01 and 192.94±6.4 µg/mL, respectively. Tens of other local medicinal plants extracts were tested for GLUT4 activity and translocation to the plasma membrane (Fig. 1). These plants include but are not limited to *Abelmoschus esculentus L., Allium cepa* (bulb), *Allium sativum* (bulb), *Asparagus aphyllus L., Atriplex halimus, Cinnamomon cassia, Crataegus azarolus L., Gundelia tournefortii, Nigella sativa* (seeds), *Ocimum Basilicum, Olea Europea, Trigonella foenum-graecum*, *Teucrium polium* and *Urtica dioica*. GC/MS phytochemical analysis for most of these plants are applied and the potential active compounds are discussed (Fig. 2). The most effective plant extracts were selected and are being tested *in vivo* in diabetic mice model. The activity of these plants will be presented and discussed.

Keywords: Phytochemicals, diabetes, GLUT4, α-glucosidase, α-amylase, lipase, GC/MS.

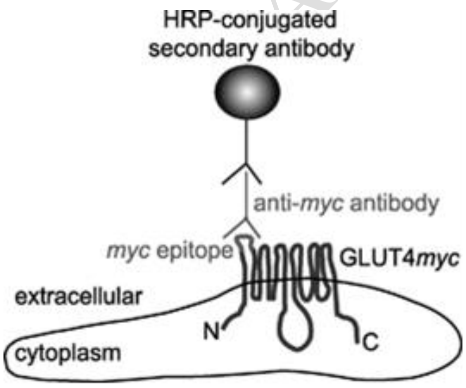

Fig.1. Model demonstrating the detection of the membrane insulin-responsive glucose transporter 4 (stably expressing *myc*-tag) -GLUT4myc [3].

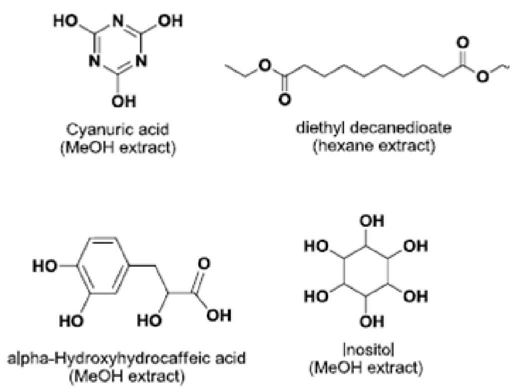

Fig.2. Some of the new detected phytochemicals

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ORAL PRESENTATION

MORPHOLOGY, YIELD AND QUALITY CHARACTERIZATIONS OF *NIGELLA SATIVA* L. AND *NIGELLA DAMASCENA* L.

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Objective/Purpose: *Nigella sativa* and *Nigella damascena* are two annual species of the family Ranunculaceae, used in traditional medicine for centuries for many diseases like cancer, cardiovascular complications, diabetes, asthma, kidney disease etc. The aim of this study is to determination the important agricultural and quality characters of 1 *N. sativa* cultivar (Çameli) and 2 *N. damascena* populations (Ankara and Rize) in Bolu ecological conditions.

Material and Methods: One cultivar of *N. sativa* and two populations of *N. damascena* were used to conduct these studies. The field experiments were performed during two successive seasons (2017 and 2018) at the experimental farm (40°44'44'' N, 31°37'45'' E, 881), which is located at an elevation of approximately 881 m altitude, belonging to Bolu Abant İzzet Baysal University. (Bolu, Turkey).

Results: On average days to 50% seedling and flowering determined as 15.67-18.0 days in 2017, 14.33-16.0 days in 2018, 69.0-75.33 days in 2017, 65.67-75.33 days in 2018 respectively. Seed weights per capsule were 17.11-27.08 g in 2017 and 11.51-12.43 g in 2018, the 1000 seeds weights were between 2.18-2.84 g in 2017 and 2.37-2.77 g in 2018. Mean seed yield in the first year (38.02-60.18 kg/da) were higher than yield in the second year (31.88-37.20 kg/da). The essential oil content of the populations and cultivar was found to be very close to each other with 0.10-0.31%, and essential oil compositions were between 5.68-51.06 %. The mean fixed oil rate in populations and cultivar varied between 20 % and 29%, and fatty acid compositions were between 66.83-91.58%. The major inorganic matters were found as K^+ (177.50-310.5 mg/g) and Ca^{+2} (58.90-128.0 mg/g) and followed by Mg^{+2} and Na^+.

Conclusion: The plant height, the number of branches, the number of capsule and 1000-seed weight of populations varied according to years. Generally, in compared to populations and cultivar the best results were obtained in *N. sativa* cultivar.

Keywords: Morphology, *Nigella* sp., quality, yield

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ORAL PRESENTATION

NATURAL INDUCED RESISTANCE IN CROP PLANTS BY PALESTINIAN INDIGENOUS PLANT EXTRACTS AGAINST PESTS AND DISEASES

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The potential beneficial roles of indigenous plant extract have been emphasized in treatment of various plant diseases and insects Plant and plant-derived products contain antioxidants, anti-inflammatory and repellents; that are thought to provide protection or treatment against many common pests and diseases. Extensive studies are now focusing on the positive role of plant extract derivatives as resistant elicitors thus insect pest and disease control. Negative cross-talk between the jasmonic and salicylic acid response pathways has been shown to increase the level of the plant’s pathogen defenses at the expense of pest defenses. Plant activators are biological or chemical-derived stimulators of plant systemic acquired resistance (SAR) mediated through the salicylic acid (SA) pathway. Examples include plant pathogen *Pseudomonas syringae* and the chemical 1,2,3-benzothiadiazole-7-carbothioate (BTH or the commercial plant activator Actigard 50WG), both of which have been shown to prime the defenses in several crops. The objective is to determine under what circumstances pathogens might be negatively affected by plant SAR inducible defenses. A series of experiments with barley cultivars are compared according to relative growth rate when the inoculated artificially with loose smut pathogen on SAR induced or untreated crops leaves in combination with inhibitors of detoxification enzymes, anti-oxidants and multidrug resistance protein (MRP) transport.

The objectives of this study was to determine the effect of several indigenous plant extracts on the plant crops and evaluate their impact as elicitor for inducing plant resistance against disease.

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ORAL PRESENTATION

INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF SOME PLANTS AGAINST *E. COLI* ISOLATED FROM BLOOD CULTURES

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Objective / Purpose: It is well known that plant-derived antimicrobials are often more efficient than antibiotics, even on resistant pathogens, likely owing to plant compounds synergistic activity [1,2].

Material and Methods: In this study, Plant samples were collected from Konalga village, Çatak/Van city, in the Eastern Anatolia on August 18th, 2018. the ground plant material was mixed with a 20-fold volume of acidified ethanol (80% ethanol, 19.9% H_2O and 0.1% trifluoroacetic acid, v/v/v), shaken for 2 h at room temperature (22°C) and centrifuged for 20 min at 4°C with the supernatant collected. The supernatants from the consecutive extractions were combined. Solvent was evaporated under reduced pressure at 40°C. The derived fraction was dissolved in purified water and freeze-dried under a vacuum at - 51°C to obtain a fine lyophilized powder used for analysis.

Thirty-seven *E. coli* were isolated from the patients in the intensive care unit. Disc diffusion and microdilution method were used to determine the sensitivity of *E. coli* isolates to plant extracts [3]. The plant extracts were dissolved in 12.5% dimethylsulfoxide and then sterile empty antibiotic discs (10 µL each) were absorbed.

Results: According to the disc diffusion test, it was observed that a low sensitivity diameter was formed against the extracts from *Helicrysum pallasii*, *Prangos meliocarpoides*, *Epilobium hirsutum.* In the *Verbascum cheiranthifolium* extract, it was determined that the sensitivity diameter did not occur. According to the microdilution method, *Helicrysum pallasii* showed sensitivity at 1000 to 250 µg/mL, *Prangos meliocarpoides* showed sensitivity at 1000 µg/mL, *Epilobium hirsutum* showed sensitivity at 1000 to 500 µg / mL and *Verbascum cheiranthifolium* showed no sensitivity.

Conclusion: The antimicrobial efficacy of the plant extracts on *E. coli* was found to be different and the antimicrobial effect was reduced in low concentrations.

Keywords: Antimicrobial, plant extracts, *E. coli*, disc diffusion test

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ORAL PRESENTATION

THE ROLE OF MEDICINAL PLANTS USED IN TRADITIONAL MEDICINE FOR DRUG DISCOVERY AND THEIR CONSERVATION IN PAKISTAN

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Pakistan has a unique diversity of medicinal plants due to its geography and diverse environmental and ecological conditions. Medicinal plants are frequently used among the different communities in the world generally and in Pakistan predominantly for treating numerous diseases. Due to recent emerging trends in traditional medicine research and interest to use medicinal plants, a large portion of the medicinal plants has been exhausted, demonstrating a lack of traditional knowledge among communities using the plants. Therefore, an attempt is made in this study to concentrate on the role of medicinal plants used in traditional medicine for drug discovery. Questionnaire using Semi-structured interviews were conducted to list the medicinal uses of plants. Results were analyzed using different quantitative indices including Informant consensus factor (ICF), fidelity level (FL), use value (UV), frequency citation (FC) and relative frequency citation (RFC). In total of 101 plants species were reported for the medicinal purposes. Asteraceae was found to be most dominant family.

The leaves were noted as most frequently used parts (66%). Powder (54%) was the most commonly used preparation method. The highest ICF was reported for Glandular diseases (Diabetes Jaundice, diabetes, tonsils, hepatitis, liver inflammation). Fidelity level varies from 55 % to 97%. Species having high UV were *Cannabis sativa*, *Oxalis corniculata*, while the plant species with the highest RFC were *Ficus palmata*. High use value of medicinal plant should be screened for the pharmacological investigation for bio active phytochemical compounds that are essential for the synthesis of new drugs for numerous diseases. Their conservation is the need of the time for sustainable use and plant biodiversity.

Keywords: Diverse environment, Medicinal plants, Traditional medicine, Quantitative indices and plant biodiversity conservation.

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ORAL PRESENTATION

ESSENTIAL OIL COMPOSITION OF *CENTRANTHUS LONGIFLORUS* STEVEN SUBSP. *LONGIFLORUS* FROM TURKEY

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Objective / Purpose: *Centranthus* DC. (Caprifoliaceae) is represented by three species in the Flora of Turkey (1). *Centranthus longiflorus* Steven subsp. *longiflorus* widely distributes Northern, Southern and Central Anatolia and is used sedative and antispasmodic in the folk medicine and plant leaves are consumed as a spice in Turkey (2,3). The aim of this study is to determine the essential oil composition of aerial parts of *C. longiflorus* subsp. *longiflorus* collected from Tokat province of Turkey.

Material and Metod: Essential oil of *C. longiflorus* subsp. *longiflorus* was hydrodistilled from aerial parts for 3 h using a Clevenger apparatus and analysed by gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS).

Results: The main constituents were identified as *neophytodiene* (19.5 %), *germacrene* D (13.8 %), *phytol* (8.9 %) and *δ-cadinene* (6.9 %) in the essential oil.

Conclusion / Discussion: The present work is the first contribution into the oil composition of *C. longiflorus* subsp. *longiflorus* from Turkey.

Keywords: *Centranthus longiflorus* subsp. *longiflorus*, Caprifoliaceae, essential oil, GC-FID, GC-MS

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ORAL PRESENTATION

INFLUENCE OF ANTIOXIDANT CAPACITY OF PLANT EXTRACTS ON ENHANCEMENT OF ADAPTIVE CELL-MEDIATED RESPONSE IN HERBIVORES

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Objective: This study monitored several alcoholic vegetal extracts from the antioxidant and immune cell stimulating adjuvant perspective in farmed herbivores, differing by their digestive physiology.

Material and Methods: Blood from randomly selected farmed ruminants (Romanian Spotted dairy cows, n=28 and Angora goats, n=19) and Romanian draft horses (n=27) was used to measure the *in vitro* effects on specific cell-mediated reactivity of alcoholic extracts of *Calendula officinalis*, *Echinacea angustifolia* and *E. purpurea*, by the *in vitro* whole blood blast transformation indices (BI%) after 60-72h of incubation. Cell growth was quantified by an orto-toluidine technique. All extracts were tested for their antioxidant capacity (free radical scavenging effect over DPPH (%). Student's t- test was used to evaluate the significance of the differences.

Results: The antioxidant activity of plants rendered as follows: *E. purpurea E. angustifolia* and *C. officinalis*. The *in vitro* growth indices were lowest in goats (*C. officinalis* - 58.52±10.02%, *E. angustifolia* - 50.06±11.67%, *E. purpurea* -50.79±10.98%) and higher in bovine (*C. officinalis* – 69.9±2.65%, *E. angustifolia* - 74.9±10.1%, $p<0.05$), and increased towards *E. angustifolia* versus *C. officinalis*. Similar BI% were obtained for the *Echinacea* species, which differed by their antioxidant capacity.
In vitro responses to *C. officinalis*, *E. angustifolia* and *E. purpurea* were the most pronounced similar for all extracts and independent on their antioxidant activity in horses.

Conclusion / Discussion: All the extracts showed immune-inhibiting effects in bovine and goats, but not in horses, supporting host-based differences. The immunological activity of the tested extracts was rather plant species than antioxidant activity dependent.

Keywords: Ruminants, equine, plant extracts, antioxidant, cell mediated immunity

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ORAL PRESENTATION

BROMELAIN: A POSSIBLE PHYTOREMEDY TOWARDS BREAST CANCER

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Objective: Bromelain is a cysteine protease present in various parts of pineapple (*Ananas comosus*) (L.Merr.). Bromelain has got clinical significance as anti-thrombotic, anti-inflammatory, agent etc. The current work aims at purification and comparative study of Bromelain activity in fruit as well as waste parts (core, crown, skin, and stem) of pineapple and their potential application as anti-cancer agent towards breast cancer.

Materials and methods: The enzyme was extracted and precipitated by ammonium sulphate (40%), dialysed and concentrated by TFF (tangential flow filtration) and further purified using IEX (ion exchange chromatography). The purified fractions were analysed by SDS-PAGE and MALDI ToF to confirm the molecular weight of the eluted protein. The activity of purified enzyme was confirmed by Casein Digestion activity assay as well as Zymography. The presence of a glycan moiety in the bromelain protease was detected using novel proteoglycan staining. The anticancer activity of potential fractions was determined by MTT assay on MCF-7 cell lines.

Results: The molecular weight of the purified enzyme from all parts fractions was 23kDa. The fold of purification for Bromelain proteases in the core, crown, fruit, skin and stem was 5.54, 3.04, 0.41, 12.58 and 18.49 respectively. Statistical analysis suggested specific activity optimum at 45⁰ C temperature while the pH range to be 6.0-7.0. The results of SRB assay state that skin and crown bromelain extracts that are waste parts of pineapple, have GI50 value ≤ 10 making them potential cytotoxic agents towards breast cancer.

Conclusions: From the above study, bromelain protease one of the constituents of bromelain complex from pineapple fruit and its waste parts was purified and recovered with highest activity and purification fold. The potential cytotoxicity on breast cancer cell line MCF-7 was obtained from bromelain present in skin and crown of pineapple which are waste parts. This gives us an opportunity to explore their potential further and harness their efficiency in anticancer drug therapy or adjuvant/targeted drug delivery.

Keywords: Protease, Purification, Zymography, anticancer.

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ORAL PRESENTATION

CHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY AND EFFECT ON HUMAN FIBROBLAST VISIBILITY OF EXTRACTS FROM OLEASTER *(ELAEAGNUS UMBELLATE)* FRUIT COLLECTED IN POLAND.

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Objectives: Oleaster is a plant come originally from Asia, where it is used in traditional medicine. Studies from Asian countries have shown that oleaster fruit extracts have a strong antioxidant activity. We wanted to investigate whether fruits growing in other climatic conditions will also exhibit these properties. The objective of this study was to determine the chemical composition of oleaster fruits grown in central Europe and the redox properties of berry extracts. In order to verify its potential use as medicine or cosmetic, cytotoxicity assays with fibroblast from human foreskin were made.

Methods: The fruit was collected from the farm located in Poland. Berry was analyzed for proximate contents according to AOAC International. Total phenols content was measured by the Folin–Ciocalteu method and mineral content by ICP-AES method.

Then, lyophilized samples were extracted with methanol. The solvent was evaporated and antioxidant activity was measured by DPPH and ferric reducing antioxidant power (FRAP) assays. Different concentrations of extract (1-6mg/ml) were used. As a reference, TROLOX standard curve (20-100µM) was made.

Furthermore, in vitro cell viability analysis was performed. Human normal fibroblast cell (HFFF-2) were incubated with oleaster extract (as 10% of medium). Cell Proliferation Kit II (XTT, Sigma-Aldrich) were made according to manufacturer protocol.

Results: Fresh berry contained 30,94% dried matter, 4,97% crude protein, 2,13% crude fat and 4,88% crude fiber. Proximate content of fruit collected in Poland was similar to fruit analyzed in Turkey [1]. Extracts showed the ability to reduce iron ions. The strength of the reaction was dose-dependent. Lyophilized extracts were an equivalent of 2,32 and 4,08 µM Trolox for 3mg/ml and 6 mg/ml. DPPH assay show 86,91% inhibition at 6 mg/ml concentration. The XTT test showed no negative effect of extracts on human fibroblast cell viability. There was a trend towards increased proliferation in cells receiving oleaster extract (n/s).

Conclusions: The results of the study indicate that oleaster can be a valuable and safe raw material for cosmetics. The use of this fruit can be much wider because fibroblasts are the most common resident cells in ordinary connective tissue.

Keywords: Oleaster, autumn olive, *Elaeagnus umbellate*, antioxidant activity, fibroblast

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ORAL PRESENTATION

WILD PLANTS USED AS FOOD IN EDIRNE CENTRAL DISTRICT AND VILLAGES

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Objective: The aim of this study is to identify the wild plants used as food by local people in Edirne province and villages, their local and Latin names, the parts used, forms of utilization.

Material and Methods: The study is executed in March-October in the years of 2013-2015 in Edirne and surrounding villages. Interviews were carried out face-to-face with the community. In this study, 18 villages of Edirne province were visited and interviews were performed with 26 persons in total. The identification of the plant species those determined to be in use was based on “Flora of Turkey and East Aegean Islands” (1,2). The plant samples identified are kept in Faculty of Pharmacy.

Results: As result of in this study, 41 taxa including in 24 families which are wild plants used as food, were recorded. In the table; the scientific names of the plants, families, local names, usable parts and utilization have been given alphabetically.

Conclusion: Previously, some ethnobotanical studies carried out in Trace (3-8). There is no study about wild plants used as food in Edirne province and villages.

Keywords: Edirne, etnobotany, food, wild plants

Acknowledgement: I thanks to all the villagers of Edirne province, who have collaborated in the realization of this study. The study was carried out with the support of Trakya University (Project 2013/22).

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ORAL PRESENTATION

CORRELATION BETWEEN CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS FROM SIX AROMATIC MEDICINAL PLANTS GROWING IN ILLIZI AND GHARDAÏA (SOUTHERN ALGERIA)

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This study provides an assessment to the relationship between the chemical composition of the essential oils and the antioxidant activity of six aromatic medicinal plants growing in southern Algeria, namely *Cymbopogon schoenanthus*, *Cymbopogon Citratus*, *Artemisia judaica*, Artemisia *herba alba*, *Pituranthos chloranthus and Pituranthos scoparius*. The essential oils were obtained by hydrodistillation and analyzed by GC/MS. Their antioxidant activity was determined by using three methods of testing (DPPH, ABTS and iron reduction test (FRAP)), the results showed that the essential oil of *A. judiaca* has the best antioxidant capacity in all tests. The statistical study allowed us to show a correlation between the chemical composition of the studied essential oils and the antioxidant activity and our results indicate that the high content of cyclic ether sesquiterpenes compounds (such as: Davana ether), the compounds have furan rings, or the existence of non-terpene alkenes/esters in the essential oil, increases its antioxidant effect.

Keywords: *C. schoenanthus*, *C. Citratus*, *A. judaica*, A. *herba alba*, *P. chloranthus*, *P. scoparius*, essential oil, antioxidant activity.

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ORAL PRESENTATION

THYMUS SIPYLEUS BOISS. SUBSP. *ROSULANS* (BORBAS) JALAS EXTRACTS AS ALTERNATIVE THERAPEUTIC AGENTS FOR THE TREATMENT OF SKIN INJURY

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Objective / Purpose: Wound can be described as a disruption in the structural and physiological continuity of skin, as a result of chemical, physical or immunological factors. In recent years, the demand for medicinal plants has increased globally in the management of wound healing, due to their less toxicity and fewer side effects. The extracts from Thymus sipyleus Boiss. subsp. rosulans (Borbas) Jalas (TS) which is an endemic plant for the flora of Turkey, is used for the treatment of skin wounds in Turkish folk medicine. In this study, it was aimed to explain the mechanisms underlying the wound healing activity of this plant and to support the traditional use of TS extracts scientifically.

Material and Methods: The effects of different solvent (ethanol, n-hexane and water) extracts obtained from TS on wound healing were investigated on *in vitro* test systems, in relation to different phases of healing process. The effects of TS extracts on the inflammatory phase were determined by tumour necrosis factor alpha (TNF-α) assay and nitric oxide (NO) assay in RAW 264.7 mouse macrophages. The effects of extracts on proliferative phase were evaluated by MTT and Scratch assays on swiss 3T3 albino mouse fibroblast. Hydroxyproline assay was performed to elucidate the influence of TS extracts on collagen production. Antioxidant activities of extracts were determined by DPPH assay and Folin-Ciocalteu assay. As well as, the cytoprotective effects of extracts against H_2O_2-induced oxidative damage were evaluated in the co-treatment and pre-treatment protocols. Finally, antimicrobial activities of extracts were investigated against the most common bacteria found in skin wounds.

Results: We observed that TS extracts influenced the proliferative and inflammatory phases of healing process by increasing the proliferation and migration of fibroblasts and down-regulating the level of TNF-α and NO. The highest increase in the level of hydroxyproline was determined in cells treated with decoction extract (1.79 to 2.40 fold) at all applied concentrations. In addition, our results showed that TS extracts have antioxidant and antibacterial potential. The percentage of DPPH radical scavenging activities of extracts were ranged from 74.32% to 93.02%, at the highest concentration. Total phenolic compounds of extracts were calculated at the range of 78,15- 147,64 mg GAE/ g. DPPH radical scavenging activities and total phenolic content of n-hexane (non-polar) extracts were too low to be detected. All Thymus extracts exhibited complete protection against H_2O_2 induced oxidative damage on mouse fibroblasts.

Conclusion / Discussion: The extracts from TS accelerated the wound healing process by increasing fibroblast proliferation and migration as well as collagen synthesis and decreasing inflammation. We also concluded that TS extracts have potent effect on the protection of cells from oxidative damage, in relation to the antioxidant activity of plant. Our results indicated that TS can be an effective source to develop a therapeutic agent against skin wounds.

Keywords: Medicinal plants, *Thymus sipyleus* subsp. sipyleus var. rosulans., wound healing

ORAL PRESENTATION

ACUTE AND DERMAL TOXICITY STUDIES OF *ADANSONIA DIGITATA* L. SEEDS OIL ON WISTAR ALBINO RATS

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Introduction: *Adansonia digitata* L. (Baobab tree) of family; Malvaceae is a multi-purpose tree species native to Africa. It is found abundantly in Western parts of Sudan and has been used extensively since ancient times in traditional medicine. Baobab seed oil has quality comparable with those of commercial oil and it has great nutritional and industrial potentials. Thus, research is recommended to validate its efficacy and safety for use.

Objectives: The present work is designed to evaluate the acute oral toxicity of *A. digitata* seeds oil and its observational dermal toxicity.

Methods: Three different doses (50, 250, 500 mg/kg) of the oil were administered to 3 groups of Wistar albino rats for 7 consecutive days. Hematology and biochemical parameters (AST, ALT, ALP, total protein, albumin, cholesterol and urea) values were investigated and compared to control un-treated rats. Dermal toxicity was assessed in another group of animals for 14 days' time.

Results: Oral administration of the oil caused a significant; dose dependent increases in AST, ALT and urea levels compared to the control. Gross pathological studies revealed congested heart and fatty changes in liver and kidney. While, dermal toxicity study revealed no observational signs of toxicity or irritation.

Conclusion: Oil extracted from *Adansonia digitat*a L. seeds is safe to be used externally, however, it showed toxicity signs on oral administration.

Keywords: *Adansonia digitat*a, Baobab oil, acute toxicity.

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ORAL PRESENTATION

DETERMINATION OF HYBRIDIZATION IN *NEPETA* x *TMOLEA* BOISS. USING DIFFERENT APPROACHES

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Objective / Purpose: The main purpose of this study is to examine morphological, micromorphological and molecular features of *Nepeta* *tmolea* Boiss. hybrid specimens and its parents. For this purpose, trichome types and density are especially studied and one nuclear and two chloroplast DNA regions were sequenced, and after that distinct characters were revealed.

Material and Methods: Plant specimens were collected during the flowering stage (2016-2018) from natural habitats in Balıkesir, İzmir, and Muğla. *N.* × *tmolea* and *N. nuda* L. subsp. *nuda* were collected from Balıkesir and İzmir, and *N. viscida* Boiss. were picked up all of these locations. Stems, leaves, calyces, corollas, and nutlets of *N. viscida*, *N. nuda* subsp. *nuda* and their putative hybrid specimens *N.* × *tmolea* were investigated and photographed using a NeoScope JCM scanning electron microscopy. DNA isolations were performed using the DNeasy Plant Mini Kit, and the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) sequences and the rpl32-trnA(UAG) and trnA(Leu)-trnA(Phe) (trnL-F) regions of the chloroplast DNA (cpDNA) were used for molecular analysis. The PCR amplifications were performed using Shaw et al. (2007) protocol. Sequenced DNA was edited using Sequencer version 4.9.

Results: Field observations are very important to find out general characters. *N.* × *tmolea* has some intermediate characters between its parents. According to our findings, *N.* × *tmolea* specimens resembled *N. viscida* as general, they have a dense indumentum structure especially on their stems and leaves, but *N.* × *tmolea* samples were not viscid, and their stem, leaf and corolla colors were quite different from *N. nuda* subsp. *nuda* and *N. viscida*. 16 different individuals were sequenced to define their nrITS DNA data, and 32 different individuals for trnL-F and 15 different individuals for rpl32-trnA were sequenced to obtain these chloroplast DNA data. The most single nucleotide polymorphisms were obtained in nrITS data, but chloroplast DNA data has more ancestral information due to their insertions and deletions.

Conclusion / Discussion: Our data showed that the genetical methods are more compatible do define hybrid specimens and their parents. But, only one region could not be informative, different genome regions should be appropriate. *N.* × *tmolea* possibly has two DNA data from its parents.

Keywords: Hybridization, trichome, molecular, *N.* × *tmolea*.

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Shaw J, Lickey EB, Schilling EE, Small RL (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am J Bot 94: 275-288.

ORAL PRESENTATION

ANTI-FERTILITY AND ABORTIFACIENT EFFECT OF AQUEOUS SPROUTED SEEDS EXTRACT OF *TRIGONELLA FOENUM GRAECUM* (L.) IN MICE.

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Introduction: *Trigonella foenum graecum* or fenugreek is one of the oldest medicinal plants; the sprouts are used as spices and condiment to improve the flavor and the nutritive value of foods. Seeds have been revealed teratogenic and anti-fertilizing as it shown in our previous study carried out in Swiss albino mice (1), reason for why, we were interested in evaluating of sprouted seeds effect in mice reproduction.

Material and methods: Female mice have been treated orally with lyophilized aqueous extract of sprouted organic fenugreek seeds, from the first day of pregnancy until delivery, at doses of 0 (control), 200, 500, 800, 1000 mg/kg/day. Animals were observed for any intoxication symptoms all over the period of pregnancy. Additionally, Pregnancy abortion and deliverance indexes have been determined as follows:

- Pregnancy index: the percentage of pregnant females which have shown a vaginal plug.
- Abortion index: the percentage of the number of aborted offspring over pregnant animals.
- Deliverance index: the percentage of females delivering over pregnant animals.

The main phenolic compounds of lyophilized aqueous extract of germinated fenugreek seeds has been analyzed by high-performance liquid chromatography.

Results: all females survived until study termination, no abnormal clinical symptoms were observed in all groups, at any time during gestation. Oppositely, for the reproduction function, we have noticed a significant decrease of pregnancy index especially at 800 and 1000 mg/kg/day with a 25 % of fertility loss. The abortive effect starts at 500 mg/kg/day with 42% and increases to 66 % at 1000 mg/kg. The deliverance index shows a big decreasing at 800 and 1000 mg/kg where only 33 % of female mice end their gestation period. Spontaneous abortion took place between gestation days 16 and 18. HPLC analysis revealed the presence of gallic acid, caffeic acid, tyrosol, syringic acid and flavonoid rutin.

Discussion and conclusion: this result is important because it shows, for the first time, one of the negative aspects of use of germinated fenugreek seeds. Basing on this finding we could suggest that consummation of sprouted seeds of fenugreek should be avoided during pregnancy.

Keywords: Fenugreek, abortive, anti-fertilizing, sprouted seeds.

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ORAL PRESENTATION

FROM A TRADITIONAL REMEDY TO MODERN THERAPY; *IN VIVO* ANTIHEMORRHOIDAL STUDY OF *MALVA SYLVESTRIS* L.

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Objective / Purpose: It is thought that about 50% of the world's population is suffering from hemorrhoids, an anorectal disease. Patients have tried to cope with the disease with both herbal and chemical products. ***Malva sylvestris*** belongs to Malvaceae family and its common English name is mallow, whereas Turkish name, is 'Ebegümeci' in Turkey. It's leaves have potent anti- inflammatory, antioxidant, anti- complementary, anticancer and skin tissue integrity activity [1,2]. In this study, *in vivo* antihemorrhoidal and antiinflammatory activities of *M. silvestris* aerial part extract was examined

Material and Methods: Hemorrhoid model was induced by croton oil in rats. Then the extract was implemented both externally (50%). A commercial herbal product was preferred as a positive control. After 7 days of the treatment, the animals were anesthetized, intracardiac blood samples were taken and divided into serum to determine tumor necrosis factor alpha (TNF-α) and vascular endothelial growth factor (VEGF). Sacrified rats were weighed, following, 2 cm long anorectal tissues were cut and weighed to calculate anorectal coefficient (ARC). The tissues were fixed in formaldehyde (10%) for histopathological evaluations [3-5].

Results: According to ARC scores, biochemical and histopathological results, *M. sylvestris* leaf extract showed significant biological activity compared the controls. Value of ARC was found 1,86±0,09. Serum TNF-α and VEGF values were found 163,22±11,15 ng/L and 246,37±12,64 ng/L, respectively.

Conclusion / Discussion: The data demonstrated that the antihemoroidal activity is directly proportional to the effect on capillary permeability. Results will be discussed in detail in the oral presentation. The effectiveness of the traditional usage has been verified by scientific studies.

Keywords: hemorrhoid, *in vivo*, croton oil, mallow, Malva silvestris

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MESMAP-5 Final Proceedings Book

ORAL PRESENTATION

ANTICANCER, ANTIPROLIFERATIVE AND CELLULAR DNA FRAGMENTATION ACTIVITIES OF *ASTRAGALUS ELONGATUS* SUBSP. *NUCLEIFERUS* ON HUMAN CANCER CELLS

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Background and Objective: The genus *Astragalus* are of important medicinal plants that known as immunostimulants, hepatoprotective, antiviral and antidepressive agents. Their wide range of pharmacological effects led us to investigate biological activities of *Astragalus* species growing wild in Turkey. In our previous study, aerial parts and root extracts of *A. elongatus* subsp. *nucleiferus* were analyzed as their potential neuroprotective effects and antioxidant capacities along with polyphenolic contents. Taking our previous results, we aimed to evaluate potential anticancer and antiproliferative activities of ethanol and water extracts from both aerial parts and roots of that plant species in the presented research. **Material and Methods:** On this bases, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and trypan blue exclusion assays were performed to investigate cytotoxicity and cell proliferation potentials of the extracts against human lung carcinoma (A549), non-small lung cancer (H1299), glioma (C6) cancer cells, and non-tumorous human umbilical vein endothelial cells (HUVECs). All the conditions and chemicals were same as described in our previous research. The release of the enzyme lactate dehydrogenase (LDH) activity in the cultured cells was also analyzed according to the method of our previous research. Based on their cytotoxicity against A549, H1299 and C6 cancer cells, the concentrations of the extracts were selected for *in vitro* micronucleus (MN) assay. Additionally, apoptotic activity in the cells treated and untreated with the plant extracts were determined in terms of DNA fragmentation using the 'Apoptotic DNA-Ladder Kit'. All the experiments were carried out at least triplicate, and a linear regression analysis was performed to calculate IC_{50} values for each cell line. **Results and Discussion:** The results showed that the roots of *A. elongatus* subsp. *nucleiferus* are able to induce growth inhibition and apoptosis in a concentration and time dependent manner. As a results of MTT and trypan blue assays, dose-dependent anticancer and antiproliferative effects were observed on human lung and brain carcinoma cells. The water extracts obtained from root was exerted higher anticancer activity, IC_{50} values ranging from 5.81±0.46 to 18.24±0.12µg/mL ($p<0.01$) than the extracts obtained from the aerial parts of the plant. Besides increasing extract concentration lead to decrease the growth rate of the cancer cells, apoptosis was observed almost in all the cultured human malignant cells, which rapidly exhibited sign of apoptotic cell death as detected by DNA fragmentation. In contrary anticancer and cytotoxicity results, the ethanol extract of the root was also caused to highest apoptosis level and DNA fragmentation in a dose and time dependently. Regarding LDH activity, which gives information about the percentage of dead and necrotic cells, the results were found a similar activity profile as observed in MTT and trypan blue assays. Briefly, the extracts obtained from aerial parts and roots of the plant were demonstrated the highest LDH activity towards H1299 cells, which was followed by A549 cells. To summarize, anticancer and antiproliferative activity assays were resulted in the superiority of the water extracts obtained from the root of the plant against lung carcinoma cells. **Conclusion:** Overall, the results revealed that the root extracts of the plant could have remarkable anticancer and antiproliferative activities through enhancement of apoptosis and necrosis. The data obtained from our laboratory studies could be assumed as the first report for the literature, and the results should be validated by using further *in vitro* and *in vivo* techniques.

Keywords: Anticancer, apoptosis, *Astragalus* sp., DNA fragmentation, micronucleus, necrosis.

Acknowledgment: This study was financially supported by Scientific Research Project Unit of Kilis 7 Aralık University, Kilis-Turkey (Project code no: 1859LTP1)

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ORAL PRESENATION

ASSESSMENT OF CHOLINESTERASE AND TYROSINASE ENZYMES INHIBITORY, PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF *MENTHA SPICATA* L.

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Background and Objective: Because oxidative damage is one of the major factor contributing to neurodegeneration, combination of antioxidant activity and enzyme inhibition potential of the herbal extracts is closely related to combat neurodegenerative diseases and disorders efficiently. Spearmint (*Mentha spicata* L., Lamiaceae), an edible aromatic plant, are of important food sources used as flavoring, tea infusions and spicing in the world. Besides its importance in food, beverage, cosmetic and confectionary industries, there has been huge ethnobotanical information on the curative properties of *M. spicata* L. against common diseases. Even though biological properties of spearmint have been documented by previous scientific reports, one less known biological activity of spearmint is its neuroprotective activity. **Material and Methods:** In this concern, neuroprotective potentials, antioxidant capacities, mineral contents and total polyphenolic compositions of aqueous and methanol extracts of the leaves from *M. spicata* L. were aimed to examine in the current research. Neuroprotective potentials of the extracts were determined against inhibition of AChE, BChE, and tyrosinase enzymes, which are associated with pathogenesis of Alzheimer's disease. *In vitro* assays including DPPH, ABTS, FRAP, and CUPRAC were carried out to reveal antioxidant capacities of the extracts. The total phenolic and total flavonoid contents were analyzed spectrophotometrically. Mineral contents (K, Ca, Mg, Fe, Zn, Cu, Mn, Cd, and Pb) in spearmint leaves were also determined by Atomic Absorption Spectrometry. **Results:** A wide variation was observed in the mineral contents of the plant that Fe was found the major mineral with the values of 1471.41±12.28 mg/kg, whilst Cu and Pb were determined as minor minerals. With regard to total polyphenolic components, the methanol extract possessed higher phenols and flavonoids contents (3.158±0.52 mg/g extract as GAE and 1.31±0.08 mg/g extract as QE, respectively). A significant correlation was found between total polyphenolic compositions and antioxidant capacities of the spearmint leaves. Correlation analysis indicated that the methanol extract, which possessed higher total phenols and flavonoids components than the water extract, was also found the more efficient in terms of antioxidant capacities and neuroprotective potentials. Both of the extracts demonstrated powerful inhibition on BChE, whereas they showed moderate inhibition on AChE and tyrosinase enzymes with the values ranging from 24.06±0.12% to 57.68±0.18%. **Conclusions:** Consequently, our results indicate that *M. spicata* L., a natural antioxidant and neuroprotective agent, can provide considerable benefits against oxidative stress-related diseases.

Keywords: *Mentha spicata* L.; Alzheimer's Disease; neuroprotection; antioxidant; polyphenolic; mineral content

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ORAL PRESENTATION

NEUROPROTECTIVE EFFECTS OF ACTIVE PHYTOCONSTITUENTS AGAINST $A\beta_{1-42}$ -MEDIATED INJURY IN HIGH GLUCOSE-INDUCED PRIMARY HIPPOCAMPAL NEUROTOXICITY: *IN VITRO* HYPERGLYCEMIC ALZHEIMER'S DISEASE MODEL

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Background: Alzheimer's Disease (AD) is the most common of dementia and characterized by progressive loss of memory and other cognitive functions among elderly people [1]. However, the underlying mechanisms of AD is still unclear. Hyperglycemia have an impact on oxidative stress pathways and neuroinflammatory signals in the brain, thereby connecting diabetes to neurodegeneration [2,3]. Four pathological free radical-related sources in the brain have been described which also feed the oxidative damage in diabetes: mitochondrial dysfunction, inflammation, AGEs/ALEs and high cytosolic ionic-calcium levels [4]. Thus, we aimed to investigate the neuromodulatory effects of well known antioxidant phytoconstituents, Oleuropein (OLE) and Rutin (RUT), in hyperglycemic (HG)-AD, *in vitro.*

Methods: AD model was designed by using embryonic primary hippocampal neurons. Cytotoxicity of OLE and RUT in various concentrations (1 nM-25 μM) were tested by MTT and NRU assay. Long incubation periods (48 h) and high glucose (150 mM) were chosen to mimick chronic hyperglycemia. For the protection experiments, cells were pre-treated with OLE or RUT (10-500 nM), then co-treated with 150 mM glucose (24 h) and followed by incubation with 500 nM oligomeric $A\beta_{1-42}$ (24 h) in high glucose conditions. MTT, NRU assays were held to evaluate toxicity and intracellular ROS generation levels were measured by DCFH-DA assay.

Results: Both OLE and RUT have cytotoxic effects at relatively high concentrations (> 10 μM). While, OLE significantly reversed the high glucose toxicity in a wide range concentration (1-500 nM), RUT exerts only at 50-500 nM. Moreover, intracellular ROS levels were drastically increased in HG-AD model ($p<0.01$), leading to a significant cell death ($p<0.01$). RUT treated cells seemed to have higher resistance to ROS-mediated cell death (up to $p<0.001$), on the other hand OLE treated cells tended to have mild antioxidant effect in that HG-AD *in vitro* model.

Conclusion: Present results suggested that neuroprotection against HG and **$A\beta_{1-42}$ induced** oxidative damage in primary hippocampal neurons may be attributable to redox modulatory effects of OLE or RUT.

Keywords: Hyperglycemia, Alzheimer Disesase's, ROS, Oleuropein, Rutin, Neuroprotection

Acknowledgements: This study was financially supported by the Scientific and Technological Research Council of Turkey (Grant No: 315S088)

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ORAL PRESENTATION

SEA BUCKTHORN TREATMENT INFLUENCE ON THE MULTI-EFFECTOR IMMUNE RESPONSE IN SWINE EXPOSED TO POTENTIALLY PATHOGENIC MICROBIOME

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Objective / Purpose: Free range systems expose swine to increased infectious pressure and further immune suppression, therefore alternative therapies, such as medicinal plant extracts could aleviate complex environmental stress.

Material and Methods: The research was performed on treated and untreated groups of extensively raised pigs, for identification of the carried microbiome and its sensitivity to antibiotics used in current therapy, subsequent to *in vivo* therapy with sea buckthorn (*Hippophae rhamnoides*) syrup. The antioxidant activity of the sea-buckthorn syrup was determined by assessing free radical scavenging effect over DPPH radical. Skin swabs were processed by classical microbiological methods, identification by RapIDTM and antibiotic sensitivity (Kirby-Bauer method, MAR) was evaluated against gentamicin, streptomycin, oxitetracycline, tylosin, amoxacillin-clavulanic acid, marbofloxacin, tulatromycin, cefotaxime and doxycycline. Immunological tests included *in vitro* blast transformation, serum MIC and MIB concentrations and N/L index.

Results: Strains from *Staphylococcus, Shigella*, *Kytococcus*, *Salmonella* and *Citrobacter* genera with MAR index of 0.22 - 0.33 were identified in the skin swabs. Although the antioxidant activity of the syrup was intermediate (54.65%) the N/L index was increased in syrup-treated piglets, similar to blast-transformation indices obtained from sea buckthorn treated piglets towards all extracts used *in vitro* indicating both an increased non-specific reactive capacity and specific cell-mediated immune potential. The sera from treated piglets showed a bacterial growth inhibiting capacity from dilution ¼ to 1/64.

Conclusion / Discussion: The relationship between antioxidant capacity and antimicrobial protective response for sea-buckthorn syrup needs further investigation to improve the protective capacity against carried potentially pathogenic microbiome in swine.

Keywords: swine, sea-buckthorn, antioxidant, immune response, carried microbiome

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ORAL PRESENTATION

IN VITRO ANTI-*HELICOBACTER PYLORI* AND ANTIMYCOBACTERIAL ACTIVITY EVALUATION OF PLANTS FROM TURKEY

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Objective: In this study, the extracts of *Ulmus minor* Mill. subsp. *minor* (Ulmaceae), *Lathyrus pratensis* L. (Leguminosae), *Glaucium leiocarpum* Boiss. (Papaveraceae) and *Echium vulgare* L. (Boraginaceae) were investigated for *in vitro* anti-*Helicobacter pylori* and anti-mycobacterial activities.

Material and Methods: The air-dried plant material was powdered and extracted with 70% ethanol by maceration. The extract was filtered and evaporated to dryness under reduced pressure in a rotary evaporator. The concentrated residue dissolved in a water-ethanol (90:10) mixture and then extracted with dichloromethane and ethyl acetate, respectively. Antibacterial activity was investigated by microdilution method against *Helicobacter pylori* ATCC 43504, *Mycobacterium smegmatis* ATCC 25291 and *Mycobacterium avium* clinical isolated[1-3].

Results: The ethyl acetate extract of *U. minor* subsp. *minor* showed MIC of 125 μg / mL activity against *H. pylori*. The other extracts showed weak or no inhibitory activity up to 2000 μg/mL concentrations against the tested microorganisms.

Conclusion: The ethyl acetate extract of *U. minor* subsp. *minor* will be further investigated to identify its active compounds that may provide a lead structure to develope a potential antibacterial drug substance for the treatment of *H. pylori* infections.

Keywords: anti-*Helicobacter pylori*, anti-mycobacterial, *Ulmus minor, Lathyrus pratensis, Glaucium leiocarpum, Echium vulgare.*

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ORAL PRESENTATION

NEMATICIDAL ACTIVITY OF ESSENTIAL OILS FROM *LAMIACEAE*

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The *Lamiaceae* family contains important aromatic plants used in traditional and modern medicine and in food and pharmaceutical industries. Plants belonging to this family are mostly exploited for the extraction of essential oils (EOs). The genus *Monarda* L. includes about eighteen species of herbaceous, annual or perennial plants endemic of North America. Several biological activities have been reported for plant EOs and their main components, including a biocidal activity against plant soilborne pathogens and pests, which also includes plant-parasitic nematodes [1]. The EOs can play an important role in crop protection and have been propose as an environmentally alternative to synthetic pesticides.

In this study, the EOs from two Italian species of *Monarda* (*M. dydima* and *M. fistulosa*) were evaluated for their *in vitro* activity on infective stages of plant-parasitic nematodes *Melodoigyne incognita*. EOs from both *Monarda* species were strongly active on *M. incognita* juveniles showing a LC_{50} value of 1 µL mL^{-1} after 24 h exposure. The main constituents of EOs, carvacrol, γ-terpinene, o-cymene and thymol were also evaluated for their *in vitro* activity. Among them, carvacrol showed higher nematicidal activity also at low concentrations and at short exposure time [2].

Keywords: Essential oils, carvacrol, *Meloidogyne incognita*, Nematicidal activity.

Reference:

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ORAL PRESENTATION

NEW BIOACTIVE GYPSOGENIN COMPOUNDS

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Objective: The saponin groups have become quite remarkable on natural products. Many substances isolated from plant that has been reported pharmacological properties. Especially, Gypsogenin aglycone obtained from *Gypsophila arrostii* plant roots which is well known saponin. Gypsogenin aglycone has important biological activities such as antiviral, antitumor, anticarcinogenic, antioxidant and anticancer. Therefore, it is aimed to obtain new bioactive compounds that are functionally enriched in gypsogenin which show bioactive properties.

Material and Method: The starting material gypsogenin compound was obtained from *G. arrostii* plant roots water extract. Then, this gypsogenin aglycone and four different amines compounds were synthesized with ethanol at room temperature.

Conclusions: The study of the structures of the compounds **(1-4)** synthesized in our study which was determined by IR, UV, ^{1}H NMR, APT and LCMS analysis.

Conclusion / Discussion: In the last part of the study, biological activities of new compounds will be investigated.

Keywords: Gypsogenin, semi synthesis.

Acknowledgements: This project is supported by EGE UNIVERSITY BAP Project (17FEN064).

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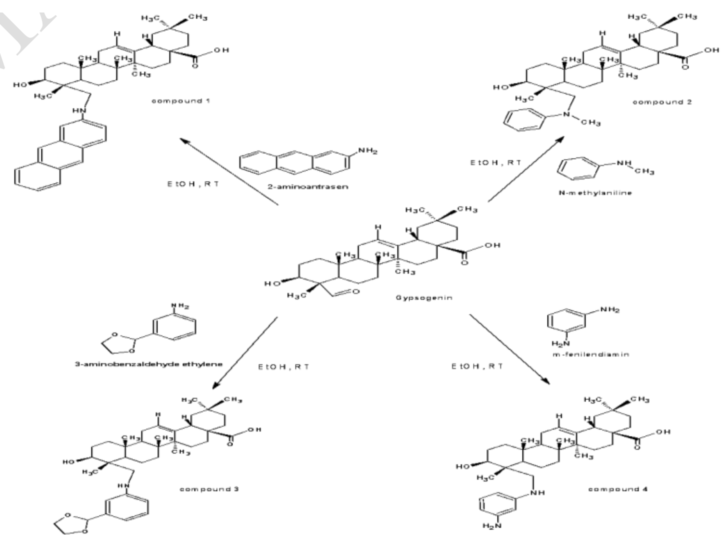

ORAL PRESENTATION

LEVELS (PRODUCTION) AND BIOLOGICAL ACTIVITY OF SOLAMARGINE AND SOLASONINE FROM *SOLANUM INCANUM*

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Solanum incanum is a species of nightshade native to the Middle East, the Sub-Sahara region and India. The plant, commonly known as thornapple and has diverse uses traditionally with reported biological activities in diabetes, parasitosis and cancer. These effects possibly arise from the steroidal alkaloid solasodine and its glycoalkaloids mainly solamargine and solasonine.

Optimal extraction of solasodine was performed using 2.5 N methanolic HCl followed by precipitation and extraction using chloroform. A fast and simple extraction method was developed and optimised to get maximum recovery of glycoalkaloids using a mixture of chloroform and methanol by sonication, in one-step. Similarly, a fast and accurate High Performance Thin Layer Chromatography (HPTLC) method for solasodine, solamargine and solasonine content was established and used to identify the developmental stages at which solasodine and its glycoalkaloids contents are maximal in the plant structures of *S. incanum* grown in Sultanate of Oman. The HPTLC method was validated for linearity, precision, recovery, LOD and LOQ.

The solasodine, solamargine and solasonine produced well separated compact bands at R_f values 0.30, 0.26 and 0.14, respectively on silica gel HPTLC plate using chloroform: methanol (9.25: 0.75 v/v) for solasodine and chloroform: methanol: 5% ammonia (7: 3: 0.5 v/v/v) for glycoalkaloids after visualization with anisaldehyde sulphuric acid reagent. The chromatograms were scanned at 530 nm wavelength in absorbance mode and simultaneous method was found linear (r^2 = 0.99) in concentration range of 40 – 2000 ng/ spot for solasodine and 50 – 2000 ng/ spot for both glycoalkaloids. The proposed method was also validated as per ICH guidelines and found precise, accurate, specific, robust and sensitive enough to carry out analysis of very minute quantity of solasodine, solamargine and solasonine in complex extracts. The method was applied for analysis solasodine and its glycoalkaloids content in small, young, immature and mature leaves with stem and root parts of *S. incanum* at intervals of 4 to 24 weeks for solasodine and up to 40 weeks for glycoalkaloids. The study revealed that the highest solasodine contents occur in the small leaves 12 – 20 weeks of plant growth and rich concentrations of glycoalkaloids with large variation at different stages of plant development.

In this study, solamargine displayed a superior cytotoxicity in human melanoma cancer cell lines at low concentration of 10 μM, however it exhibited less cytotoxicity against breast cancer cell lines, bone metastasized and brain metastasized.

Solamargine appears to be selective agent targeting highly proliferating cancer cells; its effect was dependent on the dose of the compound and the type of the cancer cell line. Solamargine induces both necrosis and apoptosis in melanoma cancer cells and breast cancer cells and the mechanism was characterized. Solamargine up-regulated the expression of external death receptor, tumour necrosis factor receptor 1 (TNFR-1).

Keywords: *Solanum* glycoalkaloids, solasonine, solamargine

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ORAL PRESENTATION

KARYOMORPHOLOGY OF SOME MEDICINAL *LEPIDIUM* SPECIES (BRASSICACEAE) FROM TURKEY

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Objective / Purpose: The genus *Lepidium* L. is one of the largest genera of the Brassicaceae, consisting of 175 species. It is distributed worldwide, primarily in temperate and subtropical regions. *Lepidium* species has been used in folk medicine for curing some ailments such as diabetes, hypertansion, asthma, bronchitis and rheumatic pains (Roughani and Miri, 2018). The aim of this study is to determine karyomorphological features of five species belonging to genus *Lepidium.* These species are also used as drugs.

Material and Methods: Many specimens were collected from different localities and populations belonging to genus *Lepidium* during the field studies in the year 2018. Mature seeds were selected and periodically germinated for chromosomal analyses. Chromosome counts were made on somatic metaphases in root tips using the squash technique. After germinating, the root tips were pretreated by 8-hydroxyquinoline and fixed by the Carnoy solution. Before staining, root tips were hydrolyzed with 5-N HCl, and stained with 1% aceto-orcein. At least 10 metaphases were examined per taxa; the best metaphase plate was photographed. Moreover, the coefficient of variation of the chromosome length (CV_{CL}), coefficient of variation of the centromeric index (CV_{CI}) and mean centromeric asymmetry (M_{CA}) were calculated. Idiograms and karyograms of these taxa were made by using the KAMERAM analysis system.

Results: The somatic chromosome number was counted as 2n=16 for all studied species (*L. campestre* (L.) R.Br., *L. perfoliatum* L., *L. ruderale* L., *L. vesicarium* L. and *L. virginicum* L.). Each of species are diploid. AI, A1, A2, CV_{CL}, CV_{CI} and M_{CA} indexes were calculated for all taxa. The value of CV_{CL} ranges between 11.189–18.226 and the M_{CA} value varies between 12.69–21.53. According to the asymmetry indices it can be concluded that most of the taxa have symmetric karyotype. The most common karyotype formulas 14m + 2sm and 12m + 4sm. Satellites were identified only in karyograms of most of *L. virginicum*.

Conclusion / Discussion: As a result of our study, we conclude that there are significant differences between chromosomal differences between chromosomal characteristics in *Lepidium* species and the reported notes about the intraspecific variation may be useful for taxonomy.

Acknowledgements: This study was financially supported by Selçuk University (BAP Project Number: 18401090).

Keywords: Asymmetry, Brassicaceae, Karyomorphology, *Lepidium*, Turkey

Reference:

Roughani, A., Miri, M.S., 2018. Lepidium species as antidiabetic herbal medicines. The First National Congress and International Fair of Medicinal Plants and Strategies of Persian Medicine that affects diabtes. MPSD 2018.

ORAL PRESENTATION

CHEMICAL AND BIOLOGICAL VALORISATION OF *CEDRUS ATLANTICA*

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The present study concerns a medicinal and aromatic plant of the Moroccan High Atlas Mountains: Cedrus atlantica (Manetti ex Endl.) Carrière 1855. This work contributes mainly to the chemical and biological study of needles and wood of Atlas cedar (Oukaimeden region).

After extractions, chromatographic analysis, antioxidant and antibacterial activities of essential oils were investigated. The essential oils (EO) of needles and Cedar wood were obtained by steam distillation and then analyzed by gas chromatography (GC-FID). Which allowed detecting some major compounds: in the case of EO of fresh needles: 3 major compounds; EO needles after 2 weeks of drying: 2 compounds, for needle EO after 3 weeks of drying: 3 compounds and finally wood HE: 4 major compounds. This analysis allowed highlighting a effect of drying on the abundance of compounds. Open column chromatography and 1H, 13C and DEPT 135 NMR analysis were performed and Di-isooctyl phthalate and stearic acid have been identified.

In the other hand, quantitative estimation of total phenols by the method of Folin Ciocalteu revealed that the ethyl acetate extract of Cedrus needles contains a phenol content of 0.52 ± 0.049 mg EAG / g MS. The antioxidant activity was evaluated by DPPH free radical scavenging method (2,2- diphenyl-1-picrylhydrazil) and by FRAP iron reduction method. The quantitative antioxidant effect showed that AcOEt extract of Cedrus needles has an IC50 value of 91.57 ± 1.05 µg / ml. This value approches the BHT positive control: IC50 = 77.18 µg / ml.

On the other hand, the essential oils of needles and wood of Cedrus have no effect on the reduction of DPPH. According to the FRAP method, those EOs show lower activity with higher IC50% values (81.35 mg / ml and 2.5 ± 0.123 mg / ml, respectively). Antimicrobial activity was determined *in vitro* on two Gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa and the Gram-positive strain Staphylococcus aureus by the disk diffusion method. The species tested were sensitive to the essential oil of Cedrus needles at concentrations of 0.5 to 20 mg / ml and for the wood at the concentrations of 0.83 to 83.3 mg / ml.

Keywords: *Cedrus atlantica*; Essential oil; GC / FID; Di-isooctyl phthalate; Stearic acid;

ORAL PRESENTATION

PHYTOCHEMICAL STUDY AND EVALUATION OF INHIBITORY POTENTIAL OF *ANGELICA PANCICII* VANDAS. ESSENTIAL OILS AND HEXANE EXTRACTS AGAINST ENZYMES INVOLVED IN ALZHEIMER DISEASE AND TYPE II DIABETES

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Objective: In the present work, the essential oils and hexane extracts obtained from roots, leaf and fruits of Balkan endemic species *Angelica pancicii* Vandas. were investigated for their chemical composition, free radical ($DPPH^{\bullet}$ and $ABTS^{+\bullet}$) scavenging potential and inhibitory effects against acetylcholinesterase and porcine pancreatic α-amylase.

Material and Methods: *A. pancicii* was collected from Rila Mountain, Bulgaria in 2016. Air-dried leaves, fruits and roots were separately used for analysis. Essential oils were obtained with Clevenger apparatus and analyzed by GC-FID/MS. Hexane extracts were obtained by Soxhlet extraction. The amount of the individual coumarins in different plant organs was determined by HPLC-DAD method. Antioxidant effect was tested for their scavenging activity against DPPH and ABTS. Acetylcholinesterase inhibition was evaluated using Ellman's method. Antidiabetic potential was assessed *via* measurement of inhibition of α−amylase enzyme using KI/I_2 method.

Results: CG-FID/MS analyses of essential oils obtained from the different plant parts of *A. pancicii* resulted in the identification of 170 components. It has been found that essential oils differed significantly in their chemical composition. The fruit essential oil was rich in monoterpene hydrocarbons (84.3%), while sesquiterpenoids dominated in the leaf (64.0%) and the root (71.7%) oils. β-Phellandrene (69.1%), germacrene D (9.7%) and elemol (9.8%) were the main components in the fruit, leaf and root oils, respectively. Isoimperatorin, imperatorin, oxypeucedanin, oxypeucedanin hydrate, angeloylpangelin and umbelliprenin were isolated from hexane extract of the fruits and used for HPLC quantification. The main component in the fruits and roots was oxypeucedanin (326.7±1.3 and 261.9±1.5 mg/gDE, respectively). These extracts differed significantly in the content of oxypeucedanin hydrate, which was 15 times more in the roots than in the fruits. The leaves were poor in coumarins. The essential oils and extracts of *A. pancicii* demonstrated good anti-acetylcholinesterase activity (between 47.3 and 88.3 %). The leaf and fruit oils were the most active with values similar to that of the standard galantamin (88 %). The essential oils were poor scavengers of DPPH and ABTS radicals when compared with the hexane extracts. The highest TEAC values were obtained for leaf and root extracts (0.5 mM). The leaf extract exhibited also the best $DPPH^{\bullet}$ scavenging activity (IC_{50} 1.2±0.06 mg/ml). The studied samples demonstrated a weak inhibitory effect (Inh. % up to 31.9±1.1) towards α-amylase.

Conclusion: This is the first report on inhibitory potential of *A. pancicii* essential oils and hexane extracts against enzymes involved in Alzheimer disease and Type II diabetes. Our data indicated that the fruit and leaf essential oils from *A. pancicii* emerged as the sources of possible cholinesterase inhibitors and deserve future studies.

Acknowledgements: Authors thank to TUBITAK (Project No 116S021) and BAS (Bilateral project) for financial support.

Keywords: *Angelica pancicii* Vandas., essential oil, coumarins, DPPH and ABTS, α-amylase, acetylcholinesterase.

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ORAL PRESENTATION

IN VIVO ANTIDIABETIC ACTIVITY OF RHINACANTHINS ENRICHED EXTRACT PREPARED BY MICROWAVE ASSISTED GREEN EXTRACTION PROCESS

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Objective: *Rhinacanthus nasutus* is utilized as an herbal tea in China and a Thai traditional medicine for various ailments including diabetes. Rhinacanthins enriched extract (REE) is a semipurified *Rhinacanthus nasutus* leaf extract obtained by microwave assisted green extraction process. The present study was aimed to evaluate the antidiabetic potential of REE in comparison with its marker compound rhinacanthin-C (RC) (Fig 1).

Material and methods: REE was prepared simply by microwave assisted extraction process using ethanol and enriched by passing through ion exchange resin (Amberlite®) column. RC content in REE was analyzed by HPLC. In the present study, REE was tested for the antidiabetic potential in comparison with its marker compound RC and positive control glibenclamide employing streptozotocin induced diabetic rat model for 4 weeks. Numerous pathological parameters including fasting blood sugar, insulin, glycated hemoglobin, lipid profile, AST/ALT, creatinine and blood urea nitrogen levels were analyzed.

Results: HPLC analysis showed that REE contains 62 % w/w of RC. REE significantly normalized the blood sugar level, glycated hemoglobin, insulin level, lipid profile, AST/ALT, creatinine and blood urea nitrogen in diabetic rats without affecting the normal control.

Conclusion: The results of the *in vivo* study suggest that after safety assessment, REE could be employed as a standardized natural remedy for diabetes treatment.

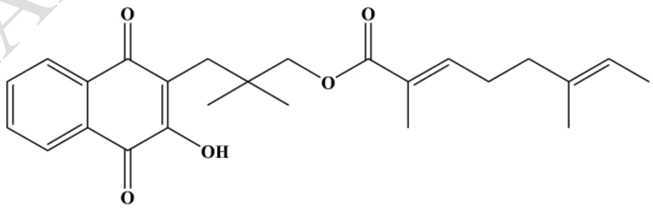

Fig 1 Chemical structure of rhinacanthin-C

Keywords: Antidiabetic, *Rhinacanthus nasutus*, rhinacanthins enriched extract

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[1] Shah, M.A., Khalil R., Haq Z.U., Panichyupakaranant, P. (2017). α-Glucosidase inhibitory effect of rhinacanthins-rich extract from *Rhinacanthus nasutus* leaf and synergistic effect in combination with acarbose. *Journal of Functional Foods, 36*(1), 325-331.
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ORAL PRESENTATION

ENZYME INHIBITORY, ANTIOXIDANT ACTIVITES AND PHYTOCHEMICAL STUDIES ON *VISCUM ALBUM* SAMPLES COLLECTED FROM DIFFERENT HOST PLANTS

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Objective/Purpose: Metabolic disorders such as diabetes and obesity are increasing day by day. In the treatment of these disorders, plants used in traditional folk medicine are also utilized. The main purpose of our study is to test these plants by *in vivo* and *in vitro* methods and isolation of active natural molecules from active plants. *Viscum album* L. (Loranthaceae) is a semi parasitic plant and traditionally used in the treatment of diabetes mellitus in Anatolia. In this study was planned to compare the *in vitro* antioxidant, antidiabetic (α-glucosidase and α-amylase enzyme inhibition), and antiobesity (pancreatic lipase enzyme inhibition) potentials of *V. album* samples collected from different host plants. The phytochemical contents of the extracts tested were examined by UV spectroscopy and HPLC techniques.

Material and Methods: *V. album* samples were collected from Beynam and Kızılcahamam (Ankara) in 2015 (March-May). α-amylase and α-glucosidase and pancreatic lipase inhibitory effects and antioxidant activities of the aqueous and ethanol extracts prepared from the samples were evaluated. Antioxidant activities of the extracts were determined by DPPH radical scavenging, ferric reducing, metal chelating and phosphomolybdenum assays. Additionally, total phenol and flavonoid contents of the extracts were investigated. Qualitative and quantitative analysis of phenolic acids in active extracts were performed by HPLC method.

Results: Ethanol extracts of *V. album* samples collected from *Salix*, *Abies* and *Pinus* species showed potent α-glucosidase enzyme inhibitor activity, especially at a concentration of 3 mg/ml. Also these extracts displayed strong ABTS scavenging (95.37%->100) and metal chelating activity (99.65-100.48%). Many of the extracts were ineffective on □-amylase and pancreatic lipase enzyme. Total phenol content calculated from gallic acid was 21.52-67.85 mg/g extract and total flavonoid content calculated from quercetin was determined as 10.31-13.11 mg/g extract.

Conclusion/Discussion: The findings obtained in the experiments demonstrated that *V. album* species possess potent antihyperglycaemic and antioxidant activity depending on host plant. Activity-guided isolation studies should be carried on *V. album* samples collected from *Salix, Abies* and *Pinus* species.

Keywords: α-glucosidase, α-amylase, pancreatic lipase, HPLC, *Viscum album* L.

Reference:

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ORAL PRESENTATION

ENCAPSULATION OF *CAPSICUM FRUTESCENS* EXTRACTS WITH β – CYCLODEXTRIN AND 2-HYDROXYPROPYL – β – CYCLODEXTRIN

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Purpose: *Capsicum frutescens* extracts and capsaicin topical formulation are used in the treatment of such diseases like: rheumatoid arthritis, diabetic neuropathy, osteoarthritis, muscular pain, etc. The aim of this study is the evaluation of recovery percent of *Capsicum frutescens* alcoholic extracts during encapsulation in β – cyclodextrin and 2 – hydroxypropyl – β – cyclodextrin forms.

Materials and Methods: The Capsicum frutescens fruits were collected from region of Korca at the southeast of Albania. The alcoholic (ethanol 95°C) extracts were obtained by the methods of maceration process. The extracts contain the capsaicin alkaloid, which is a liphosoluble molecule. For improvement of the bioavailability and solubility of this molecule is proposed the encapsulation process in β – cyclodextrin and 2 – hydroxypropyl – β – cyclodextrin. Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a somewhat lipophilic central cavity. They are able to form water-soluble inclusion complexes with many lipophilic water-insoluble drugs.
Inclusion complexes between the Capsicum extracts and β-cyclodextrin and 2 – hydroxypropyl – β – cyclodextrin were prepared by co-precipitation method with ratios: 10:90, 20:80 and 30:70 (w/w) for β – cyclodextrin and ratios: 1:1 and 1:9 for 2 – hydroxypropyl – β – cyclodextrin. For all the encapsulation complexes were evaluate the percent of recovery.

Results: From the study it was concluded that capsaicin can capsulate successfully in β-cyclodextrin and 2 – hydroxypropyl – β – cyclodextrin with a best powder recovery 92.4% in the ratio of 30:70 for the complex with β – cyclodextrin and a 90.1% recovery for the complex capsaicin: 2 – hydroxypropyl – β – cyclodextrin at the ratio 1:1.

Keywords: Capsicum extract, β-cyclodextrin, 2 – hydroxypropyl – β – cyclodextrin, recovery.

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ORAL PRESENTATION

INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF EXTRACTS OBTAINED FROM TESBI (*STYRAX OFFICINALIS* L.)

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Abstracts Book

Objective / Purpose: Tesbi (*Styrax officinalis* L.) is a shrub grows in forestland. The resin of the species S. officinalis, named by the past as 'storax', was used in traditional medicine in the Mediterranean basin, notably for antiseptic purposes and against respiratory diseases [1]. This resin was also used by Romans, Egyptians, Phenicians and Ionions to treat a variety of ailments and as an incense [2]. To our knowledge, there is no work on leaf extract of this species. The aim of this study was to determine the antibacterial activity of leaf extract.

Material and Methods: 4 g of each tesbi leaf (finely grounded with labrotary blender) was extracted with five different solvents (ethanol / methanol / acetone / ethyl acetate / chloroform) and the extracts were dried at 40 ºC by rotary evaporator. The disc diffusion technique (Kirby-Bauer method) is used for the evaluation of anti-microbial activity [3]. 7 Gram (-) bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens*, *Proteus vulgaris*, *Enterobacter cloacae* and *Klebsiella pneumoniae*) and 2 Gram (+) bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*) were used in the tests.

Results: As a result of the experiment, it was understood that tesbi leaf extracts have moderate effect on S. aureus, P. vulgaris, S. epidermidis and no effect on other bacteria.

Conclusion / Discussion: In this preliminary study tesbi leaf extract shows antibacterial activities, but more investigation is needed, especially leaf extracts should be investigated in HPLC and which component shows antibacterial activity should be determined. Also this results, should be verify with agar dilution and broth dilution methods and minimum inhibitory concentration will be determine.

Keywords: Antibacterial activity, *P. vulgaris*, *S. aureus*, *S. epidermidis*, *Styrax officinalis* L.

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ORAL PRESENTATION

ETHNOMEDICINAL STUDY OF *CORNUS MAS* L. FROM THE FOLK AND TRADITIONAL MEDICINE OF REPUBLIC OF MACEDONIA

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Objective/Purpose: This study was carried out concerning ethnomedicine on preparations obtained from *Cornus mas* L. from the folk and traditional medicine of Republic of Macedonia, an area so far less frequently studied from the perspective of folk traditional medicinal, local food and handicraft and other uses.

Results: European cornel acts as astringent and antidiaroic. Tea, juice, fresh and dry fruits because of its antimicrobic, anti-inflammatory action are used at diarrhea, colitis and other intestine disorders. Dry fruits are used directly as a medicine against diarrhea. It acts chemo static and protective on capillary. Yörüks in Republic of Macedonia collected cornels primarily for medicinal herbal infusions for internal cleansing.

Cornels as mountainous fruits were used by Macedonians as a food long time ago. Fruits are used dry as a food. Dry fruits are used for preparing compote or directly in nutrition in winter and spring period. From the fruit jam, marmalade, compote and juice are prepared at home and in the food industry. Dry fruits are kept in rakiya and sugar for obtaining liqueur. Alcohol beverage *rakiya* - dryankoitsa was prepared from cornels. Manufacture of hammer in Ohrid or stick in Mariovo (koledashki) for Christmas Eve (kolede) is made from a cornel branch. Handmade handle for Macedonian traditional instrument gajdarka, in Kumanovo is manufactured from cornel because it is smooth and not cracked. Cornel is a symbol of durability, solidity, and with its hardness influences the health.

Conclusion/Discussion: 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.

Keywords: European cornel, medicinal, food, handicraft, use

Reference:

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ORAL PRESENTATION

CYTOTOXIC FLAVONOIDS FROM THE ETHYL ACETATE EXTRACT OF THE AERIAL ROOTS OF *FICUS BENGHALENSIS* LINN

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Objective/Purpose: The aerial roots of *Ficus benghalensis* are used as an immunomodulatory medicine by traditional healers in rural areas of Maharashtra, India. Our lab has demonstrated the antioxidant and immunomodulatory activity of the roots thereby validating their traditional use. The antioxidant activity of natural phytochemicals/extracts is related to other bioactivities like antiproliferative activity hence we thought it prudent to evaluate the aerial roots for potential anticancer activity. Our earlier studies reported the presence of flavonoids in the ethyl acetate extract and literature reports indicate potent anticancer properties of flavonoids by inducing apoptosis and G1 cell cycle arrest via different pathways. The objective was to evaluate the ethyl acetate extract for potential anticancer activity, isolate and characterize some anticancer compounds from the ethyl acetate extract of the aerial roots of *Ficus benghalensis*.

Materials and Methods: Preliminary *in vitro* studies on the successive solvent extracts of the aerial roots indicated good antioxidant and anticancer activity for the ethyl acetate extract. Bioactivity guided fractionation of the ethyl acetate extract using preparative TLC resulted in four fractions. The fractions were subjected to LC-UV and LC-MS analysis to identify the flavonoids present. Cytotoxicity studies were conducted by the MTT assay using doxorubicin as the standard drug on A549 cell line (lung cancer cell line), MDA-MB-231 cell line (breast cancer cell line), Hela cell line (cervical cancer cell line).

Results: Seven compounds (kaempferol 3-O-acetyl-glucoside, quercetin 3-arabinoside-7-rhamnoside, quercetin 3-O-(6"-malonyl-glucoside) 7-O-glucoside, naringenin-feruloyl hexose, pelargonidin 3-O-rutinoside, epicatechin gallate, cyanidin 3-O-rutinoside) were identified from this extract and are reported for the first time from this plant. Cytotoxicity studies using the MTT assay indicated a significant anticancer activity on A549 cell line (lung cancer cell line), MDA-MB-231 cell line (breast cancer cell line), Hela cell line (cervical cancer cell line) with IC_{50} values comparable to doxorubicin standard for the extract and its fractions.

Conclusion/Discussion: The bioactive ethyl acetate rich extract is a potential source of anticancer flavonoids and their glycosides which could serve as templates for anticancer drug design and discovery. Quercetin and related flavonoid analogues identified are reported to induce apoptosis on different cancer cell models by affecting the intrinsic apoptotic proteins via the Erk pathway. The functional groups present in the identified flavonoids such as 2, 3 double bond, 3-OH group and 4-oxo on the C ring are reported to affect the function of mitochondria membrane by decreasing the fluidity via inhibiting the respiratory chain of mitochondria or causing uncoupling, which might partially be attributed to apoptosis.

Keywords: *Ficus benghalensis*, aerial roots, cytotoxicity, A549 cell line, MDA-MB-231 cell line, Hela cell line kaempferol 3-O-acetyl-glucoside, quercetin 3-arabinoside-7-rhamnoside, quercetin 3-O-(6"-malonyl-glucoside)- 7-O-glucoside,

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ORAL PRESENTATION

SEASONAL EDIBLE PLANTS OF MAHA RASHTRA(INDIA) - A POTENTIAL MEDICINAL TREASARY

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Objective/Purpose: India's rich biodiversity of flora offers a unique opportunity to study different plant species important to human health. Most of these are looked upon as a rich source of bioactive molecules, useful in developing therapeutics. In todays' times, the urban population is often neglecting inclusion of nutraceutically important indigenous food sources by opting for exotic ones. Leafy seasonal vegetables, is one such class of plants, with nutritional as well as therapeutic value. These leafy plants, from plant families such as Amaranthaceae, Liliaceae, Caesalpinae etc, are used for treating ailments by tribal population. They are either used as juice or eaten in cooked form by common population. This research project aimed at re-discovering some of these seasonal plants that are grown in rural areas adjoining metro city of Mumbai, India and explorİng their nutritional as well as antibiotic/antioxidant properties, that would make them, a potential target for nutraceutics or medicinal formulations.

Material and Methods: Different leafy vegetables such as Math, Shevra, Bharangi, Ghol, Mayalu, Takla, Phodshi available in monsoon season in Mumbai, were procured from local vendors. The dried leaf powders were used as sample for estimating proteins, micro, macronutrients and heavy metals by ICP-AES. For antimicrobial analysis, samples were extracted using three solvents viz, ethanol, acetone and chloroform and were tested against three laboratory strains of microorganisms, *Escherichia coli, Staphylococcus aureus, Kleibsella pneumoniae,* using Agar cup diffusion method. Antioxidant activity was checked for Phodshi with a comparison between edible and non-edible plant part and for Bharangi, the comparison was done for the cooked and uncooked leaves for their scavenging capacity by DPPH assay.

Results: In all the plants under study, Math and Bharangi showed a high % of protein which is uncommon in leaves. In elemental analysis, Shevra was found to be rich in Magnesium, Potassium, Iron and Zinc, where as Takla, Ambadi and Alu were good source of Calcium and Copper. For the study of antibacterial effect, maximum inhibition was observed by the plant extract of Mayalu while least inhibition was found by Takla. Between the three solvents used, inhibitory action was Chloroform >Ethanol> Acetone. Comparing microorganisms, maximum inhibition was seen against *S. aureus* followed by *E. coli* and least against *K .pneumoniae*. The inedible or waste part of Phodshi, was effective in controlling *Corynebacterium* and the scavenging role of its edible portion was found to be as high as 99% in 10 µg/ml of sample. In Bharangi plant, cooked sample showed increased antioxidant activity than the fresh or dried samples.

Conclusion/Discussion: All plants under study showed promising results for nutrient composition, antimicrobial efficacy and antioxidant effects, which can be considered further for identifying bioactive components of therapeutic use. Since these plants are seasonal, there is a need for creating awareness regarding their dietary and health benefits. Most of these indigenous leafy vegetables are less known to the urban population thus growers are faced with low demand. If this downtrend continues, these seasonal plants with a therapeutic potential, would soon disappear from map of available edible vegetables. It is, therefore, imperative to investigate their potential for contributing active biomolecules for medicinal purpose.

Keywords: Seasonal plants, Nutraceutics, ICP-AES, Antimicrobial, Indigenous

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ORAL PRESENTATION

BERBERIS PLANTS WITH ORANGE, RED AND PURPLE FRUITS: ANTIOXIDANT AND ANTI-HYPERTANSIVE ACTIVIES

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The genus *Berberis* belonging to the Berberidaceae family is widely grown in Europe, North Africa, Asia, Iran and in the Central Anatolia region of Turkey (1). The parts (fruit, root, leaf, branch) of this plant exhibit various health effects such as anti-hypertensive, anti-diabetic, anti-microbial, anti-cancer, and antioxidant activity (3) since it is a rich source of polyphenolics, as well as carotenoids (2). Therefore, the present study was performed to investigate phenolic and carotenoid content and some biological properties such as antihypertensive and antioxidant activity of *Berberis* plant with orange, red and purple fruits obtained from Bayburt Province in Turkey. Individual phytochemical profile was analyzed by HPLC-DAD/ESI-MS. Antioxidant activity was determined by CUPRAC and DPPH assays, antihypertansive activity was detected using Angiotensin converting enzyme (ACE) inhibitory activity. According to the results, purple *Berberis* fruit had the highest phenolic content (81.42 mgGAE/gDW), anthocyanin content (455.91 mg cyn-3-gly/100gDW) and antioxidant activity (112 mgTE/g and 290 mgTE/gDW, DPPH and CUPRAC assays, respectively). All kinds of *Berberis* leaves had phenolics in the range of 24.81-42.62 mgGAE/gDW while all *Berberis* branches contained between 15.01 and 21.36 mgGAE/gDW. There is no significant difference among all kinds of branches and leaves in terms of antioxidant activities. Chlorogenic acid was the major phenolic compound found in all fruits and leaves. However, epigallocatechingallate was the major phenolic compound in all branches. Delphinidin-3-glycoside, cyanidin-3-glycoside and peonidine-3-glycoside were the main anthocyanins found in purple *Berberis* fruit. Surprisingly, orange, red and purple fruits contained similar amount of carotenoids (~4mg/100gDW). The highest ACE inhibition activity was found in red *Berberis* fruit (73.84%). However, orange and purple fruits did not show ACE inhibition activity. *Berberis* branches and leaves had ACE inhibition activity over 50%. The order of ACE inhibition activity in *Berberis* plants was red fruit>orange branch>purple leaf~red branch>purple branch>orange leaf~red leaf ($p<0.05$). No correlation was detected between phenolics and ACE inhibition activity while a high correlation was observed between phenolics and antioxidant activity. The purple fruit exhibited the highest antoxidant activity due to anthocyanins and other phenolics while red fruit exhibited the highest anti-hypertansive activity. Therefore, *Berberis* which is unknow in Turkey but it is produced and consumed in İran is used for dietary supplemet and functional food production.

Keywords: Anthocyanins, Angiotensin converting enzyme inhibiton, Antioxidant, *Berberis*, Carotenoids, Phenolic compounds.

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ORAL PRESENTATION

EVALUATION OF THE RELIABILITY OF PLANTS USAGE IN FOODS: A REVIEW

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Functional food is a concept that have been put forward in recent years. A food given an additional function (often one related to health-promotion or disease prevention) by adding new ingredients or more of existing ingredients is known as functional. Functional foods are products that provide significant benefits on the human physiology and metabolic functions beyond the body's need for essential nutrients, thus enabling to protect from diseases and achieve a healthier life. Although the concept of functional foods is new, its application is very old. As the side effects of the drugs have been learned and health problems have increased, the orientations to functional foods have increased and the products are diversified.

Functional foods have been even more than normal food (Ozcelik, 2016). The functional food markets, whose trade volume has exceeded $ 100 billion around the worldwide, has increased in terms of quantity and diversity in developed countries such as the USA, the European Union and Japan. In Turkey, the functional products market for 2015, it is stated that the ratio reached to 600 million Turkish Liras (Anonym,2014). The usage of plants for food industry to obtain functional food products has become a notable issue in recent years. A rising interest for medicinal and aromatic plants and the active substances obtained from them has led to studies to determine the effects of these plants. The purpose of his review is to determine and evaluate the reliability of plants in terms of food and drugs effects.

Keywords: Functional food, plant, reliability.

ORAL PRESENTATION

IN VITRO CYTOTOXICITY AND *IN SILICO* MUTAGENICITY ASSESSMENTS OF THE ISOLATED COMPOUNDS FROM *SIDERITIS GERMANICOPOLITANA* BORNM. SUBSP. *GERMANICOPOLITANA*

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Objective / Purpose: The genus *Sideritis* L. belongs to the Lamiaceae family and consists of more than 150 species which are mainly distributed in Mediterranean region. In Turkey, *Sideritis* sp. are commonly known as "Dağ çayı, Yayla çayı, Ada çayı" and represented by 46 species with high endemism ratio [1]. *S. germanicopolitana* Bornm. subsp. *germanicopolitana* is a perennial herb which is endemic to Turkey, has been used as herbal tea in Anatolia [2]. The aim of the present study was to investigate the chemical constituents of *S. germanicopolitana* subsp. *germanicopolitana* and evaluate some of the isolated compounds for cytotoxic and mutagenic activities.

Material and Methods: Aerial parts of *S. germanicopolitana* subsp. *germanicopolitana* were collected from Söğüt, Bilecik, Turkey in July 2017, at the flowering period. The dried and powdered plant was extracted with methanol at room temperature. The methanol extract was subsequently fractionated by *n*-hexane, chloroform and *n*-butanol. The *n*-hexane and chloroform fractions were subjected to chromatographic methods for the isolation and purification of the compounds. The structures of the compounds were elucidated by means of spectroscopic analysis. The cytotoxic activity of some of the isolated compounds was evaluated on MCF-7, A549, HeLa and BEAS-2B cell lines by MTT assay. In addition, their *in silico* mutagenicity assessments were performed by combining of knowledge-based and statistical-based approaches.

Results: We isolated a number of diterpenoids, flavonoids and one fatty acid derivative. Among them, we have identified a diterpenoid isomer [major: 7-*epi*-candicandiol; minor: sideridiol (**1**)] from *n*-hexane fraction, and also a diterpenoid isomer [major: foliol; minor: isofoliol (**2**)] and two flavones [penduletin 4'-methyl ether (**3**) and penduletin (**4**)] from chloroform fraction. Compounds **1-4** showed inhibitory effects on cell proliferation, especially compound **1** was found to have remarkable cytotoxic activity against MCF-7 (IC_{50}=6.46 µg/mL), A549 (IC_{50}=2.26 µg/mL), and HeLa cells (IC_{50}=4.48 µg/mL). All compounds were found to be non-mutagenic in *in silico* analyses. Other isolated compounds are under research.

Conclusion / Discussion: All identified compounds were isolated from *S. germanicopolitana* subsp. *germanicopolitana* for the first time. In conclusion, *S. germanicopolitana* subsp. *germanicopolitana* was displayed to be a potential natural source for developing cytotoxic agents and furthermore, no mutagenic activity was observed in the isolated compounds.

Keywords: *Sideritis*, *S. germanicopolitana* subsp. *germanicopolitana*, diterpenoid, flavonoid, cytotoxicity, mutagenicity.

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ORAL PRESENTATION

EVALUATION OF THE *IN VITRO* WOUND HEALING ACTIVITY OF ISOLATED CONSTITUENTS FROM *CHRYSOPHTHALMUM MONTANUM* (DC.) BOISS.

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Objective: *Crysophtalmum montanum* (DC.) Boiss. is commonly distributed in the eastern and southeastern parts of Turkey, as well as the North of Iraq and Syria. In Turkey, the aerial parts of *C. montanum* is traditionally used to treat common cold by sniffing into nose as powder, and applied with cotton on injured body for wound healing by preparing decoction method [1]. In our study, we aimed to investigate the inhibitory effects of the isolated constituents from *C. montanum* on metalloproteinase enzymes using *in vitro* assays to explain the potential wound healing activity mechanism.

Material and Methods: As a part of our ongoing researches on *C. montanum*, we isolated secondary metabolites after the multistep chromatographic purifications on the chloroform and *n*-hexane subextracts of the aerial parts of *C. montanum*. We investigated the potential wound healing activity of the isolates by using inhibitory activity assays on hyaluronidase, collagenase and elastase enzymes, all of which have essential role in the wound healing process.

Results: Our phytochemical studies on *C. montanum* led to the isolation of five sesquiterpene lactones (**1-5**), namely 6α-acetoxy-4α-hydroxy-1βH-guaia-9.11(13)-dien-12.8α-olide **(1)**, 6α-acetoxy-4α-hydroxy-9β.10β-epoxy-1βH-guaia-11(13)-en-12.8α-olide **(2)**, 4α,6α-dihydroxy-1β,5α,7αH-guaia-9(10),11(13)-dien-12,8α-olide **(3)**, (4α,5α,8β,10β)-4,10-dihydroxy-1,11(13)-guaidien-12,8-olide (**4**), and (4α,5α,8β,10α)-4,10-dihydroxy-1,11(13)-guaiadien-12,8-olide (**5**), as well as a steroidal glycoside mixture (**6**) and a flavonoid, chrysosplenol C (**7**) from the chloroform subextract, together with a mixture of triterpenes, taraxasterol acetate and Ψ-taraxasterol acetate (**8**) from the *n*-hexane subextract. Compounds **7** and **8** displayed significant inhibitory effects with the value of 28.53 ad 24.41 % for collagenase, 32.28 and 29.31 % for elastase enzymes, respectively. However, none of the compounds showed any inhibitory activities on hyaluronidase enzyme.
Conclusion: Our results provide the phytochemical and biological evidence for the usage of *C. montanum* in Turkish folk medicine for wound healing. It was revealed that compounds **7** and **8** can promote wound healing process by inhibiting the collagenase and elastase enzymes.

Keywords: *Crysophtalmum montanum*, collagenase, elastase, hyaluronidase, wound healing

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ORAL PRESENTATION

ANTI-*HELICOBACTER PYLORI* ACTIVITY OF TWO MEDICINAL PLANTS; FENUGREEK (*TRIGONELLA FOENUM*-GRAECUM L.) AND CUMIN (*CUMINUM CYMINUM* L.)

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The bacterial pathogen *Helicobacter pylori* colonizes in over half of the world's population. The infection with this bacterium is an important cause of peptic ulcer disease and eradication of this organism greatly reduces the recurrence rate of ulcers. *H. pylori* also can cause chronic gastritis and gastric cancer inducing inflammatory responses and pathological changes in the gastric microenvironment. The growing problem of antibiotic resistance by the organism demands the search for novel candidates from plant-based sources. In the present study, we evaluated the *in vitro* anti-*H. pylori* activity of two medicinal plants (*Cuminum cyminum L.* and *Trigonella foenum-graecum L.*) on clinical isolates of *H. pylori.* Gastric biopsy samples were obtained from Algerian patients presenting with gastroduodenal complications. *Helicobacter pylori* was isolated from the specimens following standard microbiology procedures, characteristics of colonies were identified by Gram's stain, urease, oxydase and catalase tests. The disc diffusion method was used to determine the susceptibility of H. *pylori* isolates to methanol extracts of cumin and fenugreek at different concentrations. The results showed that methanolic extract of these plants demonstrated significant *in vitro* anti-*H. pylori* activity against the clinical isolates of *H. pylori*.

Keywords: *Helicobacter pylori*, Fenugreek, Cumin, Antibacterial effect

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ORAL PRESENTATION

ETHNOMEDICINAL AND AROMATIC SALT TOLERANT PLANTS USE VALUE IN KHEWRA SALT MINE, PUNJAB, PAKISTAN

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It is the principal quantitative investigation of halophytic vegetation with their customary therapeutic and fragrant uses in Khewra salt mine, Punjab, Pakistan. In this investigation, the reliance of nearby individuals on halophytes gives pragmatic proof about conventional social insurance frameworks. An aggregate of 151 respondents including 13 customary healers were talked with utilizing quick evaluation strategy. The reported information was mapped and examined quantitatively and in GIS too. The mineral portrayal of the dirt was completed utilizing X-beam diffraction (XRD) strategy. Ethno-restorative information was gathered for 38 salt tolerant plant species having a place with 23 families. Results showed the Fabaceae as much of the time referred to plant family (5 species) and *Acacia nilotica* (L.) Delile is the most refered to plant species. Herbs (half) were the prevailing living thing and leaf (37%) was the generally utilized plant section (24 reports). The general significance of the halophytes and homogeneity of the respondents' learning was dictated by utilizing source agreement factor (ICF) and use esteem (UV). The most noteworthy ICF (0.62) was found for gastrointestinal illness class. Relative recurrence of reference and UV ranges from 0.08 to 0.3 and 0.22 to 0.48 separately. The XRD examination demonstrated the copper sulfide nature of soil which is the pointer of halophytes dispersion in the investigation territory. Lion's shares of the halophytes have been seen to have solid pharmacological and sweet-smelling proof. It is the main recorded provide details regarding halophytes utilized for therapeutic purposes in this locale. The discoveries of this study will be valuable for the safeguarding of Ethnomedicinal and sweet-smelling legacy and for future pharmacological, sweet-smelling and phytochemical screening of salt tolerant plants to discover their potential for normal medication improvement.

Keywords: Halophytes, Medicinal, aromatic, GIS, X-ray diffraction, Khewra Salt Mines, Phytochemicals, Pakistan.

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ORAL PRESENTATION

CHEMICAL CHARACTERIZATION AND BIOLOGICAL STUDY OF THE SPECIES *SENECIO CINERARIA*

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Senecio cineraria is a perennial shrub of Mediterranean origin, belongs to the family of *Asteraceae* (Dupont et Guignard, 2015)[1], it is used in pharmaceutical preparations and in homeopathy. This study is a scientific contribution to the determination of certain phytochemicals, as well as the study of some in vitro biological activities of the methanolic extract of the plant. The analysis of the extract by colorimetric tests was revealed the presence of flavonoids, alkaloids and tannins. Qualitatively, the TLC analysis of the extract showed the presence of a multitude of flavonoid varieties. The flavonoid assay showed a significant content on the order of 60.16 mg EQ / g E. The study of the antioxidant power by the DPPH method has shown that the concentration which traps 50% of the DPPH ° (IC50) radical is 0.35 mg / ml. EMSC revealed a significant anti-hemolytic effect compared to the positive control. This is proportional to the concentration of the extract used during the test. The antibacterial potential of the extract was confirmed on strains: Staphylococcus aureus, Listeria monocytogenes, Klebsielaoxytoca, with MICs of 10 mg / ml, 20 mg / ml and 2.5 mg / ml respectively. Chronometric coagulation tests (TCK and TQ) have shown that the extract has significant anticoagulant activity.

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ORAL PRESENTATION

PHENOLIC CONTENTS AND ANTI-OXIDANT ACTIVITIES OF *ABIES NUMIDICA* LEAVES EXTRACTS

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Objective / Purpose: Recently, polyphenols have attracted a great deal of attention due to their roles in the plant and on human health. There is a strong need to find new effective anti-oxidants from natural sources to prevent human diseases. Algerian fir (*Abies numidica*) is an endemic tree, belongs to *Pinaceae* familly, used in traditionnal medicine as anti-inflammatory, for cataplasm and for the respiratory system. The objective of this study is to extract secondary metabolites from Algerian fir leaves (AFL) and evaluate the anti-oxidant activities of its extracts.

Material and Methods: the air-dried powdered leaves were extracted with 80% methanol and fractionated by dichloromethane (CH_2Cl_2), ethyl acetate, and n-butanol. The total phenolic (TPC) and total flavonoid (TFC) contents of ethyl-acetate and n-butanol fractions were estimated. Furthermore, the antioxidant activities of ethyl-acetate, n-butanol extracts were evaluated by DPPH, CUPRAC assays.

Results: the results showed that ethyl-acetate of AFL is rich in total phenol (490,76±6,65mg gallic acid equivalent/ g extract) and total flavonoids (143,19±5,59 mg quercetin equivalent/g extract) and showed a high anti-oxidant activities in DPPH and CUPRAC assays.

Conclusion / Discussion: it can be concluded that extracts of Algerian fir can be considered as strong anti-oxidant agents, and it is very important to evaluate more assays to determine the biological activities in order to exploit it in human requires.

Keywords: phenolic contents, anti-oxidant activity, leaves extracts, *Abies numidica.*

ORAL PRESENTATION

PHYSICOCHEMICAL AND PHYTOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SPINELESS CACTUS (*OPUNTIA FICUS INDICA* F. *INERMIS*) CLADODES IN THE REGION OF TIARET (ALGERIA)

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Opuntia ficus indica commonly known as barbary fig has many medicinal properties that have recently been approved. The objective of this study was to promote the therapeutic potential of *Opuntia ficus indica f. inermis* cladodes in the region of Tiaret by their physicochemical and phytochemical characterization and antibacterial activity evaluation. Physicochemical analyzes have shown that young and old *Opuntia ficus indica f. inermis L.* cladodes are rich in water, minerals (potassium and calcium) and fiber but low in crude protein.

The total phenolic compounds and flavonoids contents were 36.4 mg GAE / g DW and 16.6 mg QE / g DW for the young cladodes respectively and 17.6 GAE / g DW and 15.35 mg QE / g DW for the old cladodes respectively. Phytochemical screening revealed the presence of tannins, steroids and reducing sugars in both types of cladodes.

The results of antibacterial effect of young cladode methanolic extract carried out by the diffusion method of disks against three pathogenic strains: *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeroginosa* ATCC 27853 showed the sensitivity of *S.aureus* and *E. coli* to this extract. The cladodes of *Opuntia ficus indica inermis* L. may be a potential source of biological molecules for medicine, nutrition and biotechnology.

Keywords : Cladode, *Opuntia ficus indica* f. *inermis*, physicochemical, phytochemical, antibacterial.

ORAL PRESENTATION

DEVELOPMENT OF MICROEMULSION CONTAINING *PLANTAGO MAJOR* EXTRACTS: FORMULATION AND EVALUATION OF TOPICAL ANTI-INFLAMMATORY ACTIVITIES

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Objective / Purpose: *Plantago major* L. (Plantaginaceae) is widely used to treat various diseases. It is reported to have anti-microbial, anti-diabetic, anti-spasm, anti-inflammatory activity and wound healing properties. *P. major* contains biologically active compounds such as polysaccharides, flavonoids, iridoid glycosides, terpenoids, and alkaloids[1]. Despite several publications supporting health benefits of this plant, not much work has been done to develop an anti-inflammatory formulation containing extracts of *P. major*. Therefore, the aim of this work was to formulate extract of *P.major* into microemulsion to increase economical value of this plant and to support the society with affordable dosage form.

Material and Methods: Microemulsions are clear, optically isotropic and thermodynamically stable systems generally composed of a blend of oil, water and surfactant(s) [2]. Three formulas were developed with variation of surfactants tween 80: lecithin, Formula 1 = 10:1, Formula 2 = 11:1 and Formula 3 = 12:1 respectively, along with cosurfactant (propylene glycol) and extract of *P. major* (0.3%). Microemulsions were stored at 4°C, 25°C, and 40°C for 4 weeks, and were characterized regarding their organoleptic, homogenity, pH, and viscosity. Particle size was determined on day 0 and week 4 using Horiba®SZ-100 nano particle analyzer. Formula 3 was investigated for its anti-inflammatory activity using croton-oil induced ear edema in mice [3]. Percentage of inflammatory inhibition was calculated and confirmed by histological section of ears

Results: During 4 week of storage, all formula showed homogeneity and stability and met the particle size requirements for microemulsions.Topical application of microemulsions reduced ear edema and pro-inflammatory cells in the tissue and its activity were similar to hydrocortisone 1% ($p > 0.05$).

Conclusion / Discussion: Our data indicated that *P. major* extracts can be developed into modern formulation with potent anti-inflammatory activity.

Keywords: *Plantago major*, microemulsion, topical anti-inflammation, croton oil-induced ear edema

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ORAL PRESENTATION

UNTARGET METABOLOMICS ANALYSES OF THE NEUROPROTECTIVE POTENTIAL OF AYAHUASCA ON HUMAN NEUROBLASTOMA CELLS

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Objective/Purpose: The available treatments of Parkinson's Disease (PD) target mainly the pathology symptoms and are responsible for severe adverse effects on the host. The aim of this research was to analyse the neuroprotective role of Ayahuasca and its plant matrices: *Banisteriopsis caapi* and *Psychotria viridis* on the viability of human neuroblastoma cells line (SH-SY5Y) by using chemical metabolomic approaches.

Material and Methods: The Ayahuasca beverage and the vegetal material were obtained from a syncretic religion in Brazil 'O Santo Daime'. The *B. caapi* vines and *P.viridis* leaves were harvested in the city of Piedade-SP, Brazil in 2017. The extracts, fractions and isolated alkaloids were characterized by HPLC-UV-DAD. The neuroprotective effects of the samples were screened for 48 h and 72 h assays on the viability of SH-SY5Y cells through the MTT and Calcein-AM. Whereas the cell death was evaluated by propidium iodide staining. The 6-hydroxydopamine (6-OHDA) was used to assess neurodegeneration in PD. The UPLC-HRMS metabolome data was handled using Waters MS software MassLynxTM V4.1 and MarkerLynx XS 3.0.1, which the multivariate statistical analyses were performed. The molecular formula determination was based on the WATERS scientific i-FIT norm. The metabolites were dereplicated using Dictionary of Natural Products (DNP) and online databases.

Results: Ayahuasca as well as the crude extracts of *B. caapi* and *P. viridis,* and the hexanic fractions exhibited neuroprotective effects at the non-toxic concentrations tested. In addition, lesser neuroprotective effects were also obtained for the *B. caapi* hydroethanolic fraction and for the harmine alkaloid. The PCA-X provided an initial look at the dataset, while the cross-correlation statistical analysis of the MS data performed by OPLS-DA regression has revealed key metabolites for the activity. Thus, we identified besides the harmine others 26 potential biomarkers, indicated by S-plot dispersion and Variables Important for Projection (VIP) that have contributed most to the difference in the metabolite profiles of the active samples.

Conclusion / Discussion: The data demonstrated that the evaluated crude extracts have noteworthy neuroprotective effects. Thus, Ayahuasca based plants definitely could be considerate promising for the treatment of PD. The potential of untargeted metabolomic analysis has been underlined as it has generated chemically relevant data for the research. Therefore, the highlighted metabolites and other plant extracts containing them are likely to be in further consideration for the PD.

Keywords: Metabolomics, Ayahuasca, Neuroprotection, Neurodegenerative diseases

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ORAL PRESENTATION

ANTIOXIDANT ACTIVITY OF THE FRUIT EXTRACTS OF *WITHIANIA SOMNIFERA* AND *SOLANUM NIGRUM*

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Objective and Purpose: Antioxidants are known for the prevention and treatment of oxidative stress related disorders, such as diabetes and cardiovascular diseases. Synthetic antioxidants have considerable amount of toxicity, therefore there is an interest in developing herbal drugs. Medicinal herbs possess many phytochemicals which are efficient scavengers of free radicals.The present study was conducted to assess the antioxidant activity of two medicinal herbs,Withiania Somnifera and Solanum nigrum from the family Solanaceae.

Material and Methods: The dried berries (10g each) were extracted in alcohol, separately. Total phenolic content was determined by Folin- Ciocalteau Method and antioxidant activity was measured by DPPH radical scavenging activity.

Results: The total phenolic content and antioxidant activity of Solanum nigrum was considerably higher than Withiania somnifera. However, both herbal extracts showed good amount of total phenolic content and antioxidant activity.The alcoholic extract of Withiania somnifera shows more than 50% DPPH radical scavenging activity at 200µg/ml while the ethanol extract of Solanum nigrum showed above 80% DPPH radical scavenging activity at the same concebtration.

Keywords: Antioxidants, total phenolic content, medicinal herbs, *Withiania somnifera*, Solanum nigrum

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ORAL PRESENTATION

EVALUATION OF SOME BIOLOGICAL ACTIVITIES OF THE BULBS OF *CORYDALIS SOLIDA* (L.) CLAIRV. SSP. *INCISA* LIÉDEN SPECIES GROWING IN TURKEY

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Objective: The continuing research for the determination of bioactive secondary metabolites fron Turkish geophytes as therapeutic agents for demantia is mainly based on the need for drug candidates affected to brain areas. This presentation describes for the first time the *in vitro* anticholinesterase properties and cytotoxic activities of the bulb extracts and alkaloidal fractions of *Corydalis solida* (L.) Clairv. ssp. *incisa* Lidén. Furthermore, the content of the active alkaloid fractions of the bulbs was determined by LC-Q-TOF-MS/MS.

Material and Methods: The bulbs of *Corydalis solida* (L.) Clairv. ssp. *incisa* Lidén were collected from Edirne province. The plant species were also preserved as *ex-situ* in Yalova. The ethanolic and alkaloidal extracts were prepared from the bulbs. The anticholinesterase activities of the extracts and fractions were tested by modification of the Ellman's method. The cytotoxic activities of the extract were determined using the MTT assay against 3T3 and MCF-7 cell lines. The optimization of LC-MS conditions was used in ESI in the positive ion mode.

Results: The alkaloidal extract of the bulbs exhibited a highest activity against AChE and BChE with IC_{50} values of 0,0167 ± 0,0006 mg/ml and 0,1759 ± 0,0039 mg/ml (galanthamine 0,0068 ± 0,0005 mg/ml and 0,3444 ± 0,0082 mg/ml as positive control), respectively. Among the fractions obtained from the alkaloidal extract, protoberberine-type alkaloids exerted the most promising activity against both cholinesterases, with IC_{50} values of 0,0224 ± 0,0002 mg/ml and 0,1831 ± 0,0053 mg/ml for AChE and BChE, respectively. The ethanolic extract did not show any cytotoxicity against 3T3 normal fibroblast cell line, however it was found to be active against MCF-7 cell line at 50 □g/ml concentration as 57.38 % inhibition.

Conclusion: The present study showed for the first time the *in vitro* activity of *Corydalis solida* ssp. *incisa* alkaloidal extract and fractions from bulbs as neuroprotective potential and their metabolite profile by LC-Q-TOF-MS/MS. Besides, the anticholinesterase assays on alkaloidal extract and its fractions showed that protoberberine–type alkaloids were the most potent inhibitor of both AChE and BChE.

Keywords: *Corydalis*, isoquinoline alkaloids, anticholinesterases, cytoxicity

Acknowledgement: This study was funded by the grant of Gazi University Scientific Research Projects Unit (Grant No: 02-2017-13).

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ORAL PRESENTATION

MEDICINAL PLANTS USED IN THE TREATMENT OF SKIN DISORDERS IN TURKISH VETERINARY MEDICINE

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Over centuries, people have developed their own system of keeping animals healthy and productive using age-old home remedies, surgical and manipulative techniques, husbandry strategies and associated magicoreligious practices. In this context, ethnoveterinary medicines (EVM) are used extensively and quite effectively in many parts of the world for primary health care treatment to make domestic animals productive and healthy. These medicals, considered often safer, cheaper than conventional drugs, can provide useful alternatives to modern animal health care systems.

Medicinal plants are regarded as an integral component of EVM and have been successfully used for treatment of many diseases in traditional veterinary medicine. Turkey has very rich flora with more than 11.000 species of plants, of which around 34.5% are endemic, as a result of its ecological features and geographical location. Especially in recent years, the use of phytotherapeutic remedies has gained popularity in veterinary practice as well as human medicine, in Turkey. In this context, skin diseases and wounds (18.7%) have been reported as the most treated disorders with medicinal plants in Turkish veterinary medicine, following gastrointestinal diseases (26%).

Medicinal plants used in the treatment of skin wounds in animals can regulate the wound healing by affecting key steps for the healing process such as proliferation, migration, collagen deposition or angiogenesis. In addition, many medicinal plants can accelerate the healing process of common skin diseases in animals such as mange, ringworm or dermatitis, due to their multiple pharmacological properties such as antiparasitic, antifungal, antioxidant or antimicrobial activity.

In this review about the medicinal plants used for the treatment of skin disorders in Turkish veterinary medicine, it was aimed to present current knowledges on traditional uses of selected plants from a scientific view point.

Keywords: Medicinal plants, skin disorders, veterinary medicine

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ORAL PRESENTATION

LEAVES ANTIMICROBIAL ACTIVITY OF SOME *GLYCYRRHI* ZA L. SPECIES

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Objective / Purpose: The genus *Glycyrrhiza* includes well-known traditional medicinal plants growing in various parts of the World. Antimicrobial effect studies on leaves are limited although there are many previous studies on antibacterial activity of root extract from licorice. In the study is aimed to determine in-vitro antibacterial effect of leaves extract of *G. glabra* L., *G. flavescens* Boiss. and *G. echinata* L. species.

Material and Methods: Plant samples of these *Glycyrrhiza* species were collected from 17 different localities of the East Mediterranean part of Turkey in 2016. The taxonomic identification was carried out based on related literature. The plant leaves were shade-dried at room temperature and then ground into powder. The leaves metanolic extracts were evaluated for antimicrobial activities against 7 bacterial and 2 yeast strains using discdiffusion and minimum inhibitory concentration methods.

Results: Our results indicated that the leave extracts were more effective against Gram-positive bacteria than against Gram-negative ones. In addition, the extracts had higher antimicrobial effect against Candida species than against bacteria. Max. Inhibition zones values were determined as 20 mm in *G. flavescens*, 16 mm in *G. glabra* and 15 mm in *G. echinata*.

Conclusion / Discussion: Results indicated that different populations of same species had got different level in terms of biological activity. This study also supported the traditional use of licorice and as well as suggested that it may also be its beneficial role in the treatment of other infections. The obtained results indicated that different environmental conditions in each habitat might affect the contents of chemical compounds and the biological activity in the natural licorice populations.

Keywords: *Glycyrrhiza*, licorice, antimicrobial activity, Turkey

MESMAP-5

ORAL PRESENTATION

QUORUM SENSING INHIBITION ACTIVITY OF VOLATILE OILS APPLIED IN AROMATHERAPY

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Objective/Purpose: Quorum sensing (QS), the cell-to-cell signalling system in bacteria, is a molecular mechanism of gene regulation, in which bacteria adapt their behavior according to cell density and the surrounding environment. Multidrug-resistance bacteria commonly use this system for developing the resistance; therefore, quorum sensing inhibition (QSI) could be an effective approach for the prevention of bacterial infections, and the discovery of new anti-QS agents. In this study, it is aimed to investigate QSI activity of selected volatile oils used in ‘Aromatherapy’ on *Chromobacterium violaceum* biosensor system.

Material and Methods: Volatile oils of *Citrus limon*, *Mentha piperita*, *Eucalyptus globulus, Citrus sinensis*, *(linalol), Citrus aurantium bergamia, Melaleuca alternifolia, and their blends*, were tested for their ability to inhibit QS. The bioreporter strain *Chromobacterium violaceum* CV026 the signaling molecule N-(ß-ketocaproyl)-L-homoserine lactone (3-oxo-C6-HSL) was used with violacein pigment inhibition and diffusion assays.

Results: All of the aromatherapeutic volatile oils inhibited the violacein pigment of CV026 (11.32%-90.07%) and exhibited violacein inhibition zone varying from 7.00 to 32.33 mm. The most powerful volatile oils against CV026 were *C. aurantium bergamia, M. piperita* and *M. Alternifolia* respectively.

Conclusion/Discussion: This study indicates that the volatile oils may be the potential to be used as natural QS inhibitors for the treatment of bacterial infections in aromatherapy.

Keywords: Aromatherapeutic volatile oils, *Chromobacterium violaceum* CV026, quorum sensing, aromatherapy

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ORAL PRESENTATION

ANTIOXIDANT, ANTIGLYCATION AND ANTIDIABETIC POTENTIAL OF A NATURAL SWEETENER, *STEVIA REBAUDIANA*

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Objective / Purpose: The antidiabetic potential of leaf aqueous *Stevia* extract, a natural, non-caloric sugar substitute, is known. The mechanism is still not clear. This study proposes one of the underlying mechanisms of controlling diabetes by evaluating the extract’s antioxidant and antiglycating potential in an in vitro glycation system.

Material and Methods: The plants of *Stevia rebaudiana* Morita I variety, was obtained from organic Innovation, Guwahati, Assam. The aqueous extract, prepared from leaves of stevia were used for antioxidant assays like FRAP and DPPH. The same extracts were used for antidiabetic assays (α-glycosidase inhibitory assays and α-amylase inhibitory assays) and antiglycating assays (measurement of browning and early glycation end products).

Results The IC_{50} value for aqueous extract of *Stevia* was found to be 300µg/ml, by both DPPH inhibition and ABTS radical scavenging assays. The same concentration of *Stevia* showed 20.35±1.67% inhibition of α-amylase and 8.30±0.95% of α-glycosidase inhibition. It was observed that there was significant reduction in fructosamine formation and browning of *in vitro* glycation system with Stevia extract as compared to the control.

Conclusion/Discussion This study proved that the leaf extract from *Stevia rebaudianana* can be used as the source of antiglycating agents in alleviating the symptoms of diabetes.

Keywords: Antidiabetes, antioxidants, antiglycation, stevia, sweetener.

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MESMAP-5 Initial Proceedings Book

ORAL PRESENTATION

CHANGES IN THE WEED SPECIES IN FIELDS OF MILK THISTLE, CORIANDER, CHICKPEA AND FORAGE PEA WHICH ARE SOWN ON DAMAGED BY FROST AREAS OF WINTER OILSEED CANOLA

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Objective / Purpose: The aim of this experiment is to investigate the changes in the weed species in fields of 4 crops - milk thistle, coriander, chickpea and forage pea which are sown on damaged by frost crops of winter oilseed canola.

Material and Methods: During 2016 - 2018 was conducted a field experiment. On areas with damaged by frost winter oilseed canola, at the spring were sowed and investigated 4 spring crops: 1 milk thistle cultivar - Silmar (*Silybum marianum* Gaertn.); 1 coriander cultivar - Lozen (*Coriandrum sativum* L.); 1 chickpea cultivar - Kabule (*Cicer arietinum* L.); 1 forage pea cultivar - Mir (*Pisum sativum* L.). The same variants were planted on areas under conventional soil cultivation for each of these crops. Weed control was carried out by different herbicide combinations and herbicide tank mixtures.

Conclusion / Discussion: Coriander and milk thistle are suitable crops for sowing on areas after damaged by frost winter oilseed canola, when using herbicide combination Stomp aqua + Stratos ultra or herbicide tank mixture Zencor + Shadow. After plowing of canola crops, it is more appropriate to sow chickpea in which weed control is carried out by soil treatment with herbicide Merlin flex, followed by foliar treatment with herbicide tank mixture Challenge + Shadow. Herbicide combination Dual gold + Listego should not be used in chickpea. On plowed areas with damaged by frost winter canola hybrids without any problems can be sown forage pea, in which weed control is carried out by herbicide combinations Stomp aqua + Korum or Dual gold + Listego.

Keywords: milk thistle, coriander, chickpea, forage pea, weeds, herbicides.

MESMAP-5 Final Proceedings Book

ORAL PRESENTATION

INVESTIGATION OF VOLATILE COMPONENTS AND VARIOUS *IN VITRO* BIOLOGICAL ACTIVITIES of *Tilia tomentosa* (SILVER LINDEN) MOENCH FLOWERS

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Objective: *Tilia tomentosa* Moench. (Malvaceae) flower *n*-hexane extract was investigated for its *in vitro* biological activities and volatile constituents by Gas Chromatography / Mass Spectroscopy (GC / MS).

Material and Methods: The dried plant material was powdered and extracted with *n*-hexane by maceration. The antioxidant capacity was tested *in vitro* DPPH and ABTS radical scavenging methods. Antimicrobial activity was investigated by microdilution method against microorganisms of *Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* NRLL B-3008, *Staphylococcus aereus* ATCC 6538, *Helicobacter pylori* ATCC 43504 and *Mycobacterium smegmatis* ATCC 25291. The effect on cell viability *in vitro* was determined by measuring the metabolic activity of the cells.

Results: *T. tomentosa n*-hexane extract shows that it has moderate levels of antioxidant activity. In addition, the increased activity of the extract on *in vitro* cell viability was determined.

Conclusion: *T. tomentosa n*-hexane extract has a positive effect on cell viability, it is rich in camphor, it affects *S. aureus* at a dose of 125 mg / mL and it has a certain level of antioxidant activity. For these reasons, the extract may be used as a cell regenerator.

Keywords: Cell kulture, *Tilia tomentosa,* Antimicrobial, Cell regenerator, GC / MS.

MESMAP-5 Final Proceedings Book

ORAL PRESENTATION

ANTIDIABETIC AND ANTIGLYCATING PROPERTIES OF *SPIRULINA PLATENSIS*

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Objectives/Purpose: Diabetes is a major health concern for the world population. Natural resources are being explored for the antidiabetic and antiglycating properties to combat Diabetes. This study was carried out to evaluate both these properties in *Spirulina platensis*, well-known blue - green algae.

Material and Methods: The Antidiabetic property was checked using the extent of inhibition of alpha-amylase enzyme. The extract of *Spirulina platensis* was also incubated with the *in vitro* glycation system consisting of sugar and protein. The amount of glycation products in the presence/absence of *Spirulina* extracts were measured by established methods like NBT assay, carbonyl content, Thioflavin T assays and total AGEs. The DNA damage induced by glycation was also checked by agarose gel electrophoresis.

Results: The results obtained with alpha-amylase inhibition indicate that the *Spirulina* extracts have significant antidiabetic potential. The significant decrease in the amount of glycation in the presence of *Spirulina* extract also suggests the antiglycating potential of phytochemicals present in this organism.

Conclusion/Discussion: These results indicate the significance antidiabetic and antiglycating potential of the extract of *Spirulina platensis*. Further characterization will help in the identification of active constituent from *Spirulina* which can be used for the prevention of Diabetes.

Keywords: Antiglycation, Carbonyl content, DNA damage, Glycation, Protein aggregation, *Spirulina*

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ORAL PRESENTATION

PHYTOCHEMICAL PROFILES AND BIOLOGICAL ACTIVITIES OF ENDEMIC MEDICINAL PALNTS FROM KAZAKHSTAN

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Objective / Purpose: For thousands of years, medicine and natural products have been closely linked through the use of traditional medicines. In Kazakhstan grow over six thousand kinds of plants and these natural resources have been efficiently used in the treatments for a variety of diseases in Kazakh traditional medicine. We focused our attention on study of the bioactive chemical constituents of endemic medicinal plants such as *Ikonnikovia kaufmaniana*, *Artemisia heptapotamica*, *Artemisia albicerata* and *Limonium michelsonii* Lincz.

Material and Methods: The flowering plants of *I. kaufmaniana and A. heptapotamica* Poljak were collected in July, 2016, from Almaty region of Kazakhstan. Phytochemical profiles from ethyl acetate extract of *I. kaufmanniana* were quantified by HPLC-ESI MS/MS. The antioxidant activities of all compounds were screened using three different radical sources (DPPH, ORAC, and hydroxyl radicals). As well as, the bioactive constituents from *A. heptapotamica* obtained by using different chromatographic methods including MCI, Sephadex LH-20, silica gel, ODS, preparative HPLC and semi – preparative HPLC. The structures of all isolated compounds mainly achieved by extensive analysis of MS, 1D and 2D NMR spectroscopic data, and ECD spectrum.

Results: Isolation and structural identification processes of *I. kaufmaniana* revealed phenolic compounds having dihydroflavanonol, flavonol, isoflavone and flavanol skeletons and most compounds had significant antioxidant activity against three radical sources, and their efficacies were found to differ by their functionality and skeleton. The phytochemical investigation of *A. heptapotamica* resulted new sesquiterpene lactone dimer, together with 10 known compounds including three dimers, four guaianolides and three *seco*-guianolides.

Conclusion / Discussion: Secondary metabolites of endemic medicinal plants were investigated for the first time, and related biological activities were evaluated.

Keywords: phytochemical profile, *Artemisia heptapotamica, Ikonnikovia kaufmaniana,* biological activity.

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MESMAP-5 Final Proceedings Book

ORAL PRESENTATION

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

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Objective: In this study, measuring the inhibitory effect of seven plant species on enzymes that associated with carbohydrate and lipid metabolism was aimed. Their antioxidant activities and total phenol and flavonoid contents were also evaluated.

Material and Methods: Methanol and water extract were prepared from aerial parts of *Alcea biennis* Witerl, *Anchusa leptophylla* Roem. & Schult., *Alhagi pseudalhagi* Desv., *Berberis crataegina* DC., *Ceratonia siliqua* L., *Scabiosa rotata* M.Bieb. and inflorescence of *Tilia argentea* DC. and their in vitro α-amylase, α-glucosidase and pancreatic lipase inhibitory activities were evaluated. On the other hand their total phenol and flavonoid contents, metal chelating, super oxide dismutase, reducing power and antioxidant activities were also determined [1-11].

Results: Methanol and waters extracts of *A. pseudalhagi* (45.21 and 358.7 µg/mL IC_{50}) and *T. argentea* (81.86 and 411.6 µg/mL IC_{50}) attracted attention with their high α-glucosidase and pancreatic lipase inhibitory activities. Studied extracts were determined not to have effect against α-amylase, except methanol extract of *S. rotata.* Generally, they also showed high antioxidant activity in CUPRAC, ABTS, SOD, Metal Chelating and DPPH methods. The highest total antioxidant activity was observed in *B. crataegina* methanol extract.

Conclusion: Extracts from *A. pseudalhagi* and *T. argentea* came into prominence with their high α-glucosidase and pancreatic lipase inhibitory activities. More detailed investigations on these extracts should be done.

Keywords: α-glucosidase, α-amylase, pancreatic lipase.

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MESMAP-5 PROCEEDINGS BOOK

ORAL PRESENTATION

THE EFFECTS OF NITROGEN AND FARM MANURE APPLICATIONS ON ESSENTIAL OIL CONTENT OF DILL (*Anethum graveolens* L.)

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Proceedings Book

Objective/Purpose: *Anethum graveolens* L. or dill, belonging to Apiaceae (Umbelliferae) family, is an annual aromatic herb known for culinary and medicinal use since ancient times. It has been used in ayurvedic medicines since ancient times and it is a popular herb widely used as a spice and also yields essential oil. The present study aimed to determine the effects of different doses of chemical (ammonium nitrate) and organic fertilizer (farm manure) applications on the essential oil content of dill (*Anethum graveolens* L.).

Material and Methods: In this study, different doses of farm manure (750, 1000, 1250 and 1500 kg/da) and ammonium nitrate (3, 6, 9, 12 kg/da) with a control (no manure) were applied by sowing. The experiments were arranged in the Completely Randomized Blocks Design with three replicates. Each experimental plot consisted of five rows,with a distance of 0.3 m between rows and 0.2 m between plants, and a plot size of 3.3 m^2. The essential oil percentage was subjected to hydro-distillation for 3 hours using a modified Clevenger apparatus.

Results: The hydro-distilled essential oil content ranged from 0.28% to 6.16%. The highest essential oil content were obtained in the treatments with farm manure doses of 750 kg/da and control application, which were significantly different compared to the other treatments. Among all of the essential oils included in the study, dill seeds showed the highest essential oil yield, followed by dill leaves and dill herbs. As suggested by the present study, a suitable ratio of farm manure in the soil can increase the essential oil content.

Conclusion: This organic manure could thus be considered as suitable substitutes for chemical fertilizers when growing medicinal plants that are gaining both an increased importance and demand.

Keywords: Dill, essential oil, farm manure.

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ORAL PRESENTATION

ELEMENTAL ANALYSIS OF LEAFY VEGETABLES AS NUTRITIONAL & MEDICINAL BENEFACTORS

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Objective/Purpose: A geographical biodiversity so rich that it's considered a blessing & a source of natural wealth. This has been the outlook of Indians for generations, who have created traditional cuisines which are abundant in nutritional & medicinal benefits. Leafy vegetables form a part of meals which contain a variety of minerals. Near Mumbai are some regions of the Western ghats, a hot spot of rich biodiversity, where plants from families such as Amaranthaceae, Liliaceae, Fabaceae are often consumed as leafy vegetables. Yet there are some leafy vegetables which are lesser known to urban people. They are indigenous, occur in natural wild habitats and are available only during monsoon. Their benefits are known to the local tribes who consume them to ward off monsoon ailments. Ca^{+2}, Na^{+}, K^{+}, Cu^{+2}, Zn^{+2}, Fe^{+2} etc. are elements which are important for normal metabolism in human being. Most of the elements are quantified along with their effects. Our aim was to analyse some common and uncommon leafy vegetables for the mineral content. On understanding their mineral content, the dietary benefits of uncommon leafy vegetables can be popularised among urban population.

Materials and Methods: Different leafy vegetables; all Season/common plants: *Portulaca oleracea* (Ghol), *Basella rubra* (Mayalu), *Vigna unguiculate*(Chawli), *Colocassia esculenta*(Alu) and *Cassia tora* (Takla) and seasonal/uncommon plants (Monsoon): *Amorphophallus commutatus* (Shevra), *Amaranthus viridis* (Pandhara math), *Clerodendron indicum* (Bharangi), *Ipomoea aquatica* (Nalichi bhaji), Smithia conferta(Kauli), *Hibiscus cannabinus*(Ambadi) were procured from Mumbai local and rural vendors. The leaves were dried at a constant weight and powdered. The sample powders were acid digested and then subjected to ICP-AES analysis.

Results: The macro-elements K^{+}, Ca^{+2}, Na^{+} were present in adequate quantities in both the plant groups. Minor-elements were checked and Cu^{+2} was also present in adequate amounts but the uncommon plants had more content of Copper as compared to common ones. *Amorphophallus commutatus* (Shevra) had Zn^{+2} present in good quantity, where as all other plants showed its absence.

Discussion: The macro-elements are all essential for the normal metabolism of human body. Potassium maintains Na+/ K+ - ATPase pump, muscular activities and electrolyte balance in the body. Its deficiency results in formation of kidney stones and cardiac diseases whereas in excess it causes accumulation of calcium in bones. Calcium acts as neurotransmitter, maintains muscular functioning, helps in proper bone formation, promotes absorption of Ca+2 from intestines by calcitonin, also helps Na^{+} /K^{+} pump. Na^{+} helps to maintain homeostasis. The minor-elements are necessary for enzyme activation.

Key words: Minerals, leafy vegetables, ICP-AES.

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MESMAP-5 Final Proceedings Book

ORAL PRESENTATION

CHEMICAL ANALYSIS OF WILD PLANT *INULA HELENIUM* SUBSP. *PSEUDOHELENIUM* GRIERSON GROWING IN VAN LAKE AROUND.

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The genus *Inula* belongs to the Compositae family and comprises 27 species growing wild in Turkey. *Inula helenium* L. subsp. is of the commonly consumed wild edible plants as food and medicinal purposes inhabitants in Eastern Anatolia. It has been used in traditional Anatolia medicine for abdominal distension and pain, acute enteritis and bacillary dysentery. 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 *Inula helenium subsp. pseudohelenium* Grierson 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 in this research showed that *Inula helenium subsp. pseudohelenium* Grierson contains high value of macro elements such as sodium (1.07 mg/g), potassium (19.16 mg/g), magnesium (2.12 mg/g) and calcium (14.17 mg/g). Also, it has maximum micro-elements such as iron (246.24 mg/kg), manganese (52.98 (mg/kg), copper (40.27 mg/kg and zinc (26.84 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, *Inula helenium* L, East Anatolia

ORAL PRESENTATION

NEUROPROTECTIVE AND COGNITIVE ENHANCEMENT POTENTIALS OF XANTHONE-ENRICHED FRACTION OF *GARCINIA MANGOSTANA* AND A-MANGOSTIN

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Objective: Mangosteen (*Garcinia mangostana*), also known as “The Queen of Fruit” has been used traditionally in Southeast Asia to treat against inflammation, skin infection, wound and diarrhea. The present study aimed to investigate the neuroprotective and cognitive enhancement potentials of xanthone enriched fraction (XEF) from *G. mangostana* and its major constituent, α-mangostin.

Material and Methods: The neuroprotective effects of XEF and α-mangostin were studied in various stressors-induced neurotoxicity models in neuroblastoma cell lines and rat primary cells. Their effect on cognition was investigated in chronic cerebral hypoperfusion (CCH) rats, prepared by permanent bilateral common carotid arteries occlusion (PBCCAO). Two weeks after surgery, CCH rats were orally administered (single and 14 days repeated dose) with XEF and α-mangostin prior to locomotor activity and Morris water maze, long term potentiation (LTP) evaluation.

Results: The stressors-induced neurotoxicity caused reduction in cell viability of 25 to 45%. At lower concentration range of 0.25-1µg/mL, XEF and α-mangostin showed significant and concentration dependent neuroprotection in all test models. Among them, α-mangostin showed the most promising neuroprotective effect, especially in the glutamate-induced neurotoxicity. The *in vivo* studies showed no effect on the rat’s locomotor activity. However, α-mangostin (50mg/kg) and XEFGM (100mg/kg) significantly reversed the cognitive impairment induced by PBCCAO in the spatial learning test. In addition, α-mangostin also showed significant improvement in the reference memory. LTP results revealed that α-mangostin improved the basal synaptic transmission but has no improvement on the inhibition of LTP observed in CCH rats. Likewise, no changes were observed in the protein expressions of BDNF and CAMKIIα in hippocampus of the treated rats.

Conclusion: The present study suggests that XEF and α-mangostin are potential protective agents against oxidative stress and excitotoxicity-induced neurodegeneration and ameliorated learning and memory deficits in CCH rats, worthy of further investigation.

Keywords: *Garcinia mangostana*; α-mangostin; Xanthone enriched fraction; Neuroprotective; Cognitive enhancement; Chronic cerebral hypoperfusion.

ORAL PRESENTATION

MEDICINAL PLANTS AS IMMUNOSTIMULANTS IN FISH FARMING

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For a long time, disinfectants and antibiotics have been used effectively for the treatment of fish diseases. It has been revealed that overuse of chemicals carry potential risks such as environmental problems, residual accumulation in tissue, resistance to antibiotics, and reduction of the efficiency. These problems have led some researchers and fish farmers to consider alternative natural sources from plants that would minimize environmental harm while allowing increased aquaculture production. Main problems of aquaculture production are high stock density and consequently the decrease in water quality, the increase of stress and infection risk. After the occurrence of an infection, the control and treatment are more challenging, and it leads to mass deaths and economic losses. Because of the difficulties in treatment, prophylactic methods like vaccination or immunostimulants have gained importance in the protection of fish health.

It has been shown that the use of immunostimulants can be an effective tool for increasing the resistance of fish against disease. İmmunostimulant medicinal plants have been reported to stimulate non-specific immune mechanisms also have additional effects like stimulation appetite or weight gain and also antibacterial and antiparasitic properties due to alkaloids, flavonoids, terpenoids and saponins. The advantage of stimulating nonspecific immune response is that it acts against all pathogens since it is not selective for against only specific agents and it can be used in different kinds of aquaculture systems conveniently.
The aim of this presentation is to give information about the medicinal plants, plant extracts, application methods and the mode of actions on the nonspecific immune system of fish.

Keywords: Immunostimulant, Medicinal Plants, Aquaculture.

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ORAL PRESENTATION

STUDIES ON MEDICINAL AND AROMATIC PLANTS UNDER THE FARMERS CONDITIONS IN THE PROVINCE OF KAYSERI

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This study comprises the results of demonstrative researches on some Medicinal and Aromatic Plants carried out by Kayseri Directorate of Provincial Agriculture and Forestry under the farmers' stuations in 2015-2017 within the scope of the Project of Improving the Medicinal-Aromatic and Dye Plants Cultivations sutained by BÜGEM (General Directorate of Improving Plant Production).

In this Project, 5 different plants consisting of Lavandin, Lemon Balm, Orchis, Black Seed and Oregano were studied in 11 counties for 3 years. Favorable results were obtained from Lavandin, Lemon Balm, Black Seed and Oregano plants in terms of cultivation under the Kayseri ecological stuations. For Lavandin (*Lavandula x intermedia*) species, dry flower yield was found to be 30-127 kg/da, essential oil rate in dry flowers was found to be % 2.72-9.00, for Lemon Balm (*Melissa officinalis* L.) species, dry herb yield was found to be 525-610 kg/da, essential oil rate in dry leaf was found to be % 0.07-0.24, and for Oregano (*origanum onites* L.) species, essential oil rate was found to be % 4.64.

In the cultivation of Black Seed, although there is no any trouble in terms of the cultivation, weed problem seemed as an important problem in front of us. Since there aren't any herbicides authorised by the Ministry of Agriculture and Forestry, sufficient yield wasn't obtained. Altough applying different planting times and mulching methods in orchis plants, favorable results couldn'd be obtained in terms of cultivation because of the difficult winter conditions and frost damage.

Keywords: Farmer Conditions, Medicinal Plants, Farming.

MESMAP-5 Final Proceedings Book

ORAL PRESENTATION

SOLVENT DEPENDING IMMUNOLOGICAL ACTIVITY OF NETTLE PLANT EXTRACTS IN SHEEP WITH CONTAGIOUS ECTHYMA

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Objective / Purpose: Contagious ecthyma is a highly economically impacting disease of sheep. The purpose of this study was to evaluate the immunological effects of nettle extracts in different solvents, also depending of the age category, on the adaptive cell-mediated response in sheep with clinical disease.

Material and Methods: Twelve adult ewes and their lambs, from a 360 animal flock, during a spontaneous outbreak of orf were subject to treatment which started on day 0, while blood was sampled in parallel, from diseased lambs and their mothers and also clinically healthy controls on days 0 and 10. The antioxidant capacity (scavenging effect over DPPH,%) of the nettle extract was established. The *in vitro* blast transformation test was performed to evaluate the effects of two nettle extracts (DMSO and ethylic alcohol). The significance of the differences was estimated by Excel program.

Results: The scavenging effect in the nettle extract was of 68.5±2.03. Both the alcoholic and DMSO nettle extracts significantly ($p<0.05$-$p<0.01$) increased the *in vitro* blast transformation indices after the treatment in diseased adult sheep (4.8±1.03 and 7.06±0.92%) and lambs (13.39± 2.45 and 19.68±5.42%), when compared to initial values and those obtained in healthy animals.

Conclusion / Discussion: The *in vitro* blast transformation indices were influenced by the health status of the animals, the age and the solvent used to nettle extraction, allowing the best choice of extraction to achieve stronger adaptive immune responses.

Keywords: *Urtica dioica L.*, orf, sheep, adaptive immunity, blast trasformation.

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MESMAP-5 Final Proceedings Book

ORAL PRESENTATION

HIV-2 VPX/VPR REGULATE THEIR STABILITY USING TWO MOTIFS

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Objective / Purpose: HIV-2 and some SIVs have similar two proteins Vpx and Vpr, and expression level of Vpx is much higher than that of Vpr. The Vpx has unique two motifs, a zinc-binding site HHCC, and a polyproline motif (PPM) which consists of seven consecutive prolines from the P103 residue. In contrast, the Vpr does not have these two regions. We already showed that zinc-binding site of HIV-2 Vpx withstands the instability of the protein (1), and introduction of the zinc-binding residue to HIV-2 Vpr increases its expression level (2) in a cell. About the PPM, we reported that this motif promotes translation, multimerization, and infectivity in macrophages (3, 4).

Metarials and Methods: Expression vector for Vpx/Vpr or its mutants was transfected into 293T cells, and the cells were lysed. Immunoblotting of the lysates were then conducted. E.coli was transformed with expression vector for Vpx or its mutants, and lysed. The lysates were centrifugated, and immunoblotting was performed using the supernatants and precipitates.

Results and Discussion: The expression level of seven point PPM mutants of Vpx was low in a mammalian cell. In the presence of proteasome inhibitor or with co-expression of Gag, the expression level was recovered. This recovery was not seen in the zinc-binding site mutants, demonstrating that zinc-binding site and PPM prevent instability of the Vpx differently in a cell. Next, the ratio of H39L mutant (one of the zinc binding sites H39 was changed to L) and P109A mutant (one of PPM amino acid P109 was changed to A) of Vpx in soluble and insoluble fractions after lysis of transformed E. coli was examined, and compared to that of WT. The result showed that more amounts of proteins are insoluble in H39L and P109A, indicating instability of protein structure. Finally, PPM was introduced into HIV-2 Vpr mutant having zinc-binding site HHCC. This Vpr chimeric protein with both zinc-binding site and PPM has further higher expression level than the mutant having only zinc-binding site.

Conclusion: HIV-2 Vpx oppose the instability by carrying both zinc-binding site and PPM, in contrast, HIV-2 Vpr keeps low expression level by not having these two motifs. The motifs are dual tools to regulate their stability in Vpx/Vpr.

Keywords: HIV-2, Vpx, Vpr, zinc-binding site, poly-proline motif

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ORAL PRESENTATION

ANALYSIS OF CORRELATION BETWEEN HEAVY AND ESSENTIAL METALS IN *SALIX ALBA* L. (FAM. *SALICACEAE*) IN HIGHLY POLLUTED AREA IN KOSOVO

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Objective/Purpose: Plants, like all other living things, need essential elements for their growth and development. It is determined that different plant cultures, grown in contaminated area have indicated difference in the proportion of the metal absorption, accumulation, distribution and also showed significant difference in concentration in different parts of plant.The selected useful macroelements (Ca, Mg) play a major role in building a plant structure, while microelements (Fe, Cu, Zn, Mn), are principally involved in enzymatic process. The main aim of this study is to investigate the concentration of selected elements in willow bark samples *Salix alba* L. (Fam. *Salicaceae*) in highly polluted area in Kosovo and the correlation between heavy toxic metals in soil and selected elements in plant.

Material and Methods: In highly polluted area near Kosovo Thermo Power Plants (KTPP) in Kosovo, 30 samples of willow bark were taken from the trees in the height 1.5 - 2 m, from the ground and 30 samples of soil were taken in around area 1 - 2 m from the willow tree (30 - 40 cm depth). All samples were collected in distance 30 km along the river stream of Sitnica which passes near the KTPP. Soil samples were air dried, while bark samples initially were thoroughly washed with deionized water to remove any particles or surface impurities attached to the samples, then were dried in thermostat for 24 hours on 65^0C. Metal contents were measured by inductively coupled plasma optical emission spectrometry using Perkin-Elmer Optima 2100DV.

Results: Our results indicated accumulation of toxic heavy metals and useful elements in soil and plant. The average values of useful elements in willow bark samples ranged for Ca: 5260.00 – 22280.00 mg/kg; Mg: 840.00 - 1680.00 mg/kg; Fe: 66.79 - 910.75 mg/kg, Cu: 5.09 -28.66 mg/kg, Zn: 56.39 -140.94 mg/kg, Mn: 19.68 - 392.75 mg/kg and Al: 67.79 -894.77 mg/kg.

Conclusion/Discussion: Our data indicated that the correlation between heavy metals in soil and useful elements in plant (Ni/Zn r = 0.558; Cr/Cu, r = 0.343 and Cr/Mn, r = 0.385) and also correlation between heavy toxic metals with useful ones in plant samples Ni/Al (r = 0.883), Ni/Fe (r = 0.790) Ni/Zn (r = 0.327), Cd/Fe (r = 0.393) and Cr/Fe (r=0.793)

Keywords: Heavy metals, essential metals, correlation, willow

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MESMAP-5 Final Proceedings Book

ORAL PRESENTATION

SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING VARIOUS PLANT EXTRACTS AND THEIR VARIOUS ENVIRONMENTAL APPLICATIONS

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Objective/ Purpose: Silver nanoparticles are being used in numerous technologies and incorporated into a wide array of consumer products that take advantage of their desirable optical, conductive, and antibacterial properties. Considering the advantages of green synthesis of silver nanoparticles, the present study aims at synthesis of silver nanoparticles (AgNPs) using various plant extracts and their characterization.

Materials and methods: The AgNPs were synthesized using about 24 different plant extracts; Turmeric, Henna, Onion, *Moringa*, to name a few. The effect of varying pH and temperature of these extracts on the synthesis of AgNPs was studied. Characterization of nanoparticles was carried out by UV –Vis spectroscopy, Scanning Electron Microscopy (SEM) and XRD in order to determine the size and purity of the samples. They were screened for their antibacterial property against various bacterial cultures using agar diffusion method and zone of inhibition was measured. Minimum inhibitory concentration of silver nanoparticles against *E. coli* and *S. aureus* was determined using broth dilution method. The AgNPs were used for remediation of toxic dyes from water samples and also screened for their anticancer properties against lung cancer cell lines.

Results: The AgNPs that were synthesized using the various plant extracts turned from colourless to brown after 24hrs indicating that ionic silver (Ag^{+}) has reduced to metallic silver(Ag°). Those SNPs which had a characteristic absorption maximum between 400nm to 480nm indicating the formation of nanoparticles, were selected for further studies namely SNPs made from extracts of Arjuna, Turmeric, Betel-leaf and Henna. Effect of varying pH and temperature on the synthesis of SNPs were determined, indicating that they could be best synthesized within 24hrs at 45°C and are stable at pH 4 (Arjuna), 6 (Turmeric) and having maximum absorbance between 400nm to 480nm. The synthesized extracts were concentrated by lyophilization and characterized by SEM and XRD. The size of the SNPs was confirmed by SEM to be between 70 to 80 nm.

The AgNPs showed good antimicrobial property against the organisms used, and was found to be in the range of 20-100 µg/ml. This antimicrobial effect of the synthesized AgNPs was to disinfect water contaminated with *E. coli* and was found to be effective in eliminating *E.coli* from water samples and hence could be used for water treatment. The nanoparticles were also found to be efficient in the remediation of toxic dyes from waste water samples. The AgNPs were screened for their anticancer properties and were found to be inhibitory to lung cancer cell lines.

Conclusion: From our studies it is clear that the AgNPs synthesized using the various plant extracts namely, by the green synthesis method can be used for various environmental applications.

Keywords: Green synthesis, Silver nanoparticles (AgNPs), Antimicrobial activity, SEM, Water treatment, dye remediation, anticancer effects.

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ORAL PRESENTATION

MICROWAVE HYDRO-DIFFUSION AND GRAVITY OF ESSENTIAL OIL FROM *ZINGIBER OFFICINALE* ROSC.

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Objective / Purpose: In this study, the optimal conditions of microwave hydro-diffusion and gravity (MHG) to extract the essential oil from Zingiber officinale Rosc (Ginger) were investigated. MHG has been compared with hydrodistillation (HD) in terms of extraction time, chemical composition, cost and energy of the operation.

Material and Methods: Fresh rhizomes of Zingiber officinale Rosc. (Age about 12 months) extracted by MHG and HD methods. Analysis chemical composition of essential oil by gas chromatography – mass spectrometry (GC-MS)

Results: The optimized conditions of MHG was microwave power of 720 W and extraction time of 35 min, identified 45 compounds by GC-MS. On the other hand, HD methods takes 180 min to extraction and only identified 4 compounds.

Conclusion / Discussion: Results of this study showed that MHG better than HD: shorter extraction time, better chemical composition, reduce cost and less than energy consuming.

Keywords: Green extraction, Hydro-diffusion, Solvent-free microwave extraction, Essential oil, Ginger.

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ORAL PRESENTATION

THERAPEUTIC POTENTIAL OF OLIVE LEAF POLYPHENOLS (ZeyEX®) IN HUMAN OSTEOARTHRITIC CHONDROCYTES *IN VITRO*

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Objective: Osteoarthritis (OA) is characterized by degeneration of articular cartilage. It is among the most common diseases all over the world and leading cause of chronic morbidity in older population. OA patients have limited treatment options. Inflammation and oxidative stress plays an important role in (OA pathogenesis) the progressive loss of articular cartilage[1]. Olive leaf polyphenols like oleuropein and hydroxytyrosol have antioxidant and antiinflammatory effects[2]. Chondroprotective effects of olive leaf extracts has been shown to in animal models[3], but the cellular mechanism(s) are unclear. Thus, we aim to investigate the cellular action mechanism of olive leaf polyphenolic extract mixture (ZeyEX®) in human osteoarthritis.

Methods: For primary chondrocyte culture, joint tissues were obtained from OA patients undergoing total knee arthroplasty[4]. Isolated primary chondrocyte cells were treated with different consantrations of ZeyEX®. Cell viability (MTT assay), proliferation (RTA- Icelligence) and *ROS production (DCFH-DA assay) were analized. Anti-inflamatuar effects were revealed* with inflammatory markers, IL-1B, IL-6, TNF-a (LUMINEX) and caspase-1 levels (ELISA). Lipid peroxidation, 4-HNE-, AGE-adduct and glutathione peroxidase (GPx) were also measured by ELISA kits.

Results: Olive leaf polyphenols (ZeyEX®) increase cell viability at nanomolar concentrations and reduce intracellular ROS production. ZeyEX® also ameliorates chondrocyte proliferation via regulation of inflammatory markers, lipid peroxidation/protein oxidation (4-HNE, AGE-adducts) and antioxidant enzyme, GPx.

Conclusion: This study, which implies that olive leaf polyphenols (ZeyEX®) is a potential therapeutic and redox modulatory agent for OA *in vitro*, is leading the way for future research.

Keywords: Olive leaf, ZeyEX®, Osteoarthritis, Primary human chondrocytes

Acknowledgements: This study was financially supported by the Scientific and Technological Research Council of Turkey (Grant No: 315S088)

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ORAL PRESENTATION

EFFICACY AND SAFETY STUDY OF THE NEW FORMULATION OF HERBAL COFFEE ON WEIGHT CONTROL

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Objective / Purpose: Overweight and obesity are the majorly underlying factors in public health leading to clinical complications. A new coffee formula with natural extracts was developed to increase metabolic rate and would possibly be used as an alternative for weight control. We aimed to formulate and study on the efficacy, safety, and satisfaction of the coffee formula containing five herbal extracts compared with placebo.

Materials and Methods: Herbal coffee have been made from the mixture of five herbal extracts from Guarana (*Paullinia cupana*), Lotus leaves (*Nelumbo nucifera*), Turmeric (*Curcuma loga*), Ginger (*Zingiber officinale*) and Black ginger (*Kaempferia parviflora*) with coffee in vary proportions to obtain twelve different formulations, each herbal cofffee bag contained approximately 14 g of the product and have been selected based on the stability test and favor. 24 overweight participants were included and randomized into 2 groups, 12 participants for each in which one group received coffee only while another one received the herbal coffee. Once daily consumption for 30 days was assigned in both groups as well as the body composition that was examined on day 0, 14, and 30.

Results: The results indicated that the formulation number 5 showed the promising result in stability test and taste preferences. Study results showed the group receiving herbal coffee were statistically different in body fat percentage and serum LDL-cholesterol decrease compared within group (P=0.073, 0.036 respectively at 90% and 95% CI). The coffee were found to be safe in both group of participants. The satisfactory level was moderate.

Conclusion / Discussion: In summary, this new herbal coffee tended to be safe with the promising results for weight control in a short term study. For statistically significant difference of outcomes, further experiment on efficacy and safety in longer period should be developed.

Keywords: Overweight, obesity, herbal coffee

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ORAL PRESENTATION

PHYTOCHEMICAL PROFILE AND ANTIMICROBIAL POTENTIAL OF EXTRACTS OBTAINED FROM *THYMUS MARSCHALLIANUS* WILD

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Objective / Purpose: Previous studies highlighted for several medicinal plants belonging to the Lamiaceae family multiple valuable biological activities and therapeutic applications [1]. This study was aimed to evaluate the phytochemical profile and antimicrobial potential of two extracts obtained from *Thymus marschallianus* Willd., an Eurasian species of Lamiaceae family.

Material and Methods: Two extracts were prepared from *Thymus marschallianus* Willd. harvested from spontaneous Flora and culture, respectively. Extract preparation was made by maceration with ethanol 70%, 10 days, at room temperature, using blooming aerial part of the plants. Qualitative and quantitative characterization of the extracts (polyphenolic, flavonoid and phenylpropane derivatives profiles) was performed by chromatographic (HPLC-MS/ESI+ analysis) and spectrophotometric methods. The disk diffusion method was used for the *in vitro* antimicrobial screening, while minimum inhibitory concentrations and minimum bactericidal concentrations were established based on the broth microdilution assay.

Results: The chromatographic analysis allowed the identification of 6 flavonoids, 2 aglycons (quercetol, luteolin), 4 flavonoids (derivatives of quercetol, luteolin and apigenin) and rosmarinic acid and its derivative, methyl-rosmarinate. Spontanous flora extract presented a higher content in flavonoid aglycon, but less rosmarinic acid. The spectrophotometric results showed similar values for total polyphenols, total flavonoids, and total caffeic acid derivatives. Also, both extracts displayed promising antimicrobial potential against Gram positive and Gram negative bacteria, with the highest efficacy expressed towards the *Bacillus cereus* > *Staphyloccus aureus* > *Escherichia coli* reference strains. The MBC/MIC ratio indicated bactericidal activity against Gram positive bacteria.

Conclusion / Discussion: Our data suggested that the extracts from *Thymus marschallianus* may represent sources of antimicrobials and further studies are intended to investigate the mechanism of action and also the potential cytotoxicity.

Keywords: *Thymus marschallianus* Willd., phytochemical profile, *in vitro* antimicrobial efficacy

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ORAL PRESENTATION

HEADSPACE SOLID PHASE MICROEXTRACTION (HS-SPME) ANALYSIS OF *SIDERITIS OZTURKII* AYTAC & AKSOY

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Objective / Purpose: The genus *Sideritis* (Lamiaceae) is represented by more than 150 bushy species and annual or perennial plants. Many studies have been conducted to elucidate the chemical composition and pharmacological activities of plants of the genus. *Sideritis ozturkii* Aytac & Aksoy is an endemic plant to Turkey and used as herbal tea and folk medicine in central Anatolia. The purpose of this study was the detection of the volatile oil components of *S. ozturkii* by solid phase micro-extraction/gas chromatography/mass spectrometry (SPME-GC-MS) technique.

Material and Methods: The plant material was collected from Konya-Derebucak province in 2016. The collected plant material washed and dried without sunlight. Dried and powdered samples were used directly. Two SPME fibers, 50/30 µm DVB/CAR/PDMS and 85 µm Carboxen/PDMS were preferred for analysis. SPME procedure were analysed on a Shimadzu QP2010 ULTRA FID GC–MS system. The identification of volatile components was carried out by comparing three libraries with W9N11, SWGDR4G4, and SWGDR4G5. Analyses were performed triplicate.

Results: The volatile oil compositions were identified in *Sideritis ozturkii* samples by SPME-GC/MS analysis using two different fibers. The major constituents of dried leaves detected with 50/30 µm DVB/CAR/PDMS fiber were octanoic acid (11.70 %) and nonanoic acid methyl ester (16.68 %) for 85 µm Carboxen/PDMS fiber, respectively. On the other hand, the major constituents of flowers were detected with both fibers were hexanoic acid (24.32 %- 26.34 %) and hexanal (18.67 % -16.68 %) respectively.

Conclusion: The comparative volatile oil composition of *S.ozturkii* leaves and flowers were revealed via SPME method for the first time with this study.

Keywords: Paşa çayı, Solid phase microextraction, Turkey.

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ORAL PRESENTATION

ANTIBACTERIAL ACTIVITY AND COMPOSITION OF ESSENTIAL OILS OF LEAVES AND BASIL FLOWERS OF THREE DIFFERENT GROWING SEASONS UNDER ARID CLIMATE

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Objective / Purpose: The evaluation of the antibacterial activity of essential oils of basil grown under arid climate has been studied by many researchers [1]. We wanted to study this property in relation to the growing seasons of the plant as the first parameter of study, and what part of plant that will present a better antibacterial activity as the second parameter of study. The relationship between this activity and the majority composition of the essential oil has been developed.

Material and Methods: The antibacterial activity of our 6 essential oils was evaluated on three two Gram-negative strains: Escherichia coli ATCC 25923 and Pseudomonas aeruginosa ATCC 27853 and one gram positive: Staphylococcus aureus ATCC 25922. The culture media used are nutrient agar (GN) for the maintenance of bacterial strains and Mueller Hinton agar (MH) for the study of bacterial susceptibility to HE. The diffusion method in Agar medium (Aromatogramme) is used for the study of antibacterial activity which allows to determine the sensitivity of the different bacterial species vis-à-vis our essential oils. The majority composition of essential oils was made by a Schimadzu GC-MS – QP2020.

Results: The results obtained showed that the essential oil of the different parts of the *Ocimum basilicum* L. Had no antibacterial activity marked against the strain of Pseudomonas aeruginosa (ATCC 27853). According to our results, the essential oil of basil Autumn flowers inhibits bacterial growth with inhibition diameters of 21.5 and 25 mm against Escherichia coli and Staphylococcus aureus respectively. This has a direct relationship with its majority composition: Linalool (34.99%), methylcinnamate (21.09%) and Methylchavicol (14.53%).

Conclusion / Discussion: The essential oils of the flowers showed antibacterial activities better than that of the leaves and this in the three study seasons.

Keywords: Activité antibactérienne, *Ocimum basilicum* L, huile essentielle

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ORAL PRESENTATION

THE ESSENTIAL OIL YIELD AND COMPOSITIONS of TARRAGON *(Artemisia dracunculus* L.) CULTIVATED IN BAYBURT

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Objective / Purpose: In the study, the essential oil yield and compositions obtained from herb of tarragon *(Artemisia dracunculus* L.*)* cultivated in Bayburt, Turkey ecological conditions were investigated.

Material and Methods: The air-dried herb of tarragon was subjected to hydrodistillation for 3h using a Clevenger-type apparatus to produce essential oil. The GC-MS analysis was carried out with Agilent 7890 GC-MS system. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and NIST mass spectral library.

Results: The essential oil yield of tarragon herb was determined as 0.3%. The most important essential oil component of metil kavikol is 65.3%. The major essential oil components of tarragon were determined as (*Z*)-beta-Osimen (7.9%), (*E*)-beta-Osimen (5.7%), Timol (3.4%), respectively.

Conclusion / Discussion: Tarragon (*Artemisia dracunculus* L.) was cultivated commercially in Bayburt province. According to the results of this study, it was determined that essential oil yield and components of tarragon were determined. Tarragon, a medical and aromatic plant, is known to be rich in content in terms of seconder metabolits. This exotic plant for Turkey has been used traditionally as spices herb, cooking, and salads.

Keywords: Tarragon, *Artemisia dracunculus* L., essential oil, composition

ORAL PRESENTATION

THE EFFECTIVENESS OF THAI TRADITIONAL HERBAL DECOCTION CONSUMED IN THE NORTHEAST OF THAILAND

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Objective / Purpose: The study is studied the effectiveness of Thai traditional herbal decoction consumed in Khon Kaen and Chaiyaphum province located in the northeast of Thailand. The information on the use of decoction in the treatment of fatigue are flu-like syndrome and diabetes.

Material and Methods: The research was a gather from 10 traditional herbal healers 3 monks and the other village through 80 interviews. All of the conversations with the information were conducted in the Thai language. The data were recorded as documents, questionnaires, sound recording and photographs.

Results: The finding were 1) 86% commonly used cause of get well sooner than consumed tablets and capsule pattern. 2) 90% specific to personal up to weight, age, allergy and symptoms. 3)92% Safe without chemicals effect to their health 4) 79% Following the herbal ways by believe in ancestors 5) 84% Believe in social media.

Conclusion / Discussion: The Thai traditional herbal decoction consumed in Khon Kaen and Chaiyaphum province located in the northeast of Thailand.The information on the use of decoction in the treatment of fatigue, flu-like syndrome and diabetes. People still consumed Thai traditional herbal decoction in their daily lives. The promoting Thai traditional herbal knowledge should be transferred for younger generations.

Keywords: Thai traditional herbal decoction, Thailand, flu-like syndrome

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ORAL PRESENTATION

STABILITY STUDY OF OSELTAMIVIR EXTEMPORANEOUS SUSPENSION

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Objective / Purpose: The stability of Oseltamivir Extemporaneous Suspension (OSEL ES) used in Queen Sirikit National Institute of Child Health (QSNICH), Bangkok Thailand was investigated.

Material and Methods: Solution of 10 mg/ml OSEL ES were compounded and stored in three different temperature conditions; room temperature, refrigerated temperature (7 ± 3 °C) and accelerated temperature (45 ± 5 °C). Triplicate samples were periodically taken for 12 weeks and assayed by high-performance liquid chromatography.

Results: All of the samples stored under room temperature and refrigerated conditions showed more than 90% of their initial concentrations of OSEL remained throughout the course of the study. For the accelerated temperature 62.91% of the initial concentration of OSEL remained at 12th week, which was significantly lower than the other conditions ($p < 0.05$). In addition, samples stored at 45 ± 5 °C showed changes in color and reductions in pH over the course of the experiment.

Conclusion / Discussion: In conclusion temperature above room temperature should be included for stability testing of extemporaneously prepared suspensions in tropical climates.

Keywords: oseltamivir phosphate, extemporaneous suspension, physicochemical tests, stability test

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Thatsanand Xayavong and Theera rittirod, Stability of Oseltamivir Extemporaneous Suspension, Asia Pacific J. Sci and Tech. 24 (1) Jan-March 2019 **(**in Press**)**

ORAL PRESENTATION

EFFECTS OF OLIVE LEAF (*OLEA EUROPEA L.)* EXTRACT ON THE GROWTH PERFORMANCE AND SOME BLOOD PARAMETERS OF COMMON CARP (*CYPRINUS CARPIO*)

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Objective / Purpose: Herbs or spices have been reported to promote various functions like growth, appetite stimulation, antistress, immune functions, skin coloration, egg hatching rates, hematological and biochemical status and also increase disease resistance in fish culture due to different active components. Olive leaf is the most important source of oleuropein known in nature. Several researchers revealed the fact that oleuropein has many pharmacological qualities including antioxidant, antimicrobial, antiaterogenic and antiviral activities. The olive leaf extract that we used in study include oleuropein at the rate of 2.3%. In this study, the effects of olive leaf extract on growth performance and some blood parameters of common carp were evaluated. For this purpose, 3 different experimental diets containing rate of 0% (OLE0), 0.25% (OLE025) and 0.5% (OLE05) olive leaf extract were prepared. This study consisted of 3 repetitions for each group with 71.58±0.66 g and 200 L aquarium as 25 fish per aquarium for 60 days. The objective of this study was to determine the effects of olive leaf extract on the growth performance and some blood parameters in the common carp.

Material and Methods: *Cyprinus carpio* fishes were obtained from a local fish farm in İzmir, Turkey. Olive leaf extract was obtained from Talya Herbal product. It was incorporated to a laboratory-prepared feed rate of 0% (control group), 0.25% and 0.50% for diets designed OLE0, OLE025 and OLE05, respectively. A control group was prepared without any supplements. Proximate analyses of the diets were performed using standard methods. Haematological parameters (RBC count, Hct ration, Hgb concentration) were determined by using an automatic analyser (Mindray BC-3000 Plus, China). Creatinine, total protein, glucose, albumin, globulin, uric acid, urea, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and cholesterol were assessed by the automatic analyzer apparatus (Reflotron plus, Roche, Germany) using commercially available Reflotlon kits (Roche Diagnostics, Germany). The analyses of the data obtained in the experiment were done by using the SPSS 17 statistics program. One way variance analysis (ANOVA) was applied to the data which were then subjected to Tukey multiple comparison test. The differences between groups were evaluated to be $p<0.05$.

Results: The three diets were equally accepted by the fish and there was no mortality or disease in any treatment. There were no significant differences ($P>0.05$) in average weight gain, feed conversion ratio (FCR) and specific growth rate (SGR). The RBC count, Hgb concentration, Hct ratio, Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV) and Mean corpuscular hemoglobin (MCH) in the treatment groups were statistically similar to the values recorded for the control group ($p>0.05$). In serum biochemical findings of fish, olive leaf extract was found to be a significant difference among the groups ($p<0.05$).

Conclusion / Discussion: In conclusion, findings of present study indicate that feeding *Cyprinus carpio* with diet containing 0.5% olive leaf extract over a period of 60 days might be adequate to improve fish immune parameters.

Keywords: *Cyprinus carpio*, Olive leaf (*Olea europea L.*) extract, Blood parameter, Growth performance

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ORAL PRESENTATION

LEAF ANATOMY OF THE GENUS *ORIGANUM* L. (LABIATAE) IN TURKEY

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Objective / Purpose: The plants of the genus *Origanum* L, which is used as oregano in the Labiatae family, are mainly used as traditional folk medicine and spices. In 2018 with addition of new taxa to the flora, the genus *Origanum* is represented by 67 natural taxa in the world, 34 of them occurs in Turkey [1]. These are mainly found in the Mediterranean region. In particular, there are difficulties in distinguishing closely allied taxa. A detailed study has been carried out in order to distinguishing these taxa according to anatomical characteristics. The study is carried out in order to contribute to the identification of taxa that cannot be diagnosed morphologically if it contains missing diagnostic character.

Material and Methods: Leaf samples are taken from a collection of the specimens collected from the nature between 2001-2012 by Narin Sadıkoğlu, located in the Herbarium of İnönü University, Faculty of Pharmacy. Cross-sections are taken from the center of the leaves at 15 & 25 microns thickness with a microtome.

Results: The leaves are isolateral, covered with a layer of cuticle. Epidermis consists of single-layered rectangular cells. Cover and glandular hairs, Labiatae type glandular hairs on the epidermis can be seen. The stomata are 2 (diacytic)-3, rarely 4 (anisocytic) of Labiatae type and they are hygromorphic or mesomorphic. Palisade parenchyma cells are single layered and have much chloroplasts. Sponge parenchyma cells are in 1-5 layered and intercellular spaces are evident.

Conclusion / Discussion: *Origanum* taxa differs in the characters such as cover hair, glandular hair, shape of upper and lower epdermis, number and size of cells, stomata type, crystals, cuticle, width of the vascular bundle. According to the data obtained as a result of microscopic analysis of the taxa, the distinctive basic characters were determined.

Keywords: *Origanum*, leaf anatomy, Labiatae, Turkey

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ORAL PRESENTATION

CHARACTERIZATION OF VOLATILE COMPOUNDS OF *MENTHA PULEGIUM* VIA HS-SPME

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Objective / Purpose: *Mentha pulegium* L. is a herbaceous perennial plant that belonging to the Lamiaceae family. The plant grows up in moist areas of the mountains and plains. In therapeutic applications, this plant and/or its active constituents have been used traditionally for several disorders. Their diuretic, antispasmodic, digestive, diaphoretic, sedative, antiseptic, stimulant and expectorant effects have been reported. The aim of this study is the determination of the volatile and semi-volatile oil content of *Mentha pulegium*.

Material and Methods: The *Mentha pulegium* species was collected from its natural habitat. The powdered plant samples were analyzed directly on a Shimadzu QP2010 ULTRA FID GC–MS system. Volatile compounds were detected by solid-phase microextraction/gas chromatography/mass spectrometry (SPME-GC-MS) method. Two SPME fibers preferred for analyses; 85 µm Carboxen/PDMS and 85 µm polyacrylate. The components were identified by comparing three libraries with W9N11, SWGDR4G4, and SWGDR4G5. Analyses were performed three times and the mean values were taken into consideration.

Results: The volatile oil compositions were identified in *Mentha pulegium* samples by SPME-GC/MS analysis. According to the analyses, significant differences were observed between the two SPME fibers. The major volatile component for 85 µm Carboxen/PDMS fiber were 2-Isopropyl-5-methylhex-2-enal (30.37%) and for 85 µm polyacrylate fiber 2(3H)-Benzofuranane, 3a,4,7,7a-tetrahydro-3a-methyl (48.59%), respectively. In addition, the most common and shared components to both fibers were, p-Menthone (14.15%- 8.82 %), phenol,5-methyl-2-(1-methylethyl)(8.68 %- 7.67 %).

Conclusion: *Mentha pulegium* is a plant that is rich in essential oils and this study is the first study about the volatile content of plant with SPME method.

Keywords: Yarpuz, SPME-GC-MS, Turkey.

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ORAL PRESENTATION

CYTOTOXIC AND GENOTOXIC EFFECTS OF WATER EXTRACTS OF *ZYGOPHYLLUM CORNUTUM* AND *ATRACTYLIS GUMMIFERA* USING *ALLIUM CEPA* ASSAY

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Objective / Purpose: The interest in herbal products in the world is fueled by the rising costs of drugs. The safety of herbal products depend on the safety of its sources, and this should be established before these are developed as herbal medicinal products. The potential genotoxic effects of aqueous extracts of ***Zygophyllum cornutum*** and ***Atractylis gummifera,*** which are commonly used in traditional medicine to treat a variety of disease conditions, was investigated using *Allium cepa* assay.

Material and Methods: Both extracts of 2, 4, 6, 8, 10 and 12 mg/mL were tested on root meristems of *A. cepa* to determine the half maximal effective concentration (EC50) as a parameter of cytotoxicity. Synthetic water was used as negative control **[1]**. Based on EC50 corresponding values, three concentrations were used for each plant to evaluate the genotoxic effects on *A. cepa* **[1][2].**

Results: The results showed a dose-dependent decrease in roots growth with both plants, but it was more remarkable with ***A. gummifera***. According to the obtained EC50 values, ***Z. cornutum*** (9.38 mg/ml) is considered less toxic than ***A. gummifera*** (5.38 mg/ml). The results of genotoxic test showed that mitotic index decreased as the concentrations of the extract increased. For ***Z. cornutum***, the three tested concentrations 6, 10 and 12 mg/ml had respectively 83.56, 77.49 and 65.12 % mitotic indexes, while with ***A. gummifera,*** the three tested concentrations 2, 6 and 12 mg/ml had respectively 67.92, 58.19 and 22.38 % mitotic indexes.

A dose-dependent increase of total chromosome aberrations (TCA) was also observed. For ***Z. cornutum*** (concentration mg/ml; TCA%): (6 ; 3.85), (10 ; 5.25) and (12 ; 6.87)**.** For ***A. gummifera***: (2 ; 13.81), (6 ; 32.35) and (12 ; 48.67)**.**

Conclusion / Discussion: The results of this study suggested that the aqueous extracts of ***Zygophyllum cornutum*** and ***Atractylis gummifera*** exerted significant genotoxic and mitodepressive effects at the tested concentrations.

Key words: *Allium cepa*, *Atractylis gummifera*, Chromosome aberrations, cytotoxicity, genotoxicity, Mitotic index, *Zygophyllum cornutum*

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ORAL PRESENTATION

ADVERSE EFFECTS OF CONVENTIONAL DRUG-MEDICINAL PLANT INTERACTIONS IN VETERINARY MEDICINE

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The use of herbal remedies for the prevention and the treatment of a variety of illnesses in small animals have been increasing nowadays. Because of the increased interest in natural products in veterinary medicine, more information is needed regarding their use and potential hazards. Although extensive literatures are available on the effectiveness of herbal extracts and components, there is no profound information on the safety assessments of the combined use of herbal medicines and conventional drugs in veterinary medicine.

Particularly, the interaction of the biological active constituents contained in herbal remedies with conventional drugs is a major concern. Drug-herb interactions refer to the possibility that an herbal constituent may alter the pharmacologic effects of a conventional drug. Possible pharmacokinetic interactions include those that alter the absorption, metabolism, distribution, or elimination of a drug or herbal constituent, resulting in an increase or decrease in the concentration of active agent at the site of action. In addition, the interaction of the herbal medicines and conventional drugs may lead to potential toxic effects. Some interactions are documented in clinical trials, some are inferred from in vitro experiments, and others are suspected only on theoretical grounds. Numerous case reports in literature serious and potentially life-threatening adverse effects following human and animal exposure to herbal preparation are seen.

We are of the opinion that the review not only highlights the most updated research results available in the evaluation of the possible risks of herb-drug interactions, but also provides some essential skills for the healthcare researchers and veterinary clinicians to solve some relevant issues they may encounter in this field.

Keywords: Medicinal plants, conventional drugs, interaction, veterinary therapy

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ORAL PRESENTATION

DETERMINATION OF VOLATILE AND SEMI-VOLATILE OIL CONTENT OF *ISATIS TINCTORIA* L. VIA SPME-GC-MS

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Objective / Purpose: *Isatis tinctoria* L. is a species of the genus Brassicaceae and generally distributed in Central Asia. The plant is also used for medical purposes as well as being used as a colorant. *Isatis* is one of the most effective herbs used in herbal medicine, antiseptic and antiviral drugs in traditional Chinese medicine. The aim of this study is to determine and characterize the volatile oil components found in the leaves and flowers of *Isatis tinctoria* via the SPME-GC-MS method.

Material and Methods: The plant material was collected from the Ardıçlı Village (Konya Province) and preserved under suitable conditions. 65 µm PDMS/DVB and 85 µm polyacrylate fibers were used to determine the volatile and semi volatile oil composition of the samples. Analyzes were performed using the Shimadzu QP2010 ULTRA FID GC–MS system. The components were identified by comparing three libraries with W9N11, SWGDR4G4 and SWGDR4G5.

Results: According to the data obtained from the analysis of *Isatis tinctoria*, in leaf content 120 and 78 compounds and in flower content 123 and 87 compounds were detected respectively. According to the analysis, with 65 µm PDMS/DVB fiber, the most amount of components in the leaf benzeneetanol (4.67 %) and undecane,5-methyl (3.93 %), the most common components in the flower, morpholine, 4-octadecyl- (18.68 %), 4-Amino-furazan-3-carboxylic acid (3-morpholin-4-yl-propyl)-amide (11.91 %). With 85 µm polyacrylate fiber, the most amount of components in the leaf, benzene, 2,4-diisocyanato-1-methyl- (33.41 %) and pyrrole-3-carbonitrile, 5-formyl-2,4-dimethyl (14.47 %), in the flower content 2,2,3,3,4,4,4-Heptafluoro-N-(2-morpholin-4-yl-ethyl)-butyramide (19.67 %) and morpholine, 4-octadecyl- (18.07 %), respectively.

Conclusion: This study is the first study in terms of characterization and comparison of volatile and semi volatile oil components by SPME-GC-MS analysis from leaves and flowers of *Isatis tinctoria* L. species from Turkey.

Keywords: Çivitotu, solid phase micro-extraction, Turkey.

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ORAL PRESENTATION

THE CHEMICAL COMPOSITION OF *DELPHINIUM SZOWITSIANUM* BOISS. SPECIES SPREADING IN VAN REGION

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The family Ranunculaceae, which includes the Delphinium species, has 59 genera and 2500 species worldwide. In Turkey, it has 18 genus and 216 species and its endemism rate (23.5%) is quite a high family. *Delphinium szowitsianum* Boiss. is a perennial herbaceous species. It has been spread in the Eastern Anatolia Region of our country and it is known as "zarif hezaren".

In the study, it is aimed to determine some nutrients and mineral substance contents of *Delphinium szowitsianum* Boiss which is naturally grown around the Van lake in the Eastern Anatolia Region.. In plant samples, some nutrients and mineral contents such as total ash, crude protein, pH, crude cellulose and N, Na, Mg, K, Ca, P, S, Mn, Fe, Cu, Zn, Cd, Co have been investigated.As a result of the research; it has been determined as crude protein ratio 4.60%, pH 5.99, total nitrogen content 0.74 %, crude ash content 6.31% and crude cellulose content 28.20%. In addition, while some mineral substance contents were determined as K 5.31 g/kg, Ca 16.37 g/kg, P 1.07 g/kg, Mn 35.41 mg/kg, Fe 459.69 mg/kg and Zn 32.23 mg/kg, heavy metal contents was determined as Cr 0.49 mg/kg, Cd 0.11 mg/kg, Co 1.35 mg/kg and Pb 0.44 mg/kg.

Key Words: *Delphinium szowitsianum* Boiss, Eastern Anatolia, Heavy Metal, Medicinal Plants

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ORAL PRESENTATION

THE ESSENTIAL OIL YIELD AND COMPOSITIONS OF SUMMER SAVORY (*SATUREJA HORTENSIS* L.) COLLECTED IN TURKEY

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Objective / Purpose: In this study, ıt was investigated essential oil yield and compositions of Summer Savory (*Satureja hortensis*) collected from Artvin, Turkey. Common name of *Satureja hortensis* is Summer Savory.

Material and Methods: The air-dried herb parts of *Satureja hortensis* were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce essential oil. The GC-MS analysis was carried out with Agilent 7890 GC-MS system. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and NIST mass spectral library.

Results: The essential oil yield of Summer Savory was determined as 3.0 % . Thymol amount from essential oil composition of *Satureja hortensis* was found 42.7 %. Other main components were carvacrol (3.2 %), gamma-terpinene (25.0 %) and p-cymene (13.7 %).

Conclusion / Discussion: In this study, thymol was determined main compound from Summer savory essential oil. But in other studies, carvacrol was determined as the main compound. According to the results of this study, the observed differences in the constituents of summer savory essential oil collected from Artvin may be due to different climatic, soil factors and the cultivation conditions.

Keywords: Summer Savory,*Satureja hortensis*, Essential oil, Thymol.

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ORAL PRESENTATION

THE ESSENTIAL OIL YIELD AND COMPOSITIONS OF *NIGELLA SATIVA* L. AND *NIGELLA DAMASCENA* L. CULTIVATED IN KONYA ECOLOGICAL CONDITIONS

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Objective / Purpose: In this study, ıt was investigated essential oil yield and compositions of seeds of *Nigella sativa* L. and *Nigella damascena* L**.** cultivated in Konya ecological conditions, Turkey.
Material and Methods: The air-dried seeds of black cumins species were subjected to hydrodistillation for 3h using a Clevenger-type apparatus to produce essential oil. The GC-MS analysis was carried out with Agilent 7890 GC-MS system. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and NIST mass spectral library.

Results: Essential oil yield of black cumin seeds was determined as 0.1% in *Nigella sativa* L. and 2.5% in *Nigella damascena* L**.**. The most important essential oil component of *Nigella sativa* L., timokinon content is 15.4 % and the major essential oil components of *Nigella damascena* L**.**. were determined as beta-Elemen(45.9%) . Timokinin, is the important essential oil component, was not found in *Nigella damascena* L**.**

Conclusion / Discussion: According to the results of this study, information about essential oil yield and components of every both species was been evaluated. The black cumin seeds have a very important place due to fixed oil, volatile oil and nutrients constant. The oil obtained from the seeds of the plant is used in the treatment of many diseases with medical purposes as well as being used as a spice and flavor in food products.

Keywords: Çörekotu, *Nigella sativa* L., *Nigella damascena* L**.**, essential oil, composition, timokinon

ORAL PRESENTATION

THE ROLE AND USE OF OLIVE OIL AND OLIVE LEAVES AS A FUNCTIONAL FOOD

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Objective / Purpose: The main feature of the Mediterranean diet is the pharmaceutical and nutraceutical effects of plant-derived foodstuffs and beverages include mainly fruits, vegetables, herbs and spices used in human nutrition. These phenylpropanoid-rich foods and beverages are important as pharmanutrients due to their antioxidant, anti-inflammatory, antimutagenic, cardioprotective, antihypersensitive and antitumoral activities. Olive (Olea europaea L.) is a characteristic plant of the Mediterranean region.

The olive plant has been an important source of nutrition and medicine for centuries. Due to the recognition of its high dietetic nutritional value, olive oil and olive leaves have been used for ancient times. The leaves, fruits and fruit oil of the olive tree contain significant amounts of bioactive and phenolic compounds. Oleuropein, hydroxytyrosol, catechin, chlorogenic acids, p-coumaric acid, caffeic acid, 3-hydroxcinnamic acid are some of the important compounds. The amount of these substances varies according to factors such as tree age, fruit load, cultivar, harvest time, biotic and abiotic stress conditions, processing, and storage status of the crop. Nutritional and health benefits of olive and olive products stand out it as a functional food.

Conclusion / Discussion: Olives, olive products and olive oil used in the Mediterranean diet are becoming increasingly important in both the nutrition and the pharmaceutical industry due to the bioactive substances it contains.

Keywords: *Olea europaea* L., Olive oil, olive leaves, polyphenol, functional food

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ORAL PRESENTATION

AS A GOOD SOURCE OF MELATONIN: GRAPE AND GRAPE PRODUCTS

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Abstract: Melatonin is known to be naturally found in a wide range of mammals and birds mainly responsible for the circadian rhythm of the body. It was then identified in plants. Studies look for melatonin in higher plants have been focused on the physiological roles in plants and also beneficial health effects for humans as a bioactive plant based compound. Recent studies have been indicated that melatonin in the edible plants has health-promoting effects on human health due to its anti-inflammatory, antioxidant and anticancer features as well as the therapeutic effect on cardiovascular diseases. Melatonin has been identified and quantified in different plants such as grapes, walnuts, sweet cherries, tomatoes and cucumbers. In this context grape and grape products provide a wide range of natural products as pharmanutritional effects. However, melatonin content may alterable according to cultivars, ecological conditions and some agrochemical treatments. Grape and grape products are represented to be good melatonin sources. In this review, we focus on melatonin content of grape and grape products and its effects on human health.

Keywords: *Vitis vinifera* L., circadian rhythm, foodstuffs, phytochemicals

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ORAL PRESENTATION

EFFECTS OF TOPIRAMATE A NOVEL ANTI-EPILEPTIC DRUG IN CHRONIC CONSTRICTION INJURY INDUCED NEUROPATHIC PAIN IN RATS

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Objective / Purpose: Topiramate is a novel anti-epileptic drug, various report showed its beneficial effects in various neurodegenerative disorders namely epilepsy and neuropathic pain. Based on established extensive study of anti-epileptic drugs in neuropathic pain, the present study was design to examine the dose dependent effects study of Topiramate in chronic constriction injury (CCI) induced neuropathic pain in rats.

Material and Methods: Male Albino Wistar albino rats were divided in different groups each contain six rats. The rats were placed in a standard laboratory condition as suggested by regulatory guidelines. The experimental protocol was approved by IAEC of the Institute. Chronic constriction injury (CCI) to sciatic nerve was used as a model of induction of neuropathic pain. The characteristic symptoms of neuropathic pain were assessed by examining behavioral parameters such as allodyania and hyperalgesia. The degree of sciatic nerve injury was also examined by estimating the membrane bound enzyme, defense of oxidative stress and markers of inflammatory markers. The treatment groups received Topiramate 20, 40 and 80 mg/kg/p.o for 7 days after the development of neuropathic pain.
Results: CCI to sciatic nerve significantly produced the allodyania and hyperalgesia; increases membrane bound ATPases activities, disturbed the antioxidant defense mechanism and increased the level of inflammatory markers. Treatment with Topiramate significantly and dose dependently attenuated the CCI induced allodyania and hyperalgesia, decreases membrane bound activities, restored the antioxidant defense mechanism and decreased level of inflammatory markers.

Conclusion / Discussion: Topiramate attenuated the CCI induced neuronal, biochemical and behavioral changes in sciatic nerve which may be due its antioxidant, neuroprotective, analgesic and anti-inflammatory actions.

Keywords: AEDs, Topiramate, CCI, allodyania, hyperalgesia, inflammatory markers, antioxidants, and membrane bound enzymes.

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ORAL PRESENTATION

NEGATIVE DATA BUT NO WORRIES

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Abstract: There have been numerous studies using both individual supplements and multivitamins, and the results are mostly negative; evidence shows lack of benefit. However, omega-3 supplements, vitamin and other supplement sales soar without any stop. Fish oil sales increased 10-fold in 15 years, whereas vitamin D use simply increased fourfold. Meanwhile, 22,071 men who receive beta-carotene or placebo for 12 years in Physicians Health Study, shows no benefit for cardiovascular disease risk and neoplasms or on overall mortality. 2nd Physicians Health Study, the Women's Antioxidant Cardiovascular Study, VIDA and VITAL studies also shows no benefit for Vitamin C, D and E again and again. Then why nearly everybody still use vitamins and supplements? Unaware or not concerned? Do expectations and believes have any role in it? This review will summarize all the published surveys about these questions to find the reason behind the global non-stopping non-declining vitamin and supplement trend.

Keywords: supplements, vitamins, evidence-based results, surveys, health behavior

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POSTER PRESENTATIONS

MESMAP-5 Final Proceedings Book

POSTER PRESENTATION

THE EFFICACY OF NEW BIOAPIFIT ANTI-HEMORRHOIDAL OINTMENT VERSUS STANDARD TREATMENT PROTOCOL

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Objective / Purpose: Hemorrhoids are defined as varicosities of the veins of the hemorrhoidal plexus, often complicated by inflammation, pain, thrombosis, and bleeding. The disease correlate positively with age and more than half of the population of both sexes aged 50 years and older will develop hemorrhoid symptoms during their lifetime. The primary objective of this study was assessment of the efficacy of new multi-herbal ointment consisted of honey, *Cera flava*, glycerin, the oil macerates of *Achilea millefolium* L., *Plantago major* L., *Quercus robur* L., *Salvia officinalis* L., *Olea europaea* L., *Polygonum aviculare* L., *Calendula officinalis* L., *Matricaria chamomilla* L., essential oils of *Melaleuca alternifolia*, *Timus vulgaris* ct. Thymol and *Origanum vulgare* for the treatment of hemorrhoids of grade 1 to 3 while the second goal was the comparison of its treatment potential with standard approach applied under the same condition.

Materials and methods: The experimental group consisted of 66 participants ranging from 27 to 69 years was treated with new Bioapifit anti hemorrhoidal ointment while the control group consisted of 40 participants ranging from 25 to 89 years was treated with Faktu ointment (50 mg/g of policresulene and 10 mg/g of cinchocaine). Either Bioapifit or Faktu ointment was applied externally three times a day for ten days onto clean perianal area and rectally once a day (before bedtime) using appropriate applicator. The evaluation of the patients before and following the therapy was done in terms of pain (0-10), defecation discomfort (0-10), bleeding severity (0-4), anal itching severity (0-4) and overall subjective discomfort (0-10). For statistical evaluation Statistica 11.0 software package was employed.

Results: Before the therapy the mean values and standard deviations in the experimental group for pain, defecation discomfort, bleeding, itching and overall subjective discomfort were 6.6±0.9, 6.9±1.0, 2.2±0.7, 2.6±0.6 and 7.0±0.8, respectively. A significant decrease of all five parameters was obtained after only three days of the treatment with mean values and standard deviations of 2.2±0.8, 2.5±1.0, 0.6±0.5, 1.3±0.7and 3.0±0.8 for pain, defecation discomfort, bleeding, itching and overall subjective discomfort, respectively. After five days of the therapy, there was no bleeding while the mean value and standard deviation were reduced to 0.5±0.6, 0.9±0.7, 0.6±0.6 and 1.4±1.1 for pain, defecation discomfort, itching and overall subjective discomfort, respectively. At 10th day of the treatment all five parameters were graded 0 by all 66 patients. The control patients treated with Faktu ointment also showed a significant decrease of all five parameters after only three days of the therapy while in the end of the treatment overall subjective symptoms decreased from 3.8±1.0 to 0.9±0.9. Bioapifit anti-hemorrhoidal ointment showed better results for all treatment period compared to Faktu ointment.

Conclusion / Discussion: Presented results revealed that the preparation combining herbal ingredients with the honeybee's products could be used safely and effectively for the treatment of the symptoms of hemorrhoidal disease. The combination of the ingredients with soothing, calming, anti-inflammatory, coating, pH adjusting and lubricating effect with those with proven astringent, vasoconstrictor and haemostatic activity resulted in alleviation followed by complete disappearance of the symptoms like pain, bleeding, itching and defecation discomfort.

Keywords: hemorrhoidal disease, herbal preparations, honeybee's products, ointment

POSTER PRESENTATION

ANTIOXIDANT ACTIVITY AND FREE AGLYCONE COMPOUNDS IN AERIAL PARTS OF THREE SPECIES OF *PISTACIA* GROWING IN ALGERIA

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Pistacia a genus of flowering plants from the family anacardiaceae , contains about twenty species .In Algeria among them three are more popular including *P.Atlantica* Desf , *P.Lentiscus* L and *P.Therebinthus* L. Different parts of these species have been used in traditional medicine for various purpose like antiseptic, gastrointestinal and respiratory tract disorder. Also they have pharmalogical activities such as: antioxidant and antimicrobial.

The aim of the present study was to identify an important class of phenolic compounds: flavonoïds and specially free aglycones, contained in the leaves and seeds of the quoted three species of *Pistacia* growing in Algeria , and to determine the antioxidant activity using the
1,1-diphenyl-1-picrylhydrazyl (DPPH).

The results allowed as to identify several compounds such as: quercitine myrecitine, luteoline and kaempferol. Antioxydant capacity (%) [IC_{50} (0.0123 for leaves and 0.0121for fruits of *Pistacia terebenthus*), (IC_{50} (2.546 for leaves and 99.996 for fruits of *Pistacia atlantica*) and
(IC_{50} (11.748 for leaves and 52.292 for fruits of *Pistacia lentiscus*)].

According to the results it may be concluded that the extracts of the threee species of Pistacia revealed considerable antioxidant activity.

Key words: *Pistacia*, flavonoïds, antioxidant activity, HPLC.

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POSTER PRESENTATION

CHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF NEW SELECTIVE *ZINNIA ELEGANS* FRACTIONS

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Objective / Purpose: *Zinnia elegans* (syn. *Zinnia violacea*) is a very popular ornamental plant of the *Asteraceae* family, widely cultivated for the impressive range of flower colors and persistent bloom [1,2]. Given its easy growth and high adaptability to improper landscape conditions [1], we decided to investigate its potential use as a resource of valuable secondary metabolites with biochemical actions.

Material and Methods: Preliminary detection of classes of compounds found in a methanolic extract obtained from *Zinnia elegans* inflorescences was achieved using high-resolution LC-MS techniques. The extract has been further subjected to Solid Phase Extraction and separation using Sephadex LH-20 column chromatography, which allowed for different fractions to be obtained. The LH-20 fractions have been investigated regarding their antioxidant properties through lipoxygenase inhibition and metal chelating activity assays. Moreover, one representative fraction was further purified through preparative HPLC, and NMR spectroscopy analyses using a Bruker Avance III HD 500 MHz spectrometer were carried out for establishing the exact structure.

Results: The obtained results indicate that the investigated fractions contain several polyphenolic compounds such as chlorogenic acids and apigenin, kaempferol and quercetin glycosides, as well as nitrogen containing compounds. Antioxidant tests showed that some of the analyzed fractions had similar EC_{50} values as the positive control. The NMR analysis revealed the presence of a new kaempferol glycoside.

Conclusion / Discussion: Given the promising antioxidant results, fractions obtained from *Zinnia elegans* inflorescences justify further purifications and structural analysis of compounds, while the investigation of other biological actions could prove of significant applicability.

Keywords: *Zinnia elegans*, HR-MS, lipoxygenase, metal chelation

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POSTER PRESENTATION

IN VIVO ANTI-ULCERATIVE AND ANTIDIARRHEAL ACTIVITIES OF THE TETRACLINIS ARTICULATA SPECIES OF THE CUPRESSACEAE FAMILY

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Tetraclinis articulata (Thuya of Barbary), endemic to North Africa, is used as a traditional medicine for the treatment of many diseases. We studied the antidiarrheal properties of Tetraclinis articulata n-butanol extract in male and female Swiss albino mice to support its traditional use. The antidiarrheal activity of the plant extract was evaluated in a model of castor oil-induced diarrhea in mice and compared to loperamide (a reference inhibitor of diarrhea) .The effect of n-butanol extract on gastrointestinal motility was determined by the oral administration of charcoal and Castrol oil-induced intestinal fluid accumulation (enteropooling).

The n-butanolic extract of the species tetraclinis articulate showed antidiarrheal activity significantly inhibited gastrointestinal motility and castor oil induced enteropolysis, similar to the inhibition achieved in loperamide treated mice. Ulcer is the most common gastrointestinal disturbance resulting from an inadequate gastric mucosal defense. Several drugs are available in the market to address the disease; however, these drugs are associated with unnecessary side effects.

Previous research have confirmed the efficacy of plant extracts for possible treatment of the disease. This research aims to evaluate the anti-ulcer properties of medicinal plants. N-butanol extract from the leaves of Tetraclinis articulata is evaluated for his anti-ulcer activity. The extract showed inhibitory activity with exhibiting more than 70% inhibition. We conclude that Tetraclinis articulata extracts are potential sources of new anti-ulcer agents and antidiarrheal drugs.

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POSTER PRESENTATION

IN VIVO ANTI-INFLAMMATORY EFFECTS OF SOME EXTRACTS FROM *PELARGONIUM* SP.

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Objective: Various *in vivo* tests are used to evaluate the anti-inflammatory properties of chemical compounds [1]. The most common test is the TPA-induced oedema because it produces a strong reaction in the dermic inflammation. TPA activates the protein kinase-C, but also the nuclear factor KB and the inflammatory mediators such as TNF-α, the TL-1β and the MIP2 macrophages [2]. In our study we evaluated the anti-inflammatory effects of some methanolic extracts obtained from *Pelargonium hispidum, Pelargonium radens, Pelargonium peltatum,* and *Pelargonium zonale*.

Material and Methods: The anti-oedematous/anti-inflammatory effect of the investigated samples was demonstrated on a TPA-induced animal model, when, after the treatment, there was a reduction of the typical inflammatory parameters (ear base size, length and mass) [3].
Results: Generally, the values obtained were determined after a single topical application of TPA and are consistent with the literature data [4]. The maximum expression of oedema was observed 24 hours after the administration of TPA with important differences for the groups treated with the investigated *Pelargonium* extracts. For the same dose (5 mg/mL) applied topically, the most intense oedema reduction effect was present in the group 4 treated with methanol extract of *P. zonale*, in which inflammation compared to the negative control (TPA group) decreased by about 70%, and, compared to the positive control (treated with indomethacin), decreased by about 30%. The other groups treated with the *P. radens* extract (group 3) and *P. grandiflorum* extract (group 2) showed a reduction of the inflammatory process by about 50% compared to the negative control. *P. hispidum* methanol extract (group 1) showed an anti-edematous effect of 45% compared to the negative control.

Conclusion / Discussion: Inflammation is a pathological process whose evolution involves the presence of enzymes, proinflammatory chemical mediators, extravasation of fluids, cell migration, tissue damage, but also tissue recovery processes. Thus, our data indicated that the methanol extract of *Pelargonium zonale* has a promising potential in reducing the inflammatory processes.

Keywords: anti-inflammatory effect, *Pelargonium species*, TPA-induced assay, methanol extract.

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POSTER PRESENTATION

NEW GYPSOGENIN-AMINE COMPOUNDS

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Background / aims: Gypsogenin compound, a natural saponin of pentacyclic triterpene, belongs to the carnations family (Caryophyllaceae). The Gypsogenin compound is found in Gypsophila plant along with sugar chains that contribute to various biological properties such as antiviral, antitumor, antioxidant and anti-cancer. We have targeted some of the semi-synthesis reactions in order to make the higher activity more active.

Material and Method: We synthesized new gypsogenin derivatives (**1-3**). The progress of the reactions was followed by sodium triacetoxy borohydride in DCE. Purification was carried out using chromatographic methods.

Results: Up to now, in our continuous research, the elucidation of the synthesized compounds (**1-3**) was determined by IR, UV, ^{1}H NMR, APT and LCMS analysis.

Conclusion / Discussion: In the last part of the study, biological activities of new compounds will be investigated.

Keywords: Gypsogenin, semi synthesis

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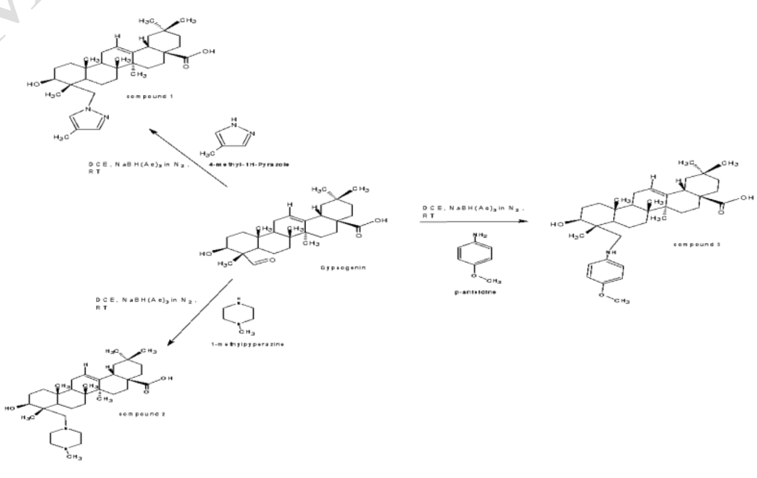

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POSTER PRESENTATION

CHEMICAL CONSTITUENTS OF *ARTEMISIA ALBICERATA*

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Objective / Purpose: *Artemisia albicerata* is of great interest and occupy an important place among the vast variety of medicinal plants in Kazakhstan due to its medical and pharmacological properties and has a rich chemical composition consisting of amino acids, fatty acids, flavonoids and other low-molecular substances. The aim of the present research was the investigation of chemical composition and developing a technology for producing a biologically active complex based on *A. albicerata*.

Material and Methods: The aerial part of *A. albicerata* utilized in the present survey was collected in Almaty region, Kazakhstan at the end of August in 2018. The elemental constituent in the ash was investigated by applying the method of multi-element atomic emission spectral analysis.Gas-liquid chromatography was used for determination of amino acids and fatty acids. Conditions for obtaining a biologically active complex from *A. albicerata* were: extractant - 70% ethyl alcohol, the ratio of raw material and extractant - 1:8, time of triple extraction - 3 days, temperature - 10 ° C.

Results: Moisture content (6.62 %), extractives (12.71 %) and total ash (7.96 %) were identified. Eleven macro-micro elements were obtained from the ash of plant, main of them were Ca (485.110 μg/ml), K (481.660 μg/ml), Mg (97.950 μg/ml), Na (46.820 μg/ml), Fe (30.2939 μg/ml). In addition, twenty amino and eight fatty acids were identified from *A. albicerata*. The major amino acid contents were glutamate (2615 mg/100g), aspartate (1296 mg/100g) and alanine (890 mg/100g), while the composition of fatty acids mainly was oleic (19.6 %) and linoleic (68.4 %) acids.

Conclusion / Discussion: The results demonstrated constituent of important macro-micro elements, amino and fatty acids, main classes of biologically active substances in the aerial part of *A. albicerata*. Consequently, the results indicate a more thorough study is needed.

Key words: *Artemisia albicerata*, macro-micro elements, amino-, fatty acids.

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POSTER PRESENTATION

LIPOSOLUBLE CONSTITUENTS FROM THE AERIAL PART OF *LIGULARIA NARYNENSIS*

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Objective: *Ligularia* is a medicinally important genus of the family Compositae that comprises about 180 Eurasian species, 17 species growing in mountains of Kazakhstan [1]. Phytochemical investigations of various *Ligularia* species showed the presence of monoterpenes, sesquiterpenes, diterpenes, triterpenes, alkaloids, steroids, flavonoids, lignans, and other skeleton type compounds, as well as some of these compounds showed various biological activities such as anticancer, antibacterial, antihepatotoxicity, antioxidant, and antithrombus [2]. In this work, fifty liposoluble constituents in chloroform extract from the aerial part of *Ligularia narynensis* from Kazakhstan have been identified by GC-MS method for the first time.

Material and Methods: To determine the liposoluble constituents' composition was made erenow of the raw material used GC/MS device. The chloroform extract from the aerial part of *L. narynensis* was analyzed by Electron Impact Ionization (EI) method on Agilent 7890A-5975C GC-MS (Gas Chromatograph coupled to Mass Spectrometer) fused silica capillary column (30m x 0.25mm; 0.25 μm film thickness), coated with HP-5MS were utilized. The carrier gas was helium (99.999 %). The column temperature was programmed from 50°C (held for 10 min), with 10°C/min rate program to increase temperature to 300℃. The latter temperature maintained for 40 min (Acquisition parameters full scan; scan range 30-1000 amu). The injector temperature was 310°C. Injection: with a 1 μl. Detector ion source (EI-70eV). Samples were injected by splitting with the split ratio 5:1.

Results: The liposoluble constituent in aerial part of *L. narynensis* was analyzed by GC-MS method. Total fifty compounds were separated and their relative contents were determined by area normalization in which the major constituents were Olean-12-ene, 3-methoxy-, (3.beta.)- (18.24 %), 12-Oleanen-3-yl acetate, (3.alpha.)- (9.70 %), Linoleic acid ethyl ester (8.62 %), Lupeol (6.29 %), and A'-Neogammacer-22(29)-en-3-ol, acetate, (3.beta.,21.beta.)- (5.71 %).

Conclusion: Presence of these bioactive constituents indicated that the plant extract possesses anti-inflammatory, anticoronary and anticancer activities. Further and comprehensive investigation is scheduled to be implemented in the next research stage.

Key words: *Ligularia narynensis*, chloroform extract, liposoluble constituents, GC–MS.

Acknowledgement: The work was supported by grants from Ministry of Education and Science of the Republic of Kazakhstan (0118PK00458).

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POSTER PRESENTATION

ANTIBACTERIAL ACTIVITY OF CONIFERS

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Objective / Purpose: The discovery of new antimicrobial agents is necessary in view of the appearance of multidrug resistant bacterial strains, allergies and other side effects of conventional antibiotic treatment, rendering difficult the therapy of many infectious diseases. This study is aimed at identification of conifers with efficient antibacterial activities against Gram-positive (GP) and Gram-negative (GN) bacteria.

Material and Methods: *Juniperus sabina* var. *balkanensis* was collected from the Rhodope Mountains.[1] *Sequoiadendron giganteum* was from the Botanic garden area of the Bulgarian Academy of Sciences. *Chamaecyparis pisifera*, *Chamaecyparis lawsoniana*, *Cupressus arizonica* and *Taxus baccata* were from the Arboretum of the University of Forestry, Sofia. The extracts, obtained as it was described,[2] were screened for antimicrobial activity towards reference strains (ATCC). Minimal inhibitory concentrations (MIC) were tested by two-fold microdilution method at concentrations ranging from 0.039 to 10 mg/ml.

Results: Antibacterial activity of various conifer extracts was studied on harmful pathogens or commensal microorganisms that can become pathogenic in immunocompromised patients. In general, GP bacteria were more sensitive than GN strains to the tested extracts that may result from different structure of their cell wall. In the group of studied conifer extracts, the best activity was determined for the leaves extracts of *Juniperus sabina* var. *balkanensis* R.P. Adams & A.N. Tashev against GP bacteria: *Micrococcus luteus*, *Bacillus subtilis* and *Bacillus cereus* (MIC=0.078 mg/ml); *Staphylococcus epidermidis* (MIC=0.156 mg/ml); *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae* (MIC=0.313 mg/ml); *Enterococcus faecalis* and *Streptococcus mutans* (MIC=0.625 mg/ml). *J. sabina* var. *balkanensis* showed growth-inhibitory effects also against some GN bacteria, such as *Klebsiella pneumoniae* (MIC=2.5 mg/ml) etc. *Chamaecyparis pisifera* (Siebold & Zucc.) Endl,, *Sequoiadendron giganteum* (Lindl.) J. Buchh., *Cupressus arizonica* Greene also exhibited efficient activities against the studied GP bacteria, compared to less active *Chamaecyparis lawsoniana* (A. Murray) Parl. and *Taxus baccata* L. leaves extracts.

Conclusion / Discussion: In the group of studied conifers, the leaves extract of *Junperus sabina* var. *balkanensis* possessed superior antibacterial activity against a panel of GP and GN bacteria. *J. sabina* varieties are easy for cultivation and therefore they are feasible for industrial exploitation. Comparatively good activities against the studied GP bacteria exhibited also the leaves extracts of *Chamaecyparis pisifera*, *Sequoiadendron giganteum*, *Cupressus arizonica*, whereas *Chamaecyparis lawsoniana* and *Taxus baccata* leaves extracts showed less activity. Identification of antibacterial conifer metabolites is in progress.

Keywords: antimicrobial activity, Coniferae

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POSTER PRESENTATION

MEDICINAL PLANTS OF BULGARIA

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Objective/Purpose: Bulgaria is a small country in Southeast Europe, but its geographic location, varied terrain and climate contribute to great floristic diversity. The medicinal plants are part of it. They represent 23.2% of the country's flora. The aim of the present paper is to present information about the full composition of the medicinal plants of Bulgarian flora as well as characteristics of the systematical structure and classification according to biological type, life form, floristic geoelements, period of flowering, and ecological groups.

Materials and methods: The complete list of medicinal plants in Bulgaria was compiled on the basis of the Medicinal Plants Act [1], extensive literature analysis and own researches [2, 3]. The life forms were identified according to Raunkiaer [4] and for floral elements the classification of Walter [5] was used. The ecological forms were identified according to Flora of Republic Bulgaria, vol. I-XI.
Results: As a result of the conducted surveys it was found that there are 950 medicinal species of vascular plants in the country belonging to 468 genera and 126 families. The richest family containing medicinal plants was Rosaceae (98 specie). The most medicinal plants existed among perennial herbaceous plants (539 species). Geoelements with an European component were predominal (408 species). The most plants bloom from June to August (121 species). The ecological structure of medicinal plants shows that mesophytes (435 species); heliophytes (545 species) and thermophylous plants (607 species) were dominated. The results of the study include also a detailed information about the national and international conservation importance of the analyzed species. 65 species were included in the Red Book of Bulgaria. There were 81 species protected by the Biodiversity Law. Among the medicinal plants are 16 Balkan and 4 Bulgarian endemic spesies. The international significance of these plants was defined by the fact that 33 of the species were under protection by different international conventions.

Conclusion/Discussion: The ratio between the biological types, the predominant geoelements and the life forms shows that it is typical for the temperate continental climate zone and the flora of our country. The total percentage of protected species (7%) is not high, which is positive because most species are subject to use by the local population, the pharmaceutical and perfumery industry.

Key words: medicinal plants, flora, systematic structure, geoelements, biological types, conservation significance, Bulgaria

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POSTER PRESENTATION

POLYPHENOLS FROM *ATHAMANTA SICULA* L.

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Introduction: The genus *Athamanta* (Apiaceae) consists of about nine species found in the South Eastern Europe and North Africa. Previous phytochemical investigation on this genus reported the isolation of the flavonoids and propenphenols. In the present work, we described the isolation of polyphenols from the dichloromethane, ethylacetate and *n*-butanol fractions of *A. sicula* L. The present work aims to determine the polyphenols of *Athamanta sicula*, collected in North-eastern Algeria

Material and methods: Air dried and powdered aerial parts (2.5 kg) of *A. sicula* were macerated with 70% MeOH then concentrated under reduced pressure. The residue was dissolved in water and sequently partitioned with light petroleum, CH_2Cl_2, EtOAc and *n*-Butanol. The CH_2Cl_2 fraction (6g) was subjected to silica gel column chromatography eluting with gradient of $CHCl_3$-Toluene (0-100% $CHCl_3$) and $CHCl_3$- EtOAc (0-100% EtOAc). Subfractions underwent further fractionnations led to a phytosterol and three propenphenols (**1-4**). The, EtOAc fraction was submitted to silica gel CC using a step gradient solvent system *n*-hexane-acetone (0-100% Acetone) to give seven fractions. The fraction 3 was also subjected to silca gel CC but eluted with an isocratic system EtOAc:MeOH:H_2O (10:1:0.5) to yield three flavonoids (**5-7**). The *n*-butanol fraction (15g) was fractionated by polyamide SC6 column chromatography followed by TLC purification leading to the isolation of one flavone glycoside (**8**). Compounds **3-5** were tested for their cytotoxicity activity against fibrosarcoma HT 1080 cells by means of the MTT method

Results. Eight known compounds were isolated *Athamanta sicula*, their structure elucidation was based on analyses of spectroscopic data 1D-2D NMR and HRESIMS techniques and by comparing data with those reported in literature . They exhibited a moderate cytotoxicity.

Discussion. Stigmasterol (**1**), anthricinol (**2**), myristicin (**3**), apiol (**4**), apigenin (5), luteolin (**6**), diosmetin (**7**), and luteolin-7-O-β-D-glucoside (**8**). Compounds **2-4** are characteristic of Apiaceae family, anthricinol (**2**) is new in *Athamanta* genus. Only myristicin exhibited a moderate cytotoxic activity with IC_{50} = 45 µM. As a conclusion: *Athamanta sicula* is a good source of polyphenols

Keywords: *Athamanta sicula* ; flavonoids ; propenphenyls ; cytotoxicity.

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POSTER PRESENTATION

ANTIBACTERIAL ACTIVITY OF *PEGANUM HARMALA* SEED EXTRACTS

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Objective / Purpose: *Peganum harmala* L. is a member of Nitrariaceae family which has traditional usages as anthelmintic, lactagogue, antispasmodic, antipyretic effects and stimulation of the motor tracts of the cerebrum in folk medicine [1,2]. Previous studies illustrate that methanolic extracts of *Peganum harmala* seed had good antibacterial activity against *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *P. vulgaris*, and *E. coli*, but it was more susceptible in *S. aureus* [2]. By contrast, *n*-hexane extract from the seed of *P. harmala* showed no antimicrobial activity against both Gram (+) and Gram (-) bacteria [3]. Furthermore, depending on the former experiments, methanolic extracts of the seeds also demonstrated antibacterial activity against *B. cereus* bacteria [4]. In addition to all these, methanolic extracts also indicated antimicrobial activity against *Branhamella catarrhalis* [5]. The objective of this study is to determine the antibacterial activity of *Peganum harmala* seed extracts.

Material and Methods: The hexane, ethyl acetate and methanol extracts from the seed of *Peganum harmala* were obtained by maceration method. Antibacterial activity of seed extracts was observed against *S. aureus* ATCC 29213, *B. cereus* ATCC 14579, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 by using a broth microdilution and disc diffusion assays. The experiments were started 8 mg/mL for broth microdilution assay and 10 mg/disc extracts were used for disc diffusion assay. Chloramphenicol was used as a positive control for both assays. All the experiments were performed in duplicate.

Results: Hexane and ethyl acetate extracts did not show activity against any of the tested bacteria. However, methanol extract showed inhibitory activity against the tested microorganisms. The highest inhibitory activity was observed against *B. cereus*, the MIC value was 1 mg/mL and the disc diffusion results were 24 mm at 10 mg/disc.

Conclusion / Discussion: The methanol extract of seed showed antibacterial activity against Gram (+) and Gram (-) bacteria. But the extract was more susceptible to *B. cereus* than other tested bacteria.

Keywords: *Peganum harmala*, extract, antibacterial activity

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POSTER PRESENTATION

DETERMINATION OF ANTIBACTERIAL ACTIVITY FROM SEED EXTRACTS OF *PISTACIA TEREBINTHUS*

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Objective / Purpose: *Pistacia terebinthus* L. is a member of Anacardiaceae family that has been used as a traditional natural remedy for different purposes all around the world. The plant has been used as an aphrodisiac and expectorant in Greece, as a diuretic and laxative in Jordan, as a hypotensive and antiseptic in Spain, antidiarrhea and astringent properties in Iran, and finally stomach ache, asthma and as an antidiabetic in Turkey [1]. Previous studies demonstrate that ethanolic extracts of *Pistacia terebinthus* showed antimicrobial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus cereus*, and *Mycobacterium smegmatis* depending on the disc diffusion tests [2]. Additionally, ethanolic extracts of the plant also showed antimicrobial activity against *S. aureus* in microdilution experiments, whereas there is no indication of antimicrobial property on *E. coli* [3]. The objective of this study is to determine the antibacterial activity of *Pistacia terebinthus* seed extracts.

Material and Methods: The plant material (350 g) was macerated with hexane, ethyl acetate, and methanol, respectively. Antibacterial activity of seed extracts was observed against *S. aureus* ATCC 29213, *B. cereus* ATCC 14579, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 by using a broth microdilution and disc diffusion assays. The experiments were started 8 mg/mL for broth microdilution assay and 10 mg/disc extracts were used for disc diffusion assay. Chloramphenicol was used as a positive control for both assays. All the experiments were performed in duplicate.

Results: The inhibitory activity was observed against the tested microorganisms for methanol extract. The methanol extract showed the highest inhibitory activity against *S. aureus* and *B. cereus.* The inhibition zone diameters were 14 mm at 10 mg/disc for *S. aureus* and *B. cereus*. The minimum inhibition concentration (MIC) values were 0.5 mg/mL for *S. aureus* and 1 mg/mL for *B. cereus*. Ethyl acetate extract showed inhibitory activity against *E. coli* and *P. aeruginosa*. The MIC values were 8 mg/mL and the inhibition zone diameters were 8 mm at 10 mg/disc for both bacteria. However, the same extract did not show activity against *S. aureus* and *B. cereus*. Hexane extract showed inhibitory activity against *P. aeruginosa*, the MIC value was 8 mg/mL and the inhibition zone diameter was 9 mm at 10 mg/disc.

Conclusion / Discussion: *Pistacia terebinthus* seed methanol extract has antibacterial activity against both Gram-positive and Gram-negative bacteria. The methanol extract was susceptible against *S. aureus* and *B. cereus.* The ethyl acetate extract has inhibitory activity against Gram-negative bacteria. All the seed extracts were susceptible against *P. aeruginosa*.

Keywords: *Pistacia terebinthus*, extract, antibacterial activity

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POSTER PRESENTATION

ANTIBACTERIAL ACTIVITY OF *SAMBUCUS NIGRA* SEED EXTRACTS

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Objective / Purpose: *Sambucus nigra* L., is 3-4 meters in length, a small tree with unified leaves and fragrant [1]. It belongs to the Adoxaceae family. The plant is located in the Northern Turkey and Iraq, Western Syria and Iran. Infusion or decoction from flowers, leaves and stem barks of *Sambucus nigra* used as diuretic, diaphoretic and laxative effects [2]. Previously, the antimicrobial activity of ethanol extract from *Sambucus nigra* was studied. The ethanol extract showed inhibitory activity against *Bacillus cereus, Escherichia coli* ve *Pseudomonas aeroginosa* [3]. The objective of this study is to determine the antibacterial activity of *Sambucus nigra* L. seed extracts against *Staphylococcus aureus* ATCC 29213, *B. cereus* ATCC 14579, *P. aeruginosa* ATCC 27853 ve *E. coli* ATCC 25922 by using a broth microdilution and disc diffusion assays.

Material and Methods: The hexane, ethyl acetate and methanol extracts from the seed of *Sambucus nigra* L. were obtained by maceration method. The experiments were started 8 mg/mL for broth microdilution assay and 10 mg/disc extracts were used for disc diffusion assay. Chloramphenicol was used as a positive control for both assays. All the experiments were performed in duplicate.
Results: Methanol extract showed inhibitory activity against tested microorganisms. The minimum inhibition concentration (MIC) value for methanol was 8 mg/mL and the disc diffusion result was 12 mm for all bacteria. Ethyl acetate extract showed inhibitory activity against *B. cereus*. The MIC values of ethyl acetate extract were 8 mg/mL for *B. cereus* and the disc diffusion result was 8 mm. Hexane extract did not show inhibitory activity against any of the tested bacteria.

Conclusion / Discussion: The methanol extract of seed showed antibacterial activity against Gram-positive and Gram-negative bacteria. Hexane extract is not susceptible to any bacteria. Ethyl acetate extract is not susceptible to tested bacteria except that *B. cereus*.

Keyworlds: *Sambucus nigra* L., extract, antibacterial activity

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POSTER PRESENTATION

DETERMINATION OF ANTIBACTERIAL ACTIVITY OF *LINUM USITATISSIMUM* SEED EXTRACTS

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Objective / Purpose : Flaxseed or linseed (*Linum usitatissimum* L.) is an annual herb belongs to the Linaceae family. Consumption of flaxseed has been demonstrated to have a multitude of positive health benefits including decreasing rate of tumor growth, reducing serum cholesterol level and decreasing the incidence of breast, prostate, and colon cancers [1]. Previously, the antimicrobial activity of *n*-hexane, ethyl acetate, butanol and water extracts from *L. usitatissimum* was studied by disc diffusion assay. The ethyl acetate and butanol extracts showed inhibitory activity against *S. aureus*, *B. cereus* and *E. carotovora* [2]. The objective of this study is to determine the antibacterial activity of *Linum usitatissimum* L. seed extracts against *S. aureus* ATCC 29213, *B. cereus* ATCC 14579, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 by using a broth microdilution and disc diffusion assays.

Material and Methods: The hexane, ethyl acetate and methanol extracts from the seed of *Linum usitatissimum* L. were obtained by maceration method. The experiments were started 10 mg/mL for disc diffusion assay and 8 mg/mL for broth microdilution assay. Chloramphenicol was used as a positive control for both assays. All the experiments were performed in duplicate.

Results: Hexane extract showed inhibitory activity against *E. coli*, *P. aeruginosa* and *S. aureus*. The MIC value was 8 mg/mL and the disc diffusion result was 10 mm at 10 mg/mL. Ethyl acetate extract showed inhibitory activity against *S. aureus* and *P. aeruginosa*. The MIC values of ethyl extract were 0.5 mg/mL for *S. aureus* and 8 mg/mL for *P. aeruginosa*. The diffusion results were 8 mm for *P. aeruginosa* and 12 mm for *S. aureus* at 10 mg/mL. The methanol extract showed inhibitory activity against *S. aureus*. The MIC value was 8 mg/mL and the disc diffusion result was 8 mm at 10 mg/mL.
Conclusion / Discussion: Seed extracts of the plant did not show activity against *B. cereus*. These extracts are not susceptible to *B. cereus*. But the extracts showed activity against *S. aureus* at different concentrations. Ethyl acetate extract was more susceptible against *S. aureus* than other extracts.

Keyworlds: *Linum usitatissimum* L., extract, antibacterial activity

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POSTER PRESENTATION

THE ANTIBACTERIAL ACTIVITY OF *ELAEAGNUS ANGUSTIFOLIA* L. FRUIT AND LEAF EXTRACTS

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Objective: *Elaeagnus angustifolia* L. (Russian olive, oleaster) belongs to Elaeagnaceae family. The plant has been used as traditional folk medicine for many years due to it is anti-inflammatory, muscle relaxant, anti-ulcerogenic, antimicrobial, antinociceptive, antitumor, and antioxidant effects [1]. Previously, the antibacterial activity of crude methanol extract, *n*-hexane, ethyl acetate, chloroform and water fractions of *E. angustifolia* L. was determined with well diffusion assay. The crude extract, hexane, ethyl acetate, and water fractions showed activity with different inhibition zone diameters against *E. coli* and *S. aureus*. And only the hexane and ethyl acetate fractions showed inhibitory activity against *P. aeruginosa*. But the chloroform fraction had no antibacterial activity against tested microorganisms [2]. The purpose of this study is to determine the antibacterial activity of *Elaeagnus angustifolia* L. fruit and leaf extracts against *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 and *B. cereus* ATCC 14579 by using broth microdilution and disc diffusion methods.

Material and Methods: Maceration method was used to obtain the hexane, ethyl acetate and methanol extracts from the fruit and leaf of *Elaeagnus angustifolia* L. The experiments were began with 8 mg/mL for broth microdilution assay and 10 mg/disc extracts were used for disc diffusion assay. Chloramphenicol was used as a control antibiotic for both methods. All the experiments were performed in duplicate.

Results: The ethyl acetate extract of fruit was more effective than the other extracts for tested microorganisms. The minimum inhibition concentration (MIC) values of fruit ethyl acetate extract were 1 mg/mL for *B. cereus*, 2 mg/mL for *E. coli* and *S. aureus* and 8 mg/mL for *P. aeruginosa*. The inhibition zone diameters of fruit ethyl extract were 15 mm for *E. coli*, 14 mm for *S. aureus*, 12 mm for *P. aeruginosa* and 10 mm for *B. cereus*. The hexane and ethyl acetate extracts from leaves showed inhibitory activity against tested microorganisms at 8 mg/mL. But the fruit hexane extract did not show activity against *E. coli*. The leaf methanol extract did not show activity *E. coli* and *S. aureus*. The fruit methanol extract did not show antibacterial activity against *B. cereus.*

Discussion / Conclusion:

In the current study reveals that the fruit and leaf extracts of *E. angustifolia* had inhibitory activity against different bacteria at different concentrations. And the antibacterial activity may change according to the plant part and extract type which is used. As an example, the leaf methanol extract was not susceptible against *E. coli* and *S. aureus* but fruit methanol extract was susceptible against these two bacteria.

Keywords: *Elaeagnus angustifolia* L., extract, antibacterial activity

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POSTER PRESENTATION

MUTAGENIC *IN VITRO* ACTIVITY AND GENOTOXIC EFFECT OF *ZYGOPHYLLUM CORNUTUN* METHANOLIC EXTRACT

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Objective/Purpose: *Zygophyllum cornutun coss* is an Algerian medicinal plant widely used in the treatment of different diseases mainly diabetes. The purpose of our study was to determine possible mutagenic/genotoxic effect of this plant.

Materials and Methods: Three concentrations (5, 15 and 50 mg/ml) of *Zygophyllum cornutum* methanolic extract were screened to the presence of mutagenic activity by Ames test using TA100 and TA98 strains of *Salmonella tiphymurium* with and without metabolic activation (S9 microsomal fraction) according to the method of K. Mortelmans and E. Zeiger, 1983 [1]. The micronucleus assay on human lymphocytes was used to evaluate genotoxic effect according to the method described by Fenech et morley, 1985 [2]. The frequency of the micronucleus (MN) and the proliferation index (PI) were calculated according to Fenech et morley, 1985.

Results: The Ames test showed negative results without microsomal activation while mutagenic activity was observed in the presence of microsomal activation. The number of the revertant colonies observed at 5, 15 and 50 mg/ml was respectively 49, 77 and 89 with TA98 and 88, 95 and 55 with TA100. In the micronucleus test, a non significant increase of the micronucleus frequency was observed at 15 mg/ml 8.66 with PI of 1.70 . At 50 mg/ml, the frequency of MN increased (24.76) and PI decreased (1.55) significantly.

Conclusion/Discussion: Even that further studies must be carried out, the mutagenic activity and the genotoxic effect of *Zygophyllum cornutum* should be taken in consideration when used as therapeutic plant.

Key words: Ames test, genotoxicity, micronucleus test, *Zygophyllum cornutum*.

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POSTER PRESENTATION

BIOLOGICAL ACTIVITIES OF *THYMUS FONTANESII* POLYPHENOLIC EXTRACT: *IN VITRO* AND *IN VIVO* EFFECT ON BACTERIAL ADHESION AND COLONISATION

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Objective / Purpose: Polyphenols are plants secondary metabolites that are not absorbed in the small intestine but can be degraded by colonic microbiota to simpler and more easily absorbable bioactive compounds with potential physiological effects. They can act as potent antioxidants and have diverse effects on microbes. They appear to be effective antimicrobial agents against pathogens and to improve probiotic growth and metabolisms. A polyphenol-rich diet may then improve the intestinal status and possibly attenuate oxidative stress and suppress inflammations. These attributes express the potential antioxidant and microbial effects of those molecules. Furthermore, these compounds have technological aspects as they can enhance metabolism of LAB in dairy industry. The aim of this study was therefore to assess the *in vitro* and *in vivo* influence of *Thymus fontanesii* polyphenolic extract on the bacterial adhesion and colonization.

Material and Methods: The polyphenolic content of *Thymus fontanesii* was extracted in methanol by sonication. For the assessment of *in vitro* polyphenolic effet on bacterial adhesion and biofilm formation, bacterial cultures, at fixed density (~10^7 germs/ml), are supplemented by the polyphenolic extract of *T. fontanesii* in increasing final concentrations: 0µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml. For the in *vivo* data**,** against negative control group (no treatment has been achieved), test group was force-fed 4 weeks with 100µl of polyphenolic extract.

Results: Cynamic, caffeic, carboxylic, furilic, M anisic and vanilic acids, berberine, catechine, resorcinol and rutine were the important phenolic acids and flavonoids identified by HPLC in the extract. Considerable anti-adhesive activity of extract has been recorded; the adhesion of the pathogenic strains tested was reduced in presence of the polyphenolic extract of *Thymus fontanesii* at lethal concentrations ranging from 100 µg / ml to 200 µg / ml, the strains *Bacillus cerius 11798, Bacillus subtilis 3633, and Escherichia coli 10536* proved to be more sensitive, they completely lost their ability to form a biofilm. At sublethal concentrations (from 50 to 100µg/ml), *Staphylococcus aureus 25922* and *Pseudomonas aerugenosa 27853* develops a very high propensity to form a biofilm protector against the toxic effect of extract. The *in vivo* tests were shown that the polyphenolic extract shaped Wistar Rat gut microbiota in favour of beneficial bacteria and in detriment of pathogenic bacteria, and prevented bacterial translocation.

Conclusion / Discussion: Therefore, these natural molecules from medicinal plant can be used as alternative or additive agents to prevent the oxidative stress and modulate the bacterial activity and their adhesion, colonization and translocation capacity.
Keywords: Microbiota; bacterial translocation; polyphenols; bacterial adhesion; *Thymus fontanesii.*

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MESMAP-5 Final Proceedings Book

POSTER PRESENTATION

IN VITRO TOXICOLOGICAL EVALUATION OF SOME MEDICINAL PLANTS USED BY WOMEN IN NORTHERN MAPUTALAND, SOUTH AFRICA

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Purpose: Medicinal plants are still used as a primary source of healthcare in northern Maputaland because of the strong cultural beliefs in medicinal plants. This increases the potential exposure to plant toxicity. Women in this area use also medicinal plants to maintain healthy pregnancy/delivery and to treat gynaecology problems [1]. There are very few scientific reports on the toxicity of medicinal plants in this area. The lack of toxicological information on these plants raises concerns about the safety for the unborn child. The aim of this study was to evaluate the safety of 33 medicinal plants used by women in northern Maputaland to treat gynaecology and obstetrics complaints.

Material and Method: An Ethnobotanical survey was conducted using a structured questionnaire to investigate medicinal plants used to treat gynaecology and obstetrics complaints. All plant material was collected on site, dried and ground into powder. Methanol-dichloromethane (1:1) and aqueous extracts were prepared. An acute cytotoxicity study was conducted using an *in-vitro* assay called the Brine Shrimp Lethality Assay (BSLA). Cytotoxicity was determined by the death of *Artemia franciscana* after the exposure to the plant extract. In cases where roots were being used by the people, leaves (17) were simultaneously collected for comparison. The results were recorded after 24 and 48 hours.

Results: The ethnobotanical survey resulted in the documentation of 33 medicinal plant species. The people in northern Maputaland use mostly water to prepare their herbal medicine. Eleven aqueous extracts were cytotoxic, and the same number was also observed with the ethanol-dichloromethane extracts. However, in some cases, only the aqueous extracts were toxic or only the organic extracts. Aqueous extract of *Euphorbia tirucalli* stem was considered the most cytotoxic by killing 100% of the brine shrimps within 24 hours in all replicates. However, this plant was only used by one interviewee. The most mentioned plants were *Bridelia cathartica* (18 times to treat dysmenorrhoea, amenorrhoea, menorrhagia, oligomenorrhoea infertility and to prevent miscarriage) and *Ranunculus multifidus* (13 times for genital warts). The aqueous root extract of *B. cathartica* was non-cytotoxic but the organic extract and the leaves were cytotoxic. Both the aqueous and organic extracts of *R. multifidus* indicated signs of cytotoxic activities. Overall leaf extracts were highly cytotoxic when compared to their respective root extracts, only three of the leaf extracts were non-cytotoxic in this experiment.

Conclusion: Literature showed that the majority of medicinal plants reported to have medicinal use in northern Maputaland have not been assessed for their safety. The number of cytotoxic extracts encountered indicate the importance of evaluating safety of traditional medicine. Subsequently, the communities need to be aware of such adversities.

Keywords: Cytotoxicity, Medicinal plants, Women problems, northern Maputaland, South Africa

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POSTER PRESENTATION

IN-VITRO SUSCEPTIBILITY TESTING OF TEA TREE OIL ACNE CREAM IN COMBINATION WITH SALICYLIC ACID AND NICOTINAMIDE

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The tea tree oil acne cream was prepared in Negiban dispensing pharmacy according to SOP by levigating the specific amount of ingredients; tea tree oil (2%), nicotinamide(3%) and salicylic acid(3%). The formulation is then tested for its susceptibility by microwell dilution method on 15 samples of *Staphlococcus aureus (S. aureus)* and five samples of *Propionibacterium acnes (P. acnes)* collected from the out-patient department of Skin, Mayo Hospital, Lahore. After finding the susceptibility, minimum inhibitory concentration of the cream was estimated to establish its effectiveness, by 96 well plate method. The results of the samples (1 to 15) against *S. aureus*, majority of the samples have showed maximum zone of inhibitions at dilution 1:5. For *P. acnes*, samples (1 to 5) majority no of the samples depicted maximum zone of inhibition at dilution ratio 1:10. The results suggests that the tea tree oil acne cream is effective in a narrow dilution range and shows potent anti-acne effect. While, the results of the MIC of the *S. aureus* and *P. acnes*, the cream showed 0.074, 0.069 at concentration 12.5 mg/ml and 0.023 at concentration 12.5 mg/ml, 0.007 at concentration 6.25 mg/ml, 0.073 at concentration 3.125 mg/ml and 0.072 at concentration 0.02 mg/ml, respectively. The results confirm the susceptibility and effectiveness of the formulation against the bacterial strains responsible for acne.

Key words: Tea Tree Oil, *Staphylococcus aureus*, *Propionibacterium acnes*

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POSTER PRESENTATION

IMPACT OF SOME HERBICIDES AND HERBICIDE COMBINATIONS ON SOWING CHARACTERISTICS OF CORIANDER (*CORIANDRUM SATIVUM* L.)

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Objective / Purpose: The aim of this investigation was to establish the influence of some soil-applied herbicides, foliar-applied herbicides and herbicide combinations on sowing characteristics of the coriander seeds and the quantity of waste grain.

Material and Methods: The research was conducted during 2013 - 2015 on pellic vertisol soil type. Under investigation was Bulgarian coriander cultivar Lozen 1 (*Coriandrum sativum* L.). Factor A included no treated control, 6 soil-applied herbicides – Tender EC (S-metolachlor) - 1.5 l/ha, Silba SC (metolachlor + terbuthylazine) - 3.5 l/ha, Sharpen 33 EC (pendimethalin) - 5 l/ha, Merlin flex 480 SC (isoxaflutole) - 420 g/ha, Smerch 24 EC (oxyfluorfen) - 1 l/ha, Raft 400 SC (oxidiargil) - 1 l/ha and 5 foliar-applied herbicides – Kalin flo (linuron) - 2 l/ha, Eclipse 70 DWG (metribuzine) - 500 g/ha, Sultan 500 SC (metazachlor) - 2 l/ha, Corrida 75 DWG (tribenuron-methyl) - 20 g/ha, Lontrel 300 EC (clopyralid) - 500 ml/ha. Factor B included no treated control and 1 antigraminaceous herbicide – Tiger platinium 5 EC (quizalofop-P-ethyl) - 2.5 l/ha. Soil-applied herbicides were treated during the period after sowing before emergence. Foliar-applied herbicides were treated during rosette stage of the coriander.

Conclusion / Discussion: Herbicides Merlin flex and Lontrel and herbicide combinations Merlin flex + Tiger platinium and Lontrel + Tiger platinium proven decrease germination energy of the coriander seeds. Laboratory seed germination and lengths of primary germ and primary root are decreased by herbicide Merlin flex and herbicide combination Merlin flex + Tiger platinium only. Herbicide Merlin flex and herbicide combination Merlin flex + Tiger platinium do not proven decrease waste grain quantities. High yields of coriander seeds are obtained by foliar treatment with antigraminaceous herbicide Tiger platinum after soil-applied herbicides Raft, Smerch, Sharpen, Silba and Tender. Tank mixtures of Tiger platinum with foliar herbicides Kalin flo, Eclipse, Sultan, Corrida and Lontrel also lead to obtaining of high seed yields. The use of the soil-applied herbicide Merlin flex does not increase the seed yield, due to its higher phytotoxicity against coriander. Alone use of soil-applied or foliar-applied herbicides leads to lower yields due to they must to combine for full control of weeds in coriander crops.

Keywords: coriander, herbicides, herbicide combinations, seed yield, sowing characteristics

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POSTER PRESENTATION

PROFILING OF PHENOLIC COMPOUNDS BY HPLC–DAD, ANTIOXIDANT AND ENZYME INHIBITORY ACTIVITIES OF *MARRUBIUM VULGARE* L. AND *MARRUBIUM PARVIFLORUM* FISCH. & MEY. SUBSP. *PARVIFLORUM*

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Objective: The genus *Marrubium* L., belonging Lamiaceae, is represented by 20 species in Turkey, twelve of which are endemic. *Marrubium* species is traditionally used in the treatment of various diseases such as asthma, pulmonary inflammation and hypoglycemi; for analgesic, sedative and cholagogue effects. According to the literature, it was found that *Marrubium* species have various therapeutic effects such as hypoglycemic, antihypertensive, antispasmodic, antiproliferative, antioxidant, hepatoprotective, hypolipidemic, gastroprotective, antibacterial and vasorelaxan as well as bioactive secondary metabolites, such as diterpenes, sterols, phenylpropanoids and flavonoids. We aimed to investigate the antioxidant and enzyme inhibitory activities of the methanol and water extracts of *M. vulgare* L. (MV) and *M. parviflorum* Fisch. & Mey. subsp. *parviflorum* (MP) growing in Turkey.

Material and Methods: We conducted *in vitro* preliminary bioassays on the plant extracts for their evaluation of antioxidant activities (DPPH, ABTS, iron chelating, β-carotene/linoleic acid system, TPC and TFC) and enzyme inhibition effects (acetylcholinesterase, butyrylcholinesterase, and tyrosinase). We also analysed the plant extracts for the qualitative and quantitative determination of phenolic compounds by HPLC-DAD using various mobile phases having different elution gradients and run times.

Results: According to our results, the methanol extract of MP was found the highest antioxidant properties for DPPH and ABTS assays while the water extract of MV having the most iron chelating and lipid peroxidation capacity. In addition, while the methanol extracts of MP and MV showed more inhibition effects on the cholinesterase enzymes, it was found that water extracts of MP and MV had more antityrosinase activities. When the plant extracts are compared in terms of phenolic substance contentof MP and MV, we observed that trans-ferulic acid, elagic acid, caffeic acid, and 1,2-dihyrdoxy benzene were determined as the main components in the extracts.
Conclusion: As a conclusion, our study can provide scientific results for further investigation on the developing novel antioxidant, anticholinesterase, and antityrosinase agents from natural sources.

Keywords: Antioxidant activity, enzyme inhibition, HPLC, *Marrubium vulgare*, *Marrubium parviflorum*,

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POSTER PRESENTATION

PRELIMINARY PHYTOCHEMICAL SCREENING OF FOUR SAHARAN PLANTS USED IN SOUTH WEST OF ALGERIA

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Phytochemical surveys are now acted as the first step towards the discovery of useful drugs. The present investigation was carried out to assess the qualitative phytochemical analysis of differents parts of four Saharan plants belonging to Solanacea and Asclepidiacea family namely: *Daturastramonium*, *Hyoscyamusmuticus*, *Pergulariatomentosa* and *Calotropisprocera.*

Qualitative phytochemical analysis of this plant confirms the presence of various phytochemicals like saponins, flavonoids, steroids, unsaturated sterols and terpenes and alkaloids in both the plants extracts, where as unsaturated sterols, cardinolides and tannins were found to be absent in some extracts of parts of plants tested.

Keyword: qualitative phytochemical analysis, *Calotropis procera, Pelgularia tomentosa, Datura staramonium and Hyoscyamus muticus*.

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POSTER PRESENTATION

PHYTOCHEMISTRY AND CYTOTOXIC POTENTIAL OF *LITSEA DECCANENSIS* BARK.

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Objective: The genus *Litsea*, family Lauraceae, contains approximately 200 plant species mainly distributed in the tropical and subtropical regions of the world. *Litsea* species have been reported to possess a wide variety of pharmacological activities such as anticancer, anti-inflammatory, antimicrobial, antioxidant, antidiabetic, anti-HIV, insecticidal to name a few. There are very few phytoconstituents reported from the bark of *Litsea deccanensis* Gamble, viz. caryophyllene oxide, p-sitosterol, actinodaphnine and litsomentol. Given the varied pharmacological activity of the leaves and stem of this plant we thought it prudent to investigate the bark for secondary metabolites with potential biological activity. These are the first reports of biological activity of the bark of *Litsea deccanensis* and identification of new phytoconstituents.

Material and methods: The bark was collected from Amboli Ghats, Maharashtra state, dried and powdered. Successive soxhlet extraction was carried out using petroleum ether 60-80ºC, toluene, dichloromethane, ethyl acetate, methanol and water. Alkaloid rich fraction of the bark powder was prepared using reported method. The successive solvent extracts and the alkaloid rich fraction were screened for cytotoxic activity using Adriamycin as standard. LC-MS analysis of the solvent extracts and the alkaloid rich fraction were conducted. The total alkaloid content in the bark was determined using aluminium chloride assay.

Results: The successive solvent extracts exhibited significant cytotoxic activity on the selected cell lines. The alkaloid rich fraction showed cytotoxic activity on Human Breast Cancer cell line MCF-7, Human Breast Cancer cell line MDA-MB-231, Human Cervical Cancer cell line HeLa, Human Leukemia cell line U-937. The alkaloid rich fraction exhibited the most potent activity against all the selected cell lines. The LC-MS data of alkaloid rich fraction indicated the presence of alkaloids such as 3,4-methylene dioxy methamphetamine (MDMA), arecoline, salsolidine, boldine, hydrocodone, nedocromil, phytosphingosine and colchicine. The total alkaloid content was found to be 0.13 µg of atropine equivalent/100 mg of extract.

Conclusion: The successive solvent extracts and the alkaloid rich fraction were found to exhibit cytotoxic activity on breast, cervical and leukemia cell lines with the alkaloid rich fraction being the best and potentially responsible for the observed cytotoxic effect. This paper presents the first report on new phytoconstituents identified in *Litsea deccanensis* bark. It also presents for the first time the cytotoxic potential of the bark opening new avenues for drug design and development.

Keywords: Cytotoxicity, *Litsea deccanensis*, alkaloid rich fraction, alkaloids, MCF-7, HeLa, U-937.

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POSTER PRESENTATION

IN VITRO ANTIOXIDANT PROSPECTION OF ONION OIL FROM ROMANIAN SOURCE

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Objective / Purpose: Onion is botanically known as *Allium cepa* and it belongs to the Liliaceae family. It has been used as a medicinal remedy from time immemorial, that's probably because its chemical composition. The main organic compounds are flavonoids and organosulfur compounds. This study was designed to evaluate the antioxidant activities of Romanian onion oil.

Material and Methods: The essential oil of *Allium cepa* was obtained by hydrodistillation and then it was analyzed by GC-MS.In vitro antioxidant activity was done using DPPH scavenging activity, reducing power and hydrogen peroxide scavenging assay

Results: Onion oil was able to reduce DPPH radicals, with IC50 value of 0.781mg/mL; the results indicate that onion oil exhibited a significant DPPH radical scavenging activity, about 8 folds more active than ascorbic acid. For all investigated concentrations, the absorbance of the mixture was high, thus the reducing power was significant. Hydrogen peroxide scavenging activity showed that the antioxidant components of onion oil (probably sulfur compounds) are good electron donors, IC 50 being 0.36 mg/mL.

Conclusion: Taking into account the obtained results, we can state that *Allium cepa* oil can play a major role in alleviating the number of oxidative stress by reducing the oxidative damage of cellular component caused by ROS.

Keywords: onion oil, antioxidant activity

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POSTER PRESENTATION

PROFILING ENZYME INHIBITORY POTENTIAL OF CYCLOARTANE TYPE OF TRITERPENE GLYCOSIDES FROM THE ROOTS OF THREE *ASTRAGALUS* SPECIES

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Objective / Purpose: *Astragalus* L., the largest genus in the family Fabaceae, is represented by 64 sections and about 475 taxa with 202 endemic species in the flora of Turkey. The roots of various *Astragalus* species represent very old and well-known drugs in traditional medicine, used for antiperspirant, diuretic, and tonic purposes as well as for the treatment of nephritis, diabetes, leukemia, and uterine cancer. In the present study, the isolated compounds from the roots of different *Astragalus* species growing in Turkey were subjected to high-throughput screening against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase (TYR), the fundamental enzymes associated with the pathology of Alzheimer's and Parkinson's diseases, also collagenase and elastase enzymes, related to inflammation and cosmetic purposes.

Material and Methods: Eleven cycloartane triterpene glycosides were isolated from the roots of *Astragalus melanophrurius* (astrasieversianin II, astrasieversianin X, astragaloside I, astragaloside IV, astragaloside VI, cyclocanthoside E, and cyclocanthoside G), *A. oleifolius* (macrophyllosaponin A, macrophyllosaponin B, macrophyllosaponin C, and macrophyllosaponin D), and *A. cephalotes* var. *brevicalyx* (cyclocanthoside E). Structures of the isolated compounds were elucidated by means of following spectroscopic methods; UV, 1D NMR (^{1}H NMR, ^{13}C NMR, and DEPT-135) and 2D NMR (COSY, HSQC, and HMBC). ChE, TYR, collagenase, and elastase inhibitory activity of the compounds were screened using ELISA microtiter assays at 1 mg/mL stock concentration.

Results: Our results indicated that the isolated compounds had a low ChE inhibitory activity, whereas they were inactive against TYR and elastase. The most active compound against collagenase was macrophyllosaponin B (35.41 ± 3.96 %), while the other compounds had a modest level of collagenase inhibitory activity.

Conclusion / Discussion: Cycloartane type of triterpene glycosides isolated from the roots of three *Astragalus* species were herein shown to have only no to low or moderate level of enzyme inhibitory effect against the mentioned enzymes, which represent the first study on them in this regard.

Keywords: Cholinesterase, tyrosinase, elastase, collagenase, enzyme inhibition, cycloartane glycosides, *Astragalus*

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POSTER PRESENTATION

ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF *PEGANUM HARMALA* USED IN ALGERIAN FOLK MEDICINE

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The aim of this study is to determine the total phenolic content and the antioxidant potential of *Peganum harmala* (Zygophyllaceae), medicinal plant widely used in Algerian folk medicine.
Air-dried and powdered aerial parts of *Peganum harmala*, harvested from the area of Tébessa (North-Eastern Algeria), were extracted by percolation using solvents with increasing polarity, successively: petroleum ether (PE), dichloromethane (DM), ethyl acetate (EA) and methanol (ME), to yield dry extracts. The capacity of the obtained extracts to inhibit the free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was measured according to the method of Loo et *al*. (2008). Total phenolic content was estimated as gallic acid equivalents per milligram of dried plant extract, according to the Folin-Ciocalteu phenol reagent method (Li et *al*., 2007).

The ME extract showed, on the one hand, the best DPPH inhibition percentage at test-concentration, and, on the other hand, the highest yield which was 4.3, 5.6 and 15.8-fold higher than that of PE, DM and EA extracts, respectively. Its total phenolic content was 2-fold higher than that of PE, DM and EA extracts.

Peganum harmala may be suggested as a new potential source of natural antioxidant *via* its ME extract justifying the Algerian folk medicine use of this plant. Further investigations are necessary in order to refine its antioxidant potential and to determine its phytochemical composition.

Keywords: *Peganum harmala*, percolation, extracts, antioxidant, phenolic content.

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POSTER PRESENTATION

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *PITURANTHOS SCOPARIUS* AERIAL PARTS HARVESTED FROM THE AREA OF TÉBESSA (ALGERIA)

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The aim of this study is to determine the phytochemical screening and the antibacterial potential of aerial parts from *Pituranthos scoparius* (Coss. & amp; Dur) Benth. & amp; Hook. (Apiaceae), endemic species widely used in Algerian folk medicine.

Air-dried and powdered aerial parts of *Pituranthos scoparius*, harvested from the area of Tébessa (North-Eastern Algeria), were extracted by percolation using solvents with increasing polarity, successively: petroleum ether (PE), dichloromethane (DM), ethyl acetate (EA) and methanol (ME), to yield dry extracts. The plant aerial parts were screened for the presence of key families of phytochemicals according to the standardized methods (Dohou *et al.*, 2003; Rizk, 1982; Razafindrambao, 1973 and Bouquet, 1972). The antibacterial activity of the obtained dried plant extracts was evaluated against selected pathogenic bacteria, using the well agar diffusion method.
The phytochemical screening of the plant aerial parts highlights a variety of secondary metabolites mainly represented by flavonoïds, saponins and alcaloïds. The ME extract, on the one hand, showed against the tested pathogenic bacteria, the best antibacterial effect which was either similar or better than that of some antibiotics-controls and, on the other hand, it exhibited the highest yield which was nearly 2-fold higher than that of PE, DM and EA extracts all together. The good antibacterial effect of PE extract was relativized at view of its extraction lower-yield.

The *Pituranthos scoparius* ME extract may be suggested as a new potential source of natural antibacterial, justifying the Algerian folk medicine use of this endemic species. Further investigations are necessary in order to refine its antibacterial potential and to determine its phytochemical composition.

Keywords: *Pituranthos scoparius*, percolation, extracts, antibacterial activity, phytochemical screening.

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POSTER PRESENTATION

STUDY OF THE VARIETAL BEHAVIOR OF 6 BASIL VARIETIES (*OCIMUM BASILICUM* L) GROWN FOR THE FIRST TIME IN ALGERIA UNDER ARID CLIMATE

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Objective / Purpose: In Algeria the production of aromatic and medicinal plants is very small and difficult to evaluate and almost all of our needs is imported. The objective of our trial is to study the behavior of six (06) Varieties of basil (*Ocimum basilicum* L.) (O.B.) in the edapho-climatic conditions of the Ouargla region (southeast Algeria).

Material and Methods: Our experimentation took place at the station of the Technical Institute for Development of Saharan Agriculture (I.T.D.A. S) of Hassi Ben Abdallah, which is at an altitude of 157 m, latitude of 32 °. 52 ' north and longitude of 5 °. 26 ' East. The plant material consist 6 varieties of basil: *Ocimum basilicum* V1, *O. B. purperescens* V2, *O. B. Minimum* V3, *O. B. Marceillais* V4, *O. B. cinnamon* V5 and *O. B. citriodora* V6. The experimental device adopted [1] is in complete random blocks, consisting of 6 treatments and 4 repetitions. Each block contains 6 elementary plots of 2 m in length on 1.5 wide. To achieve the plotted objectives we studied a number of growth parameters (stem height, branch width and leaf area), flowering (flowering date), yield (number of leaves per plant, total biomass,...), The cycle time and the mortality rate.

Results: The mortality rate is very low and sometimes nil in some varieties, purple (V2), Dwarf Compact (V3) and cinnamon (V5) showed a lower mortality rate of 3.12% against the highest mortality rate is marked by OM (V4) with a E average of 40.62% this can be explained by the fact that Marseille basil (V4) is less suited to the climatic conditions of arid zones.

Conclusion / Discussion: The statistical study of our agronomic results gave us significant values to highly significant, which confirms the success of the culture of the six varieties of basil of European origin under arid climate.

Keywords: Varietal behavior, Ocimum basilicum L, growth parameters, yield parameters, arid climate.

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POSTER PRESENTATION

NEUROPROTECTIVE EFFECT OF *HYPERICUM THYMOPSIS* AGAINST CHRONIC EXPOSURE TO ALUMINUM CHLORIDE AND ALZHEIMER'S DISEASE

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The effect of Aluminum chloride was investigated to describe the associated behavioral and brain modifications. We don't know enough about the biological chemistry of chronic and sub- acute exposure to Aluminium to be able to predict its impact on human health. Although the hypothesis of a link between Aluminium and Alzheimer's disease (AD) has been supported by several epidemiological studies. Extract of Hypericum thymopsis(HTE), a well known medicinal plant, is used for the treatment of depression, has been explored in the present study for its protective role against Aluminum neurotoxicity. This data suggests that HTE may be a candidate for application in neurodegenerative diseases such as Alzheimer's disease. Results of this study demonstrate that Aluminiumneurotoxicity play an important role in the development of anxiety disorders, depression and memory deficit in mice, these alterations of behavioral activities can be a cause of development of Alzheimer's disease. HTE possesses significant antioxidant activity and renders neuroprotection which was more pronounced at the dose of 200mg/ kg against Al induced neurotoxicity. Chronic administration of Hypericum thymopsis significantly improved retention in both tasks, attenuated oxidative damage, Aluminum concentration in Aluminum treated mice (p 0,05). Hypericum have neuropotective effects against Aluminum-induced cognitive dysfunction and oxidative damage.

Keywords: Aluminum, Alzheimer's Disease, Hypericum Thymopsis Extract (HTE) Neuroprotection, Mice

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POSTER PRESENTATION

IN VITRO ENZYME INHIBITORY ACTIVITY OF THREE *FERULAGO* SPECIES GROWING IN TURKEY

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Ferulago species are named as "Çakşır" or "Çağşır" in Turkey and represented with 35 taxa (18 of them are endemic). In this study, hyaluronidase, collagenase and elastase inhibitory potential relevant to wound healing activities of the ethanol extracts of the roots of three *Ferulago* species were investigated.

Material and Methods: 80% ethanol extracts were prepared from the roots of 3 *Ferulago* species [*F. cassia* Boiss., *F. humilis* Boiss,, *F. mascrosciadia* Boiss. & Balansa,] and their *in vitro* hyaluronidase, collagenase and elastase inhibitory activities at 100 μg/ml concentration were evaluated [1-5].
Results: Ethanol extract of *F. mascrosciadia* was the most active one against collagenase and elastase with 34.83% and 41.20% inhibition values, respectively. None of the studied extracts exerted significant inhibitiory activity on hyaluronidase.

Conclusion: To the best of our knowledge, we herein report wound healing activity of three *Ferulago* species for the first time. According to the data we obtained, the most effective species in respect to collagenase and elastase inhibition tests was found to be *F. mascrosciadia*.

Keywords: Apiaceae, Collagenase, Elastase, *Ferulago*, Hyaluronidase

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POSTER PRESENTATION

COMPARATIVE ANALYSIS OF RARE AND ENDANGERED PLANTS OF HIRKAN DENDROFLORA

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In the research work has been comparatively analyzed the Hirkan dendroflora and have been collected herbariums of rare and endangered trees and shrubs. There are identified 26 families of 40 genuses 60 species of rare and endangered trees and shrubs naturally spreading areas, distribution of individuals in populations, have been performed dendrochronical analyzes on old samples.

As a result of the observations have been determined, that in the Hirkan flora the majority of species in the areas have been diminished and decreased compared to previous years, many species are in danger of being disappeared.

From this point of view by the monitorings of the area were studied threat criterias, the reasons of decline in plant varieties, those plants have been identified based on version of IUCN 3.1.
In the research work have been processed out the protection measures, the biological features of species, the causes of change in natural resources.

Keywords: Hirkan National Park, 3rd relict, endem plants, forest, flora

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MESMAP-5 Final Proceedings Book

POSTER PRESENTATION

ROS SCAVENGING AND REDUCING CAPACITY OF AROMATIC HERBS

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Objective / Purpose: The regular use of many aromatic herbs has been reported for prevention and treatment of numerous chronic conditions[1,2]. Despite their widespread utilization, their antioxidant properties have not been studied in detail yet. Moreover, their regular use in prevention from diseases associated with excess of free reactive oxygen species (ROS) lacks scientific background. The purpose of the current study is to examine the antioxidant potency of frequently used aromatic herbs in diet and traditional medicine: tuber of *Allium ascalonicum* L. (AAR), *Allium cepa* L. (ACR), *Allium sativum* L. (ASR) and *Orchis mascula* (L.) L. (OMT), herba of *Melissa officinalis* L. (MOH), seeds of *Nigella sativa* L. (NSS) and *Trigonella foenum-graecum* L. (TFGS).

Material and Methods: Ethanol extracts were evaluated for their antioxidant activity by the following *in vitro* assays: DPPH (2,2'-diphenyl-1-pycrylhydrazyl), FRAP (ferric reducing antioxidant power), NSSOH (non-site-specific-) and SSOH (site-specific hydroxyl radical-2-deoxy-d-ribose degradation) assays. Additionally, contents of total polyphenols and flavonoids were measured by Folin-Ciocalteu and $AlCl_3$ method.

Results: Obtained results showed prominent antioxidant activity *in vitro* and significant amount of polyphenols in the extracts. MOH was identified as extract with highest radical scavenging (IC_{50} 1.54 mg/mL) and ferric reducing ability (28.3%). The ferric reducing power of extracts is decreasing as follows: MOH > ASR > NSS > TFGS > OMT > AAR > ACR. Additionally, MOH and ASR also manifested metal-chelating ability. Moreover, high correlation was established between total polyphenols and the antioxidant assays showing the evident role of polyphenols in the antioxidant defense.

Conclusion / Discussion: Overall, the data obtained for ethanol extracts of *Melissa officinalis* and *Allium sativum* in the current study demonstrate they could be considered as potential source of highly bioactive compounds against oxidative stress in biological systems. Additionally, they could provide protective role from chronic diseases associated with excessive amount of ROS.

Keywords: antioxidant activity, plant extracts, traditional medicine, aromatic herbs

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POSTER PRESENTATION

ANTIOXIDANT ACTIVITY OF FOUR NITROGEN ORGANIC COMPOUNDS: CORRELATION BETWEEN THEORETICAL AND EXPERIMENTAL DATA

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Objective / Purpose: The antioxidant compounds searching, continues to attract scientists, pharmaceutical and industrial actors. In the present study, we evaluated both theoretically and experimentally, the antioxidant activity of a set of four nitrogen organic compounds, one semicarbazone, one oxime and two phenylhydrazones, noted SEM, OXN, SIP and NIP, respectively.

Material and Methods: First, our four compounds were synthesized and characterized. Then, DPPH, CUPRAC and FRAP tests were used to evaluate their antioxidant activity. Furthermore, a DFT study (geometry optimization and frequency calculations) was carried out on the four molecules permitting the calculation of some molecular descriptors (BDE, IP and PA). All computations were performed using Gaussian 09 software.

Results: The tests of antioxidant activity were very fulfilling showing a well-established antioxidant power for our compounds. The SIP compound exhibits the highest DPPH scavenging activity which was even greater than that of the used standard antioxidants butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT), with an IC_{50} of 3.81□0.26 against 6.14□0.41 and 12.99□0.41 µg/ml, respectively. On the other hand, the resulting BDE values are in a good agreement with the experimental results.

Conclusion / Discussion: The obtained results show an excellent correlation between the theoretical and the experimental data, which may allow these compounds to be used either in pharmaceutical or food industries. Farther tests are recommended in order to reinforce these findings.

Keywords: Antioxidant activity, DFT, molecular descriptors, nitrogen organic compounds.

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POSTER PRESENTATION

STUDY OF THE HYPGLYCEMIC ACTIVITY OF THE COMMON FIGS OF THE REGION OF AIN DEFLA ALGERIA

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Objective / Purpose: The fig tree is mentioned in the Quran in surah "At-Tine", which is the only one to have the title of a fruit or even a plant. Our work contributes to the study of the hypoglycemic activity of the common fig tree of the region of El Amra (Ain Defla) Algeria.

Material and Methods: To do this, 20 Wistar rats female, divided into 4 lots, were used:
Lot 1: 5 normal controls; Lot 2: 5 normal controls treated with dry fig juice at 20%; Lot 3: 5 diabetic controls; Lot 4: 5 diabetic controls treated with dry fig juice at 20%. The rats are rendered diabetic by intra-peritoneal injection of a streptozotocin solution. The blood glucose is measured after 1 hour, 2 hours, 3 hours and after 4 hours of the administration of the fig juice; it's measured also on the 5th day, 8th day and 9th day of the beginning of the experiment.

Results and discussions: On the 9th day, we recorded a very significant decrease of the blood sugar level of diabetic rats treated with dry fig juice. This blood glucose level normalized for 3 rats / 5rats. In the short term (for 4 hours), an increase of blood sugar level, one hour after administration, for normal and diabetic rats. This increase is probably due to the high level of sugar content in the preparation. The blood glucose level is then corrected, four hours later. This may be the result of anti hyperglycemic effect of the active ingredients contained in the figs.

Conclusion/Discussion: The figs have despite their high sugar content a significant hypoglycemic activity. Whose mechanism of action still is unknown. The extract aqueous of the figs may imitate the action of insulin, or stimulate the synthesis of insulin.

Keywords: Figs, hypoglycemic activity

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POSTER PRESENTATION

QUALITATIVE AND QUANTITATIVE DETERMINATION OF MONO- AND DICAFFEOYLQUINIC ACIDS IN *INULA* SPECIES FROM BULGARIA

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Objective: Chlorogenic acid (CQA) and dicaffeoyl esters of quinic acid (DCQA) are widely distributed in Asteraceae family. These compounds are known to possess various biological activities - antioxidant, antiviral, antibacterial, anti-inflammatory activities and can reduce the relative risk of cardiovascular, diabetes type II and Alzheimer's diseases. The aim of this study was to compare HPTLC and HPLC methods for qualitative and quantitative analysis of CQA and four isomeric DCQA in the methanol extracts of six *Inula* species, growing in Bulgaria - *I. aschersoniana, I. conyza, I. ensifolia, I. oculus-christi, I. bifrons* and *I. germanica*.

Material and Methods: The aerial parts of the six *Inula* species were collected in full flowering stage from natural habitats in Bulgaria in 2016. Air-dried and powdered plant material was extracted with CH_3OH and the resulting extracts were further used for HPTLC and HPLC analyses. HPTLC was performed on HPTLC plates (Merck) on CAMAG system with toluene/ethyl acetate/formic acid/water (5:25:100:10:10, v/v/v/v) as mobile phase. Densitometric determination was performed at 325 nm. A gradient RP HPLC method was carried out at 325 nm.

Results: A preliminary HPTLC comparison of the methanol extracts with commercial standards showed presence of chlorogenic (CQA), 4,5-, 3,4-,1,5- and 3,5-dicaffeoylquinic acids (DCQA). On the HPTLC plate, phenolic acids were observed under 366 nm UV light as blue bands at R_f values of 0.26, 0.49, 0.50, 0.63 and 0.66, respectively,. Good separation was achieved for the main compounds CQA, 1,5- and 3,5- DCQA, which were further densitometrically determinated at 325 nm. Additionally, HPLC method was applied for quantitative determination of CQA and DCQA isomers. The amount of CQA varied from 14.27 to 29.94 mg/g DE and the highest amount was detected in the methanol extract of *I. ensifolia*. 1,5- and 3,5-DCQA were the most abundant dicaffeoyl esters of quinic acid, but differed in their quantity in different species (20.32 - 65.0 and 1.91 - 22.92 mg/g DE, respectively). The content of 4,5- and 3,4-DCQA was relatively low (3.38-9.88 and 0.30-5.11 mg/g DE, respectively). The methanol extract of *I. germanica* was characterized with high amount of 1,5-DCQA. Similarly, *I. conyza* methanol extract was also rich in 1,5-DCQA, but contained subsequent amounts of 3,5-DCQA. Methanol extracts of *I. ensifolia*, *I. aschersoniana, I. oculus-christi* and *I. bifrons* contained almost equal amounts of 1,5-DCQA and 3,5-DCQA. There was no statistically significant difference between the mean values for CQA and 1,5- and 3,5-DCQA in studied extracts measured by HPTLC and HPLC.

Conclusion: HPTLC was found to be effective for qualitative analysis, while HPLC was more accurate for quantitative analysis. A combination of the two methods may be useful in a quality control setting, as it would allow rapid qualitative analysis of herbal material while maintaining accurate quantification of extract composition.

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Key words: *Inula*, Asteraceae, phenolic acids, HPTLC, HPLC-PDA

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POSTER PRESENTATION

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF THE *n*-BUTANOL EXTRACT OF *MENTHA ROTUNDIFOLIA* FROM ALGERIA

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Objective / Purpose: The genus *Mentha* (mint) (Lamiaceae family) is represented by 25 to 30 species grown in different parts of the world. Fifteen *Mentha* species are distributed in Algeria [1]. *Mentha* has been applied in the traditional medicine as stimulant, carminative, antispasmodic, antitussive and diuretic. It has been used as antiseptic, antimicrobial and antioxidant agents [2]. Phenolics, flavonoids and terpenoids [3] have been identified from *Mentha* species. As part of our interest in Lamiaceae species [4-6], the objective of the present study was to evaluate total phenolic content and the antioxidant activity, of the *n*-butanol extract of *Mentha rotundifolia* L.

Materials and Methods: *Mentha rotundifolia* was collected at Hammam knif (North eastern Algeria). The powdered aerial part of the plant was extracted by a mixture of ethanol/water 80/20, v/v. The *n*-butanol extract was obtained by liquid-liquid extraction. Total phenolic content was determined with Folin–Ciocalteu reagent. Three antioxidant tests were carried out with DPPH Free Radical Scavenging assay, β-carotene/linoleic acid test and ABTS cation radical decolorization assay.

Results: The *n*-butanol extract of *Mentha rotundifolia* has a high content of polyphenols (356.75 ± 2.58 mg EAG./g). The greatest activity was obtained with the bleaching of β-carotene test (IC_{50}: 1.75±0.37 µg/mL). An excellent potential with the DPPH free radical scavenging test is also noted (IC_{50}: 9.33±1.42 µg/mL). This extract also possesses a good antiradical activity towards the $ABTS^{+\cdot}$ radical (IC_{50}: 9.12±0.74 µg/mL).

Conclusion/Discussion: The *n*-butanol extract of *Mentha rotundifolia* L. possesses a great antioxidant potential with the three tests carried out (DPPH Free Radical Scavenging, β-Carotene/Linoleic Acid Bleaching and ABTS cation radical decolorization). The high content of polyphenols, in this extract, certainly contributes to this great antioxidant power.

Keywords: *Mentha rotundifolia*, antioxidant activity, DPPH, CUPRAC, $ABTS^+$.

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POSTER PRESENTATION

RADICAL SCAVENGING AND ANTI-BACTERIAL ACTIVITIES OF METHANOLIC EXTRACT FROM ALGERIAN FIR (*ABIES NUMIDICA*) LEAVES

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Objective / Purpose: Algerian forests are rich in plants due to its geographic position. It represents a high biodiversity that seems in different found species that are endemic in major, for example Algerian fir (*Abies numidica*) tree. It is a conifer belongs to *Pinaceae* familly, used in folk medicine to treat cataplasm and respiratory system diseases. Nowadays, there is a huge interest to find new molecules from natural resources to prevent human diseases. For that, the objective of the present research is to investigate anti-oxidant and anti-bacterial activities of Algerian fir leaves crude extract.

Material and Methods: The air dried powdred leaves were extracted with methanol solvent using Soxhlet apparatus. The extract was evaporated under reduced pressure by rotary vacum-evaporator type Buchi R-215 at 40°C. DPPH and ABTS assays were used to evaluate the anti-oxidant activity. The Anti-bacterial activity was also tested using the disc diffusion method against five bacterial strains; *Escherichia coli, Pseudomonas aeruginosa, Morganella morgani, Staphylococcus aureus* and *Bacillus subtillus.*

Results: The results showed that methanolic extract of Algerian fir leaves by DPPH method can reduce radicals (IC_{50}=18.08±0.21) lower than ABTS (IC_{50}=23.51±0.54). The mathanolic extract exhibited no anti-bacterial activity against Gram negative bacteria; *Escherichia coli, Pseudomonas aeruginosa as well as Morganella morganii*. However, it represented a moderate activity against Gram positive; *Staphylococcus aureus* and *Bacillus subtillus.*

Conclusion / Discussion: *Abies numidica* is a threatened species in Algeria and according to the results obtained, it has a beneficial effect. So it is very important to protect it and exploit it in pharmaceutical mains and use it in food industry.

Keywords: methanolic extract, Algerian fir, radical scavenging, anti-bacterial activity.

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POSTER PRESENTATION

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF THE ESSENTIAL OILS FROM TWO CHEMOTYPES OF *CYMBOPOGON SCHOENANTHUS* (L.) SPRENG. GROWING IN SOUTHERN ALGERIA.

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The chemical composition of the essential oils of two populations of *Cymbopogon schoenanthus* collected in Ghardaïa and Illizi (Southern Algeria), were identified by (GC-MS), this study allowed us to identify the presence of two chemotypes of *C. schoenanthus* essential oil, the first one was characterized by the high content of piperitone, and the second group was characterized by other compounds. The screening of antimicrobial activity of oils was individually evaluated against representatives of gram-positive, gram-negative bacteria and fungi, using the agar diffusion method, whereas, the antioxidant activity of the essential oils was evaluated using the DPPH, ABTS and FRAP assay. The results of in vitro testing of the antimicrobial activities of *C. schoenanthus* essential oil of Ghardaïa region proved the existence of strong activity against all the tested strains, specially the growth of *E. faecium , S. aureus and S. agalactiae*. However , the same species growing in Illizi region shown less power in the inhibition of microorganisms. The essential oil obtained from *C. schoenanthus* growing in Illizi is markedly rich in non-phenolic constituents, due to this fact the essential oil is relatively weak antioxidant, which the same remark in the essential oil extracted from Ghardaïa.

Key words: *C. schoenanthus*, essential oil, chemotype, antimicrobial activity, antioxidant activity.

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POSTER PRESENTATION

EVALUATION OF THE ANTIOXIDANT POTENTIAL, PHENOLIC AND FLAVONOID CONTENTS OF THE METHANOL EXTRACT OF *AMMOIDES ATLANTICA*

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Objective / Purpose: The genus *Ammoides* (Apiaceae), tribe of Ammineae, includes two species in Algeria, from which the endemic species *A. atlantica* (Coss. Et Dur.) Wolf [1]. In Algerian traditional medicine, the aerial parts of *A. atlantica* are used in infusions for the treatment of headache, fever and diarrhea. It is also used in compresses, alone or soaked in alcohol or vinegar and mixed with henna, to treat children affected by mental debility [2].

Materials and Methods: The plant was collected during the flowering period in the North Eastern region of Algeria (Jijel) in May 2016. The aerial parts of *Ammoides* wee extracted with methanol-water (80:20, v/v). *In-vitro* antioxidant activity was evaluated for the methanol extract using different antioxidant tests, namely, total antioxidant (DPPH scavenging, ($ABTS^{·+}$) scavenging, superoxide radical scavenging by alkaline DMSO, reducing power, cupric reducing antioxidant capacity (CUPRAC) and ferrous ions chelating assays compared to the synthetic antioxidants (BHA, BHT and ascorbic acid).

Results: The methanol extract of *Ammoides atlantica* exhibited a high antioxidant activity in DPPH essay (IC_{50}: 23.31 ± 0.99 µg/mL), $ABTS^{+}$ (IC_{50}: 11.31 ± 2.49 µg/mL) ,O_2^{-} DMSO alkaline assay (IC_{50}: 3.19±0.17 µg/mL), Reducing power assay ($A_{0.50}$: 92.70±1.37 µg/mL), CUPRAC assay ($A_{0.50}$: 13.56±1.06 µg/mL) and Ferrous ions chelating assay (IC_{50}:102.35±2.91 µg/mL), respectively compared with BHA, BHT and ascorbic acid which were used as positive controls.Total Phenolic content (371.57±7.80 mg/g) and Total Flavonoid content 41.02±6.16 mg/g were also interesting

Conclusion / Discussion: From the results, *Ammoides atlantica* can be used as a source of antioxidants

Keywords: Apiaceae, *Ammoides atlantica*, antioxidant activity, DPPH, CUPRAC, $ABTS^{+}$.

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POSTER PRESENTATION

EXTRACTION OF NATURAL PRODUCT FROM PLANT BY USING A MICROWAVE

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Objective/Purpose: Plants have unique range of fine chemicals known as secondary metabolites. Shikimic acid is found widely in plants but generally occurs in low concentration. It is a hydro aromatic intermediate of common pathway of aromatic amino acid biosynthesis. This work basically discusses the extraction of natural product that is shikimic acid from plant material by using a microwave. Shikimic acid has several benefits including its use in treating fungal infection and act as antioxidant, antibacterial, antiviral medication.

Material and Methods: Plant materials like seeds of Chinese star anise (*Illicium verum*) can be a source of shikimic acid. Shikimic acid extracted for 10, 20, 30, 40, 50 and 60 seconds by using micro oven. It is cheapest and simplest method and less time consuming.

Results: Most plants contain water in more amounts so this is effective means of extraction. Plants with low water content take longer to heat in microwave. The temperature of the plant material heated in a microwave cannot exceed the boiling point of water (100°C). Hence, the shikimic acid is in stable from.

Conclusion: Microwave can be used to isolates flu drug raw material from star anise while minimising pigment contamination. Shikimic acid can be extracted easily, and optimization of base material for production of ***oseltamivir (Tamiflu)***, which is an effective drug against the H5N1 influenza virus. Shikimate can also be used to synthesize **(*6S)-6 Fluoroshikimic acid***, *an* antibiotic which inhibits the aromatic biosynthetic pathway.

Key words: Shikimic acid, star anise, microwave.

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POSTER PRESENTATION

URSOLIC ACID AS A BIOCHEMICAL MARKER IN FAMILY *MYRTACEAE*

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Objective /Purpose:-Over the decades, biochemistry has become remarkably important to resolve the dispute of different taxon in plants. Different chemical markers can be used to differentiate plant families, genus and species, Ursolic acid a potentially important molecule from the family Myrtaceae can be used for the better understanding of different taxon. Apart from taxonomic possibilities, it also shows many pharmacological effects such as Reducing oxidative stress, anti-Inflammation, Anti-oncogenic, Inducing Apoptosis and hepato-protective nature.

Material and method: A detailed study on secondary metabolites was observed by screening different plants from *Myrtaceae*. HPTLC method was performed for the qualitative and quantitative analysis of different compounds mainly ursolic acid.

Results: HPTLC analysis resulted in presence of many secondary compounds in association with ursolic acid. Using pairing affinity for different molecules dendrogram was prepared to understand a phylogenetic relation between species of the family *Myrtaceae*.

Conclusion: Ursolic acid was found to be present in family *Myrtaceae* as a biochemical Marker.

Key words: Ursolic acid, *Myrtaceae*, Biochemical compound, HPTLC.

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POSTER PRESENTATION

DETERMINATION OF ESSENTIAL OIL COMPOSITION, PHENOLIC AND FLAVANOID CONTENTS OF *PHLOMIS LONGIFOLIA* VAR. LONGIFOLIA COLLECTED FROM HATAY DISTRICT OF TURKEY

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Objective / Purpose: *Phlomis* is a large genus in the *Lamiaceae* family, with over 100 species distributed throughout Euro-Asia and North Africa countries. Their flowered parts are generally used as an herbal tea to treat gastrointestinal troubles and to promote good health by protecting the liver, kidney, bone and cardiovascular system. Phytochemical investigations of *Phlomis* species were the subject of several studies, and, consequently, essential oils, flavonoids, iridoids, phenylethylalcohol glycosides and other components were isolated.

Material and Methods: *Phlomis longifolia* var. longifolia was collected from Hatay district of Turkey. Its essential oil was obtained by using Clevenger apparatus. Essential oil composition was determined by using GC-MS/FID device. Total phenolic and flavanoid contents were determined by using spectrophotometer device.

Results: Essential oil rate was determined as 0,33%. The main essential oil components were determined as germacrene (34,27%), *β*-caryophyllene (20,97%), *trans*- *β*-farnesene (7,76%), *trans*-cadine-1,4-diene (6,79%) and *α*-copaene (5,61%). Total phenolic content was determined as 11,87 mg GAE/g plant. Total flavanoid content was determined as 10,26 mg CE/g plant.

Conclusion / Discussion: *Phlomis longifolia* essential oil can be used for cosmetic industry because of germacrene which is a good-scent main component.

Keywords: *Phlomis*, essential oil composition, total phenolic and flavanoid content

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POSTER PRESENTATION

EFFECTS OF MULBERRY (*MORUS ALBA* L.) LEAF EXTRACT (Immunflex®) ON MMP-9, BMP-2 AND COMP IN HUMAN OSTEOARTHRITIC CHONDROCYTES *IN VITRO*

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Objective: Osteoarthritis (OA) is a common disease of older individuals that is characterized by loss of articular cartilage. In OA, an inflammatory process occurs and cytokines and enzymes develop that further damage the cartilage. Matrix metalloproteinases (MMP-9, MMP-13 etc) are proteolytic enzymes, responsible for destruction of collagen and matrix components.[1] COMP, is structural protein of extracellular matrix, is expressed in higer level in OA patients.[2] Bone morphogenetic protein-2 (BMP-2) signaling is involved in maintaining the normal function of synovial joints.[3] The redox modulatory regimens, anti-inflammatory and anti-oxidant rich diet, can help to improve OA. The mulberry plant contain a variety of phenolic compounds, steroids and triterpenes. *Morus alba* leaf extracts has been shown to have antiinflammatory and antioxidant effects in a different cell type.[4] Based on these findings, we aim to investigate the potential anti-osteoartritic and chondroprotective effects of two different *Morus alba* leaf extracts in human OA chondrocytes.

Methods: Primary chondrocytes were isolated from cartilage tissue of OA patients (grade 4). Primary chondrocytes were treated with different concentration of (1) aqueous and (2) ethanolic extract of *Morus alba* leaf extracts as Immunflex®. Cell viability (MTT assay, neutral red) and *ROS levels (DCFH-DA assay) were tested.* MMP-9, COMP and BMP-2 protein expression levels were examined by Western blot.

Results: We found that **both** ethanolic and aquous extracts had no cytotoxicity at lower concentrations. When compared to untreated OA chondrocytes, ethanolic extract treated chondrocytes showed a reduced ROS levels significantly ($p<0.01$). Ethanolic extract also have a potential modulating effects on MMP-9, COMP and BMP-2.

Conclusion: The results provide evidence for the potential use of Immunflex® as an anti-osteoartritic complex against OA pathology.

Keywords: *Morus alba*, mulberry leaf, osteoarthritis, primary chondrocytes.

Acknowledgements: This study was financially supported by the Scientific and Technological Research Council of Turkey (Grant No: 315S012)

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POSTER PRESENTATION

ASTRAGALOSIDE IV AND FORSYTHOSIDE B MODULATE HYPERGLYCEMIA-MEDIATED NEUROTOXICITY IN PRIMARY HIPPOCAMPAL NEURONAL CELLS

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Background: Recent studies have revealed that cognitive deficits and hippocampal abnormalities are the complications of Diabetes Mellitus (DM) that is characterized by chronic hyperglycemia, hyperlipidemia, insulin resistance, oxidative stress and other alterations [1]. The well known mechanisms for the progression of hippocampal neuronal loss in DM, includes oxidative damage and mitochondrial dysfunction [2]. Unfortunately, current therapeutic strategies are not sufficient enough to overcome hyperglycemia induced neurotoxicity. So, we aimed to explore the effects of Astragaloside IV (AS-IV) and Forsythoside B (FST-B) on high glucose induced neurodegeneration in hippocampal neurons.

Methods: Primary hippocampal neurons were isolated from Wistar albino pups (E17-19). Cells were exposed high glucose (150 mM) for 16 h to induce glucotoxicity. For the neuroprotection experiments cells were pre-treated with various concentrations (1 nM-1 μM) of AS-IV or FST-B for 8 h, then post-treated with high glucose (150 mM) for 16 h. Cell viability was assessed both MTT and NRU assay. Moreover cytotoxicity and intracelullar ROS were monitored using Trypan blue exclusion assay and fluorescent probe DCFHDA, respectively.

Results: Our results indicated that 150 mM glucose exposure caused significantly decreased cell viability ($p<0.01$) along with a significant increased toxicity ($p<0.05$) and high ROS generation levels ($p<0.01$). On the other hand, pre-treatment with AS-IV and FST-B ameliorated the glucose induced neurotoxicity (>50 nM) in terms of increased viability (1 nM-500 nM), improved membrane integrity and lowered the ROS generation capacity (≥50 nM).

Conclusion: We demonstrated that AS-IV and FST-B showed neuroprotective effects against hyperglycemia mediated neurotoxicity via inhibiting neuronal loss, strengthen membran integrity and impairing oxidative damage.

Keywords: Hyperglycemia, hippocampal neurons, Astragaloside IV, Forsythoside B, neuroprotection.

Acknowledgements: This study was financially supported by the Scientific and Technological Research Council of Turkey (Grant No: 315S088 and 215S197)

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POSTER PRESENTATION

THE ESSENTIAL OIL YIELD AND COMPOSITIONS OF LEMON VERBANA *(LIPPIA CITRODARA* KUNTH.*)* CULTIVATED IN ORDU

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Objective / Purpose: In the study, essential oil yield and its components leaves obtained from lemon verbana *(Lippia citrodara* Kunth.*)* cultivated in Ordu were investigated.

Material and Methods: The air-dried leaves of lemon verbana were subjected to hydrodistillation for 3h using a Clevenger-type apparatus to produce essential oil. The GC-MS analysis was carried out with Agilent 7890 GC-MS system. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and NIST mass spectral library.

Results: The essential oil yield of lemon verbana was determined as 1.45%. The most important essential oil component of Geranial was 20.8 %. The major essential oil components of lemon verbana were determined as Limonen (18.1 %), Neral (13.8 %), respectively.

Conclusion / Discussion: According to the results of this study, it was determined that essential oil yield and components of lemon verbana *(Lippia citrodara* Kunth.*)* were determined. In addition to the use of essential oil, leaves of the plant are used as herbal tea. Lemon verbana is used appetizing, relaxing the stomach, colds, cough and digestion problems in folk medicine.

Keywords: Lemon verbana, *Lippia citrodara* Kunth., essential oil, composition

POSTER PRESENTATION

THE ESSENTIAL OIL YIELD AND COMPOSITIONS OF LAURIER *(LAURUS NOBILIS* L.) CULTIVATED IN SAMSUN

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Objective / Purpose: In the study, essential oil yield and its components obtained from leaf of laurier *(Laurus nobilis L.),* cultivated in Samsun ecological conditions were investigated.

Material and Methods: The air-dried leaves of laurier were subjected to hydrodistillation for 3h using a Clevenger-type apparatus to produce essential oil. The GC-MS analysis was carried out with Agilent 7890 GC-MS system. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and NIST mass spectral library.

Results: Essential oil yield was determined as 0.4 % in dried leaves. The major essential oil component of 1,8-cineol was 45,7 %. The other major essential oil components of laurier leaf were determined as α-Terpinyl acetate (15.6 %), sabinen (8.4 %) , α -pinene (4.8 %) and β-pinene (4.1 %), respectively.

Conclusion / Discussion: According to the results of this study, it was determined that essential oil yield and components of laurier *(Laurus nobilis L.)*leaf were determined. Essential oil yield and compositions of laurier leaf were one of the significant quality factors. Laurier leaf are used to antibacterial, diaphoretic, pain relieving, discomfort, antiseptic and stomach discomfort,diabetic, migraine, weakness in folk medicine. At the same time, Laurier is used as spices in the worldwide.

Keywords: Laurier, *Laurus nobilis* L., essential oil, composition, 1,8-cineole

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POSTER PRESENTATION

A STUDY ON ESSENTIAL OIL YIELD and COMPONENTS OF LEMON BALM *(MELISSA OFFICINALIS* L.*)* CULTIVATED DIFFERENT PROVINCES IN TURKEY

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Objective / Purpose: In the study, essential oil yield and its components obtained from Lemon balm (*Melissa officinalis*) cultivated different provinces (in Konya and Elazığ) were investigated.

Material and Methods: Lemon balm belonging to the family Lamiaceae were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce essential oil. The GC-MS analysis was carried out with Agilent 7890 GC-MS system. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and NIST mass spectral library.

Results: The essential oil yield of lemon balm was 0,34 % in Konya province, while It was 0.30 % in Elazığ province. The major essential oil components of lemon balm were determined as Geranial. The amount of gerenial in Konya province and in Elazığ province were 33.4% and 46.0%, respectively.
Conclusion / Discussion: The aim of this research attempts to contribute to knowledge of differences essential oil yield and components of lemon balm produced in different provinces of our country were determined. In addition to used as essential oil, leaves of the plant are used as herbal tea. Lemon balm is used sedative, gastric, antitussive, diaphoretic and antiseptic effects in folk medicine.

Keywords: Lemon balm, *Melissa officinalis* L., essential oil, component

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POSTER PRESENTATION

THE ESSENTIAL OIL YIELD and COMPOSITIONS of *LAVANDULA ANGUSTIFOLIA* MILLER and *LAVANDULA INTERMEDIA* EMERIC EX LOISEL. CULTIVATED IN BAYBURT ECOLOGICAL CONDITIONS

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Objective / Purpose: In this study, ıt was investigated essential oil yield and compositions of *Lavandula angustifolia* Miller and *Lavandula intermedia* Emeric ex Loisel., belong to Lamiacea, cultivated in Bayburt ecological conditions, Turkey.

Material and Methods: The air-dried flowers of lavender species were subjected to hydrodistillation for 3h using a Clevenger-type apparatus to produce essential oil. The GC-MS analysis was carried out with Agilent 7890 GC-MS system. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and NIST mass spectral library.

Results: Essential oil yield of lavender species was determined as 1.5 % in *Lavandula angustifolia* Miller and 6.5 % in *Lavandula intermedia* Emeric ex Loisel. The most important essential oil component of *Lavandula angustifolia* Miller were linalol (42.8 %) linalyl acetate (13.9 %) and borneol (2.6 %) and *Lavandula intermedia* were determined as linalol (45.4 %), linalyl acetate (22.2%) and borneol (4.6 %). The amount of camphor of *Lavandula intermedia* Emeric ex Loisel. was determined 2.4%.

Conclusion / Discussion: According to the results of this study, it was determined that significant amount essential oil yield and components of the lavender species (Lavandula angustifolia Miller and *Lavandula intermedia* Emeric ex Loisel. were determined. Lavender varieties are promoted primarily in essential oil as an linalol and linalil asetat that give frangrance and also used antimicrobial, antioxidant and similar aims.

Keywords: Lavender, *Lavandula angustifolia* Miller, *Lavandula intermedia* Emeric ex Loisel., essential oil, composition.

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POSTER PRESENTATION

THE ESSENTIAL OIL YIELD and COMPOSITIONS of FENNEL (*Foeniculum vulgare* MILL.) CULTIVATED in KONYA, TURKEY

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Objective / Purpose: In this study, it was investigated essential oil yield and compositions of fennel (*Foeniculum vulgare* Mill.) cultivated in Medicinal and Aromatic Plants Farm of Selcuk University, in Konya, Turkey.

Material and Methods: The air-dried seeds of *Foeniculum vulgare* Mill. were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce essential oil. The GC-MS analysis was carried out with Agilent 7890 GC-MS system. The relative percentages of the separated compounds were calculated from total ion chromatograms. The identification of the oil components was based on the Wiley and NIST mass spectral library.

Results: The essential oil yield in fennel was determined as 2.16 % . The major essential oil components of *Foeniculum vulgare* Mill. were determined as (E)-anethole (83.7%) and limonene (5.5%).

Conclusion / Discussion: Fennel commonly is known as important medicinal and aromatic plant cultivated for its essential oil, spices herb, phytotherapy and aromatheraphy applications. According to the results of this study, the observed significant differences in the major components of fennel essential oil cultivated in Konya.

Keywords: Fennel, *Foeniculum vulgare* Mill., Essential oil, Composition

POSTER PRESENTATION

THE POLYPHENOLS EXTRACTED FROM THE *ARTEMISIA ABSINTHIUM L* PLANT COMBINE BRAIN OXIDATIVE STRESS, NEURO-INFLAMMATION AND NEURODEGENERATION INDUCED BY HEAVY METALS (ALUMINUM AND MERCURY).

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The objective of our study is the analysis of the polypholic extract of *Artemisia absinthium L* (stems and leaves) by HPLC and to evaluate the effect of its administration on the alterations of the cerebral structures (cortex, cerebellum hippocampus and striatum) in aluminum and mercury intoxicated rats [(30 mg AlCl3 / kg body weight), (5 mg HgCl2 / kg body weight)].

Thus, the analysis of extracts of *Artemisia absinthium L* allowed the identification of nine compounds of which: catechin which represents the dominant flavonoid (35,50%), followed by quercetin (31,37%), then the caffeic acid (14.49%), chlorogenic acid (10.18%), caticin (4.02%), vanillic acid (2.55%), hydroxycianamic acid (0.18%) , rutin (0.72%) and gallic acid (0.36%).

In the rat, the results obtained demonstrate that treatment with the polyphenol extract of *Artemisia absinthium L.* attenuates Al and Hg-induced alterations by decreasing lipid peroxidation and protein oxidation, increasing activity of antioxidant enzymes (SOD, CAT, GPx, GR, and trxR). In addition, the polyphenol extract of *Artemisia absinthium L.* inhibits the release of pro-inflammatory cytokines (IL1, IL6 and TNFα), nitric oxide (NO) and also prevents neuronal apoptosis by inhibition of activation of caspase-3 and the reduction of mDNA oxidation.

These results indicate that treatment with *Artemisia absinthium L.* extract may be part of an effective therapeutic strategy to reduce neuronal death and combat the oxidative stress, neuro-inflammation and neurodegeneration induced by certain heavy metals.

Key words: Polyphenols, Artemisia absinthium L, Neurodegeneration.

POSTER PRESENTATION

PEST SPECIES DETERMINED ON ARTICHOKE, *CYNARA SCOLYMUS* L. (ASTERACEAE) IN ADANA / TURKEY

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Objective: Artichoke, *Cynara scolymus* L. (Asteraceae) is one of the oldest medicinal plant worldwide. It is Mediterranean originated with high nutritional value, widely used in human nutrition and pharmaceutical industries. In recent years, a significant increase has been observed in the amount of production and cultivation area in Turkey. This study was carried out to determine the pest species on *C. scolymus* cultivated in Karaisalı and Sarıçam districts of Adana in 2016-2017.

Material and Methods: Pest monitoring was carried out weekly during spring-autumn and monthly during winter in the vegetation period. Adults were collected with different methods according to their families; via visual inspection, shaking branches into a standard sweeping net, sucking by a handheld aspirator. Immature stages were collected with their preys and cultivated in the laboratory in order to obtain adults (Lodos et al., 1978).

Results: As a result, 23 pest species belonging to 3 classes (Gastropoda, Insecta, Arachnida), 8 orders and 16 families were determined. Among the pest species determined, damage of *Tetranychus urticae* Koch (Acari: Tetranychidae), *Brachycaudus (Acaudus) cardui* (Linnaeus) (Hemiptera: Aphididae), *Cassida* sp. (Coleoptera: Chrysomelidae) on leaves, damage of *Larinus* sp. (Coleoptera: Curculionidae) on flowers was concluded to be important.

Conclusion: It was revealed that the pests identified have the potential to cause problems for artichoke production.

Key Words: Medicinal plant, artichoke, *Cynara scolymus*, pest

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POSTER PRESENTATION

INSECT PEST COMPLEX OF MINT SPECIES (*MENTHA* SPP.) (LAMIACEAE) IN ADANA / TURKEY

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Objective: *Mentha* spp. (Lamiaceae) have been economically cultivated in many countries due to its widely used as food and medicine. Mint production is facing significant yield losses because of pest attacks including arthropods. This study was carried out to determine the pest species of mint species (*Mentha spicata*, *M. piperita*, *M. arvensis*) in Karaisalı and Sarıçam districts of Adana in 2016-2017.

Material and Methods: Pest monitoring was carried out weekly during spring-autumn and monthly during winter in the vegetation period. Adults were collected with different methods according to their families; via visual inspection, shaking branches into a standard sweeping net, sucking by a handheld aspirator. Immature stages were collected with their preys and cultivated in the laboratory in order to obtain adults (Giray, 1982).

Results: Totally 24 pest species belonging to 3 classes, 7 orders and 12 families were determined. *Aphis affinis* deI Guercio (Hem.: Aphididae), *Exolygus pratensis* (L.) (Hem.: Miridae), *Eupteryx* sp. (Hem.: Cicadellidae) and *Chrysolina* sp. (Col.: Chrysomelidae) were determined as serious insect pests.

Conclusion: It was revealed that the pests identified have the potential to cause problems for mint production in Turkey.

Key Words: Medicinal plant, mint, *Aphis affinis*, *Exolygus pratensis*, pest

References:

Giray, H. (1982). Ege Bölgesi'nde nane (*Mentha*) türleri zararlı böceklerine ait liste ve önemlilerinin zarar şekilleri hakkında notlar. Türkiye Bitki Korma Dergisi, 6, 249-259.

POSTER PRESENTATION

PRELIMINARY INVESTIATIONS ON *CORIANDRUM SATIVUM* CULTIVATED IN NORTHERN CYPRUS

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Objective / Purpose: *Coriandrum sativum* L.(Apiaceae) is known as “Gollandro” and used in a large amount in Cyprus kitchen. Coriander fresh leaf and dried fruits are well known spice recommended for healthy life, also possessed lead-detoxifying potential besides several medicinal uses. The purpose of this research to identify of phenolic compounds and determine anticancer activity on breast cancer cell lines of *Coriandrum sativum* of Northern Cyprus.

Material and Methods: Fresh Corianders were collected from two different regions (Yeşilırmak-Y) and Maraş-M) of Northern Cyprus and separeted to roots(R) and leaves(L). Dried roots(YR,MR) and leaves(YL,ML) were exracted with MeOH.and evaporated. These exracts were used for anticancer activity studies and also idendification of the phenolic compounds. Phenolic compounds were determined by HPLC using Agilent Technologies 1200 series HPLC, Eclipse XDB-C18 column. Dried MeOH exracts were re-extracted with ethanol for anticancer activity studies. Different concentrations of these extracts were incubated for 24 h and 48 h with MCF-7 and M4A4 cells. Cell growth and cytotoxicity were measured by MTT assays.

Results: Caffeic acid, chlorogenic acid, rutin,quercitrin,vitexin-2-rhamnoside were determined and analysed quantitatively. According to the MTT results, all concentrations of sample extracts had not inhibition effect in M4A4 cells. Only YL(Yeşilırmak-Leaf) at 100 µg/ml concentration was more effective at inhibiting MCF-7 cell growth when compared with other dilutions.
Conclusion / Discussion: This preliminary studies indicated that Yeşilırmak leaves of *Coriandrum sativum* will be a source of cancer-preventive - food.

Keywords: *Coriandrum sativum*, Coriander, phenolic compounds, Northern Cyprus, anticancer activity, MTT analysis

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POSTER PRESENTATION

VACCINIUM VITIS-IDAEA L., PICKED IN BULGARIA INDICATE *IN VITRO* ANTITUMOR ACTIVITY ON HUMAN CERVICAL AND BREAST CANCER CELLS

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Objective: Cancer is a socially significant disease. Along with efforts to understand the complex genetic/epigenetic factors that trigger a carcinogenesis, it is also necessary to analyze the potential natural active substances that may delay or even stop neoplastic transformation. Promising candidates are Bulgarian cranberries from high mountain plant populations, which are rich in phenolics and anthocyanins and have proven beneficial effects on human body. The present study aims to evaluate *in vitro*, antitumor activities of total extracts and purified fractions of *Vaccinium vitis-idaea L.*, picked in Bulgaria on human cervical (HeLa) and breast (MCF7) cancer cell lines, as well as to examine some of the mechanisms underlying them.

Materials and methods: Four methanol extracts and respective number purified fractions (B- nonanthocyanin / C- anthocyanins) of Bulgarian lingonberry were used. Antitumor effect was established by Trypan Blue method and MTT cell viability assay. Assessment of apoptotic activity was performed using DNA fragmentation method.

Results: The results from MTT analyses showed that B- nonanthocyanin fractions of Bulgarian lingonberry have well expressed inhibitory effect on survival of tested tumor cells. The observed effect dependent of the dose administered and were stronger in relation with the high-mountain populations and HeLa cell line. The integrity of the extracted DNA from treated survival cells indicates possible apoptosis mechanisms under the action of biologically active ingredients from lingonberries.

Conclusion: Evaluation of antitumor activities of Bulgarian lingonberries using modern molecular methods, could contribute to establish the natural substances useful for human health in general and modern oncology.

Key Words: *Vaccinium vitis-idaea L*, antitumor activity, HeLa, MCF7

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POSTER PRESENTATION

CYTOTOXICITY GUIDED FRACTIONATION STUDIES ON *FERULAGO SYRIACA* ROOTS AGAINST VARIOUS CANCER CELL LINES

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Objective / Purpose: *Ferulago syriaca* Boiss. is a species belonging to the Apiaceae family. Apiaceae is a large family containing mainly coumarins and volatile oils [1]. The ethanolic extract of the roots of *F. syriaca* showed potent cytotoxic activity against various carcinoma cell lines [2]. In this study, the bioactivity guided fractionations studies are aimed to determine the cytotoxic active compound(s).

Material and Methods: Ethanolic extract of the roots of *F. syriaca* showed potent cytotoxic activity against SW480 (colorectal carcinoma), PC3 (prostate carcinoma) and MCF-7 (breast carcinoma) cell lines in our previous study [2]. Therefore, its dimethyl chloride, ethylacetate, *n*-butanol sub-extracts along with the aqueous residue were prepared from the ethanolic extract, and they were tested for their cytotoxic activities against the same carcinoma cell lines prior to the bioactivity guided fractionation. Dimethyl chloride extract was subjected to a silica gel column chromatography employing with *n*-hexane and ethyl acetate mobile phase gradients. The obtained fractions were tested for their cytotoxic activities against A549 (lung carcinoma), SW480, PC3 and MCF-7 carcinoma cell lines.

Results: The dimethyl chloride sub-extract and some of its fractions, *viz.* Frs. 46 – 93 and 164 – 206, were found to be more active than the other sub-extracts and fractions.

Conclusion/Discussion: Five known coumarins heraclenol, isoimperatorin, oxypeucedanin and two marmesin derivates were isolated from the active fractions of the dimethyl chloride sub-extract. These compounds have not yet been tested for their cytotoxicities; however, they are projected to be responsible for the observed activity. All isolated compounds were identified by means of spectral data (NMR and MS). Bioactivity guided isolation on the active fractions are in progress.

Keywords: Apiaceae, *Ferulago syriaca*, cytotoxic activity, bioactivity guided fractionation.

Acknowledgement: This study is financially supported by TUBITAK (Project no: 115S364).

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POSTER PRESENTATION

CYTOTOXICITY GUIDED FRACTIONATION STUDIES ON *FERULAGO MACROSCIADIA* ROOTS AGAINST A549, MCF-7, PC3 AND SW480 CANCER CELL LINES

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Objective / Purpose: *F. macrosciadia* Boiss & Balansa, an endemic species for Turkey, belongs to the family Apiaceae [1]. The ethanolic extract and its sub-extract of the roots of *F. macrosciadia* demonstrated the cytotoxic activity against MCF-7 and SW480 cell lines [2]. Therefore, it is aimed to determine the compound(s) responsible for the cytotoxic activity of the plant by bioactivity guided fractionation studies.

Material and Methods: The ethanolic extract of the roots of *F. macrosciadia* Boiss & Balansa, and its dimethyl chloride, ethylacetate, *n*-butanol sub-extracts and the aqueous remainder were tested for their cytotoxic activities against MCF-7 (breast carcinoma) and SW480 (colorectal carcinoma) cell lines. The dimethyl chloride sub-extract was found to be more active than the other sub-extracts and the total extract in our previous fractionation study [2]. Thus the dimethyl chloride sub-extract was selected for further fractionation. A silica gel column chromatography employed with *n*-hexane and ethyl acetate gradient was performed. The obtained fractions were tested for their cytotoxic activities against MCF-7, SW480, A549 (lung carcinoma) and PC3 (prostate carcinoma) cell lines. In addition, two compounds were isolated through the isolation process. The compounds were identified based on the spectral data (NMR and MS).

Conclusion / Discussion: All fractions of the silica gel column showed various cytotoxic activities against the cancer cell lines; however, two were found to be more prominent. Two furanocoumarin compounds, namely prantschimgin and isoimperatorin, were isolated from the cytotoxic sub-extract. The other compounds isolated from the active fractions are still being evaluated for the complete structure elucidation. Moreover, the bioactivity guided fractionation studies on the active fractions are in continuation.

Keywords: Apiaceae, *Ferulago macrosciadia,* cytotoxic effect, bioassay guided fractionation.

Acknowledgement: This study is financially supported by TUBITAK (Project no: 115S364).

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FULLTEXT PAPERS

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FULL PAPER

STUDIES ON MEDICINAL AND AROMATIC PLANTS UNDER THE FARMERS CONDITIONS IN THE PROVINCE OF KAYSERİ

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Abstract: This study comprises the results of demonstrative studies on some medicinal and aromatic plants carried out by Kayseri Directorate of Provincial Agriculture and Forestry under the field conditions in 2015-2017 within the Project of Improving the Medicinal-Aromatic and Dye Plants Cultivations, funded by General Directorate of Plant Production (BÜGEM). In this project, five different plants consisting of lavandin (*Lavandula x intermedia*), lemon balm (*Melissa officinalis* L.), oregano (*origanum onites* L.), salep (*Orchis sancta and Serapias womeraceae*), and black cumin (*Nigella sativa*) were studied in 11 counties for three years. Desired results were obtained from lavandin, lemon balm, black cumin and oregano plants under the ecological conditions of Kayseri. For Lavandin species, dry flower yield was 300-1270 kg ha^{-1}, and essential oil rate in dry flowers was found 2.72-9.00 %. For Lemon Balm species, dry herbage yield and essential oil rate in dry leaf were 5250-6100 kg ha^{-1}, and 0.07-0.24 %, respectively. For Oregano species, essential oil rate was found 4.64 %. In terms of cultivation of black cumin, there aren’t any considerable problems except for weeds affecting sufficient yields. The weed problem was primarily orginated by not having herbicides authorized by the Ministry of Agriculture and Forestry. Although application of different planting times and mulching methods in salep plants, desired results were not obtained because of the harsh winter conditions and frost damage.

Key words: Field Conditions, Medicinal Plants, Cultivation

1. INTRODUCTION

Medicinal and aromatic plants are existing in nature for centuries and versatile plants used by human being for different aims. These plants, used as food, medicine, paint and spices since ancient ages, are utilized in different fields because of containing of divergent active ingredients. Although these plants, being talked about, lost their former significances, they have been nearly discovered again and become the center of attraction in recent years. These plants, undertaken in the scope of alternative, traditional or supplementary medicine and being in the position of herbal treatment, have many advantages. Being cultivated in marginal and high-altitude-areas, without irrigation, fertilizing, using pesticides are the preeminents of them. Also, having little soil selectiveness and a suitable species to every kind of geographical conditions because of the species richness and enabling us to cultivate economically with a very low inputs are the other main advanteges.

This project has been carried out in the province of Kayseri since 2015 within the scope of the Project of Improving the Medicinal-Aromatic and Dye Plants Cultivations, sustained by BÜGEM (General Directorate of Plant Production), carried out on 5 plants (Lavandin, Lemon Balm, Oregano, Salep, and Black Cumin) in 11 counties and furthermore it has been expanding yearly by means of adding new plants. In this area by the means of increasing the plant species, determining the suitable alternative plants to the ecological conditions in Kayseri area providing more income, enabling the grower to learn and spread out their cultivation are aimed.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in demonstrations were provided from the Directorate of Aegean Agricultural Research Institution/Menemen/İZMİR, the Directorate of Directorate of Fruit Research Institute/Eğirdir/İSPARTA, The Directorate of Transitional Zone Agricultural Research Institute/ESKIŞEHIR, and the Directorate of Horticultural Central Research Institute/YALOVA. The species used in the study; *Lavandula x intermedia, Melissa officinalis L., Origanum onites L., Orchis sancta, Serapias vomeraceae, Nigella sativa.*

2.2. Methods:

In the demonstrations; the distance on the line was 50 cm, between the lines were 120 and 150 cm for Lavandin. Sewings of the seedlings were carried out in May. Lemon Balm seedlings were planted in May like Lavandin and the distances were 30 x 70 cm. For Oregano plants, seedlings were planted as 20-30 cm distance on the line, 40-60 cm distance between the line. Black Cumin seed were sown in April as 15-20 kg ha^{-1}. In the fields of Lavandin, Lemon Balm, Oregano, Salep, and Black Cumin fertilizer applications were carried out almost similarly as 20-20-0 Compound Fertilizer, 200-250 kg ha^{-1} to the ground and 10-20 tons of fertilizer burnt were added in some locations. Salep plants were planted in October in the first year. Salep tubers were planted within a burnt manure-enriched soil bed, 25-30 cm in height and 80-100 cm in width, in 15-15 cm soil depth and 10-20 cm row spacing. Unsuccessful results were obtained in the firsth year. Therefore, planting time was changed to 3 different planting times as October (a month later than the firsth planting time), Novomber and December to observe the result of different planting times. Also, different mulching method made of wheat straw and burnt manure, greenhouse and low tunnel methods were tried to see the developments under cover.

3. RESULTS AND DISCUSSION

3.1. Lavandin *(Lavandula x intermedia):* Lavanding seedlings were obtained in the years of 2015 and 2016. Demonstrative activity researches were carried out at totally 14 locations with a total of 7 locations firsth year and 7 locations second year. Harvestings were carried out in 2016-2017. Accordingly, while good results were taken from some locations in all plants, it was not possible to get any results from some of them since just firsth year or in the other years because of the reasons originated from the growers. In Lavandin, demonstrations were estabilished in 3,35 decares in 7 locations in 6 counties in 2015, in 5,2 decares in 7 locations in 7 counties in 2016, totally in 8,55 decares The harvest wasn’t made in the firsth year because of plant growth being slow, however to enhance the tillering in flowering plants, flowers were dissevered from their stems.

In lavandin demonstrations, constituted in 2015, however even if just a bit desiccations in plants originated from cold wheather and frost in some locations in 2015-2016, plants recovered again by tillering with the warming wheather in Spring.

In three designated locations below, the results of dry flowers yield, essential oil rate and essential oil yield could be obtained.

Table 1. According to the 2. Years (2016) Harvests in Some Locations, Dry Flowers Yields, Essential Oil Rates and Essential Oil Yields.

Queue Number	Location Name	Dry Flowers Yield (kg ha^{-1})	Essential Oil Rate (%)	Essential Oil Yield (L ha^{-1})
1	Yahyalı/Mustafabeyli	1270	6,1	77
2	Yeşilhisar/Merkez	300	5,7	17
3	Yeşilhisar/Kayadibi	680	6,0	48

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When examining Table 1, dry flowers yields, essential oil rates and essential oil yields are seen regarding Lavandin trials results on the grower basis in Mustafabeyli/Yahyalı, Yeşilhisar and Kayadibi/Yeşilhisar. The highest dry flowers yield became in Yahyalı, Mustafabeyli Location as 1270 kg ha^{-1}, the lowest dry flowers yield became in Yeşilhisar location as 300 kg ha^{-1}. Essential oil rates in dry flower were very close to eachother between 5,7-6,1%. While essential oil yields were 77 L ha^{-1} because of high dry flowers yield in Mustafabeyli/Yahyalı location, it was stayed at a lower level of 17 L ha^{-1} because of low dry flowers yield in Yeşilhisar. The reasons why dry flowers rate was so low were those grower retarted the harvest becasuse of his beekeeping and shadows of the trees around flower yields increased more in the following years, but these values could not be measured.

Table 2. The Amount of Essential Oils Obtained in Dry Flower in 2017 Year Harvest in Lavandin Locations (%)

Queue Number	Location Name	Essential Oil Rate (%)	Queue Number	Location Name	Essential Oil Rate (%)
1	Yahyalı Karaköy	6,00	6	Felahiye Merkez	9,00
2	Yeşilhisar Merkez	5,70	7	Yahyalı Mustafabeyli	6,80
3	Develi Sindelhöyük	5,67	8	Tomarza Işıklar	4,50
4	İncesu Garipçe	5,20	9	Kocasinan Yazır	2,82
5	Kocasinan Akçatepe	5,80		**Average Value**	**5,72**

As examined in Table 2, the essential oil ratios of the locations in the dry flower ranged between 2,82-9,00%. The highest Felahiye Center is located at the lowest Kocasinan Yazır location. Average essential oil ratio of the locations in dry flowers was 5,72%. The emergence of a essential oil rate of 9% in the Felahiye Center is an important indicator for this location. The altitude of this location is 1330 m, the south open face, the sun is good and the climatic open days are high. It is a known fact that sunbathing increases the rate of essential oil. In Kocasinan Yazır and Tomarza Işıklar, essential oil ratios were lower than other locations because of the mixing of some lavender flower stalks in dry flower sample and lack of good sample preparation. These essential oil ratios in dry flowers are a good indicator. In the scope of the support given to young farmers in the province, the producers removed the essential oils from the products they obtained in the essential oil distillation system and placed them in small glass bottles and marketed them.

Arabaci and Bayram, (2005), (1340-4430 kg ha^{-1}), and Arslancan et al. (2014) (1620-3410 kg ha^{-1}) reported that low yields of lavender were lower in producers of lavender. the inadequacy, harvest method and the methods of removing the dry flowers from stalks, the lack of sufficient infrastructure in this issue has been due to. Deaf oil yields were also low. Otherwise, when we look at the essential oil ratios, we see a rate of 5.7% which is much higher than the values indicated by Arabaci and Bayram (2005) (1.54-2.34%).

3.2. Lemon Balm (*Melissa officinalis* L.): In 2015, 4 activities were carried out in 0.9 decares area, in 4 locations in 4 counties, in 2016 in 2.05 decares, in 3 locations and in 3 counties. In total, 2,95 decare area demonstrations have been established and followed up.In the demonstrations established in 2015, a harvest was made in the autumn plants before entering the winter, but there was not much yield. In the winter, although the above-ground parts of the plants completely desiccated in all locations with the arrival of the spring with the new shoots occurred in the plant development was not a problem. Due to winter and cold weather in Kayseri, there was no negative situation. Plant growth and yields were very good after the first year. A harvest in the flowering period in June, and another harvest before the winter in September-October, plants was harvested twice per year. While a high herbage yield was obtained in the first in June, a lower herbage yield was obtained in the second.

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Table 3. Total Dry Herbage, Essential Oil Ratio and Essential Oil Yield Values of Some Locations According to the Harvest Results of the Lemon Balm

Queue Number	Location Name	Dry Herbage Yield (kg ha^{-1})	Essential Oil Rate (%)	Essential Oil Yield (L ha^{-1})
1	Yahyalı/Mustafabeyli	5250	0,08	4,2
2	Kocasinan/Yazır	6100	0,07	4,2

In Table 3, while the total dry herbagea yield was 5250 kg ha^{-1}, the essential oil rate was 0.08% and the essential oil yield was 4,2 L ha^{-1} in the Yahyalı Mustafabeyli location, these were 6100 kg ha^{-1}, 0.07% and 4,2 L ha^{-1} in the Kocasinan Yazir location at the 2nd year harvest. The dry and fresh herbage yields for the second year were higher than in the first year, but these data could not be recorded since the yield values could not be weighed. According to this, it can be said that the lemon balm plant is in compliance with the ecological conditions in Kayseri and it has no climatic, disease or harmful problems.

Table 4. Essential Oil Ratio of Dry Leaf in Lemon Balm According to 2017 Harvest

Queue Number	**Location Name**	**Essential Oil Rate (%)**	**Queue Number**	**Location Name**	**Essential Oil Rate (%)**
1	Yahyalı Mustafabeyli	0,17	3	Tomarza Işıklar	0,09
2	Kocasinan Yazır	0,13	4	İncesu Garipçe	0,24
				Average Value	0,16

Table 4 shows the rates of essential oil in four locations in the lemon balm plant. According to this, the highest essential oil ratio was found at İncesu Garipçe location with the highest rate of 0,24% and the lowest value was found at 0,09% Tomarza Işıklar location. Values are close (0.1-0.35%), to the values reported by Uzun et al., (2014), some higher than the values reported by Uyanık and Gürbüz (2014) (0.03-0.08%) and Koç, H., (2002). According to the results of Abdellatif et al. (2014) (% 1,54-2,34), it is slightly lower. Essential oil ratios are close to the findings obtained in our country.

3.3. Oregano (*Origanum onites* L.): Demonstrations in oregano were established in 2016 in 5 locations and 5 counties in a total area of 2.5 decares. Plant growth was very good in locations. A superficial harvest was made before the first year of winter and no significant yield was obtained. In the second year flowering period, the herbage yields of the plants harvested in June were taken but could not be measured. In some locations 1 and 2 reaps were taken in 2 years, in second reaps in September and October, a very low herbage yield was mentioned. No disease, no harmful organism or no cold and frost damage in winter in the plants was observed. Producers in Yahyalı Kopçu and İncesu Garipçe locations sold their products in the markets and generated certain amounts of income. In the İncesu Garipçe location, the amount of essential oil detected in the 2nd year (2017) Origanum onites L. leaves was 4.64%. S ince our producer is an organic farming producer, it has been able to pack and sell medicinal and aromatic plants (lavandin, lemon balm, oregano and sage) in the organic market. He is still doing this job.

3.4. Salep *(Orchis sp.)*

Demonstrations in the Salep plant were established in 2015 in 3 counties in 0.9 decares area, in 2016 in 6 locations in 6 counties in 3.2 decares, in a total of 4.1 decares. The first year plantings were made in October. In autumn there was no outflow of tubers. In the spring, there was some output in the Kocasinan location as in March, but they also disappeared with the effect of late frosts in spring. It was planned to investigate different applications such as different planting time, mulching methods and production conditions under cover for 2 years. In order to see the results of different planting time

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in the 2nd year, 3 different planting time applications were made in a way that the suturing times of the tubers were one month after the first planting time, from September to October-November. Different mulching methods were tried. To keep the pillows warm, burnt farm manure was laid in straw-straw and some were planted in the greenhouse and some were planted in the form of a low tunnel cover. After planting, demonstrative follow-ups were performed regularly. No improvement was observed in plants in autumn and winter Only in the greenhouse and under the cover planted some output was observed in winter, but in the later stage they disappeared. A few plants were found in the Felahiye location, which overlooks the southern slope of the open area.

It is thought that the winter conditions in Kayseri are hard and long, the damages of late frosts in spring and the salep species used in the spring are unsuccessful due to the cold and frost sensitive species *Orchis sancta* and *Serapias vomeraceae*. Salep plant is a difficult plant culture. Arabacı et al. (2014), in their study on the Effect of Different Cultural Practices in Salep Orchids, reported that many of the studies conducted for culturing Salep orchids were in vitro studies and that the plants were failing at the stage of adaptation to outdoor conditions. We are of the opinion that in the conditions of Kayseri cultivation of salep plants can be made in other species compatible with the Central Anatolia Region. Tutar et al. (2012) in our opinion in accordance with the salepte each region of their own species and work with their ecological conditions can be achieved with successful results stated.

3.5. Black Cumin (*Nigella sativa* L.): The demonstrations in Black Cumin were implemented in 3 locations (13 decares) in 3 counties in 2015, and in 5 locations (14 decares) in 4 counties in 2016, totally in 27 decares. Seed of 15-20 kg per hectare were used for seed decantation in April and sprinkling irrigation was done. Sprouts and exits occurred in locations. However, due to the large number of weed populations, black cumin did not show any improvement. Since the Ministry is not a licensed herbicide and mechanically it is not suitable for anchoring, it is not possible to obtain a product at the level of economic efficiency in black cumin. Because the yield values of the decay remained at very low levels of 100-200 kg ha^{-1}, farmers did not even need to harvest because they could not remove them.

4. DISCUSSION AND CONCLUSION

The following conclusions can be drawn from these studies conducted in 45.1 decares area in 43 locations in 11 counties in 5 different medicinal and aromatic plant species (Lavandin, Lemon Balm, Oregano, Salep and Black Cumin) for 3 years between 2015-2017. As the studies were conducted under farmer conditions, the desired results could not be obtained from each demonstration depending on the farmers. In the trials and demonstrations carried out in the farmer's conditions, the choice of the wrong farmers, the lack of equipment and equipment infrastructure at the desired level, the willingness to appear at first, and then the difficulty in raising their work intensity or cultivation in these plants, the lack of interest and the lack of enough results could not be achieved. For these reasons, some demonstrations were disabled at the first stage. However, a certain result has been reached in the studies. We tried to reduce this risk by establishing the demonstrations at more locations. Under the conditions of Kayseri, these plants can be cultivated economically and they have an idea about their adaptation to Kayseri ecology, their efficiency and quality. These results shed light on local people for those who will work or invest in these plants. As a matter of fact, the Provincial Directorate of Agriculture and Forestry has a lot of people who are interested or not interested in agriculture and want to invest in these matters. These outputs are important in informing and referring them to the right direction.

Under the conditions of farmers, making and conducting these demonstrations cause the producers to recognize and learn these plants, and to recognize, see and be interested in the farmers of the neighboring farmers and other nearby villages. In the next stage, these plants gradually enter the crop

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rotation in this area and make it become agricultural. There are many examples of this, but this can take many years. Before the start of this project, none of the medicinal and aromatic plants in Kayseri were cultivated and produced. Now some of the producers still continue to grow existing plants, new producers, new plants are added, they are included in municipalities under the leadership of Provincial Directorate of Agriculture and Forestry. According to this study; In the ecological conditions of Kayseri, positive results were obtained in the cultivation of lavandin, Lemon Balm and oregano plants. Although we do not see a problem in plant growth in the sowing of Black Cumin plants, weed problem has emerged as an important problem. Due to the lack of a medicinal product licensed from the Ministry of Agriculture and Forestry, it was not possible to obtain sufficient yield. In order to be successful in the agriculture of black cumin in Kayseri situations, we think it is necessary to plant more broadly (40-50 cm) in order to allow the weeding of the weeds mechanically, it would be appropriate to make different sowing times, including autumn, winter and early spring. Due to the fact that the winter conditions in Kayseri are hard and long, and the species used are not compatible with this region, appropriate results could not be obtained. Salep plant is a plant that is difficult to culture, but we believe that other types of studies compatible with the Central Anatolia region can be done.

Acknowledgments
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Proceedings Book

FULL PAPER

CURCUMIN, A POLYPHENOL DERIVED FROM *CURCUMA LONGA*: CLINICAL EVIDENCE AND MOLECULAR MECHANISMS IN PREVENTION AND MANAGEMENT OF CANCER

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Abstract: Cancer is the second leading cause of death globally, and it has been a major health problem in the past decades. Several strategies are involved in treatment and prevention of cancer cases such as chemotherapy, immune therapy, radiation, hormone therapy, targeted therapy and herbal therapy. Among them, herbal therapy with medicinal and aromatic plants has been gained great importance in recent years. Medicinal plants and their bioactive compounds may offer clinically useful anticancer agents, due to their rich anticarcinogenic and chemoprotective potentials. Curcumin, the rhizome of Curcuma longa, is widely used in therapeutic purposes because of its several biological properties such as anti-inflammatory, anticancer, antioxidant, and etc. From this point of view, this review is aimed to comprehensively highlight the effects of curcumin for the prevention and treatment of cancer. **Methods:** This report reviews the pharmacological and anticancer effects of curcumin with a focus on its molecular mechanisms in prevention and management of various cancer cases. For this purpose, electronic databases, including PubMed, Scopus, ScienceDirect, Cochrane library, Google Scholar and MedlinePlus, etc. were searched to summarize *in vitro*, *in vivo* and clinical studies on anticancer effects of *Curcuma longa* and curcumin, polyphenolic bioactive compound of the plant up-to-date. **Results and Conclusion:** To sum up, curcumin, the active ingredient of turmeric and exerts remarkable therapeutic potential for the treatment of various types of cancers. Curcumin is strong anti-oxidant and anti-inflammatory properties and therefore it possesses anticancer potentials through various molecular mechanisms. Based on documented data this natural compound could be a key source of anticancer agent in modern anticancer therapy after clinical confirmation.

Key Words: *Curcuma longa*; curcumin; anti-cancer agents; molecular mechanism; natural products; medicinal plants.

Graphical Abstract

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INTRODUCTION

Cancer has become a growing health problem around the world with the high numbers of cases and deaths. Meanwhile, it is predicted that it will be more critical health problem in both developed and developing countries for the next years. More recently, the researchers have mainly focused on examining the use of plant-derived natural products with less or no side effects for prevention and treatment in the cancer cases, since an increase in the incidences of drug resistance and side effects of drugs used for cancer therapy (Gezici, 2018; Gezici and Sekeroglu, 2019). Curcumin (turmeric), a widely used spice, food preservative and coloring material, is isolated from *Curcuma longa* Linn. belonging to Zingiberaceae (ginger) family. This plant is originated from southeastern Asia, and distributed throughout tropical and subtropical regions. The rhizome of the plant is known as 'Golden Spice' because of its brilliant yellow color, and it is a very popular spice in Africa, Thailand, Pakistan and India (Verma et al., 2018; Rai et al., 2018; Li et al., 2019).

A B C

Fig. 1. Pictures of plant (A), rhizomes (B) and rhizome powder (C) of turmeric (*Curcuma longa* L.)

Chemical Structure of Curcumin: *C. longa* L. has included curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin, the most important constituent of *C. longa* L., is responsible for its yellow color (Fig. 1). Chemically, curcumin is a bis-α, β-unsaturated β-diketone with keto-enol tautomerism and its chemical structure was given in the Fig. 2. (Akram et al., 2010; Rai et al., 2018; Li et al., 2019).

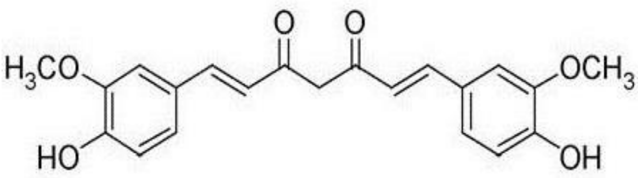

Fig. 2. Chemical structure of Curcumin

Properties and Health Benefits of Curcumin: Curcumin has a long history of use in traditional medicine for treatment of various disease and disorders including cough, diabetic ulcers, hepatic diseases, biliary disorders, rheumatism, sinusitis and anorexia (Li et al., 2019). It has been used to cure acne, heal wound, prevent skin damage, reduce cholesterol, treat diabetes, control blood pressure, prevent inflammation and cancer cell growth (Fig.3). It behaves as a free radical scavenger in the human body, thus it is able to prevent oxidative damage as blocking the free radicals. It also decreases the production of reactive oxygen species and stimulates antioxidant capacities in the body (Farzaei et al., 2018; Pagano et al., 2018). It has been used for treatment of various diseases, owing to its wide range of biological effects including not only antitumor activities but also as antioxidant, antimicrobial, anti-inflammatory, anticoagulant, antidiabetic and immunomodulatory activities (Aggarwal et al., 2007; Pan et al., 2010, Verma et al., 2018).

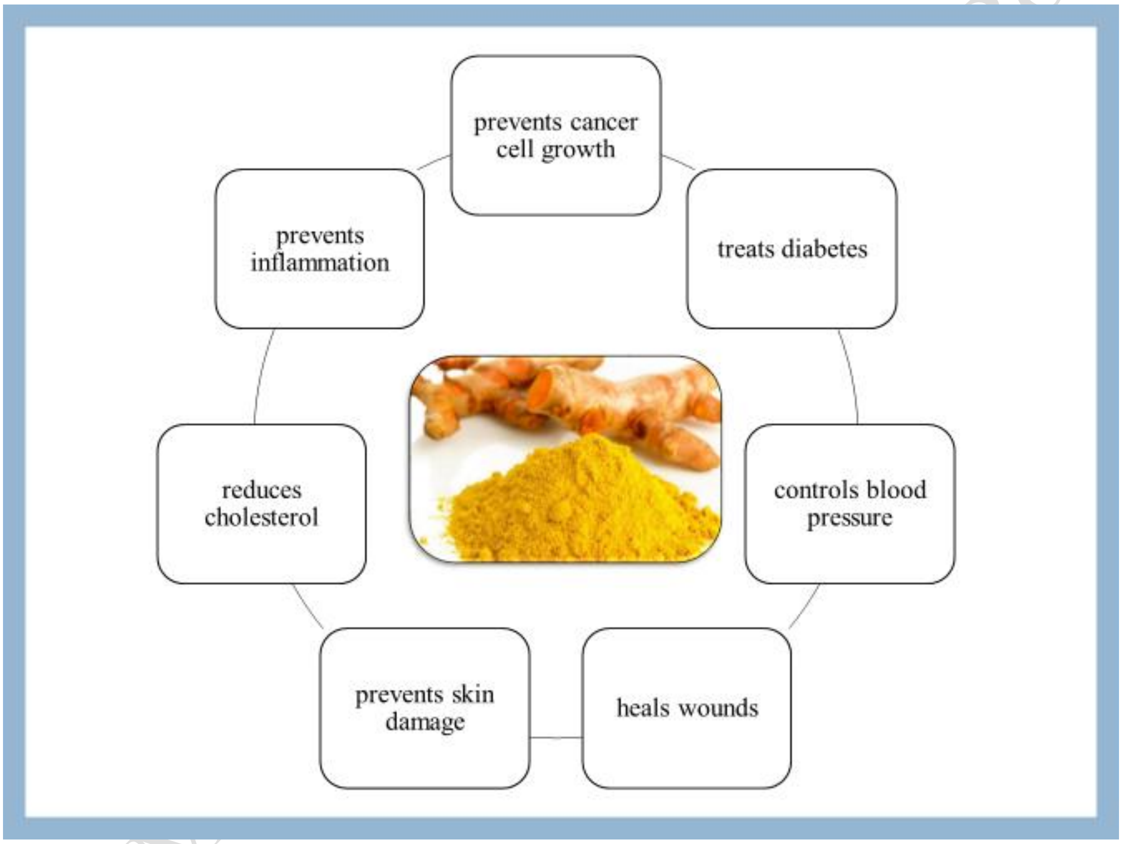

Fig. 3. General benefits of curcumin

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Role of Curcumin in Carcinogenesis and Cancer Pathways: Cancer is a multifactorial disease that occurs when a normal cell loses its cellular division control. At the molecular level of carcinogenesis, curcumin targets numerous pathways at multiple levels including activator protein 1 (AP-1), Nuclear Factor Kappa B (NF-κB), Peroxisome Proliferator-Associated Receptor Gamma (PPAR-γ), Signal Transducer and Activator of Transcription (STAT), Wnt/β-catenin, transcription factor Nrf-2-Keap, Tumor Necrosis Factor-alpha (TNF-α), Interleukins (IL), and oncogenic kinases (Li et al., 2019; Shanmugam et al., 2015).

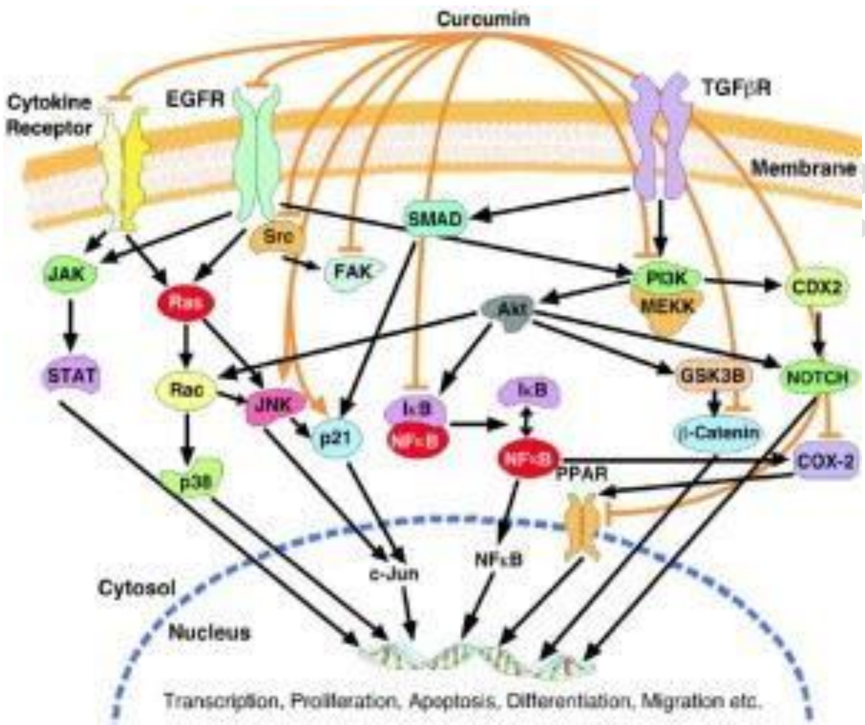

Fig. 4. Curcumin and cancer pathways (http://www.cancermind.com/curcumin-anticancer-cure-cancer-review/)

Curcumin are able to interact with molecular targets involved in inflammation, thus it modulates inflammatory response by reducing the activity of cyclooxygenase-2 and lipoxygenase, inducing nitric oxide synthase enzymes, inhibiting tumor necrosis factor-alpha and interleukins. Moreover, it interferes with NF-κB, modulates growth of tumor cells through regulation of multiple cell signaling pathways including cyclin D1, c-myc, Bcl-2, Bcl-x, cFLIP, XIAP, c-IAP1, and caspase activation pathway (Jurenka, 2009; Yan et al., 2019). Curcumin related cancer pathways are figured out in the Fig. 4.

Curcumin effects carcinogenesis process through various mechanisms as increasing of growth suppressors and apoptosis, decreasing of proliferative signals and angiogenesis, and restricted replicative immortality (Fig. 5). Curcumin targets protein kinases and growth factors which are involved in carcinogenesis process. ErbB-2 (avian erythroblastosis oncogene B) or HER2/neu is a member of the epidermal growth factor receptor (EGFR) family is the main targets of curcumin. Moreover, curcumin is known as a potential anti-inflammatory agent that able to inhibit inflammatory enzymes namely nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and lipooxygenase (LOX) (Shanmugam et al., 2015; Farzaei et al., 2018; Yan et al., 2019).

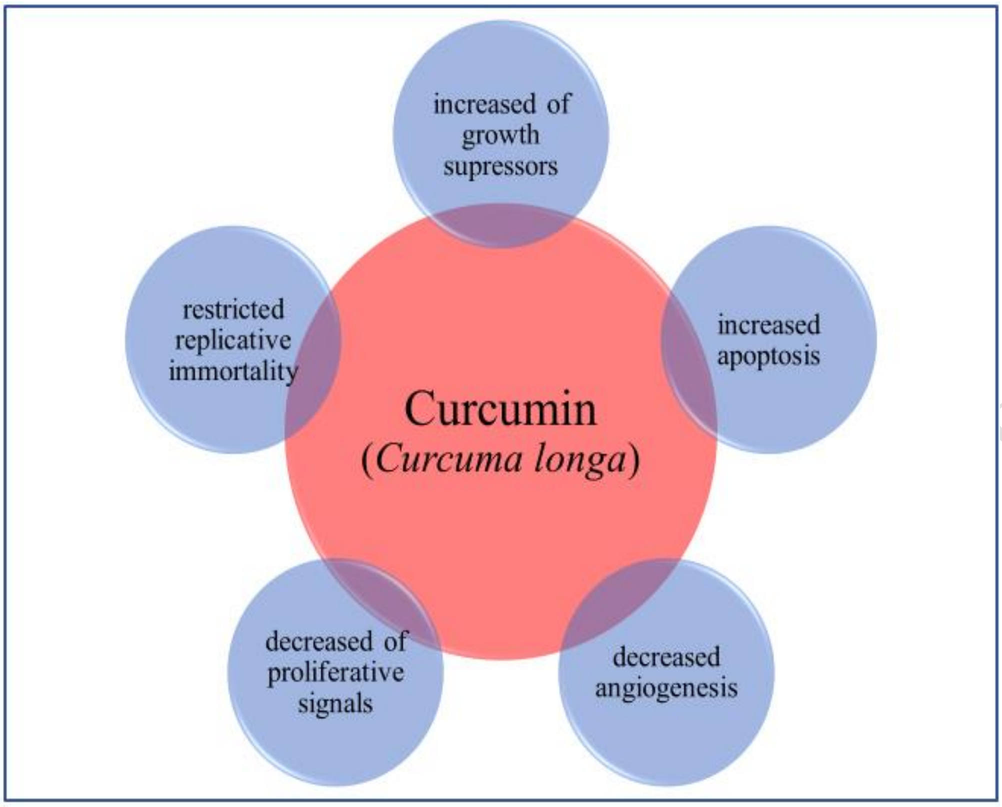

Fig. 5. Role of curcumin in carcinogenesis

Clinical Trials and Cancer: Numerous clinical studies including pilot clinical studies and Phase I, II and III clinical trials are currently in progress for revealing of the therapeutic potentials of curcumin. The clinical trials have been summarized in Table 1. The anti-cancer potential of curcumin has been successfully showed in various types of cancers such as oral, lung, breast, prostate, pancreatic, colorectal, multiple myeloma, head and neck squamous cell carcinoma. Clinical trials with curcumin indicate safety, tolerability, non-toxicity (even up to doses of 8000 mg/day), and efficacy (Shanmugam et al., 2015; Yan et al., 2019). Researches showed that curcumin is capable of inhibition of carcinogenesis even at three stages including initiation, promotion, and progression (Fig. 5). Dhillon et al., [4], at the M.D. Anderson Cancer Center in Houston determined that curcumin has potential activity to inhibit of malignant cell proliferation in pancreatic cancer in phase II clinical trial, and also, found that it is utilized for chemoprevention of colon cancer (Johnson and Mukhtar, 2007). Moreover, Dai et al. [3] designed and synthesized curcumin mono-carbonyl analogs for cancer therapy against human lung cancer A549 cells (Gezici and Sekeroglu, 2017, 2019).

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Table 1. Clinical trials the use of curcumin in cancer cases

Cancer Cases	Organization / Cancer Center	Trial Phase	Date
Colon cancer	Chao Family Comprehensive Center	Phase II	Unknown
Colon cancer	University of Pennsylvania	Phase II	2019, June
Rectal cancer	MD Anderson Cancer Center	Phase II	2010, July
Pancreatic cancer	Tel-Aviv Sourasky Medical Center	Phase III	Unknown
Pancreatic cancer	MD Anderson Cancer Center	Phase II	2009, December
Multiple myeloma	MD Anderson Cancer Center	Pilot study	2008, December
Osteosarcoma	Tata Memorial Hospital	Phase I and Phase II	2012, May
Familial adenomatous polyposis	John Hopkins University	Phase II	2013, March
Colorectal cancer	MD Anderson Cancer Center	Phase I	Unknown

Conclusions and Future Directions: To conclude, curcumin is a pharmaceutical agent that possess various therapeutic applications in cancer prevention and treatment. However, curcumin was limited in clinical use because of its low bioavailability, which might be led by poor absorption and rapid metabolism. To increase its bioavailability, several curcumin formulations have been developed namely powder, tablets, capsules, liposomal encapsulation, emulsions, and nanoparticles. Nonetheless, further clinical trials are required to validate their safety. It will be a great potential to be a dietary supplement with various formulations that is able to prevent tumorogenesis through altering different molecular pathways.

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FULL PAPER

THE EFFECTS OF NITROGEN AND FARM MANURE APPLICATIONS ON ESSENTIAL OIL CONTENT OF DILL (*ANETHUM GRAVEOLENS* L.)

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Abstract: *Anethum graveolens* L. or dill, belonging to Apiaceae (Umbelliferae) family, is an annual aromatic herb known for culinary and medicinal use since ancient times. It has been used in ayurvedic medicines since ancient times and it is a popular herb widely used as a spice and also yields essential oil. The present study aimed to determine the effects of different doses of chemical (ammonium nitrate) and organic fertilizer (farm manure) applications on the essential oil content of *Anethum graveolens* L. (dill). In this study, different doses of farm manure (750, 1000, 1250 and 1500 kg da^{-1}) and ammonium nitrate (3, 6, 9, 12 kg da^{-1}) with a control (no manure) were applied by sowing. The experiments were arranged in the Completely Randomized Blocks Design with three replicates. Each experimental plot consisted of five rows, with a distance of 0.3 m between rows and 0.2 m between plants, and a plot size of 3.3 m^2. The essential oil percentage was subjected to hydro-distillation for 3 hours using a modified Clevenger apparatus. The hydro-distilled essential oil content ranged from 0.26% to 6.18%. The highest essential oil content were obtained in the treatments with farm manure doses of 1250-1500 kg/da and control application, which were significantly different compared to the other treatments. Among all of the essential oils included in the study, dill seeds showed the highest essential oil yield, followed by dill leaves and dill herbs. As suggested by the present study, a suitable ratio of farm manure in the soil can increase the essential oil content. This organic manure could thus be considered as suitable substitutes for chemical fertilizers when growing medicinal plants that are gaining both an increased importance and demand.

Keywords: Dill, essential oil, farm manure

INTRODUCTION

Anethum graveolens L. (dill) has been used in ayurvedic medicines since ancient times and it is a popular herb widely used as a spice and also yields essential oil. It is an aromatic and annual herb of apiaceae family. There are various essential components of dill seeds and herb; carvone being the predominant odorant of dill seed and α-phellandrene, limonene, dill ether, myristicin are the most important odorants of dill herb. Other compounds isolated from seeds are coumarins, flavonoids, phenolic acids and steroids (Bailer et al., 2001). Dill is used as an ingredient in gripe water, given to relieve colic pain in babies and flatulence in young children (Pulliah, 2002). The seed is aromatic, carminative, mildly diuretic, galactogogue, stimulant and stomachic (Hornok, 1992; Sharma, 2004). The essential oil in the seed relieves intestinal spasms and griping, helping to settle colic (Fleming, 2000; Duke, 2001). The carminative essential oil improves appetite, relieves gas and aids digestion. Fertilization is one of the most significant agricultural practices used to improve the yield and quality of traditional crops. The need to use renewable forms of energy and reduce costs of fertilizing crops has revived the use of organic fertilizers worldwide (Seifritz, 1982). Furthermore, organic manure activates many species of living organisms which release phytohormones and may stimulate the plant growth and absorption of nutrient (Naguib and Aziz, 2004).

The present study aimed to determine the effects of different doses of chemical (ammonium nitrate) and organic fertilizer (farmyard manure) applications on the essential oil contents of dill. We have chosen dill for our study because it is one of the most popular culinary herbs worldwide, has a variety of cultivars available, and is often commercially produced in greenhouses, used by local people for medicinal purposes or as a nutrient.

MATERIAL AND METHODS

The present experiment was carried out during growing season in 2017 at Bolu Abant İzzet Baysal University, Bolu. The field experimental site was located at research and application area of

Agriculture and Natural Sciences Faculty. Seeds of dill were obtained from Erzurum province. The experiment was designed in a randomized complete-block design with a split-plot arrangement, with three replications being conducted in open-field conditions. Farmyard manure (FYM) and ammonium nitrate (AN) were in the main plots, whereas different AN levels (3, 6, 9, 12 kg da^{-1}) and different FYM levels (750, 1000, 1250 and 1500 kg da^{-1}) with a control (no manure) were in sub-plots. Each experimental plot consisted of five rows, with a distance of 0.3 m between rows and 0.2 m between plants, and a plot size of 3.3 m^2. Average climatic data were recorded 16.08 °C temperature; 41.37 kg m^{-2} rainfall; 69.2% humidity during the vegetation period for 2017 (Anonymous, 2017). Experimental area soils are clay-loam with a pH value of 7.8, organic matter content of 4.7%, phosphorus ratio of 10.3 ppm and potassium ratio of 235 ppm (Anonymous, 2016a). Dill was regularly irrigated to demonstrate good progress in its period vegetation since irrigation is a very important factor for cultivation of dill. The dill seeds were sown on the 20th day of april (2017). As experimental factors, the different doses of FYM (750, 1000, 1250, 1500 kg da^{-1}) was applied a week before sowing. Also, As a base fertilizer, 12 kg da^{-1} diammonium phosphate (DAP) were applied with sowing to the conventional plants. AN (3, 6, 9, 12 kg da^{-1}) was applied to the conventional plants in two splits in April and July. It was uniformly spread on the plots and lightly worked into the soil with harrow. The nutrient composition of the soil and the FYM were analyzed before use. The properties of FYM are provided in Table 1. FMY has the highest organic matter, calcium, magnesium and iron content. No chemicals were used in the experiments.

Table 1. Chemical Analyses of Farmyard Manure (Anonymous, 2016b).

Analysis Parameters	Unit	Analysis results
Organic matter	%	**40.68**
Total Nitrogen (N)	%	1.80
Moisture	%	59.50
pH (potentiometric)		7.61
EC (1 10^{-1})	mS cm^{-1}	3.26
Total phosphorus (P)	%	0.52
Water soluble phosphorus (P_2O_5)	%	0.32
Total potassium (K)	%	1.36
Water soluble potassium (K_2O_5)	%	1.04
Total calcium (Ca, ICP EPA 3052)	ppm	7758.00
Total magnesium (Mg, ICP EPA 3052)	ppm	5099.00
Total iron (Fe, ICP EPA 3052)	ppm	10606
Total copper (Cu, ICP EPA 3052)	ppm	24.97
Total zinc (Zn, ICP EPA 3052)	ppm	93.53
Total manganese (Mn, ICP EPA 3052)	ppm	368.30
Total lead (Pb, ICP EPA 3052)	ppm	2.33
Total cadmium(Cd, ICP EPA 3052)	ppm	DLA
Total cobalt (Co, ICP EPA 3052)	ppm	DLA

Note: DLA: Detection limits Cd<0.03ppm, Co<0.08 ppm,Pb<0.09 ppm

The experiment harvest was started on 02 July and the final harvest made on 28 July. The data were analyzed using XLSTAT statistic software program and obtained results were subjected to analysis of variance and Least Significant Difference Test (LSD) in order to find differences among the dill populations and controls at p=0.05.

Isolation of essential oil: Essential oil analyses were carried out in accordance with method TS 8882. A sample of approximately 20 g was taken from the dried plants of each species and placed into glass Clevenger flasks. Approximately 200 mL (ten times the sample weight) of distilled water was added and samples were then subjected to hydro-distillation for 3h. The essential oil accumulated on top and separated from the rest of the sample. The amount obtained was recorded (in mL) from the graduated section of the flask and weights were then used to calculate percentage essential oil yields.

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RESULT AND DISCUSSIONS

Organic FYM demonstrated statistically significant difference ($P<0.05$) among all of the treated plots with respect to essential oil yield except herb essential oil (Table 2). The trend in essential oil yield was slightly different compared to that in FYM applications. In general, it was higher in all of the treated plots compared to AN fertilizers. The hydro-distilled essential oil content ranged from 0.26% to 6.18%. The essential oil content was found as 0.26% to 0.72% in dill herbs, 0.27% to 1.70% in dill leaves, 4.58% to 6.18% in dill seeds. The highest essential oil content were obtained in the treatments with FYM doses of 1250-1500 kg da^{-1} and 3 kg da^{-1} AN application, which were significantly different compared to the other treatments. Also, control application exhibited significantly higher essential oil content compared to the all AN applications in dill herbs.

Among all of the essential oils included in the study, dill seeds showed the highest essential oil yield, followed by dill leaves and dill herbs. The essential oil yield (0.26- 6.18%) in the present study was comparable to that in a study by Kruger and Hammer (1996) who found that essential oil percentage of different dill cultivars (fruits) vary from 1.91% to 7.25%. Similarly, Badoca and Lamartib (1991) observed that the oil content of European dill seeds varied from 1.75-5.8%. Also, Said-al and Omer (2016) reported that the essential oil content of Egyptian dill herbs varied from 1.933-3.267%. The ssential oil content of dill herbs, leaves, seeds determined in the present study was within the range reported for dilll in all of these previous studies.

By contrast, Stanojević et al., (2015) reported the yield of the dill essential oils from seeds and herbs to be 4% and 2.80%, respectively. Such variations in the essential oil content of dill across countries might be attributed to the varied agroclimatic conditionsoftheregions, as well as different fertilizer applications.

Table 2: Essential oil of herb, leaf and seed in dill.

Doses (kg da^{-1})	Herb EO (%)	Leaf EO (%)	Seed EO (%)
Control	0.65	1.47C	5.59AB
3	0.34	1.33D	5.98AB
6	0.26	0.23H	4.63C
9	0.26	0.30F	5.26BC
12	0.35	0.30F	5.23BC
750	0.30	0.27G	4.58C
1000	0.64	0.55E	5.56AB
1250	0.55	1.50B	5.91AB
1500	0.72	1.70A	6.18A
LSD (5%)	0.71	0.03	0.77

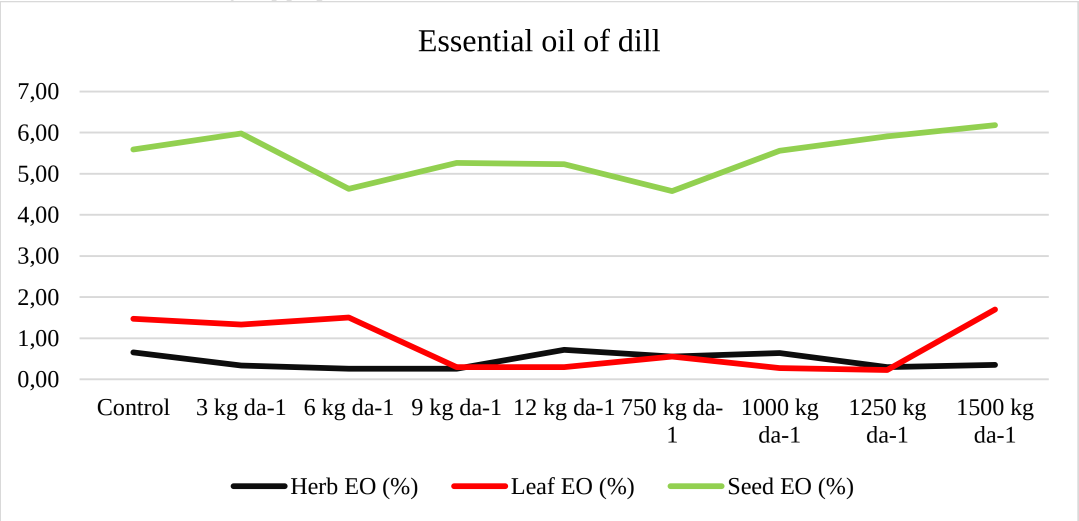

Figure 1. Essential oil of herb, leaf and seed in dill.

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CONCLUSION

Anethum graveolens L. is a very popular and widely grown herb worldwide. Organic and inorganic fertilization studies have been carried out with respect to cultivation, breeding, high herbage yield and high essential oil content, etc., although few studies have been conducted concerning the organic FYM effect on essential oil content and the quality of dill. Especially, FMY doses 1250-1500 kg da^{-1} had the highest essential oil content. As suggested by the present study, a suitable ratio of organic FYM in the soil can increase essential oil content. FMY could thus be considered as suitable substitutes for chemical fertilizers when growing medicinal plants that are gaining both an increased importance and demand.

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FULL PAPER

COMPOUNDS FROM AN AROMATIC ANNUAL PLANT GROWING IN ARID ZONE OF ALGERIA AND SCREENING OF ITS ANTIBACTERIAL ACTIVITY

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Abstract: The Asteraceae*Matricaria pubescens* (Desf.) is a spontaneous species from arid regions of Algeria. To get its volatile fraction, the aerial parts of this plant were subjected to an hydrodistillation using a cleavenger apparatus. A yield of 0.1% was obtained. The sample was analyzed by GC-MS on a non polar capillary column. Among identified components in this volatile fraction were; spathuleol (10.59%), decanoic acid (5.03%), transisoelemicin (4.70%), cadinol (1.59%). Beside, the purpose of the present study was to continue the exploration of the biological effects of *M. pubescens*. Indeed, the prepared essential oil and its fragrant water have been the subject of an antibacterial investigation. However, most of the microorganisms tested were resistant to the tested samples.

Keywords: GC-MS, volatile composition, bioactivity, spontaneous plant, arid zones

INTRODUCTION

Matricaria pubescens (Desf.) belongs to the *Asteraceae* (Chemli R., 1997), it is an annual plant and in spring it begins to appear spontaneously in its native areas in the Sahara of Algeria. Guertoufa is its vernacular name; it is widely appreciated in these regions for its agreeable fragrance. Nomad and cities habitants used it to preserve and to give a good smell to their food; they collect it from its native areas and sale it in week-markets. For traditional medication, the species is used alone or with other medicinal plants to treat various ailments such as spasm, colic and digestive troubles. In previous studies, it has been demonstrated that the plant showed interesting biological activities such as antimicrobial anti-inflammatory and anticancer effects (Makhloufi A. et al., 2014, Gowsiya S. et al., 2014, Maiza K. et al., 2014). To the best of our knowledge, no previous investigations were performed about the volatile composition of this plant from Biskra city except some ones which reported a study of the essential oil of *M. pubescens* growing in other region of Algeria (Tadrent W. et al., 2014).
The present paper presents a continuance to our work on the spontaneous species of Algerian Sahara; it aims at the exploration of the volatile chemical composition of this plant. Furthermore, an investigation of the antimicrobial activity was performed for this fraction and the resulted fragrant water.

MATERIAL AND METHODS

Extraction of the essential oils: *M. pubescens* plant material was collected at the flowering stage from Biskra city (Algeria) where latitude longitude coordinates are respectively, 34°51’1.37’’N, 5°43’40.98’’E (http://latitudelongitude.org/dz/biskra). Dr O. Oulad Belkhir agronomist from the faculty of biology, university of Ouagla, carried out plant identification. The aerial parts of *M. pubescens* were air-dried at room temperature in the dark. The essential oil was obtained by hydrodistillation using a Clevenger apparatus for 3 h. The essential oil (sample1) was collected directly by simple physical separation of the distillate, while the odorant water (sample2) was the remaining aqueous phase after decantation. Both samples were stored at 4 ºC.

GC-MS Analysis: The essential oil sample was analyzed using gas chromatography with MS detection. In fact, an Agilent HP6890 GC-MS system was used; it was equipped with a mass-selective detector using electron impact ionization. The separations were performed on HP-5MS a non polarcapillary column (30 m x 0.25 mm; film thickness 0.25 µm). The temperature program of the analysis started at 60°C that was maintained for 8 minutes and then increased to 220°C by 2°C / minute, secondly it ramps at 10°C to reach 295°C and held constant for25 min. Injector temperature was set at 250 ºC. Helium was the carrier gas at 1 ml/min flow rate and the Splitless mode was applied. Concerning MS detection, it was used 70 eV for the electron impact ionization energy and the scan mode ranged from 20 to 550 (m/z). Kovats indices (Ir) for all the compounds were determined according to Van Den Dool equation (Van Den Dool H. and Kratz P. D., 1963) using retention times of n-alkanes serie (C5-C31), that had been injected after the essential oil under the same chromatographic conditions. Detected components were confirmed by comparison of their retention indices with those reported in published works (AdamsR.P., 1989, http://www. flavornet. org/d_kovats_ov101. html, Zhu M. et al, 2008, Bélanger A. et al., 2008) as well as by comparison of their mass spectra with those of standards in Wiley, NIST libraries. Quantitative data were obtained electronically from TIC %without the use of correction factors.

ANTIBACTERIAL ASSAY

Microbial strains: The antimicrobial activity was evaluated by paper disc diffusion and dilution method towards Gram-negative and Gram-positive referenced bacteria; *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 70060, *Proteus mirabilis* ATCC 49452, *Enterobacter cloacae* ATCC 13047, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212. Moreover, against the following microorganisms that were taken from patients; *E. coli, K. pneumonia, S. aureus, Streptococcus, Aspergillus niger and Candida albicans.*

Disc diffusion method (aromatograms): The qualitative antibacterial test was performed using culture growth at 37°C for 18 h and adjusted to approximately 108 colony forming unit per milliliter (CFU/ml). The Mueller Hinton Agar (MH) medium was used for the bacteria culture and Sabouraud agar medium for yeasts. 500µl of the inoculums were spread over plates containing (MH) and a sterilized Whatman paper discs (0.5 cm) impregnated with 5 µl of the essential oil (sample 1) or the aromatic water (sample 2) were placed on the surface of the well dried media. The plates were left for 20 min at room temperature to allow the diffusion of the samples. Plates were incubated at 37°C during 24 h for those containing bacteria and 5 days for those of yeasts. The inhibition zone observed around the antibiograms after incubation was measured. All experiments were repeated in triplicate. (Boussaada et al.(2008) and Yeşil-Çeliktaş et al., (2007).

Dilution method and determination of MIC: The following dilutions in DMSO were prepared from the essential; 25%, 50% and 75% and a disc containing only DMSO was the blank of this assay. The minimum inhibitory concentration (MIC) is the lowest concentration of the essential oil giving no visible growth in the naked eye (Kaloustian et al., 2008).

RESULTS AND DISCUSSION

GC-MS analysis: The hydrodistillation of *M. pubescens* aerial parts provided a yellow essential oil (sample 1) with an agreeable smell with a yield of 0.1 % v/w. From the data obtained from GC-MS analysis, we were able to identify the main composition of sample 1, as it is shown on table 1. Among them the following major compounds were revealed: spathulenol (10.59%), isoelmicin (4.70%), decanoic acid (5.03%), terpineol (3.09%), cadinol (1.59%), and in a second order it was present Z-ocimene (0.53%) and α-pinene (0.23%). As it can be seen on the table 1, other constituents were also present in the studied sample.

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Table 1: Compounds from *Marticaria pubescens* essential oil.

Ir (exp)	Ir (ref)	Tic (%)	Compound	SM (exp)	SM (ref)
801	801	0,64	Hexanal	44(100), 41(99), 43(84), 56(83), 57(68), 27(50), 55(32)	44(100), 56(82), 41(66), 43(53), 29(38), 27(33),72(19)
928	932	0,23	α-pinene	93(100),91(36),92(29),77(28), 41(23),79(22),105(12),121(11)	93(100),91(45),92(44),77(36), 41(32),79(27),105(12),121(11)
1040	1040	0,53	Z-ocimene	93(100),91(52),79(41),92(39), 41(34),77(33)	93(100),79(50),91(42),41(40), 77(36)
1119	1121	3,09	Terpineol	43(100),79(36),139(34),93(34),71(32) ,69(31),111(30)	43(100),93(46),139(35),41(33),79(3 3),1(32),111(30)
1162	1162	0,43	Borneol	95(100),41(25),110(20),43(19),55(16) ,39(15),67(1),93(11)	95(100),41(30),110(18),43(16),39(1 4),55(13),67(12),93(10)
1193	1203	0,74	Trans-*p*-menth-2-en-1-ol	84(100),83(39),41(35),139(35),43(30) ,91(20),55(2)	84(100),41(56),43(38),83(37), 55(34),139(31),91(28)
1281	1285	0,36	Bornylacetate	95(90),43(70),93(46),121(39), 41(32),55(20),69(19)	95(100),43(75),93(45),121(34),41(2 7),55(15),69(13)
1399	1373	5.03	Decanoic acide	60(100),73(91),41(60),43(54), 129(47),55(45),71(34),29(30)	60(100),73(88),41(55),43(55), 55(40),129(38),71(36),29(34)
1415	1441	0.56	Ionon-5,6-epoxide	123(100),43(57),41(15),39(10),55(7)	123(100),43(59),41(25),39(15),55(1 4)
1571	1578	10.59	Spathulenol	43(100),41(67),91(63),205(58),119(5 27),93(47),79(44),105(43),159(34)	43(100),41(69),91(42),119(42),93(4 0),105(34),159(33),205(33)
1573	1573	0,97	Caryophyllene oxide	43(100),41(94),79(30),93(64),91(60), 69(56),95(42),55(42), 67(41)	43(100),41(93),79(56),93(66), 91(57),95(42),69(40),55(39), 67(37)
1599	1600	0,83	Humulene epoxide2	43(100),41(53),55(35),67(25),109(21	43(100),109(44),67(302),55(23)
1608	1592	0,82	Calaren epoxide	41(100),43(37),69(34),55(30), 91(26),93(24)	41(100),69(53),93(41),55(40), 91(39),109(37)
1631	1612	0.69	Viridiflorol	43(100),41(78),81(50),93(49), 55(47),79(43)	43(100),41(68),81(33),55(32), 93(30),79(28)
1636	1640	1.59	Cadinol	161(100),43(44),204(36),105(26),41(25),81(24),119(19),79(18)	161(100),43(94),41(47),204(47),10 5(39),81(36),79(29),119(26)
1656	1654	4.70	Isoelemicin	208(100),193(89),77(20),79(17),133(15),209(14),165(13)	208(100),193(69),209(16), 79(10),77(9),133(7),165(7)
1664	1675	0.54	Asaron	208(100),193(48),77(20),91(19)	208(100),193(49),91(17),77(15)
1981	1962	2,65	Palmitic acide	43(100),41(87),73(83),55(75), 60(71),57(69),69(48),71(36),	43(100),41(75),60(58),55(53), 73(52),57(48),69(24),71(19),
2303	--	0,55	Heptacosane	57(100),43(79),71(64),85(47), 41(40),55(39),69(21),56(19), 29(18)	57(100),43(80),71(62),85(42), 55(28),41(28),69(18),56(14), 29(13)

It can be noticed that the most of the relative percentages of other compounds are insignificant. But in spite of these low values, the chromatogram shows that they represent a great part of the separated compounds. In fact, these substances constitute also the essential oil of the studied plant and give it certainly the pleasant characteristic odor. More careful studies are required to clarify this chemical composition. As it can be seen on the table 1, more than 90 % of main composition of *M. pubescens* essential oil is constituted by oxygenated terpenes. Spathulenol (10.59%) was identified as the major constituent, in addition of isoelemicin (4.70%) and decanoic acid (5.03%). In previous study (Tadrent W. et al., 2014) spathulenol, with a close percentage (0.1%-19.4%) is the only common compound in the main composition of the essential oil of *M. pubescens*. Also, it was reported other main constituents in this oil: *α*-bisabolol oxide B (0.1%-7.0%), *α*-bisabolol (5.2%-56.9%) and *α*-bisabolol oxide A (10.2%-53.6%). That probably returns to the different collection origins of this plant, which are related to abiotic factors such as the specific region climate and geographical factors such as altitude and the nature of the soil of Biskra and its surroundings.

Antimicrobial assay: Essential oil of *M. pubescens* presented a variable efficiency against the tested germs; results are reproduced on table 2. In fact, *E. cloacae, E. coli and S. aureus* revealed the great

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sensitivity to sample 1 but *E. faecalis* and *K. pneumonia* strains were more resistant towards this sample. Table 2 shows also that only the pure oil has presented medium to weak action against these last bacteria in addition of *P. mirabilis*, when the other dilutions from sample 1 were inactive. Generally, the absence of a zone of inhibition does not necessarily mean the inactivity of the tested sample, sometimes some product propagates slowly in the culture medium that delays their effect (Bensizerara et al., 2013). Otherwise, as it is shown on table 3 all bacteria isolated from patients were strongly resistant to both tested samples; except a moderate effect of sample 1 against *E. coli*. In comparison with the previous results, it is possible that this negative action is not due to the inefficiency of our samples but to these microbial strains themselves. In fact, they are frequently used in our hospitals where they show this strong resistance to the prescribed antibiotics. Concerning the inefficiency of the fragrant water, it returns probably to its hydrophilic composition that could not penetrate through the lipophilic membranes of microorganisms. Other studies such as (Mighri et al. (2001) reported that essential oils from an Asteraceae had an antibacterial activity against *S. aureus* and *P. aeruginosa*.

Table 2: Diameter (mm) of the inhibition zone.

% of E.O	***E. cloacae***	***P. mirabilis***	***E. faecalis***	***K . pneumoniae***	***E. coli***	***S. aureus***
100%	+++	++	+	+	++	++
75%	+++	-	-	-	++	++
50%	++	-	-	-	++	++
25%	++	-	-	-	++	++

(+++): 20mm; (++) 10 ≤ D ≤ 20mm; (+): D ≤ 10mm; (-): negative

Table 3: Results of microbial sensibility (microbial strains from patients).

	Samples	
Microbialstrains	**E.O**	**Odorant water**
E.coli	S (++)	R
Streptocoque	R	R
S. aureus	R	R
K . pneumoniae	R	R
Aspergillus niger	R	R
Candida albicans	R	R

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CONCLUSION

The composition of the essential oils of *M. pubescens* species growing in Biskra (Algeria) was partially determined. A screening of the antimicrobial activity of the isolated essential oil and its fragrant water were also determined. GC-MS analysis revealed the presence of spathuleol (10.59%), decanoic acid (5.03%), isoelemicin (4.70%), terpineol (3.09%), palmitic acid (2.65%), cadinol (1.59%). Microbial strains of reference were more sensitive to the essential oil however; microbial strains taken from patients were strongly resistant. In both cases, fragrant water was inactive against tested microorganisms. The specific chemical composition influenced clearly such activity.

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FULL PAPER

CHARACTERISATION OF VOLATILE COMPOUNDS OF *MENTHA PULEGIUM* VIA HS-SPME

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Abstract

Mentha pulegium L. is a herbaceous perennial plant belonging to the Lamiaceae family. The plant grows up in moist areas of the mountains and plains. In therapeutic applications, this plant and its active constituents have been used traditionally for several disorders. Their diuretic, antispasmodic, digestive, diaphoretic, sedative, antiseptic, stimulant and expectorant effects have been reported before. The aim of this study is the determination of the volatile and semi-volatile oil content of *Mentha pulegium*. The studied species was collected from its natural habitat. The powdered plant samples were analysed directly on a Shimadzu QP2010 system. Two SPME fibres preferred for analyses; 85 µm Carboxen/PDMS and 85 µm polyacrylate. The volatile oil compositions were identified in *Mentha pulegium* samples by SPME-GC/MS analysis. According to the analyses, significant differences were observed between the two SPME fibres. The major volatile component for 85 µm Carboxen/PDMS fiber were 2-Isopropyl-5-methylhex-2-enal (30.37%) and for 85 µm polyacrylate fiber 2(3H)-Benzofuranone, 3a, 4, 7,7a-tetrahydro-3a-methyl (48.59%), respectively. In addition, the most common and shared components to both fibers were, p-Menthone (14.15%- 8.82 %) and thymol (phenol, 5-methyl-2-(1-methyl ethyl)) (8.68 %- 7.67 %). In conclusion, *Mentha pulegium* is a plant that is rich in essential oils, and this study is the first study about the volatile content of plant with SPME method.

Keywords: Yarpuz, SPME-GC-MS, Turkey.

INTRODUCTION

Mentha is a member of the Lamiaceae family and is distributed throughout the world. *Mentha pulegium* L. is one of the *Mentha* species and is mostly known as pennyroyal (Chalchat et al., 2000). Mentha is a perennial herb and has a pungent odour, is grown over a wide area in Turkey (Eryigit, 2006). The plant is native to North Africa, Europe and the Middle East (Aghel et al., 2004; Debbab et al., 2009). The plant contains a variety of secondary metabolites, such as essential oils, resins, tannins, and pectins (Simon et al., 1984; Zargari, 1990). *Mentha pulegium L.* is a plant rich in terms of essential and volatile oils, especially. As far as we know the volatile content of pennyroyal have reported at several studies before and pulegone is the principal volatile component (Zargari, 1990; Lorenzo et al., 2002; El Fadl & Chtaina, 2010; Benayad et al., 2008; Boughdad, 2011). And also the chemical composition of pennyroyal oil is defined by various studies (Mkaddem et al., 2007; Benayad, 2008; Hajlaoui et al., 2009; Derwich et al., 2010; Boughdad, 2011). Pennyroyal oil is characterised by the preponderance of pulegone (70-90%) along with the other monoterpenic ketones such as menthone, isomenthone and piperitenone (Bruneton, 2009). The pennyroyal oil and its constituents exhibit interesting biological activities such as antibacterial, antioxidant, antifungal and insecticidal (Jazani et al., 2009; Derwich et al., 2010; Hmiri et al., 2011; Alpsoy et al., 2011; Benayad et al., 2008; Boughdad, 2011).

Firstly, to understand the biological effect of the plants, it is necessary to know its volatile components. Gas chromatography–mass spectrometry (GC–MS) is commonly used to determine the volatile components of various aromatic and medicinal plant species. According to the literature, headspace solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) has gained broad approval as an effective extraction technique for various samples in the last twenty years (Cuevas-Glory et al., 2007; Panighel, & Flamini, 2014; Xu et al., 2016). Even though the

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volatile composition of *Mentha pulegium* has been studied before, there is not enough research about SPME-GC / MS in the literature. At present study aims to reveal the volatile constituent of *Mentha pulegium* species naturally grown in Turkey by using HS-SPME technique.

MATERIAL AND METHODS

Material: The *Mentha pulegium* samples were collected from its natural habitat. The plant samples are dried at room temperature without sunlight. Dried plants pulverised by helping a hand grinder. The ground samples (3gr) sealed in a 10 ml vial. The SPME fibres preferred for analysis were 85 μm Carboxen/PDMS and 85 μm polyacrylate. The SPME apparatus was directly injected into the upper space of the vial to adsorb volatile substances and then directly injected into the Shimadzu QP2010 ULTRA GC-MS apparatus using a Restek Rxi-5 MS capillary column.

HS-SPME-GC/MS Analysis: The volatile components were separated for about 15 minutes with different SPME fibers attached to a GC injector (the temperature of the injector was 250 °C). The plant material analysed on a Shimadzu QP2010 ULTRA FID GC–MS system. A Restek Rxi-5 MS column (30 m×0.25 mm id, film thickness 0.25 μm) used with helium as a carrier gas. The GC oven temperature was programmed to hold at 40 °C for 3 min and then to increase to 240 °C at 5 °C/min, finally holding at 240 °C for 3 min. The column flow rate was 1.8 mL/min. The ion source temperature was 200 °C, and the interface temperature set at 250 °C.

Evaluation of data: After the analysis, each sample was demonstrated with GC–MS chromatograms. Chromatograms of all samples were exposed to noise reduction before peak area integration, and then the peak areas of compounds in the chromatogram were combined. Compounds were identified by comparison with three libraries, W9N11, SWGDR4G4 and SWGDR4G5. Compounds were mostly found in the W9N11 library and specified with other libraries. Identification was performed based on the retention time (RT) of the components in the sample. The relative percentage of the volatile oil constituents was calculated from peak areas. All analyses were carried out triplicate, and all data are means of three independent analyses.

RESULTS AND DISCUSSION

The results obtained from the analysis of pennyroyal by two different SPME fibres are shown in **Table 1.** Totally 102 compounds were separated and identified from the studied samples. The major constituents of the investigated taxa with 85 μm Carboxen/PDMS fiber were 2-Isopropyl-5-methyl-2-hexenal (30.37 %), p-menthone (14.15 %) and thymol (8.68 %). For the other fiber 85 μm polyacrylate; 2(3H)-Benzofuranone, 3a, 4, 7, 7a-tetrahydro-3a-methyl (48.59 %), p-menthone (8.82 %) and thymol (7.67 %). 24 compound is common for two fibres. As reported earlier, the volatile components in *Mentha* taxa have various biological activities, usage areas and medical values (Su et al. 2014). One of the most common compounds is 2-isopropyl-5-methyl-2-hexenal, which is used as a flavouring component. Another compound menthone is a mono terpen, which naturally has a minty taste in some essential oils. Menthone is used in flavour, perfume and cosmetic products with its characteristic aromatic and tiny scent. According to our analyses, there were precious components available in the content. For example, α-pinene (0.12 %), β-pinene (0.11 %), Carvacrol (1.28 %), Limonene (0.13 %) and 1,8-Cineole (0.15 %). Our results are compatible with previous studies. Zekri et al., (2013) reported the volatile content of *Mentha pulegium* L. oils from from three regions of Morocco and they found similar results with us. Díaz-Maroto et al., (2007) studied the volatile composition of pennyroyal oil extracts via SDE method. In this study, 41 components were determined, and rates were a little more than our results. This difference may result from the used method. The SPME method which we preferred, is much more sensitive in identifying the compounds, and this sensitivity allows identification of small components in the content. Furthermore, the essential oil content of a plant can vary greatly depending on the plant's habitat, chemotype and process.

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Table 1. The volatile composition of pennyroyal plant via HS-SPME (RT=Retention time, nd=not detected)

		Fibres			
		85 µm Carboxen/PDMS		**85 µm polyacrylate**	
	Compound Name	**RT**	**% area**	**RT**	**% area**
1.	Hexanal	4,357	0.53	nd	-
2.	Heptanal	7,834	0.14	nd	-
3.	Alpha pinene	9,009	0.12	nd	-
4.	Beta pinene	10,675	0.11	nd	-
5.	3-Octanol	11,596	2.31	11,620	1.18
6.	Octanal	11,843	0.07	nd	-
7.	Benzene, methyl(1-methylethyl)	12,645	1.51	nd	-
8.	Limonene	12,812	0.13	nd	-
9.	1,8-Cineole	12,897	0.15	nd	-
10.	2-Ethylhexanol	12,970	0.06	nd	-
11.	Ocimene	13,285	0.07	nd	-
12.	Cyclopentane, 1-methyl-2-propyl-	13,595	0.07	nd	-
13.	p-menth-2-en-1-ol	14,037	0.27	nd	-
14.	trans sabinene hydrate	14,368	0.40	14,388	0.11
15.	Cis-sabinene hydrate	15,599	0.26	nd	-
16.	Linalool	15,737	3.84	15,746	1.52
17.	Nonanal	15,888	0.12	nd	-
18.	1-(3,4-methylenedioxyphenyl) ethane-1-ol	16,374	0.06	nd	-
19.	Octyl cyclobutanecarboxylate	16,725	0.12	nd	-
20.	Cis-Verbenol	17,450	0.14	nd	-
21.	2-Isopropyl-5-methyl-2-hexenal	17,797	30.37	nd	-
22.	Nonenal	18,029	0.17	nd	-
23.	p-Menthone	18,166	14.15	17,772	8.82
24.	Borneol l	18,259	1.74	18,243	0.64
25.	Isopulegone	18,615	5.96	18,623	1.86
26.	Z-citral	19,160	0.23	nd	-
27.	1-alpha-terpinyl acetate	19,271	0.71	nd	-
28.	3-Decanol	19,595	0.06	nd	-
29.	1-Tetradecene	19,832	0.11	nd	-
30.	2-Cyclohexen-1-one, 2-(2-methyl-2-propenyl)	19,930	0.24	19,915	0.04
31.	Cis piperitone oxide	20,191	6.86	20,187	2.73
32.	Carveol	20,465	0.45	20,365	0.35
33.	Alpha sinensal	20,836	0.27	20,719	0.37
34.	1,2-cyclohexanediol, 1-methyl-, trans-	21,439	0.38	nd	-
35.	Piperitone	21,562	0.32	21,551	0.17
36.	2-Decenal	21,840	0.07	nd	-
37.	2,6,6-trimethyl-cyclohex-1-enecarboxylic acid	21,904	0.51	21,904	0.25
38.	Carvone	22,192	0.18	22,192	0.06
39.	2-Acetyl-4, 4-dimethyl-cyclopent-2-enone	22,616	0.12	22,619	0.12
40.	Bicyclo[3.2.0]heptan-2-one, 5-formylmetyl-6-hydroxy-3,3-dimethyl	22,824	3.04	22,828	2.40
41.	Thymol	23,050	8.68	23,058	7.67
42.	Linalyl butyrate	23,244	0.07	nd	-
43.	Carvacrol	23,366	1.28	23,376	1.13
44.	1-hexadecyl-3-ol, 3,7,11,15-tetramethyl	23,571	0.10	23,578	0.05
45.	2,4-Decadienal	23,795	0.30	nd	-
46.	Cyclohexasiloxane, dodecamethyl-	24,360	0.26	nd	-
47.	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyldiene)	24,644	1.99	24,653	1.69
48.	Trans, cis,-3-ethylbicyclo[4.4.0]decane	24,840	0.40	24,947	0.30
49.	2-Heptyl furan	24,937	0.50	nd	-
50.	2-Hexen-1-ol, propanoate	25,060	0.20	nd	-
51.	2-Cyclohexen-1-one, 2-hydroxy-6-methyl-3-(1-methylethyl)	25,289	0.19	nd	-
52.	Cyclohexanone, 2-(1-methylethylidene)-	25,524	0.06	nd	-
53.	Butyric acid, dodecyl ester	25,770	0.07	nd	-

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54.	Copaene	25,894	0.15	nd	-
55.	Beta bourbonene	26,199	0.18	nd	-
56.	Beta funebrene	26,400	0.23	nd	-
57.	Cinerolone	26,706	0.24	nd	-
58.	Caryophyllene	27,360	0.69	27,371	0.05
59.	Carane, 4,5-epoxy-, trans	27,855	1.44	nd	-
60.	Aromadendrene	28,007	0.53	28,018	0.05
61.	2(1H)-Naphthalenone, octahydro-3-methyl-, (3alpha,4abeta,8aalpha)	28,157	0.26	nd	-
62.	Alpha humulene	28,485	0.91	28,496	0.07
63.	Hotrienol	29,144	0.16	nd	-
64.	Gamma muurolene	29,238	0.18	nd	-
65.	Germacrene-D	29,381	0.34	nd	-
66.	2_hydroxy-4’, 5’- dimethylacetophenone	29,762	0.37	29,772	0.42
67.	Beta bisabolene	30,248	1.89	30,255	0.46
68.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-	30,434	0.09	nd	-
69.	Delta cadinene	30,723	0.13	nd	-
70.	Mint furanone II	30,859	0.75	29,849	0.78
71.	Limonen-6-ol, pivalate	31,858	0.20	31,868	0.07
72.	Caryophyllene oxide	32,575	0.07	32,585	0.02
73.	12-Oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl-	33,371	0.12	nd	-
74.	Laurinsaeure, 4-octylester	46,362	0.07	nd	-
75.	Phospine oxide, trihenyl-	55,882	0.44	nd	-
76.	M-Cymene	nd	-	12,669	0.11
77.	Bicyclo[3.1.1.] hept-3-en-2-ol, 4, 6, 6-trimethyl	nd	-	17,444	0.06
78.	p-Menthan-3-one	nd	-	18,165	3.28
79.	Trans-p-Mentha-(7), 8-dien-2-ol	nd	-	19,110	0.05
80.	Alpha terpineol	nd	-	19,224	0.34
81.	Cyclohexanone, 2-isopropyl-2,5-dimethyl	nd	-	20,000	0.34
82.	Methanone, (1-hydroxycyclohexyl)phenyl	nd	-	20,296	0.30
83.	1,6,6-Trimethyl-8-oxabicyclo[3.2.1.]	nd	-	20,630	0.04
84.	2(3H)-Benzofuranone, 3a, 4, 7, 7a-tetrahydro-3a-methyl	nd	-	21,133	48.59
85.	benzene,1-methoxy-4-methyl-2-(1-methylethyl)	nd	-	21,226	1.74
86.	1-(1,1-Dimethylethoxy)-6-methyl-1-cyclohexene	nd	-	21,414	0.25
87.	13-Octadecenal	nd	-	23,818	0.04
88.	Benzene, 2,4-diisocyanato-1-methyl-	nd	-	25,082	5.66
89.	2-cyclohexen-1-one, 3,4,4-trimethyl-	nd	-	25,534	0.09
90.	7-Isopropyl-7-methyl-nona-3,5-diene-2,8-dione	nd	-	26,730	0.54
91.	Pyrrole-3-carbonitrile, 5-formyl-2,4-dimethyl	nd	-	26,793	0.56
92.	2-hydroxymethyl benzimidazole	nd	-	26,890	1.47
93.	Pulegone	nd	-	27,864	0.73
94.	Cyclohexane 1-methyl-4-(1-methylethylidene)	nd	-	28,166	0.21
95.	2,5-methano-1H-inden-7-ol, octahdyro-	nd	-	29,154	0.04
96.	Beta bisabolene	nd	-	30,255	0.46
97.	Mint furanone I	nd	-	30,871	0.19
98.	Spathulenol	nd	-	32,410	0.02
99.	12-oxabicyclo[9.1.0]dıdeca-3, 7-diene	nd	-	33,392	0.06
100.	1(2H)- Quinolinecarboxylic acid, 6-amino	nd	-	36,197	0.11
101.	4-Isopropoxybutanol	nd	-	40,862	1.12
102.	2,5-Dimethyl-4-hydroxy-3-hexanone	nd	-	52,186	0.66

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CONCLUSION

In this study, *Mentha pulegium* species from Turkey was analysed via HS-SPME-GC/MS method. As far as we know this study is the first study about the volatile composition of the plant with the SPME method. It is crucial to reveal the valuable natural compounds which have a large number of effects in the different plant species. Such studies, which are attempting to determine the active substances from plants, are of great importance in terms of more specific studies to be carried out in the future.

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FULL PAPER

EFFECTS OF OLIVE LEAF (*OLEA EUROPEA* L.*)* EXTRACT ON THE GROWTH PERFORMANCE AND SOME BLOOD PARAMETERS OF COMMON CARP (*CYPRINUS CARPIO)*

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Abstract: Olive leaf is the most important source of oleuropein known in nature. Several researchers revealed the fact that oleuropein has many pharmacological qualities including antioxidant, antimicrobial, antiaterogenic and antiviral activities. In this study, the effects of olive leaf extract on growth performance and some blood parameters of common carp were evaluated. For this purpose, 3 different experimental diets containing rate of 0% (OLE0), 0.25% (OLE025) and 0.5% (OLE05) olive leaf extract were prepared. Olive leaf extract were obtained from Talya Herbal product. The olive leaf extract that we used in study include oleuropein at the rate of 2.3%. This study consisted of 3 repetitions for each group with 71.58±0.66 g and 200 L aquarium as 25 fish per aquarium for 60 days. The objective of this study was to determine the effects of olive leaf extract on the growth performance and some blood parameters in the common carp. At the end of the experiment, the weight increase, specific growth rate and feed conversion rate were found to be similar to the trial groups ($p>0.05$). The dietary supplementation of OLE did not affect the RBC count, hemoglobin concentration, hematocrit ratio, mean corpuscular hemoglobin concentration, mean corpuscular volume and mean corpuscular hemoglobin ($p>0.05$). In serum biochemical findings of fish, olive leaf extract was found to be a significant difference among the groups ($p<0.05$). Results in the present study showed that the olive leaf extract can use in common carp diets without adverse effects on growth performance, hematological and serum biochemical parameters of fish.

Key words: *Cyprinus carpio*, Olive leaf (*Olea europea* L.) extract, Blood parameter, Growth performance

INTRODUCTION

Olive (*Olea europaea* L.) and its derivatives are very important products in both Turkey and the surrounding Mediterranean and Middle East regions (Bircan, 2006). Olive leaf is the most important source of oleuropein known in nature. Several researchers revealed the fact that Oleuropein has many pharmacological qualities including antioxidant, antimicrobial, anti-inflammatory, antiaterogenic and antiviral activities (Visioli et al., 1998; Owen et al., 2000). In addition, it is stated that oleuropein has a strong antioxidant effect with its quality of bonding endogenous peptides in addition to its anti-inflammatory, anti-atherosclerotic and anticarcinogenic properties (Gikas et al., 2007). Recent studies determined that the medical plants are effective on the growth parameters of fishes (Yilmaz et al., 2017a; Yilmaz et al,. 2017b). Also, It has been documented that natural products such as, *Azadirachta indica* extract (Harikrishnan et al., 2003), *Astragalus radix* and *Ganoderma lucidum* (Yin et al., 2009), stimulate the immune system and disease resistance in *Cyprinus carpio*.

MATERIAL AND METHODS

Olive leaf extract used in the present study was obtained from Talya Herbal product, and was supplemented in test diets at 0%, 0.25% and 0.5%, which were then named as OLE0,OLE025,OLE05 groups, respectively. In this experiment commercial fish diet (48% crude protein, Gümüşdoğa, Muğla) was used (Table 1). A control group was prepared without any supplements. The addition of olive leaf extract to feed was carried out by the spraying method. Feeds were prepared weekly and stored in capped glass bottles at +4ºC to preserve the efficacy of the components of the olive leaf extract. During the experiment that lasted 60 days, fishes were manually fed 3 times a day in a ratio of 2% of their body weight.

Table 1. Proximate analysis of the commercial diet (2 mm) and olive leaf extract *

Nutrient	Diet percentage%
Crude Protein (%)	48
Crude Lipid (%)	18
Crude Cellulose (%)	3
Crude Ash (%)	12
Moisture (%)	12
Macro elements (%)	
Calcium (%)	1.2
Total Phosphore %	1.5
Meth+Cyst (%)	1.8
Lysine (%)	2
Compound	Olive leaf extract (OLE) percentage %
Oleuropein	2.3
Epicatechin	4.7
Quercitrin	5.2
Rutin	4.1
Quercetin	7.5
Vanillic acid	2.3

Ingredients: Fish oil, fish meal, soybean and by products, wheat and by products, yeast and by products, amino acids, vitamins and minerals. The ratios of phenolic compounds in the extracts of olive leaf was taken from Talya Herbal Company (Certificate of analysis 2018)

Cyprinus carpio fishes were obtained from a local trout fish farm in İzmir, Turkey. The US-EPA (United States Environmental Protection Agency) guidelines for qualitative assessment of fish health were followed for the visual and external inspection of each fish (Klemm et al., 1993). At the start of the trial, a total of 225 fish with initial mean weight of 71.58±0.66 g were randomly distributed into nine 200-L aquariums (25 fish per aquarium). During the experimental period water quality identified as follows: temperature 24.40±0.29°C, pH 7.11±0.39, salinity 0.5 ppt, total dissolved solid (TDS) 11.90 mg/l and dissolved oxygen 8.48±0.21 mg/L. Proximate analyses of the diets were performed using standard methods (AOAC, 2000). Moisture was detected after drying at 105˚C until a constant weight was achieved. Crude protein was analyzed by the Kjeldahl method, and crude ash by incineration at 525˚C in a muffle furnace for 12 h. Crude fat was analyzed by methanol/chloroform extraction. The haematological and biochemical analyses were conducted in the private Detay Analysis Laboratory. At the end of the 60-day feeding trial, blood was taken from a total of 9 fish/ group, 3 fish from each aquarium. After the fish were caught randomly from each aquarium, they were anesthetized with clove oil (Mylonas et al., 2005). They were well wiped and cleaned in order to avoid mucus mixing into the blood, and blood was taken from the fish through the caudal vein by a 5-mL plastic syringe without harming the fish (Val et al., 1998). The blood samples were put into the K_3EDTA tubes and haematological and biochemical analyses were done. Haematological parameters, such as RBC count, HCT, hemoglobin (Hb) and mean cell hemoglobin concentration (MCHC), were determined by a blok cell counter (Mindray BC-3000 Plus, China) within 2 h. Blood serum was separated by centrifugation (3000g, 20 min). The clear supernatant was aspirated carefully for the estimation of various blood chemical parameters in serum. The biochemical parameters like creatinine, total protein, glucose, albumin, globulin, uric acid, urea, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and cholesterol were assessed by the automatic analyzer apparatus (Reflotron plus, Roche, Germany) using commercially available Reflotlon kits (Roche Diagnostics, Germany) for the above mentioned blood chemical parameters (Bain et al., 2006). The analyses of the data obtained in the experiment were done by using the SPSS 17 statistics program. One way variance analysis (ANOVA) was applied to the data which were then subjected to Tukey multiple comparison test. The differences between groups were evaluated to be $p<0.05$. Fish experiments were performed in accordance to the guidelines for fish research from the animal ethic committees at Adnan Menderes University (Protocol Number:2018/085).

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RESULTS

It was determined that the specific growth rate (SGR) values among the growth parameters showed similarity for all groups. With regard to survival rate and feed conversion ratio (FCR), the groups were similar to each other. Nutrient component analysis results showed that there was no statistically difference between moisture, lipid, crude protein and crude ash amounts of all groups (Table 2.)

Table 2. The growth performance and proximate composition of common carp

	Control	OLE 0.25%	OLE 0.50%
Initial fish weight (g)	71.82±0.88	71.26±0.81	71.65±0.40
Final fish weight (g)	134.71±4.04	136.44±0.82	137.39±2.66
Weight gain (g)	62.88±3.16	65.18±0.80	65.74±2.87
FCR	0.81±0.05	0.80±0.00	0.80±0.01
SGR (%/day)	1.04±0.03	1.08±0.01	1.08±0.03
Survival (%)	100	100	100
Crude Protein	12.32±0.25	12.77±0.87	12.55±0.25
Crude Lipid	3.29±0.38	3.80±0.56	3.38±0.06
Crude Ash	2.44±0.83	2.58±0.58	2.10±0.34
Moisture	76.29±0.99	75.27±0.88	75.78±0.79

*The different letters on the lines indicate that differences are important ($p<0.05$)

The hematological parameters were presented in Table 3. Olive leaf (*Olea europea* L.) extract used in study did not significantly affect on the hematological parameters ($P>0.05$).

Table 3. Hematological parameters of *C. carpio* fed diets containing olive leaf extract (OLE)

Blood Parameter	Control	OLE 0.25%	OLE 0.50%
Hb (g/dL)	13.74±0.20	14.06±0.18	13.96±0.19
Hct (%)	22.53±3.05	24.64±1.24	26.13±4.65
RBC (10^6 mm^3)	1.48±0.15	1.49±0.09	1.55±0.16
MCV (μm3)	153.70±26.55	165.52±3.45	168.60±30.84
MCH (pg $cell^{-1}$)	93.66±9.57	94.69±6.76	90.50±10.08
MCHC (%)	61.79±7.36	57.18±3.36	54.81±9.64

Values are mean ±SE (n=9) Hct: hematocrit; Hb: hemoglobin; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration

The serum biochemical parameters were presented in Table 4. It was found that the values of albumin, globulin, glucose, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, total protein, cholesterol, urea, uric acid and creatinine among serum biochemical parameters have statistical difference among groups ($P<0.05$). Also, urea and uric acid levels were similar in all the treatment groups ($p>0.05$).

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Table 4. Serum biochemical parameters of *C. carpio* fed diets containing olive leaf extract (OLE)

Parameter	Control	OLE 0.25%	OLE 0.50%
ALB (g dL^{-1})	1.04±0.13^{b}	1.18±0.03^{ab}	1.24±0.05^{a}
GLO (g dL^{-1})	3.70±0.09^{c}	4.06±0.20^{b}	4.36±0.11^{a}
TPROT (g dL^{-1})	4.80±0.03^{c}	5.31±0.08^{b}	5.64±0.11^{a}
GLU (U l^{-1})	192.±8.74^{a}	174.20±7.94^{b}	160.20±8.49^{b}
GOT (U l^{-1})	60.92±5.82^{a}	52.32±6.24^{ab}	44.47±5.12^{b}
GPT (U l^{-1})	27.59±1.56^{a}	20.34±1.00^{b}	18.37±1.00^{b}
CHOL (mg dL^{-1})	138.40±3.84^{a}	122.40±2.30^{b}	116.80±4.65^{b}
Urea (mg dL^{-1})	0.16±0.01^{a}	0.16±0.01^{a}	0.14±0.00^{a}
Uric acid (mg dL^{-1})	1.32±0.05^{a}	1.30±0.08^{a}	1.32±0.10^{a}
Creatinine (mg dL^{-1})	0.39±0.02^{a}	0.28±0.02^{b}	0.22±0.02^{b}

Values are mean ± SE (n=9). Different letters represent the significant differences at $P<0.05$
ALB.albumin; GLO.globulin, TPROT.total protein; GLU.glucose; GOT=glutamic oxaloacetic transaminase; GPT=glutamic pyruvic transaminase; CHOL.cholesterol ment. Values are mean ± SEM (n=3)

DISCUSSION

In the present study, olive leaf (*Olea europea* L.) extract did not change significantly average weight gain, specific growth rate (SGR) and feed conversion rate (FCR) (p>0.05). Similarly, in a study by Baba et al. (2018), it was observed that there is no change in the final body weight (FW), spesific growth rate (SGR) and feed converstaion ratio (FCR) of rainbow trout fed by a diet containing 0.25% and 0.50% olive leaf (*Olea europea* L.) extract (p>0.05). Haematological analyses are important in determining the fish health and decreases in the amount of erythrocyte and haematocrit as well as haemoglobin destruction are considered to be indicator of anemia. The amount of erythrocyte sedimentation gives information about the existence of diseases. Total leucocyte amount and types of it are used in the diagnosis of diseases (Blaxhall and Daisley, 1973). In this study, the olive leaf (*Olea europea* L.) extract used in study did not significantly affect on the red blood cell count, hematocrit, hemoglobin, mean corpuscular hemoglobin concentration, the mean corpuscular volume and mean corpuscular hemoglobin level (p>0.05). In our study, the serum biochemical parameters, i.e. the values of albumin, globulin and total protein were found to be higher in the groups fed with olive leaf extract than the control group (P<0.05). In a different study, low utilization of garlic decreased total protein (Sahu et al. 2007a). In a different study which involved addition of mango to feed, a decrease of albumin in the ratio of 0.5 g/100g was reported (Sahu et al., 2007b). In our study, it was observed that the blood glucose level in fishes fed by olive leaf extract were lower in the trial groups than the control group. In the study of Baba et al., (2018), it was observed that the blood glucose level didn't change in fishes fed by olive leaf. When we look at the studies involving the use of vegetable resource, it is noticed that the plants of *Cynodon dactylon*, *Aegle marmelos*, *Withania somnifera*, ginger, mango and garlic decreased the blood glucose values. The serum enzymes including GOT and GPT are the indicators of damages that occurred in tissue and organs (Campbell, 2004). Our study shows that olive leaf extract causes decrease in the amounts of GOT and GPT. Similarly, the study by Baba et al. (2018) found that the amounts of GOT and GPT decreased in *O. mykiss* fishes fed by the olive leaf extract. Another study found decrease in the amounts of GOT and GPT in the *O. niloticus* fishes fed by garlic (Shalaby et al., 2006). The present study found that the olive leaf extract decreased the cholesterol amount in *Cyprinus carpio*. In a study which involved the addition of *Cynodon dactylon, Aegle marmelos, Withania somnifera* and ginger to the feeds of the *Oreochromis niloticus,* it was found that cholesterol significantly decreased (Immanuel et al., 2009). In another study involving the addition of olive leaf extract to the trout feeds, it was seen that there was no change in the cholesterol amount (Baba et al., 2018). This shows that different plant species may have different effects on fat metabolism. The reason of it may be explained by the fact that the components of plants and their mechanisms of action are different. Therefore, the increases of urea in blood are considered to be an

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indicator of gill and liver diseases rather than kidney (Stoskopf, 1993; Campbell, 2004). Creatinine in fishes consists of creatine and excreted by kidneys (Campbell, 2004). Uric acid in fishes consists of purine nucleotides as exogen and endogen. Later on, uric acid is converted to urea by liver. Kidneys have small effect in this conversion and urea is removed lastly from gills (Stoskopf, 1993). The present study demonstrated that the urea and uric acid amounts in *Cyprinus carpio* fed by olive leaf extract didn't change and that the creatine amounts decreased.

CONCLUSION

In conclusion, it was found that olive leaf extract has no negative effect on the metabolism of *Cyprinus carpio*. The positive effect of adding olive leaf extract to feed in the ratios of 0.25% and 0.50% on immunity shows that it can be used as a feed additive.

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FULL PAPER

EVALUATION OF THE RELIABILITY OF PLANTS USAGE IN FOODS: A REVIEW

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Abstract: Functional foods is a concept that have been put forward in recent years. A food given an additional function (often one related to health-promotion or disease prevention) by adding new ingredients or more of existing ingredients is known as functional. Functional foods are products that provide significant benefits on the human physiology and metabolic functions beyond the body's need for essential nutrients, thus enabling to protect from diseases and achieve a healthier life. Although the concept of functional foods is new, its application is very old. As the side effects of the drugs have been learned and health problems have increased, the orientation to functional foods have increased and the products are diversified. Functional foods have been even more than normal food (Ozcelik, 2016). The functional food markets, whose trade volume has exceeded $ 100 billion around the worldwide, has increased in terms of quantity and diversity in developed countries such as the USA, the European Union and Japan. In Turkey, the functional products market for 2015, it is stated that the ratio reached to 600 million Turkish Liras (Anonym,2014). The usage of plants for food industry to obtain functional food products has become a notable issue in recent years. A rising interest for medicinal and aromatic plants and the active substances obtained from them has led to studies to determine the effects of these plants. The purpose of his review is todetermine and evaluate the reliability of plants in terms of food and drugs effets.

Key Words: functional food, plant, reliability

1.INTRODUCTION

The function of functional, medically useful, regulating, special-purpose nutrients, which are not nutrients, but which have benefits and functions like nutrients, are called "phytochemicals" and the number of these chemicals is expressed in thousands. Basic functions of this type of foods are to collect the free radical-containing metabolites and inactivate them as well as to stimulate the immune system and to regulate antibacterial and antiviral effects in the cellular and intercellular environment activities. (Aksoy, 2007; Dundar, 2001). The purpose of this study is to look for the variety of plants or their ingredients for being used to produce functional food.

1.1. Evaluation In Terms of Food Industry: Approximately 1/3 of the 300 plant families grown in nature contain essential oil. The most known families with their volatile oils are: Pinaceae, Laureceae, Myrtaceae, Rutaceae, Laminaceae (Labiatae), Apiaceae (Umbelliferae), Zingiberaceae, Asteraceae (Compositae), Piperaceae, Irridaceae, Chenopodiaceae, Verbenaceae, Brussicaceae and Ranunculaceae. Some of these families have a special importance. For example, in the Labiatae family, Thymus, Lavandula, Melissa, Mentha species and some other plants found in many Mediterranean and European countries are valuable volatile oil sources (Ceylan, 1996). The amount and composition of volatile oils in Lamiaceae plants depends on the light (Johnson et al. 1999), the availability of nutrients from the plant (Skoula et al., 2000) and season (Kokkini et al., 1997).

The antioxidant activity of some aromatic plants is related to the amount of secondary components in plant structure and phenolic compounds in the structure (Skerget et al., 2005). The amount of these components varies from plant to plant due to individual factors (morphogenetic, ontogenetic, diurnal and ecological factors), genetic and genome differences (Ceylan, 1996). Among these compounds, the most common are flavonoids, phenolic acids and phenolic terpenes (Javanmardi et al., 2003). Antioxidant effect of phenolic compounds is to clean the free radicals (Rice-Evans et al., 1995; Pekkarinen et al., 1999),to compound with the metal ions (metal chelating) and to prevent or reduce singlet oxygen formation (Rice-Evans et al. (1995).

Flavonoids and other phenolic compounds are mostly found in the leaves, flowers and woody parts of the plant (Kähkönen et al., 1999). For this reason, generally aromatic plants are used as a form of extracts of volatile oil obtained by methods such as extraction, drying. (Baytop, 1999) or distillation (Botsoglou et al., 2003a). There are many companies exporting products from plants in the Aegean Region, where has a great potential for aromatic plants. The primary criterias formed for many plants and plant products found in our food chain are important for researchers and firms. For example, the basic phytochemical groups found in fruits and vegetables are commonly used in daily life; Polyphenols, flavones, phenytoids, are the most powerful microchemicals in terms of antioxidant activity. Because of these properties, they protect cells by inhibiting LDL oxidation. At the same time, it has been determined that, primarily polyphenols, isoflavones and flavonoids have a change effect on metabolic profile of steroids and p450 substrates. (Fuhrman et al., 1997).

In vitro studies have shown that flavonoids have a wide spectrum of physiological, pharmacological activity ranges including the antiallergic, anti-inflammatory, antibacterial, antifungal, antiviral and anticancer. Carrot, citrus, strawberry, apple, raspberry, broccoli, ginko bloba, black and green tea, parsley, soybeans, cereals, cabbage, squash, potatoes, tomatoes, cucumbers, vegetables and fruits are rich sources of flavonoids (Van Hethof et al., 1997). There are many studies that test the efficacy of plant products or plant origin products against pathogens and saprophyte microorganisms; These studies have shown that microbial safety of foods can be protected in this way. For this purpose, the plants themselves (leaves, body, bud, flowers, fruits, seeds, onions and rhizomes) as well as the compounds obtained from plants (extracts, essential oils) are used.

1.1.1. Fruit and Vegetable Juices: It has been known that it has an inhibitory effect on microorganisms due to its low pH environment and / or its antimicrobial components that depend on the origins. Many fruit and vegetable juices, especially lemon juice, which is frequently used as acidifying and flavoring in foods, have antimicrobial activity on various microorganisms. Lemon juice obtained from lime; *Serratia spp.*, *Salmonella paratyphi*, *Shigella lexnerri*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter spp.*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus feacalis*, *Clostridium spp.*, *Bacteroides spp*., and *Porphyromonas spp*. It has been reported that it has inhibitory activity in varying proportions (Aibunu et al, 2007).

1.1.2. Spices: It is also known that it is used to extend the shelf life of foods by showing antimicrobial activity on pathogenic and saprophyte microorganisms as well as flavor and aroma enhancing properties. (Hsieh, 2001). According to the researchs, it was observed that he cinnamon (960,1, 0.2 and 0.3) added to the pasteurized apple juice decreased the L. *monocytogenes* by 4 - 6 log cfu / ml, and the number of *E. coli* O157: H7 was decreased by around 1.6-2 log cfu / ml; It was reported that the vanilla (260.10, 0.15 and 0.20) decreased the number of *L. monocytogenes* in orange juice by 2-3 log cfu / ml and the mint added to tomato juices decreased the microbial flora by 4.77 - 8.34 log cfu / ml.

Clove and garlic were tried against to *Bacillus sphaericus, Staphylococcus aureus, Si. epidermidis, Enterobacter aerogenes, E. coli, P. aeruginosa, Salmonella Typhi and S. flexneri* microorganisms and it was found that garlic nd cleve had he inhibitory effect on these microorganisms. When it comes to mint, 11 different gram negative bacteria strains were tried. (*E.coli, K. pneumoniae, P. aeruginosa, S Typhi, S. Paratyphi A, S. Paratyphi B, Proteus mirabilis, Proteus vulgaris, E. aerogenes, Shigella dysenteriae, Y. enterocolitica*). It was found to have inhibitory effect against these microorganisms.

Mustard flour used in mustard prepartion was tested against pathogens of *S. typhimurium*, *E. coli* O157: H7 and *L. monocytogenes* at two different storage temperatures at a concentration of 0.10; At 5 * C on 1st, 3rd and 5th days, at 22 ° C, 12th and 24th hours, respectively, it was seen that it reduced the number or microorganisms below the detectable level (Oncul and Karabiyikli 2014).

1.1.3. Essential Oils: The antimicrobial activities do not depend on a single mechanism, but are based on the presence of many target points on the cell and different groups of chemical components. The mechanisms of essential oils on the bacterial cell is degradation of the cell wall, separation of the cytoplasmic membrane and membrane proteins, leakage of cell components, coagulation of the

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cytoplasm, and loss of proton repellent force. The environmental and genetic factors such as species and / or sub-species of the plant, part of the plant used, isolation methods, harvesting time, growing conditions cause modifications in chemical composition (Oncul and Karabiyikli 2014).

1.1.4. Plant extracts Most of the plant extracts, which are evaluated as GRAS, are thought to have a wide range of application in the food industry as a flavoring agent and preservative, and many in vitro studies have been carried out on some extracts with high antimicrobial activity. Several studies have shown that extracts can offer alternative solutions to conventional food additives, preservatives and disinfectants. It was observed that the amount of 0,1%- 0,2% roseberry and its exracts had the inhibitory effect on the some food borne microorganisms (*Listeria innocua, L. monocytogenes, Lactobacillus sake, Leuconostoc mesenteroides, Leu. dexiranicum, Leu. carnosum, Lb. curvatus Brochothrix thermosphacla, Br. thermosphacta, Lactococcus lactis)*. The effect of cranberry and cinnamon mixture (1: 1) was test on *L. Monocytogenes* and it has been reported that the mixture had inhibition effect on the microorganism. The antimicrobial activity of the sumac extracts were tested on different microorganisms (*B. cereus, B. subitilis, St. aureus, H. pylori, L. monocytogenes, E. coli, E. coli Il, Salmonella spp.*) was tested and it was stated that *B. cereus* and *H. pylori* were highly sensitive among the tested microorganisms and inhibition value of the extracts containing sumac was higher than the others. The antimicrobial activity of the plant extracts prepared with ethanol was tested against *S. typhimurium, E. coli* 0157: H7, *L. monocytogenes* at various ratios of 6-7 log, and it was stated that clove was the most effective plant for inhibition among the others. At the next stage of the study, the lettuce samples were contaminated with pathogen cultures at different inoculation levels (6.80 log cfu / g, 6.70 log cfu / g and 5.31 log cfu / g) and then treated with 1, 3, 5 and 10 minutes with clove extracts. . It has been reported by researchers that clove extract provides 3 log inhibition within 3 minutes. The contents of these extracts obtained from different foods depend on the origin; It has been reported that antimicrobial activity is generally caused by compounds that have the polyphenol structure (Oncul and Karabiyikli 2014).

1.2. Evaluation In Terms of Pharmaceutical Industry: Nowadays, there is a tendency for the natural products, this idea is increasing in order to avoid the health problems caused by advancing technological processes. Pharmaceutical companies based on the use of traditional plants, have identified and synthesized the active ingredients of some of them and have been able to predict their effects in the body with the dose standardization.

Treatment with plants is known as complementary-salternative medicine (CAM). In spite of this widespread usage, the definition of side effects of phytotherapy have not been yet available in the clinical side of the modern medicine and it is estimated that few of the unwanted situations occured due to plant based treatment are reported. There are also no obligation regarding explaining the side effects of these products (Gozum et al, 2007). There very few data about the interaction of plants used for treatment purposes with other drugs. For example, the interaction of some plants with anticoagulants has begun. Plants can change the effects of the drugs in the body and the bioavailability rates. Their interaction with nutrients is an issue that has never been evaluated (Bush et al, 2007).

1.3. The Side Effects of Medicinal Plants: There is an evidence that some endemic plants have toxic effects on the liver. For example, they may increase the liver enzyme levels. *Sauropus androgynus* and *Aristolochia* species are reported to cause hepatitis, bronchiolitis obliterans, renal failure and even death. The hepatotoxic effect of a plant taken with a drug may be higher than expected. *Echinacea purpurea* (echinacea), which can be used for colds, flu, and nausea, can cause vomiting and cogulation of blood cells. *Ephedra* (ephedra, ma huang) species, which are called as Herbal ectasi, are found in slimming teas, blends and energy drinks and they are used for alternative purposes against drugs such as fenfluramine, dexfenfluramine (TC Health Ministry, Turkish Hygiene and Experimental Journal of Biology, 2009).

Also, some plants cause unwanted side effects when they are consumed with drugs. For example, herbs used for depression (*Hypericum perforatum*, St. John's wort, yellow centaury) are sold as edible herbal Prozac and may inhibit the enzyme monoamino oxidase (MAO) as well as increase serotonin, dopamine and norepinephrine levels. Therefore, they should not be used with prescribed

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antidepressants. In the literature, it is reported that this herb can cause adverse events such as gastrointestinal disorders, weakness, confusion, dizziness, dry mouth, hair loss, manic disorder, hyperactivity, irritation, allergy and sensitivity to light (Schey et al, 2000). Table 1 shows the information about some plants and part of plants known as positive (P) or negative (N) and used for medical purposes.

Table 1. Some plants and part of plants known as positive (P) or negative (N) and used for lots of purposes.

NO	LATIN NAME	USED PART	POZITIVE/ NEGATIVE	FUNCTION	USAGE AREA	SOURCE
1	*Abies sp.*	Whole plant	P	Containing volatile oil	Aroma Industry	Park J,2005
2	*Acer saccharum*	Plant Juice	P	High content of glucose	Glucose Syrup Production	Kork K et al,2002
3	*Achillea millefolium*	Flower	P	Effective on circualation system	Pharmaceutical Industry	Candan F et al,2003
4	*Acorus calamus*	Risome	N	Natural painkiller	Pharmaceutical Industry	Balakumbahan R et all 2010
5	*Acorus tatarinowii*	Risome	N	Effective on nervous system	Medicine	Liao W ve Zhang X,2005
6	*Actinidia chinensis*	Fruit	P	High content of Vitamin C	Food Extract Production	Huang S. et al,2013
7	*Adonsonia digitata*	Fruit	P	Antioxidant	Bakery Product Production	Kabbashi A.et al ,2014
8	*Aesculus hippocastanum*	Plant body shell	P	Microvaxuloki netic Activity	Pharmaceutical Industry	Gitelson A and Merzlyak M,1994
9	*Aframomum meleguetta*	Seed	N	Antimicrobial Activity	Pharmaceutical Industry (extracts are solved in the alcohol)	Okigbo RN and Ogbonnaya UO,2006
10	*Alchemilla alpina*	Plant Body	P	High content of nitrogen phosphorus	Transgenic Plant Research	Micheal D et al,1996
11	*Alchemilla vulgaris / A. arvensis*	Plant Body	P	High content of bioactive biochemicals	Herbal Medicine	Altameme H et all,2015
12	*Alkanna tinctoria*	Root	N	High content of alkaline esters	There is no enough research	Assimopoulou A et al,2004
13	*Allium cepa*	Seed	P	Antimicrobial	Food Industry as an addtive	Benkeblia B,2004
14	*Allium ursinum* L.	Leaf	P	High content of volatile compounds	Inhibitory agent	Djurdjevic L,2004
15	*Alpinia galanga*	Risome	P	Bio adhesive effect	Medicine	Oonmetta-aree, J et al,2006

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4. Conclusion and Suggestions: Nowadays, the production of funtional foods containing the bioactive components found in the plants become more popular. Especially directly plant tea consumption is one of the most pereferred way. Agreat deal of plants has beneficial extracts for health and using these extracts with a certain limits provide more healthy food products. However, the use of these products together with the drugs directly and inadequate information about these plants can lead to a chain of events that cause death. Unfortunately, the potential damages are not discussed in scientific environments. As a solution, academicians intereseted in this field should inform the people with their scientific based researches. Further studies must be done about plant mostly used for healing purposes as well.

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FULL PAPER

STUDY OF THE VARIETAL BEHAVIOR OF 6 BASIL VARIETIES (*OCIMUM BASILICUM* L) GROWN FOR THE FIRST TIME IN ALGERIA UNDER ARID CLIMATE

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Abstract: The objective of the present research is to study the behaviour of six varieties of basil, *Ocimum basilicum* L., in the edaphic-climatic conditions of Ouargla region. **Methods**: To achieve the plotted objectives, we have taken a number of parameters of growth, bloom, and yield. **Results:** The ANOVA analysis of all parameters showed that our tests were significant to very highly significant with the F computed above the theoretical F of 1%. **Conclusion**: The climate of the Algerian Sahara is adapted to the cultivation of new varieties of basil.

Keywords: Varietal behaviour, *Ocimum basilicum* L, growth parameters, yield parameters, arid climate.

INTRODUCTION

The history of aromatic and medicinal plants is associated with the evolution of civilizations with the aim of emphasizing the growing economic importance of the cultivation of aromatic and medicinal plants in the industrial investigation. And as part of the valorisation of the natural resources of arid zones, we have chosen to study the varietal behaviour of 6 varieties of basil of European origin in a Saharan climate, Hassi Ben Abdellah-Ouargla south-east of Algeria. Few agronomic studies have been carried out on basil. On the site, the test of Carron et al, which is a test of culture of basil in Mountain, ~~this test~~ was carried out in Belgium on five varieties of basil: great green, lettuce, green, purple, and exotic in 1996 and 1997 [1]. The objective of our trial is to study the behavior of six varieties of basil *Ocimum basilicum* L. in the edaphic-climatic conditions of the Ouargla region. In order to achieve the plotted objectives, we have taken a number of parameters of growth, bloom, and yield. We evaluated the obtained results using the statistical study of ANOVA.

MATERIAL AND METHODS

1.Climatic Data of the Agricultural Companion: The maximum temperature during the cycle of the crop does not exceed 33.8°C, and the minimum temperature is in the order of 4.2 °C. Humidity is high in January (68.1%) and with a significant evaporation in May [2].

Study Material:

Soil: The soil is characterized by a sandy texture, that is a basic pH with a very low organic matter rate. Soil salinity is low, C. E varies from 0.43 to 0.41 DS/m.

Irrigation Water: Irrigation water is pumped from the Alwelle aquifer which is at a depth of 1450 m. According to the Riverside classification, irrigation water belongs to the C_4S_4 class. The use of this water is very delicate and it requires a very permeable ~~soil~~, well drained, and washed out soil.

Characteristics of Organic Fertilization: Organic fertilization is characterized by an acid pH (6.11) and a high electrical conductivity of the order of 10.10 DS/M.

Experimental Protocol

Experimental Device: The adopted experimental device is in complete random blocks, consisting of 6 treatments and 4 repetitions. Each block contains 6 elementary plots of 2 m in length on 1.5 wide.

Plant Material: The material studied is composed of six (06) Varieties of basil *Ocimum basilicum* L. (c.f. table 1)

Table 1: Presentations of Plant Material

Variety	Common name	Scientific name
V1	Great Green Basil	*Ocimum basilicum* L. Genovese
V2	Purple Basil	*Ocimum basilicum* L. Purpurescens
V3	Dwarf Basil Compact	*Ocimum basilicum* L. Minimum
V4	Basil Marseille	*Ocimum basilicum* L. Marceillais
V5	Basil Cinnamon	*Ocimum basilicum* L. Cinnamon
V6	Lemon basil	*Ocimum basilicum* L. Citriodora

Conduct of the Cultivation: 1. Tillage*:* The tillage is carried out on a ploughing of 25 to 30 cm depth using a hoe. **2. Fertilization:** The organic and mineral bottom fertilization is applied to standards of 9 kg of manure from cattle per elemental parcel and 3 qx/ha of 15-15-15 per ha; therefore, (45 u N, 45 u P_2O_5 and 45 u K_2O) with the addition of phosphorus in the form of TSP: 55U/ha. **3. Nursery Seedlings:** Seedlings are made in the "nursery area" with containers filled with peat at a weight of 1.20 g of seeds to monitor our culture well. The irrigation is done on a daily basis. **4. Pre-irrigation:** Pre-irrigation has a dual purpose, to wash the salts and moisten the soil with its holding capacity. **5. Transplanting:** The plants we have planted have been of good vigour and biometrics of a height of 10 cm. Planting density was 11 plants with a spacing between the 30-cm planes and between the 40-cm lines per elemental plot.

Statistical Analysis of the Results: The obtained results for the studied parameters of the six varieties of *Ocimum Basilicum* L. were subjected to an analysis of ANOVA. This analysis allows testing the similarity of variables in statistical terms. The calculated F study and its comparison to the theoretical F, in particular the treatments, makes the meaning of the test out.

RESULTS AND DISCUSSION

Duration of the Cycle: The date of flowering is an important parameter as it allows judging the maturity of the varieties. Two periods are considered, the sowing-flowering period and the planting-flowering period. The duration of the seedling-flowering cycle of the experimented plants varies between 74 days for Purple (V2), which has the longest duration and 100.5 days for the Compact Dwarf (V3) with the most limited duration (Figure 1). Similar results are obtained for the parameter (transplanting-flowering cycle time) (Figure 2).

Plant Height: Based on the reported results in Figure 3, plant height varies very highly significance as a result of variety. The Great Green (V1) gave the best height (40.92 cm) against Marseille (V4) which is the variety that gave the lowest height (18 cm). The works of Philip and Staci [3] show that the height of the great green can reach 60 to 90 cm. According to another study, the height of basil varies from 20 to 50 cm, which reinforces the obtained results in the experimental range [4].

Mortality Rates : In general, plants with high mortality rates are the least suitable for edaphic-climatic conditions or those with low vigour at the time of transplantation. The analysis of variance reveals a very highly significant difference in the mortality rate parameter between the studied varieties. The highest rate is presented by Marseille (V4) with 40.62% which proves to be the least adapted to the Saharan conditions. The other varieties gave values below 3.12% (large green) or even zero (purple, dwarf compact and cinnamon) (Figure 4).

Total Biomass (leaves and stems): Total plant biomass is influenced by a number of factors including climatic conditions, fertilization and variety. The results show a very highly significant variation in the total biomass of the different tested varieties, varying between 82.84 g and 19.70 G.

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The best weight is obtained with the Dwarf Compact (V3) 82.84 G; however, the lowest weight is recorded by the Purple (V2) 19.7 g (Figure 5).

Number of Branches per Plant: The number of branches per plant is variety function and water supply and fertilization. The analysis of variance reveals a very highly significant difference between treatments. The highest number of ramifications is presented by the Lemon variety (V6) with 13.24 branches per plant but the lowest value is recorded by the Dwarf Compact (V4) and cinnamon (V5) with 7.47 and 9.6 ramifications per consecutive plant (Figure 6).

Leaf Weight/Total Biomass Ratio: The variance analysis reveals a highly significant difference between the varieties. The results presented in Figure 7 show that Purple (V2) and Marseille (V4) provided the most important ratio (0.87) while the Lemon (V6) gave the lowest ratio (0.74).

Number of Leaves per Plant: The number of leaves is an important parameter because the more important the plant has a large number of leaves, the more important the vegetative weight is. The results shown in Figure 8 show that the Compact Dwarf (V3) recorded the most important number of leaves (2861.12) while Purple (V2) provided the lowest number (176.37). The analysis of variance reveals a highly significant difference in this variable.

Leaf Area: The analysis of variance reveals a very highly significant difference in the tested varieties. Leaf area of leaves varied between a minimum of 9 cm 2 obtained with the Compact Dwarf and a maximum of 17.62 cm 2 obtained for the large green (V1) (Figure 9). According to Carron et al [1], the Great Green leaves are 3 to 4 cm in length.

Branch Width: The results shown in Figure 10 show that the width of the variety ramifications varies between 36.55 and 19.02 cm. The Great Green (V1) recorded a significant value; however, the basil Marseille (V4) provided the lowest width. The analysis of variance reveals a very highly significant difference between the studied varieties.

CONCLUSION

The statistical study of the varietal behaviour of six varieties of *Ocimum basilicum* L., 4 of them grown for the first time in Algeria under the edaphic-climatic conditions of the Ouargla region revealed the success of our trial. The lack of similar agronomic work has not allowed us to develop a comparative study and in particular to draw distinctive conclusions. This test was performed by studying some parameters of yield, morphological, and early maturity. The analysis of all the studied parameters allowed us to conclude that the climate of the Sahara is adapted to the cultivation of new varieties of basil. These results remain preliminary but encouraging for the importance that this culture can present on the economic level given the diversity of the use of basil in the fields of aromatherapy, the food and cosmetics industry, and in culinary use as a condiment plant.

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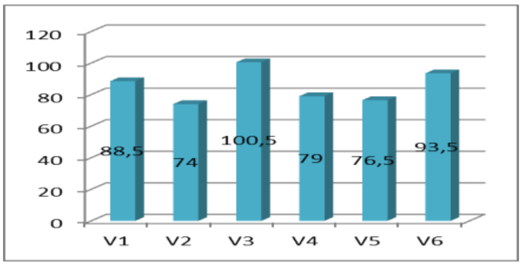

Figure 1: Effect of the variety on the duration of the sowing-flowering cycle (days)

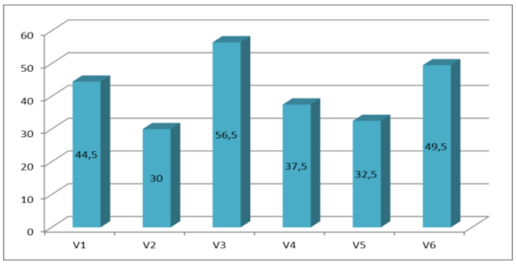

Figure 2: Effect of the variety on the duration of the transplant-flowering cycle (days)

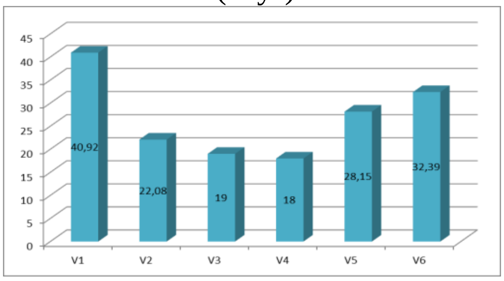

Figure 3: Variety effect on plant height (cm)

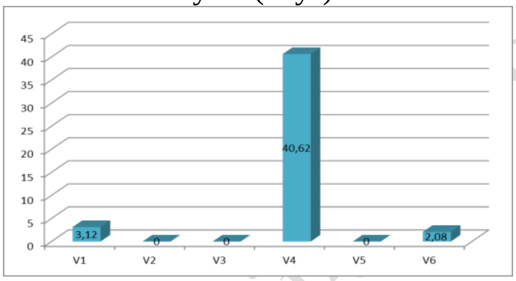

Figure 4: Effect of variety on mortality rate (%)

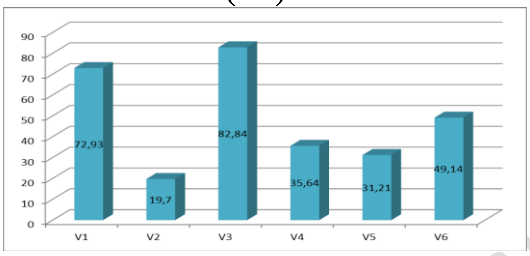

Figure 5: Effect of variety on total biomass

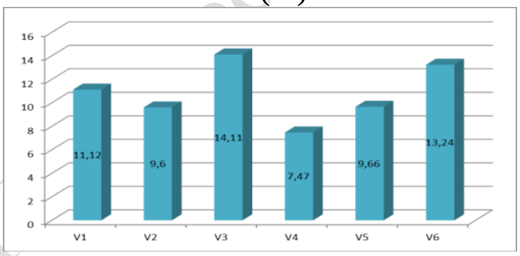

Figure 6: Effect of variety on the number of branches

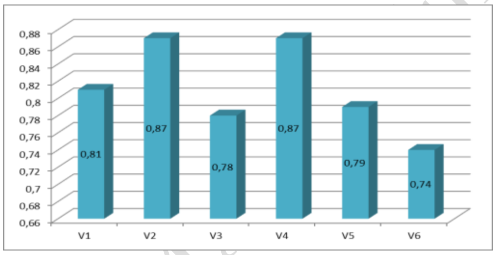

Figure 7: Effect of variety on total leaf/biomass ratio

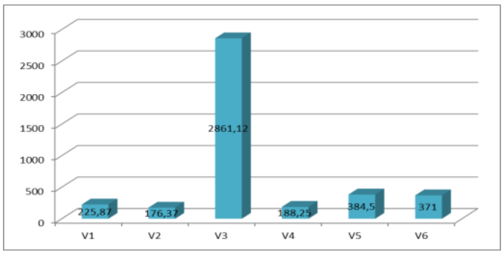

Figure 8: Effect of the variety on the number of leaves per plant

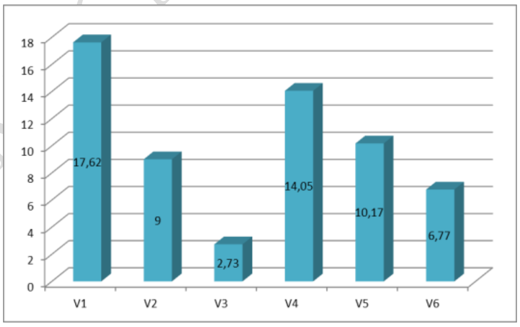

Figure 9: Effect of variety on leaf surface

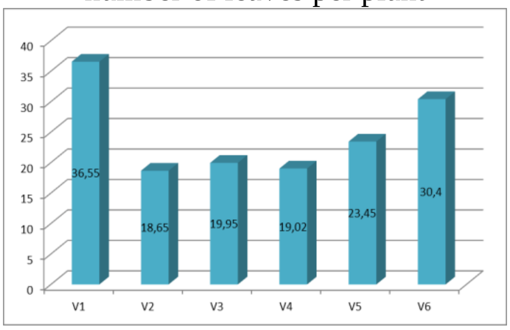

Figure 10: Effect of variety on branch width

FULL PAPER

ETHNOMEDICINAL STUDY OF *CORNUS MAS* 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 *Cornus mas* L. from the folk and traditional medicine of Republic of Macedonia, an area so far less frequently studied from the perspective of folk traditional medicinal, local food and handicraft and other uses. European cornel acts as astringent and antidiaroic. Tea, juice, fresh and dry fruits because of its antimicrobic, anti-inflammatory action are used at diarrhea, colitis and other intestine disorders. Dry fruits are used directly as a medicine against diarrhea. It acts chemo static and protective on capillary. Cornels as mountainous fruits were used by Macedonians as a food long time ago. Fruits are used dry as a food. Dry fruits are used for preparing compote or directly in nutrition in winter and spring period. From the fruit jam, marmalade, compote and juice are prepared at home and in the food industry. Dry fruits are kept in rakiya and sugar for obtaining liqueur. Alcohol beverage *rakiya* - dryankoitsa was prepared from cornels. Manufacture of hammer in Ohrid or stick in Mariovo (koledashki) for Christmas Eve (kolede) is made from a cornel branch. Handmade handle for Macedonian traditional instrument gajdarka, in Kumanovo is manufactured from cornel because it is smooth and not cracked. Cornel is a symbol of durability, solidity, and with its hardness influences the health. The district, from the ethnobotanical point of view, shows traces of the influences of the neighboring regions. Some medicinal uses are linked to beliefs or residual forms of magic prescriptions.

Key words: European cornel, medicinal, food, handicraft, use

INTRODUCTION

During the past decade, the Balkans have been the focus of a number of ethnobotanical studies that have revealed the complex relationships of humans and plants at the location of a geographical cross roads where the complex features of their history and the structure of their ethnical and religion meet. Most of the previous studies were mainly aimed at medicinal and edible plants and traditional handicrafts in the countries on the Balkans [1-8] and shed new light on existing ethnological and anthropological data.
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 *Cornus mas* 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 European cornel are described.

LOCAL NAMES

Republic of Macedonia: дрен, дренка, дренушка, петров цвет, тајане, равнец, месечник [9] [10].
Yörüks: Karamluk [11].
English: Cornelian cherry, European cornel, dogwood [10].

BOTANY AND ECOLOGY

European cornel is a deciduous shrub or small tree, reaching 3-8 m tall or slightly larger, shape is rounded. *Bark* is dark gray to reddish brown with scaly, exfoliating patches. Each node has two oppositely positioned flower buds on one-year old shoot. Leaves are ovate or ovate-elliptic up to lanceolate and narrowly elliptic, with a sharp top and round base dark green (4-11 cm long and 3-5 cm wide). Leaves are entire, and with distinctively parallel lateral *veins (up to 6 pairs of side)* arching strongly as they approach the margins. Inflorescences resembled the umbel structure. Each umbel is surrounded at the base by small, yellowish, petaloid bracts. Yellow flowers on short stalks bloom in early spring before the leaves emerge. The flowers have 4 rudimentary sepals, 4 petals, 4 stamens, and one pistil. Fruits are ellipsoid, fleshy, single-seeded, stone fruit type (drupes to 2-3 cm long) which mature to cherry red in mid-summer. Fruits (*Corni fructus*) are collected from September to October. European cornel grows most in the dry and stony places exposed to the sun, at the end of the deciduous forest, among the shrubbery throughout the entire territory of the Republic of Macedonia [9]. European cornel is a wide spread plant, beside the bushes, the roads, in the oak and beech forests and other habitats up to 1200 m above the sea level [12]. It is not indicate the exact degree of danger of the European cornel in Republic of Macedonia.

LOCAL MEDICINAL USES

Cornus mas act as astringent and antidiaroic. Tea, juice, fresh and dry fruits because of its antimicrobic, anti-inflammatory action are used at diarrhea, colitis and other intestine disorders. Dry fruits are used directly as a medicine against diarrhea [13]. It acts chemo static and protective on capillary [9].

Yörüks in Republic of Macedonia collected primarily for medicinal herbal teas (infusion) for internal cleansing [11].

LOCAL FOOD USES

Cornels as mountainous fruits were used by Macedonians as a food long time ago [14]. Fruits are also used dry as a food. Dry fruits are used for preparing compote or directly in nutrition in winter and spring period [13]. From the fruit jam, marmalade, compote and juice are prepared at home and in the food industry. Dry fruits are kept in brandy (rakiya) and sugar for obtaining liqueur [9]. Alcohol beverage *rakiya* - dryankoitsa (дренкојца) is prepared in region of Struga [14].

LOCAL HANDICRAFT AND OTHER USES

Manufacture of hammer in Ohrid or stick in Mariovo (коледашки) for Christmas Eve (коледе) was reported [15]. In Kumanovo from *Cornus mas* handmade handle for Macedonian traditional instrument gajdarka, because it is smooth and not cracked is manufactured [16].

Cornus mas is a symbol of durability, solidity, and with its hardness influences the health. The cornel tree is considered as the strongest and most healthy tree and as such is a symbol of health. Sometimes it is considered as the longest living tree, and it is an imaginable symbol of the long life. The cornel tree blooms the earliest, and because of that is regarded as a symbol of joy, youth and strength. *Cornus mas* is used during wedding rituals in order to cause agreement in marriage and fertility among spouses. *Cornus mas* is used for the health of the cattle, especially for the lambs. Early in the morning they hit the lambs, and with cornel stick cows are forced to the bulls. It is believed that such a cow will host a male calf [15].

In some areas in Republic of Macedonia people carry with them cornels stick, because they believed that the vampires run away from it. They are sheltered from witches and other unclean strengths. Gates and windows are decorated with cornel twigs, which sheltered from thunders [15]. In Porechje people collect *Cornus mas* on St. George’s day (Gjurgovden) on 6th of May, because of it durability, to be given to them [17]. When there are many cornels (cornel fruits) there will be a great winter (long winter with lots of snow). Cornel tree is small because part of it was used for the cross on which Jesus Christ was crucified, or from other traditional forecasting the bear walked out of its winter bearing earlier, after noticing that the cornels blossomed. Bear was irritated and broken the cornel tree and from than it doesn’t grow high [15].

Ritual acts with cornel twigs are carried out with the assurance that they will bring health to the participants. Such practices are connected with some holidays, mainly with Gjurgjovden (Ѓурѓовден) Saint George's Day, when people are decorated with cornel twigs, or they are sealed with the twigs

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and are swinging on *Cornus mas* to be health like it [18] [19]. Swinging on *Cornus mas* twigs is performed on Easter in Kumanovo and in Shopsko-bregalnic ethnographic entity as a symbol of new begining on Gjugjovden, to be health as cornel [20]. Also, swinging on swings tied up on green tree i.e. cornel, walnut etc is done on Gjurgjovden in the same entity [21]. People are bathing in the warm water in which cornel twigs are placed [15].

Surova or Vasilica (on 14th of January) is a holiday that marks the beginning of the Old New Year according to the Julian calendar in Shopsko-bregalnic ethnographic entity. The stick named *surovica* [суровица] or *surovaska* [суроваска] made of oak branch, or of cornel or hazelnut, served as a ritual requisite through which the magical power of the tree was transferred with the hit. Everyone in the house is hit to be healthy as a cornel. On Четерсе (St. Forty), 40 мученици, Младенац (22nd of March) from 39 twigs from *Vitis vinifera* and one cornel branch fire burns, and with the smoke the house is fumigated for moths and other animals to get away [21]. Domestically produced and hand-made brooms consisting of *Cornus mas*, nettle and *Euphorbia* are made in March and they are used for sweeping early in the morning before the sun rises [19]. Fruits from cornels are used as a nafora (antidors prosfor) [15]. Flower buds in several areas in Republic of Macedonia are used on Easter. Holy Communion was practiced with cornel flower buds and wine [15]. In the village Gorna Pcinja because of its great distance from the church, sacrament named as komka "комка" is given with cornel flower buds, nettle, *Geranium* and wine in the house [20]. Ritual act is done with cornel flower buds, nettle, wine and bread for giving the sacrament to all in the family, on the great Thursday on Easter, for God to give health [19].

DISCUSSION AND CONCLUSION

The usage of preparations obtained from *Cornus mas* 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 this area [22]. Some medicinal uses are linked to beliefs or residual forms of magic prescriptions. The use of cornels remains well alive nowadays.

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FULL PAPER

DEVELOPMENT OF MICROEMULSION CONTAINING *PLANTAGO MAJOR* EXTRACTS: FORMULATION AND EVALUATION OF TOPICAL ANTI-INFLAMMATORY ACTIVITIES

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Abstract: *Plantago major* L. (Plantaginaceae) is widely used to treat various diseases. It is reported to have anti-microbial, anti-diabetic, anti-spasm, anti-inflammatory activity and wound healing properties. *P. major* contains biologically active compounds such as polysaccharides, flavonoids, iridoid glycosides, terpenoids, and alkaloids.[1] Despite several publications supporting health benefits of this plant, not much work has been done to develop an anti-inflammatory formulation containing extracts of *P. major*. Therefore, the aim of this work was to formulate extract of *P.major* into microemulsion to increase economical value of this plant and to support the society with affordable dosage form. **Material and Methods:** Microemulsions are clear, optically isotropic and thermodynamically stable systems generally composed of a blend of oil, water and surfactant(s).[2] Three formulas were developed with variation of surfactants tween 80: lecithin, Formula 1 = 10:1, Formula 2 = 11:1 and Formula 3 = 12:1 respectively, along with cosurfactant (propylene glycol) and extract of *P. major* (0.3%). Microemulsions were stored at 4°C, 25°C, and 40°C for 4 weeks, and were characterized regarding their organoleptic, homogeneity, pH, and viscosity. Particle size was determined on day 0 and week 4 using Horiba®SZ-100 nano particle analyzer. Formula 3 was investigated for its anti-inflammatory activity using croton-oil induced ear edema in mice.[3] Percentage of inflammatory inhibition was calculated and confirmed by histological section of ears. **Results:** During 4 weeks of storage, all formula showed homogeneity and stability and met the particle size requirements for microemulsions. Topical application of microemulsions reduced ear edema and pro-inflammatory cells in the tissue and its activity was similar to hydrocortisone 1% ($p > 0.05$). **Conclusion / Discussion:** Our data indicated that *P. major* extracts can be developed into modern formulation with potent anti-inflammatory activity.

Keywords: *Plantago major*, microemulsion, topical anti-inflammation, croton oil-induced ear edema

INTRODUCTION

Ethnopharmacological studies show that *Plantago major* is used in the treatment of various diseases. It is reported to have anti-inflammatory,[1–3] anti-microbial [4] anti-diabetic,[5] anti-spasm activity,[6] and wound healing properties.[7] *P. major* contains biologically active compounds such as polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides, terpenoids, alkaloids and some organic acids.[6,8–11] *P. major* shows anti-inflammatory properties by reducing pro-inflammatory cytokine levels[1], inhibiting cyclooxygenase-2[9], and inhibiting neutrophil ROS production.[12] Despite several publications supporting health benefits of this plant, not much work has been done to develop the formulation of this plant into anti-inflammatory dosage form.

One of the practical dosage forms is microemulsion, which was first introduced by Hoar and Schulman in 1943. Microemulsion is a dispersion system comprising aqueous phase, oil, surfactant, and co-surfactant. It forms a single liquid solution that is clear, thermodynamically stable and optically isotropic having a droplet diameter usually ranging from 10 to 100 nm. Several studies have proved the potential of microemulsion to rise cutaneous delivery of hydrophilic and lipophilic drugs. Microemulsion has such advantages as the ability to increase the solubilization, dissolution rate, and bioavailability of poorly soluble drugs, the good thermodynamic stability and the easiness to produce.[13–16] Due to these advantages, it is promising to deliver the *P. major* extract into microemulsion.

MATERIALS AND METHODS

General: Unless explicitly stated otherwise, all chemicals used were of “pro analysis” quality. Ethanol, isopropyl myristate, Tween 80, Span 80, propylene glycol, methyl paraben, and propyl paraben were supplied from Sigma-Aldrich. Distilled water was used in the preparation of microemulsion. Ethanol, croton oil, ether, Phosphate Buffered Saline (PBS) and Hematoxyline-Eosin (HE), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hydrocortisone (1%) was obtained from Kimia Farma.

Ethics: All animals used in this study were obtained from the Laboratory of Pharmacology, Department of Pharmacy Universitas Islam Indonesia. Male Balb/c mice (32-36 g) were used and allowed one week to adapt to environment before experiments. Animals were maintained on a 12 h light/dark cycle in an air-conditioned room and given standard chow and water *ad libitum*. All animal experiments were approved by Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Universitas Gadjah Mada, Yogyakarta, Indonesia with reference number KE/FK/345/EC and exactly performed according to the “Principles of Laboratory Animal Care” (NIH Publication No. 86-23).

Preparation of plant extract: P. major dried herbs was purchased from Directorate Research and Development of Medicinal Plants and Traditional Medicine (B2P2TO2T Tawangmangu, Indonesia). *P. major* were macerated for 3 days using ethanol as the solvent. Extract was evaporated using rotary evaporator (Heidolph L4000) until most of its solvent evaporated.

Preparation of microemulsion: Three formulations were produced for the same percentage but different ratios of Tween 80 and Span 80, with Formulation 1 (8:1), Formulation 2 (9:1), and Formulation 3 (10:1). First, the aqueous phase, consisting of ethanol extract of *P. major*, Tween 80, methyl paraben, and distilled water, were mixed using magnetic stirrer at 50˚C. Separately, the oil phase that consisted of isopropyl myristate, propyl paraben, and Span 80 was stirred at the same condition. After that, the oil phase was added to the aqueous phase using magnetic stirrer at 50˚C to produce microemulsion.

Microemulsion characterization: All three formula were then analyzed for its characteristics including the homogeneity, viscosity, globule size, pH, and stability. Particle Size Analyzer SZ100 (Horiba, Japan) was used to measure the average size of microemulsion droplet by loading it into a clear zeta cell at 25˚C. The pH values for microemulsion were determined at 25ºC by pH meter (Horiba LaquaAct, Japan). The viscosity of optimized microemulsion was determined using Brookfield viscometer (Brookfield DV2T, USA) with spindle number 61 at 60 rpm. The physical stability of microemulsion was evaluated by visual inspection such as by phase separation, transparency, drug precipitation and color change. Three batches of microemulsion were stored at 4°C, 25°C and 40°C for 4 weeks and examined for physical stability. All measurements were carried out in triplicate.

Croton-oil induced ear edema test: Formula 3 was investigated for its anti-inflammatory activity using croton-oil induced ear edema in mice according to Patel et al., 2012. Adult male Balb-c mice (25-35 g) were randomly divided into 5 groups of 7 animals. Ear edema was induced on the anterior and the posterior surfaces of the right ear of the mice by topical application of 2 µl croton oil dissolved in 20 µl of acetone. Microemulsion based, microemulsion formula 3, and hydrocortisone (1 mg) were also dissolved in acetone and were applied with croton oil. The inflammations were measured on 4 hours after the application of croton oil. Animals were euthanized and two ear punches (6 mm diameter) were taken from each mouse. Percentage of inflammatory inhibition was then calculated and confirmed by histological section of ears.

Ear biopsies from croton oil-induced mouse ear edema: Ear biopsies were collected and fixed in 70% ethanol for 24 h and then preserved in 10% formalin. Subsequently, the ears were dehydrated, blocked in paraffin, and then sectioned with a microtome (4 μm). The cross-sections were stained with HE for the evaluation of leukocyte infiltration, vasodilatation, and edema intensity. A representative area was selected for qualitative light microscope analysis (40 and 400 x magnification).

RESULTS AND DISCUSSION

P. major microemulsion characteristic and stability: All the formulations with various ratios of surfactants formed transparent microemulsion. The microemulsion was brown and has specific odor due to its ethanolic extracts of P. major. The clarity of the microemulsion is caused mainly by the activity of surfactants and co-solvent in the formulation. The surfactants as well as the co-solvent (propylene glycol) lower the interfacial tension between the oil phase (isopropyl myristate) and the aqueous phase, so the oil droplets can disperse in the aqueous solution and yield a clear microemulsion system. The various characteristics of each formulation can be seen in table 1.

Table1. Evaluation of various characteristics of *P. major* microemulsion

Characteristics	Formulation1	Formulation 2	Formulation3
Organoleptic	Brownish color, specific odor, transparent	Brownish color, specific odor, transparent	Brownish color, specific odor, transparent
Homogeneity	Homogeneous	Homogeneous	Homogeneous
Viscosity (cP)	85.2 ± 25.3	115.2 ± 12.4	122.8 ± 6.0
pH value	6.85 ± 0.01	6.86 ± 0.02	6.88 ± 0.03
Droplet size	93.3 ± 3.06 nm	75.8 ± 1.93 nm	53.6 ±4.73 nm
Polydispersity index	0.382	0.428	0.402

Since previous studies have found that rheological property of microemulsion is of Newtonian type, the viscosity measurement in this study was performed using one shear rate for all the formulations. This resulted in the viscosity of the microemulsion that is also of Newtonian type and suitable for topical use (Table 1). In addition, there are differences in the viscosity among the three formulations due to different ratio of surfactants. The more amount of Tween, the more viscous microemulsion.

The droplet size shown in Table 1 indicated that the microemulsion had a droplet size ranging from 10 to 100 nm. The ratio of standard deviation to the mean of droplet size is shown as polydispersity index. It indicates the uniformity of microemulsion droplet size within the formulation. The lower the polydispersity index, the better the uniformity of the droplet size in the microemulsion. The polydispersity values of all formulations are about 0.4 indicating that uniformity of droplet size within each formulation is good. From stability test, the visual observation confirmed that all the formulations did not significantly change in their appearance over the storage period at 4˚C and 25˚C although there was a slight change in their viscosity, pH value, droplet size, and polydispersity index. There was a tendency for the droplet size to become smaller over 4 weeks storage. This phenomenon possibly occurred due to the solubilization of some of the extract and/or of the oil phase in surfactant micelles during the storage.

Meanwhile, Formulation 1 and 2 stored at 40˚C were not stable during the 4 weeks storage. There was phase separation in these microemulsions, which did not occur in Formulation 3 because the high storage temperature affected the activity of Tween 80. Previous study reported that in emulsions stabilized by non-ionic surfactants (e.g. Tween 80) elevated temperatures raise droplet coalescence through dehydration of non-ionic head groups. This process alters the solubility of the surfactant in the oil and water phases that can lead to phase separation.

Anti-inflammation activities of P. major microemulsion: In the topical anti-inflammatory test using croton oil induced ear edema, the negative control group demonstrated the greatest degree of edema, while the mice group treated with microemulsion of *P. major* extracts demonstrated significant reduction of the edema (Figure 1).

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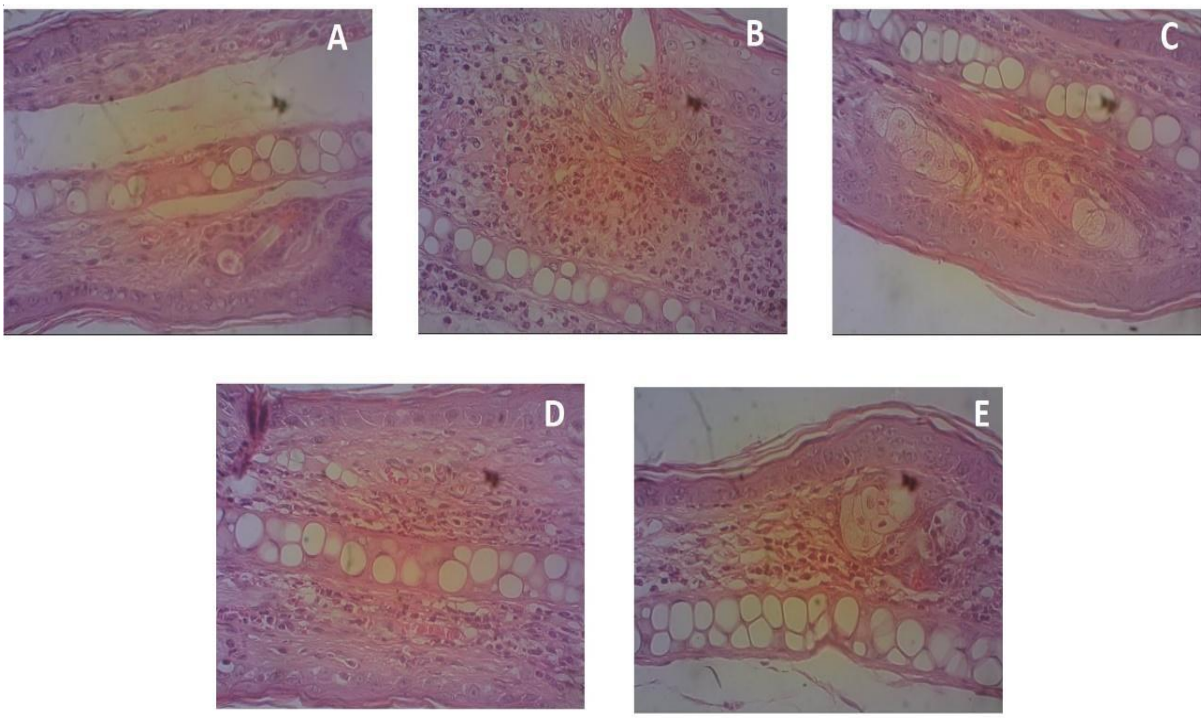

Figure 1. Photomicrograph of transverse sections of mice ears (treatments: normal group (A), croton oil (B) hydrocortisone 1% (C), microemulsion base (D) and microemulsion of *P. major* extracts (E)), stained with hematoxylin-eosin and examined under light microscopy (magnification 400x).

Croton oil (contains 12-*o*-tetracanoilphorbol-13-acetate (TPA)) topical application triggered local inflammation with edema formation and polymorphonuclear leucocytes infiltration hence suitable for studying anti-inflammatory agent that act on acute phase of inflammation [15,17] It induces an inflammatory response by activating phospholipase A2 and initiating arachidonic acid metabolites, followed by COX-1 and COX-2-induced prostaglandin production and 5-lipoxygenase-induced leukotriene production, the inflammatory mediators involved in edema and leukocyte migration.[18,19] Application of croton oil increased ear edema in negative control group strengthening the right design of this models. The histopathological analysis from ear edema exhibited dermal inflammatory reactions noted by decreasing of infiltration of leukocyte and vascular dilatation. Inflammatory cells were also counted (data not published) from the histological sections. The inflammatory cells were decreased in *P. major* microemulsion treatment group significantly different with croton oil group.

CONCLUSION

P. major microemulsion had been successfully produced by combining Tween 80 and Span 80 as the surfactants. Compared to Formulation 1 and Formulation 2, Formulation 3 containing Tween 80-Span 80 ratio of 10:1 was the most stable microemulsion after four weeks of storage at various temperatures. Furthermore, *P. major* microemulsion possess topical anti-inflammatory activities strengthened the notation that this plant may develop into a new anti-inflammatory agent.

ACKNOWLEDGEMENTS

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FULL PAPER

ANTIBACTERIAL ACTIVITY AND COMPOSITION OF ESSENTIAL OILS OF LEAVES AND BASIL FLOWERS OF THREE DIFFERENT GROWING SEASONS UNDER ARID CLIMATE

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Abstract: We wanted to study antibacterial activity of essential oils in relation to the growing seasons of the plant as the first parameter of study, and what part of plant that will present a better antibacterial activity as the second parameter of study. The relationship between this activity and the majority composition of the essential oil has been developed. **Material and Methods:** The antibacterial activity of 6 essential oils was evaluated on three two Gram-negative strains: Escherichia coli ATCC 25923 and Pseudomonas aeruginosa ATCC 27853 and one gram positive: Staphylococcus aureus ATCC 25922. The diffusion method in Agar medium (Aromatogram) is used for the study of antibacterial activity which allows determining the sensitivity of the different bacterial species towards our essential oils. The majority composition of essential oils was made by a Schimadzu QP2020 GC-MS. **Results:** According to our results, the essential oil of basil autumn flowers inhibits bacterial growth with inhibition diameters of 21.5 and 25 mm against Escherichia coli and Staphylococcus aureus, respectively. This has a direct relationship with its major composition: Linalool (34.99%), methylcinnamate (21.09%) and Methylchavicol (14.53%). **Conclusion / Discussion:** The essential oils of the flowers showed antibacterial activities better than that of the leaves and this in the three study seasons, summer, autumn, and winter.

Keywords: antibacterial activity, *Ocimum basilicum* L, essential oil, growing seasons.

INTRODUCTION

The south of Algeria is rich in its natural resources which are not well exploited. The plants with medicinal character constitute most of these resources. Spontaneous plants and aromatic plants in Algeria present a growing interest for researchers in the world. The traditional uses of plants for medicine were studied in south Algeria. Until now, 85 spontaneous medicinal plants have been identified [1]. To our knowledge, only two varieties of *Ocimum basilicum* are cultivated and marketed on a national scale in Algeria. These are: *Ocimum basilicum* and *Ocimum basilicum* minimum. The genus *Ocimum* belonging to the Lamiaceae comprises annual and perennial herbs and shrubs native to the tropical and subtropical regions of Asia, Africa, and South America [2]. Apart from culinary use, basil has traditionally been employed as a medicinal herb in the treatment of headaches, cough, diarrhoea, and constipation. It is also considered to be a source of aroma compounds and essential oils containing biologically active constituents that possess insect repellent, nematocidal and antibacterial activity. Many *Ocimum basilicum* varieties contain primarily phenol derivatives, such as eugenol, methyl eugenol, chavicol, methylcinnamate often combined with various amounts of linalool [3]. In the realm of the natural resources valorisation of arid regions in Algeria, our study, first interested in evaluating the antibacterial activities of essential oils of *Ocimum basilicum* from three different seasons and the relationship between this activity and the majority composition of these oils.

MATERIAL AND METHODS

Plant Material: The plant material used was harvested in the state of Ouargla exactly in the village of Oum Erraneb in southern east of Algeria. Oum Erraneb, agricultural zone, is a village in the municipality of Sidi Khouiled, located 18 km northeast of the Ouargla city, at latitude of 32° 03 ' 53.24 north and at a longitude of 5 ° 22 ' 33.34 east, elevated to 129 m in relation to sea level. We proceeded to separate the different organs, leaves and flowers.

Essential Oils Extraction: Samples, 1 kg of fresh material, were extracted by steam distillation for 2.5 hours. The essential oils were stored in dark glass bottles at 4°C [4] until the beginning of the analyses.

Antibacterial Activity Determination: The antibacterial activity was evaluated on three strains: two gram-negative, Escherichia coli ATCC 25923 (digestive tract infections) and Pseudomonas aeruginosa ATCC 27853 (the commensal flora of the skin in humans), and a Gram positive, Staphylococcus aureus ATCC 25922 (nosocomial infections). They are maintained by replanting on nutrient agar (NI) favourable to their growth for 24 hours, in darkness and at 37 °C [5]. The microorganisms used are pathogenic bacteria of the American type culture collection (ATCC) frequently involved in the contamination and alteration of food and often responsible for infections contracted in Hospitals [6]. The method of diffusion in agar medium (Aromatogram) is used for the study of the antibacterial activity which allows determining the sensitivity of the different bacterial species towards the essential oils studied. Petri dishes (90 mm) containing Muller-Hinton agar (MHA) are inoculated, sterile discs (6 mm diameter filter paper) impregnated with different essential oils (5µl per disc) are then gently deposited on the surface of the agar using a clamp. The whole is incubated at 37 °C in the oven for 24 hours [7]. After 24 hours of incubation, an area or a clear Halo is present around the disc. This inhibition diameter reflects the antibacterial activity of the essential oil. The result is expressed by measuring the diameter of the inhibition zone where the bacteria could not develop. The latter will be qualified: Not sensitive (-) or resistant, diameter < 8mm; sensitive (+), diameter from 9 to 14 mm; very sensitive (+ +), diameter from 15 to 19 mm; and extremely sensitive (+ + +), diameter > 20mm [8].

Determination of Essential Oils Composition by GC-MS: GC-MS analysis was performed in National Polytechnic School of Constantine, Algeria using a Shimadzu System comprising a model QP 2020. GC was equipped with non-polar capillary column HP-5 (30m * 0.25 mm, film thickness 0.25 µm). Helium was the carrier gas at a flow rate of 1.0 ml/min. The oven temperature was held at 45°C for 10 min, and increased from 45°C to 180°C at a rate of 3°C/min to be maintained at 180°C for 5 min, and then increased from 180°C to 280°C at a rate of 5°C/min to be maintained at 280°C for 5 min. For GC-MS detection, an electron ionization system with ionization energy of 70 eV, was used. Injector temperature was 280°C. Diluted samples, in dichloromethane of 1 µl, were injected in the split/splitless (100:1 split) mode. Oil identification of components analysed was accomplished based on comparison of their Arithmetic index (AI), calculated from GC-FID analysis, with those of literature [9], and by comparison of their mass spectral fragmentation patterns with those of databases.

RESULT AND DISCUSSION

***Antibacterial Activity*:** The results of antibacterial tests with essential oils (different parts each season) summarized in table 1. The results obtained showed that the essential oil of the different parts of the *Ocimum basilicum* L. had no antibacterial activity marked against the strain of Pseudomonas aeruginosa (ATCC 27853), the least susceptible to antibiotics tested. The microorganisms most susceptible to these essential oils were Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922). According to Bassol, gram-positive bacteria are more sensitive than gram-negative bacteria with essential oils [10]. The zones of inhibition of the antibiotics tested are around 19 and 30 mm. This sensitivity varies from one antibiotic to another. This activity is very strong compared to that of the oils studied. Indeed, the Autumn E.O. (essential oil) of *Ocimum basilicum* L. showed an important inhibitory effect against the microorganisms studied. According to our results, it is concluded that autumn promotes this strong activity compared to the other seasons. The inhibition diameters ranged from 8 to 18 mm for the leaves and from 12 to 25 for the flowers. According to our results, the essential oil of the flowers of *Ocimum basilicum* L. inhibits bacterial growth more than that of leaves. The results of Ijaz Hussain demonstrate that the autumn and winter E.O. are more active than in the spring and summer months: Staphylococcus aureus (24.4 ± 1.1; 24.0 ± 1.0; 23.2 ± 1.4; 22.2 ± 1.3 mm) and Escherichia coli (13.2 ± 0.8; 16.2 ± 1.0; 13.5 ± 0.8; 11.4 ± 0.6 mm) [7]. The activity of the essential oils tested could be related to their chemical compositions. According to other studies, the analysis of essential oils, flowers, and leaves of O. *basilicum* L. showed a variation in the chemical composition of one organ to another. In other words, the study of the antibacterial activity of certain constituents of essential oils made it possible to distinguish that phenolic compounds have strong antimicrobial activity, such as thymol, carvacrol, and eugenol. Constituents with low antibacterial activity are pulegone, menthone, 1.8-cineole, p-cymene, isomenthone, myrcene, α-pinene, piperitone, limonene, linalool, terpinene, sesquiterpenes, and non-terpene compounds [7]. According to these literature data, the variation in the essential oils' antibacterial activity of *Ocimum basilicum* L's leaves

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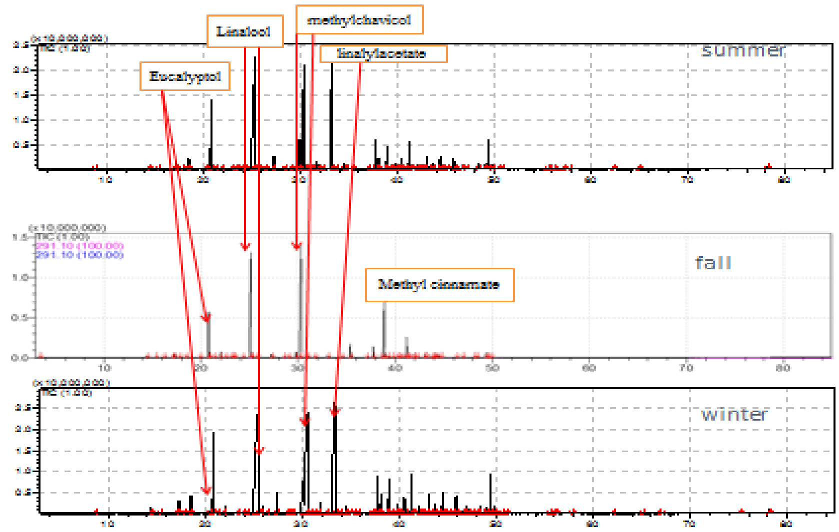
and flowers is due to their difference in the content of active compounds which must be determined by GC-MS.

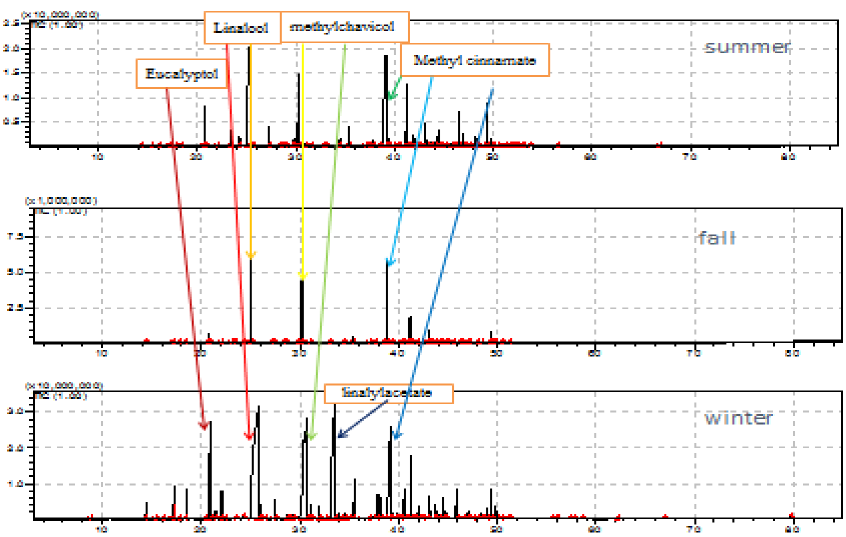
Table 1: Diameters of inhibition zones (including 6 mm disk) of *O. basilicum* L. essential oils (E.O)

The strains	Summer		Fall		Winter	
	Leaves	**flowers**	**Leaves**	**flowers**	**Leaves**	**flowers**
Escherichia coli (G-) ATCC 25922	8 mm –	12 mm +	16 mm ++	21.5 mm +++	6 -	13 mm +
Staphylococcus aureus (G+) ATCC 25923	10 mm +	14 mm +	18 mm ++	**25** mm +++	6 -	20 mm +++
Pseudomonas aeruginosa (G-) ATCC 27853	6 -	6 -	6 -	6 -	6 -	6 -

Essential Oils Major Composition: The GC-MS Chromatograms of the 6 essential oils are illustrated in Figure 1 where very different profiles are observed. The identified major compounds as well as their respective outages are summarised in table 2.



GC-MS Chromatograms of leaves essential oils in three seasons



GC-MS Chromatograms of flowers essential oils in three seasons

Figure 1: GC-MS chromatograms of leaves and flowers essential oils in three seasons

Table 2: Major composition of essential oils

Majority Composition	Summer		Fall		Winter	
	Leaves	**Flowers**	**Leaves**	**Flowers**	**Leaves**	**Flowers**
linalool	**30,26%**	**32,14%**	30,75%	**34,99%**	**29,13%**	**26,66%**
methylchavicol	17,58%	08,05%	**44,08%**	14,53%	20,43%	18,00%
linalyl acetate	17,03%	-	-	-	16,15%	15,18%
methylcinnamate	-	18,66%	17,06%	21,09%	-	-

The essential oils of the autumn season consist of the three major compounds: linalool, methylchavicol, and methylcinnamate. The absence of linalylacetate is clearly noted by comparing it with the essential oils of the other seasons (c.f. table 1).

CONCLUSION

The study of the essential oils' antibacterial activity of *O. Basilicum* L. on three bacterial strains (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Staphylococcus aureus ATCC 25923). And according to the results of the Aromatogram, our E.O. presented an important antibacterial activity against two bacterial strains, with the exception of Pseudomonas aeruginosa ATCC 27853 which is known from literature by its extensive antibiotic resistance, flowers' essential oil inhibited bacterial growth more than that of leaves. Besides that autumn promotes this activity. This variability is due to the impacts of environmental factors on the chemical composition of E.O. and their antibacterial activities. The study of essential oils' composition by GC-MS has allowed the identification of the major compounds: linalool, methyl chavicol, linalylacetate, and methylcinnamate with rates that change from one season to another and from one part to another.

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FULL PAPER

IMPACT OF SOME HERBICIDES AND HERBICIDE COMBINATIONS ON SOWING CHARACTERISTICS OF CORIANDER (*CORIANDRUM SATIVUM* L.)

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Abstract: The research was conducted during 2013 - 2015 on pellic vertisol soil type. Under investigation was Bulgarian coriander cultivar Lozen 1 (*Coriandrum sativum* L.). Factor A included no treated control, 6 soil-applied herbicides – Tender EC (S-metolachlor) - 1.5 l/ha, Silba SC (metolachlor + terbuthylazine) - 3.5 l/ha, Sharpen 33 EC (pendimethalin) - 5 l/ha, Merlin flex 480 SC (isoxaflutole) - 420 g/ha, Smerch 24 EC (oxyfluorfen) - 1 l/ha, Raft 400 SC (oxidiargil) - 1 l/ha and 5 foliar-applied herbicides – Kalin flo (linuron) - 2 l/ha, Eclipse 70 DWG (metribuzine) - 500 g/ha, Sultan 500 SC (metazachlor) - 2 l/ha, Corrida 75 DWG (tribenuron-methyl) - 20 g/ha, Lontrel 300 EC (clopyralid) - 500 ml/ha. Factor B included no treated control and 1 antigraminaceous herbicide – Tiger platinium 5 EC (quizalofop-P-ethyl) - 2.5 l/ha. Soil-applied herbicides were treated during the period after sowing before emergence. Foliar-applied herbicides were treated during rosette stage of the coriander. Herbicides Merlin flex and Lontrel and herbicide combinations Merlin flex + Tiger platinium and Lontrel + Tiger platinium proven decrease germination energy of the coriander seeds. Laboratory seed germination and lengths of primary germ and primary root are decreased by herbicide Merlin flex and herbicide combination Merlin flex + Tiger platinium only. Herbicide Merlin flex and herbicide combination Merlin flex + Tiger platinium do not proven decrease waste grain quantities. High yields of coriander seeds are obtained by foliar treatment with antigraminaceous herbicide Tiger platinum after soil-applied herbicides Raft, Smerch, Sharpen, Silba and Tender. Tank mixtures of Tiger platinum with foliar herbicides Kalin flo, Eclipse, Sultan, Corrida and Lontrel also lead to obtaining of high seed yields. The use of the soil-applied herbicide Merlin flex does not increase the seed yield, due to its higher phytotoxicity against coriander. Alone use of soil-applied or foliar-applied herbicides leads to lower yields due to they must to combine for full control of weeds in coriander crops.

Key words: coriander, herbicides, herbicide combinations, seed yield, sowing characteristics

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is an important essential-oil plant, referring to the *Apiaceae* family. The technology for coriander growing over time needs to be updated, as a result of ever-changing weather conditions. Specify and significantly improve crop rotations, tillage, timings of sowing and harvesting (Delibaltova et al., 2012). All of this leads to changes in the conditions for the weeds development, to the emergence of resistant or new weed species that were previously marginal or were not harmful.

The weeds occurring in coriander crops of are mainly from the group of the winter-spring species and from the group of early spring species. The most frequent winter-spring weeds are: *Alopecurus myosuroides* L., *Apera spica-venti* P.B., *Bromus arvensis* L., *Avena ludoviciana* Durien., *Lolium multiflorum* L., *Anthemis arvensis* L., *Chamomilla recutita* Rauchert, *Consolida regalis* Gray, *Viola tricolor* L., *Lithospermum arvense* L., *Centaurea cyanus* L., *Papaver rhoes* L., *Agrostemma githago* L. The most frequent early spring weeds are: *Avena fatua* L., *Galium aparine* L., *Sinapis arvensis* L., *Falopia convolvulus* Leve, *Myagrum perfoliatum* L. (Mitchell and Abernethy, 1993; Atanasova et al., 2012). Biological peculiarity of coriander is its slow growth rates in the first stages and therefore it is highly vulnerable to the competitive impact of weeds at the beginning of its vegetation and weed infestation significantly (Atanasova and Gospodinov, 2005, Gramatikov et al., 2005). Effective weed control is one the important conditions for full realization of the biological potential of culture (Macko, 1990; Lugo and Santiago, 1996; Vaculik, 2007; Meena and Mehta, 2009; Delchev and Georgiev, 2015, 2015a; Delchev, 2018). The presence of weeds in coriander crops requires carrying out of their chemical control to preserve coriander yield at a maximum level (Zheljazkov and Zhalnov, 1995; Smart et al., 1996; Mathukia et al., 2014). The aim of this investigation was to establish the influence of some soil-applied herbicides, foliar-applied herbicides and herbicide combinations on sowing characteristics of the coriander seeds and the quantity of waste grain.

MATERIALS AND METHODS

The research was conducted during 2013 - 2015 on pellic vertisol soil type. Under investigation was Bulgarian coriander cultivar Lozen 1 (*Coriandrum sativum* L.). Two factors experiment was conducted under the block method, in 4 repetitions; the size of the crop plot was 15 m^2. Factor A included no treated control, 6 soil-applied herbicides – Tendar EC, Silba SC, Sharpen 33 EC, Merlin flex 480 SC, Smerch 24 EC, Raft 400 SC and 5 foliar-applied herbicides – Kalin flo, Eclipse 70 DWG, Sultan 500 SC, Corrida 75 DWG, Lontrel 300 EC. Factor B included no treated control and 1 antigraminaceous herbicide – Tiger platinium 5 EC. Active substances of herbicides and their doses are shown in Table 1.

Table 1. Investigated variants

№	Variants	Active substance	Doses	Treatment period
Antibroadleaved herbicides				
1	Control	-	-	-
2	Tendar EC	S-metolachlor	1.5 l/ha	ASBE
3	Silba SC	metolachlor + terbuthylazine	3.5 l/ha	ASBE
4	Sharpen 33 EC	pendimethalin	5 l/ha	ASBE
5	Merlin flex 480 SC	isoxaflutole	420 g/ha	ASBE
6	Smerch 24 EC	oxyfluorfen	1 l/ha	ASBE
7	Raft 400 SC	oxidiargil	1 l/ha	ASBE
8	Kalin flo	linuron	2 l/ha	rosette
9	Eclipse 70 DWG	metribuzine	500 g/ha	rosette
10	Sultan 500 SC	metazachlor	2 l/ha	rosette
11	Corrida 75 DWG	tribenuron-methyl	20 g/ha	rosette
12	Lontrel 300 EC	clopyralid	500 ml/ha	rosette
Antigraminaceous herbicides				
1	Control	-	-	-
2	Tiger platinium 5 EC	quizalofop-P-ethyl	2.5 l/ha	rosette
ASBE – after sowing, before emergence				

Soil-applied herbicides were treated during the period after sowing before emergence. Foliar-applied herbicides were treated during rosette stage of the coriander. Introduction of herbicide combinations during rosette stage is done as herbicide tank mixtures – the mixing is done in the spray tank. All of herbicides, herbicide combinations and herbicide tank-mixtures were applied in a working solution of 200 l/ha. Mixing of foliar-applied herbicides was done in the tank on the sprayer.

The grain gained after every variant was cleaned through a sieves and the quantity of the waste grain was defined (siftings). All version seeds for sowing were defined for their germination energy and lab seed germination. It was studied intensity of early growth of seeds, expressed by the lengths of primary germ and primary root definite on the eighth day after setting the samples. Each index was determined in two repetitions of the year. Averages in each of the years of experience were used as repetitions in mathematical data processing were done according to the method of analysis of variance.

RESULTS AND DISCUSSION

One of the important conditions for obtaining a normal crop and a good harvest is the use of quality seeds. Apart from the high-yield cultivar which is resistance to diseases and pests, it must have the necessary sowing properties, the main of which are high germination energy and seed germination. Germination energy is one of the most important characteristics of the sowing properties of the seed. The low germination energy is the reason for slower development of primary germ and primary root

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after seed germination and is associated with later germination in field conditions, less tempering of plants and a higher risk of frost in the winter. Its lead to lower seed yields. The obtained results show that the treatment of the coriander with herbicides Merlin flex and Lontrel and herbicide combinations Merlin flex + Tiger platihium and Lontrel + Tiger platinium lead to the decrease in the germination energy (Table 2). Analysis of variance, in which the years have taken for replications, shows that these decreases are mathematically proven.

Table 2. Influence of some herbicides and herbicide combinations on sowing characteristics of the coriander seeds (mean 2013 - 2015)

Herbicides		**Germinative energy, %**	**Germination %**	**Length, cm**		**Waste grain, %**
Antibroadleaved	**Antigraminaceous**			**Primary germ**	**Primary root**	
-	-	76.5	87.0	7.8	8.2	16.9
	Tiger platinium	90.0	95.5	10.0	12.0	14.1
Tendar	-	90.0	95.0	10.1	12.1	14.4
	Tiger platinium	90.5	95.0	10.4	12.5	14.0
Silba	-	90.0	96.0	10.6	12.5	14.5
	Tiger platinium	90.0	95.5	10.3	12.7	14.0
Sharpen	-	90.0	96.0	10.7	12.3	14.4
	Tiger platinium	90.5	96.5	10.4	12.5	14.4
Merlin flex	-	75.0	88.0	7.0	8.6	15.6
	Tiger platinium	75.0	87.5	7.2	8.3	15.7
Smerch	-	89.5	94.5	10.3	11.9	13.9
	Tiger platinium	90.5	95.0	10.2	11.7	14.1
Raft	-	91.0	96.0	10.2	11.5	14.3
	Tiger platinium	91.0	95.0	10.2	11.4	14.0
Kalin flo	-	90.5	96.5	10.8	12.7	14.1
	Tiger platinium	89.5	95.5	10.0	12.8	14.4
Eclipse	-	91.0	96.5	10.3	11.3	14.0
	Tiger platinium	91.0	96.0	10.4	12.0	13.8
Sultan	-	90.5	95.0	10.5	11.8	13.0
	Tiger platinium	91.5	96.0	10.3	11.5	13.6
Corrida	-	89.5	95.5	10.1	11.3	14.1
	Tiger platinium	90.0	96.5	10.3	11.6	14.0
Lontrel	-	76.0	94.5	9.1	11.3	14.8
	Tiger platinium	75.5	94.0	9.3	11.0	14.1
LSD 5 %		6.2	5.3	1.8	2.1	2.0
LSD 1 %		8.3	7.0	2.6	2.9	3.3
LSD 0.1 %		10.5	9.2	3.7	4.0	4.8

Germination is the most important index who characterizing the sowing properties of the seeds. At low laboratory germination sowing should be done with higher sowing rate, which increases the cost production. Laboratory germination of the seeds at all variant during the three years of study is above the requirements of the standard, although in different years account for some variation of its values. This is the positive effect of their use, because it is not necessary to increase the sowing rate (in kg/ha) and the cost of necessary seeds. At herbicide Merlin flex and herbicide combination Merlin flex + Tiger platinium seed germination is lower than untreated control. The coriander seeds germinate normally by influence of the herbicide Lontrel and herbicide combination Lontrel + Tiger platinium, although the initial rate of development is lower due to lower germination energy. Other soil-applied herbicides, foliar-applied herbicides and their combinations increase the indexes germination energy and seed germination. This means that they help for joint and fast germination of the coriander sowing-seeds. The obtained results for germination energy and seed germination are a prerequisite continue to investigate the effect of herbicides and their combinations on initial intensity of the growth of seeds, expressed by the lengths of primary germs and roots. It was found that the lengths of primary germ and primary root of coriander are decreased by herbicide Merlin flex and herbicide combination Merlin flex + Tiger platinium. These decreasing are proven by analysis of variants. This two variants

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difficult young plants developments, reduces their resistance to cold and increase risk of frost damages during winter months. Herbicide Lontrel and herbicide combination Lontrel + Tiger platinium does not increase proven length of primary germ, but increase proven length of primary root. Other tank mixtures between soil-applied and foliar-applied herbicides led to increase of the lengths of primary germ and primary root of the coriander and recommended for use in seed production crops of coriander.

Table 3. Influence of some herbicide and herbicide combinations on seed yield of coriander (2013 - 2015)

Herbicides		2013		2014		2015		Mean	
Antibroadleaved	Antigraminaceous	kg/ha	%	kg/ha	%	kg/ha	%	kg/ha	%
-	-	2150	100	2333	100	2060	100	2181	100
	Tiger platinium	2227	103.6	2433	104.3	2142	104.0	2267	103.9
Tendar	-	2288	106.4	2492	106.8	2182	105.9	2321	106.4
	Tiger platinium	2417	112.4	2678	114.8	2348	114.0	2481	113.8
Silba	-	2288	106.4	2499	107.1	2188	106.2	2325	106.6
	Tiger platinium	2423	112.7	2685	115.1	2348	114.0	2485	113.9
Sharpen	-	2333	108.5	2550	109.3	2245	109.0	2376	108.9
	Tiger platinium	2526	117.5	2753	118.0	2408	116.9	2562	117.5
Merlin flex	-	2008	93.4	2240	96.0	1840	89.3	2029	93.0
	Tiger platinium	2176	101.2	2384	102.2	2017	97.9	2192	100.5
Smerch	-	2311	107.5	2522	108.1	2227	108.1	2353	107.9
	Tiger platinium	2498	116.2	2734	117.2	2396	116.3	2543	116.6
Raft	-	2356	109.6	2563	110.7	2250	109.2	2390	109.6
	Tiger platinium	2537	118.0	2767	118.6	2433	118.1	2579	118.2
Kalin flo	-	2376	110.5	2592	111.1	2268	110.1	2412	110.6
	Tiger platinium	2537	118.0	2755	118.1	2414	117.2	2569	117.8
Eclipse	-	2301	107.0	2522	108.1	2217	107.6	2347	107.6
	Tiger platinium	2481	115.4	2702	115.8	2385	115.8	2523	115.7
Sultan	-	2301	106.0	2508	107.5	2200	106.8	2336	107.1
	Tiger platinium	2473	115.0	2690	115.3	2377	115.4	2513	115.2
Corrida	-	2270	105.6	2492	106.8	2184	106.0	2315	106.1
	Tiger platinium	2457	114.3	2685	115.1	2369	115.0	2504	114.8
Lontrel	-	2258	105.0	2473	106.0	2167	105.2	2299	105.4
	Tiger platinium	2451	114.0	2676	114.7	2365	114.8	2497	114.5
	LSD 5 %	80	3.7	85	3.6	79	3.8		
	LSD 1 %	104	4.8	110	4.7	100	4.7		
	LSD 0.1 %	133	6.2	147	6.3	128	6.2		

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At the evaluation of the sowing characteristics we have to consider not only the characteristics of the sowing seeds but also the quantity of the waste grain (siftings) which are gained at the preparation of these seeds. Bigger quantity screenings lead to higher cost of the seed and reduce the economic effect of seed production of coriander. Herbicide Merlin flex and herbicide combination Merlin flex + Tiger platinium do not lead to are mathematically proven increase in the quantity of waste grain. Other herbicides and herbicide combinations lead to decreasing in the quantity of waste grain. Differences between them and untreated control are mathematically proven.Decreases in the values of germination energy and laboratory seed germination, changes in the intensity of the initial growth, expressed by the lengths of the primary root and primary germ at germination and changes in the quantity of waste grain by the influence of the relevant herbicides and herbicide combinations are explained by the depressing effects on growth and development of the coriander during its vegetative period.

To done a full evaluation of the sowing characteristics needed to establish not only the quality of seeds, but also the quantity of grain which will be received this seeds. Data for the influence of investigated herbicides and herbicide combinations on seed yield show that the lower yield is obtained by alone use of herbicide Merlin flex, followed by the untreated control (Table 3). The use of soil-applied herbicide Merlin flex do not increases seed yield despite its very good herbicidal effect against both graminaceous and broadleaved weeds. The reason for this is its higher phytotoxicity against coriander.Alone use of foliar-applied herbicides Kalin flo, Eclipse, Sultan, Corrida and Lontrel and as well as alone use of soil-applied herbicides Tendar, Silba, Sharpen, Smerch and Raft increases seed yields because the big numbers of weeds are destroyed by these herbicides. The increases of the yield by alone use of antigraminaceous herbicide Tiger platinium is less than the increase by other herbicides, because prevailing weeds in the trail are broadleaved.

Foliar treatment with Tiger platinum after soil-applied herbicides Tendar, Silba, Sharpen, Smerch and Raft increases the seed yields during the three years of the investigation. Tank mixtures of Tiger platinium with herbicides Kalin flo, Eclipse, Sultan, Corrida and Lontrel also lead to bigger increase in seed yields compared to yield at alone use of respective herbicides. Herbicide combination Merlin flex + Tiger platinum increases seed yield compared to alone use of Merlin flex, but not proven increase yield compared to no treated control.

CONCLUSION

Herbicides Merlin flex and Lontrel and herbicide combinations Merlin flex + Tiger platinium and Lontrel + Tiger platinium proven decrease germination energy of the coriander seeds.

Laboratory seed germination and lengths of primary germ and primary root are decreased by herbicide Merlin flex and herbicide combination Merlin flex + Tiger platinium only.

Herbicide Merlin flex and herbicide combination Merlin flex + Tiger platinium do not proven decrease waste grain quantities.

High yields of coriander seeds are obtained by foliar treatment with antigraminaceous herbicide Tiger platinum after soil-applied herbicides Raft, Smerch, Sharpen, Silba and Tender.

Tank mixtures of Tiger platinum with foliar herbicides Kalin flo, Eclipse, Sultan, Corrida and Lontrel also lead to obtaining of high seed yields.

The use of the soil-applied herbicide Merlin flex does not increase the seed yield, due to its higher phytotoxicity against coriander.

Alone use of soil-applied or foliar-applied herbicides leads to lower yields due to they must to combine for full control of weeds in coriander crops.

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FULL PAPER

CHANGES IN THE WEED SPECIES IN FIELDS OF MILK THISTLE, CORIANDER, CHICKPEA AND FORAGE PEA WHICH ARE SOWN ON DAMAGED BY FROST AREAS OF WINTER OILSEED CANOLA

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Abstract: During 2016 - 2018 was conducted a field experiment. On areas with damaged by frost winter oilseed canola, at the spring were sowed and investigated 4 spring crops: 1 milk thistle cultivar - Silmar (*Silybum marianum* Gaertn.); 1 coriander cultivar - Lozen (*Coriandrum sativum* L.); 1 chickpea cultivar - Kabule (*Cicer arietinum* L.); 1 forage pea cultivar - Mir (*Pisum sativum* L.). The same variants were planted on areas under conventional soil cultivation for each of these crops. Weed control was carried out by different herbicide combinations and herbicide tank mixtures. Coriander and milk thistle are suitable crops for sowing on areas after damaged by frost winter oilseed canola, when using herbicide combination Stomp aqua + Stratos ultra or herbicide tank mixture Zencor + Shadow. After plowing of canola crops, it is more appropriate to sow chickpea in which weed control is carried out by soil treatment with herbicide Merlin flex, followed by foliar treatment with herbicide tank mixture Challenge + Shadow. Herbicide combination Dual gold + Listego should not be used in chickpea. On plowed areas with damaged by frost winter canola hybrids without any problems can be sown forage pea, in which weed control is carried out by herbicide combinations Stomp aqua + Korum or Dual gold + Listego.

Keywords: milk thistle, coriander, chickpea, forage pea, weeds, herbicides

INTRODUCTION

Weed control is very important in the initial stage of milk thistle, coriander, chickpea and forage pea developments, when they are less competitive. The use of herbicides creates favorable conditions for germination, growth and development of these crops and also for the creation of well topped and high-yielding crops (Dann et al., 1987; Andrzejewska and Lamparski, 2006; Vaculík, 2007; Khan et al., 2009; Wágner, 2015; Delchev, 2018).In the crops of milk thistle, coriander, chickpea and forage pea occurring weeds of various biological groups. The implementation of the biological potential of these crops is closely related to the removal of the harmful effects of weeds (Meena and Mehta, 2009; Tanveer et al., 2010; Ratnam and Rao, 2011; Drapalova and Pluhackova, 2014; Mathukia et al., 2014; Tidemann et al., 2014). The aim of this experiment is to investigate the changes in the weed species in fields of 4 crops - milk thistle, coriander, chickpea and forage pea which are sown on damaged by frost crops of winter oilseed canola.

MATERIAL AND METHODS

During 2016 - 2018 was conducted a field experiment on pellic vertisol soil type. It was carried out a field experiment as a block method in 4 repetitions, on a 15 m^2 harvesting area.On areas with damaged by frost winter oilseed canola, at the spring were sowed and investigated 4 spring crops: 1 milk thistle cultivar - Silmar (*Silybum marianum* Gaertn.); 1 coriander cultivar - Lozen (*Coriandrum sativum* L.); 1 chickpea cultivar - Kabule (*Cicer arietinum* L.); 1 forage pea cultivar - Mir (*Pisum sativum* L.). These variants have been sown also on the areas with traditional for each of those crops soil tillage.

Weed control in milk thistle was carried out with herbicide combination Stomp aqua (pendimethalin) - 3 l/ha + Stratos ultra (cycloxydim) - 2 l/ha and herbicide tank mixture Zencor 70 WG + Shadow 3 EC (metribuzine + clethodim) - 500 g/ha + 1.6 l/ha. Soil-applied herbicide Stomp aqua was treated during the period after sowing before emergence. Foliar-applied herbicides Stratos ultra, Zencor and Shadow were treated during rosette stage of the milk thistle.

Weed control in coriander was carried out with herbicide combination Stomp aqua (pendimethalin) - 3 l/ha + Stratos ultra (cycloxydim) - 2 l/ha and herbicide tank mixture Zencor 70 WG + Shadow 3 EC (metribuzine + clethodim) - 500 g/ha + 1.6 l/ha. Soil-applied herbicide Stomp aqua was treated during the period after sowing before emergence. Foliar-applied herbicides Stratos ultra, Zencor and Shadow were treated during rosette stage of the coriander. Weed control in chickpea was carried out with herbicide combinations Dual gold 960 EC (S-metolachlor) - 1.5 l/ha + Listego 40 (imazamox) - 1.2 l/ha and Merlin flex 480 SC (isoxaflutole) - 420 g/ha + herbicide tank mixture Challenge 600 SC + Shadow 3 EC (aclonifen + clethodim) - 4 l/ha + 1.6 l/ha. Soil-applied herbicides Dual gold and Merlin flex were treated during the period after sowing before emergence. Foliar-applied herbicides Listego, Challenge and Shadow were treated during 6 - 8 real leaf stage of the chickpea.

Weed control in forage pea was carried out with herbicide combinations Dual gold 960 EC (S-metolachlor) - 1.5 l/ha + Listego 40 (imazamox) - 1.2 l/ha and Stomp aqua (pendimethalin) - 3 l/ha + Korum (bentazone + imazamox) - 1.25 l/ha. Soil-applied herbicides Dual gold and Stomp aqua were treated during the period after sowing before emergence. Foliar-applied herbicides Listego and Korum were treated during 6 - 8 real leaf stage of the pea. Due to of low adhesion of the herbicides Listego and Korum were used in addition with adjuvant Dash HC – 1 l/ha. It was investigated the changes occurring in the weed species in 4 crops - forage pea, chickpea, coriander and milk thistle. It was appointed the efficacy of the herbicides according to 100 % visual scale of EWRS (European Weed Research Society). It was followed the selectivity of the herbicides by the scale of EWRS (rating 1 - without damages, rating 9 - culture is completely destroyed).

RESULTS AND DISCUSSION

On the area of frozen winter canola was sown milk thistle cultivar Silmar. The cultivar was sowed as soon as possible to enter in the field. The same cultivar was sown at the same time and on area with traditional coriander soil tillage. Weed control was done with herbicide combination Stomp aqua + Stratos ultra and with herbicide tank mixture Zencor + Shadow (Tables 1 and 2). In both weed control variants, the efficacy of the used herbicide combinations is very good. A small reduction in the efficacy of herbicide combination Stomp aqua + Stratos ultra against some annual broadleaf weeds such as *Galium aparine* L., *Papaver rhoes* L. and *Sinapis arvensis* L., when sowing milk thistle after frozen canola has been found. Used herbicide combination and herbicide tank mixture enable effective weed control during crop growing against graminaceous and broadleaf weeds. This makes the milk thistle a suitable crop for sowing on areas after frozen winter oilseed canola.

On the area of frozen winter canola was sown coriander cultivar Lozen. The cultivar was sowed as soon as possible to enter in the field. The same cultivar was sown at the same time and on area with traditional coriander soil tillage. Weed control was done with herbicide combination Stomp aqua + Stratos ultra and with herbicide tank mixture Zencor + Shadow (Tables 3 and 4). A small reduction in the efficacy of herbicide combination Stomp aqua + Stratos ultra against some annual broadleaf weeds such as *Galium aparine* L., *Papaver rhoes* L. and *Sinapis arvensis* L., when sowing coriander after frozen canola has been found. In both weed control variants, the efficacy of the herbicide combinations used is very good. This means that coriander is a suitable crop for sowing on areas after damaged by frost winter oilseed canola.

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Table 1. Efficacy of herbicide combination and herbicide tank mixture against broadleaved weeds at milk thistle according to the 100 % visual scale of EWRS (mean 2016 - 2018)

Variants	Weeds									
	Galiun aparine	*Chamomilla recutita*	*Papaver rhoes*	*Sinapis arvense*	*Raphanus raphanistrum*	*Anthemis arvensis*	*Capsella bursa-pastoris*	*Cirsium arvense*	*Convolvulus arvensis*	*Cardaria draba*
Milk thistle – Sowing after canola										
Stomp aqua + Stratos ultra	98	100	99	98	100	100	100	0	0	0
Zencor + Shadow	15	100	98	98	100	100	100	0	0	0
Milk thistle – Normal sowing										
Stomp aqua + Stratos ultra	100	100	100	100	100	100	100	0	0	0
Zencor + Shadow	15	100	100	100	100	100	100	0	0	0

Table 2. Efficacy of herbicide combination and herbicide tank mixture against graminaceous weeds and self-sown plants at milk thistle according to the 100 % visual scale of EWRS and selectivity according to the 9-rate scale of EWRS (mean 2016 - 2018)

Variants	Weeds									
	Avena fatua	*Avena ludoviciana*	*Alopecurus myosoroides*	*Apera spica-venti*	*Bromus arvensis*	*Triticum durum* *	*Sorghum helepense*	*Cynodon dactylon*	*Agropyrum repens*	Selectivity
Milk thistle – Sowing after canola										
Stomp aqua + Stratos ultra	100	100	100	100	100	100	100	100	100	1
Zencor + Shadow	100	100	100	100	100	100	100	100	100	1
Milk thistle – Normal sowing										
Stomp aqua + Stratos ultra	100	100	100	100	100	100	100	100	100	1
Zencor + Shadow	100	100	100	100	100	100	100	100	100	1
* - self-sown plants of durum wheat										

On the area of frozen winter canola was sown chickpea cultivar Kabule. Sowing was done in late February. The same cultivar was sown at the same time and on area with traditional chickpea soil tillage. Weed control was done with two herbicide combinations: Dual gold + Listego and Merlin flex + herbicide tank mixture Challenge + Shadow (Tables 5 and 6). It was found a stronger phytotoxicity of herbicide Listego to the chickpea plants, although Listego provides better control of perennial broadleaf weeds compared to the herbicide tank mixture Challenge + Shadow. Combining of soil-applied herbicide Merlin flex with herbicide tank mixture Challenge + Shadow does not lead to phytotoxicity at chickpea plants.

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Table 3. Efficacy of herbicide combination and herbicide tank mixture against broadleaved weeds at coriander according to the 100 % visual scale of EWRS (mean 2016 - 2018)

Variants	Weeds									
	Galium aparine	*Chamomilla recutita*	*Papaver rhoes*	*Sinapis arvense*	*Raphanus raphanistrum*	*Anthemis arvensis*	*Capsella bursa-pastoris*	*Cirsium arvense*	*Convolvulus arvensis*	*Cardaria draba*
Coriander – Sowing after canola										
Stomp aqua + Stratos ultra	98	100	98	98	100	100	100	0	0	0
Zencor + Shadow	14	100	98	97	100	100	100	0	0	0
Coriander – Normal sowing										
Stomp aqua + Stratos ultra	100	100	100	100	100	100	100	0	0	0
Zencor + Shadow	15	100	100	100	100	100	100	0	0	0

Table 4. Efficacy of herbicide combination and herbicide tank mixture against graminaceous weeds and self-sown plants at coriander according to the 100 % visual scale of EWRS and selectivity according to the 9-rate scale of EWRS (mean 2016 - 2018)

Variants	Weeds									
	Avena fatua	*Avena ludovicianaa*	*Alopecurus myosoroides*	*Apera spica-venti*	*Bromus arvensis*	*Triticum durum* *	*Sorghum helepense*	*Cynodon dactylon*	*Agropyrum repens*	Selectivity
Coriander – Sowing after canola										
Stomp aqua + Stratos ultra	100	100	100	100	100	100	100	100	100	1
Zencor + Shadow	100	100	100	100	100	100	100	100	100	1
Coriander – Normal sowing										
Stomp aqua + Stratos ultra	100	100	100	100	100	100	100	100	100	1
Zencor + Shadow	100	100	100	100	100	100	100	100	100	1
* - self-sown plants of durum wheat										

At these two technologies, the high efficiency of the using herbicides is the reason for a good control of weeds in both sowing - after frozen canola and after deep plowing. These results lead to the conclusion that after plowing canola crops is appropriate to sow chickpea in which weed control should be carried out by soil treatment with herbicide Merlin flex, followed by foliar treatment with the herbicide tank mixture Challenge + Shadow. On the area of frozen winter canola was sown in early spring forage pea cultivar Mir. The cultivar was sowed as soon as possible to enter in the field. It was done a pre-sowing cultivation accompanied with harrowing. Weed control was done with two herbicide combinations: Dual gold + Listego and Stomp aqua + Korum (Tables 7 and 8). Herbicide Korum has a better effect than herbicide Listergo.

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Table 5. Efficacy of herbicide combinations against annual broadleaved weeds and self-sown plants at chickpea according to the 100 % visual scale of EWRS (mean 2016 - 2018)

Variants	Weeds									
	Galiun aparine	Chamomilla recutita	Papaver rhoes	Consolida regalis	Amaranthus retroflexus	Anthemis arvensis	Falopia convolvulus	Veronica hederifolia	Lamium purpureum	Helianthus annuus*
Chickpea – Sowing after canola										
Dual gold + Listego	93	100	94	100	92	92	93	100	100	0
Merlin flex + Challenge + Shadow	100	100	100	100	98	99	100	100	100	100
Chickpea – Normal sowing										
Dual gold + Listego	98	100	100	100	100	100	100	100	100	0
Merlin flex + Challenge + Shadow	100	100	100	100	100	100	100	100	100	100
* - self-sown plants of ClearField and ExpressSun sunflowers										

Table 6. Efficacy of herbicide combinations against perennial weeds, annual graminaceous weeds and self-sown plants at chickpea according to the 100 % visual scale of EWRS and selectivity according to the 9-rate scale of EWRS (mean 2016 - 2018)

Variants	Weeds									
	Cirsium arvense	*Convolvulus arvensis*	*Sorgum helepense*	*Cynodon dactylon*	*Avena fatua*	*Lolium multiflorum*	*Alopecurus myosoroides*	*Bromus arvensis*	*Triticum durum**	Selectivity
Chickpea – Sowing after canola										
Dual gold + Listego	98	100	94	95	100	100	100	98	100	3
Merlin flex + Challenge + Shadow	0	0	100	100	100	100	100	100	100	1
Chickpea – Normal sowing										
Dual gold + Listego	98	100	97	100	100	100	100	98	100	3
Merlin flex + Challenge + Shadow	0	0	100	100	100	100	100	100	100	1
* - self-sown plants of durum wheat										

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Korum has longer control over the secondary-emerged weeds until the pea plants cover the whole soil surface, competes with weeds and almost prevents secondary weed infestation. These results showed that after plowing of areas with frozen canola hybrids without any problems can be planted forage pea.

Table 7. Efficacy of herbicide combinations against annual broadleaved weeds at forage pea according to the 100 % visual scale of EWRS (mean 2016 - 2018)

Variants	Weeds									
	Galiun aparine	*Chamomilla recutita*	*Papaver rhoes*	*Consolida regalis*	*Sinapis arvense*	*Raphanus raphanistrum*	*Anthemis arvensis*	*Falopia convolvulus*	*Veronica hederifolia*	*Lamium purpureum*
Forage pea – Sowing after canola										
Dual gold + Listego	93	100	94	100	95	98	100	93	100	100
Stomp aqua + Korum	100	100	100	100	100	100	100	100	100	100
Forage pea – Normal sowing										
Dual gold + Listego	100	100	100	100	100	100	100	100	100	100
Stomp aqua + Korum	100	100	100	100	100	100	100	100	100	100

Table 8. Efficacy of herbicide combinations against perennial broadleaved, annual graminaceous weeds and self-sown plants at pea according to the 100 % visual scale of EWRS and selectivity according to the 9-rate scale of EWRS (mean 2016 – 2018)

Variants	Weeds									
	Cirsium arvense	*Convolvulus arvensis*	*Cardaria draba*	*Sonchus arvensis*	*Avena fatua*	*Lolium multiflorum*	*Alopecurus myosoroides*	*Bromus arvensis*	*Triticum durum**	Selectivity
Forage pea – Sowing after canola										
Dual gold + Listego	99	100	100	100	100	100	100	98	100	1
Stomp aqua + Korum	100	100	100	100	100	100	100	98	100	1
Forage pea – Normal sowing										
Dual gold + Listego	100	100	100	100	100	100	100	98	100	1
Stomp aqua + Korum	100	100	100	100	100	100	100	98	100	1
* - self-sown plants of durum wheat										

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CONCLUSIONS

Coriander and milk thistle are suitable crops for sowing on areas after damaged by frost winter oilseed canola, when using herbicide combination Stomp aqua + Stratos ultra or herbicide tank mixture Zencor + Shadow.

After plowing of canola crops, it is more appropriate to sow chickpea in which weed control is carried out by soil treatment with herbicide Merlin flex, followed by foliar treatment with herbicide tank mixture Challenge + Shadow. Herbicide combination Dual gold + Listego should not be used in chickpea.

On plowed areas with damaged by frost winter canola hybrids without any problems can be sown forage pea, in which weed control is carried out by herbicide combinations Stomp aqua + Korum or Dual gold + Listego.

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FULL PAPER

CORRELATION BETWEEN CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS FROM SIX AROMATIC MEDICINAL PLANTS GROWING IN ILLIZI AND GHARDAÏA (SOUTHERN ALGERIA)

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Abstract
This study provides an assessment to the relationship between the chemical composition of the essential oils and the antioxidant activity of six aromatic medicinal plants growing in southern Algeria, namely *Cymbopogon schoenanthus, Cymbopogon Citratus, Artemisia judaica, Artemisia herba alba, Pituranthos chloranthus and Pituranthos scoparius*. The essential oils were obtained by hydrodistillation and analyzed by GC/MS. Their antioxidant activity was determined by using three methods of testing (DPPH, ABTS and iron reduction test (FRAP)), the results showed that the essential oil of *A. judiaca* has the best antioxidant capacity in all tests. The statistical study allowed us to show a correlation between the chemical composition of the studied essential oils and the antioxidant activity and our results indicate that the high content of cyclic ether sesquiterpenes compounds (such as: Davana ether), the compounds have furan rings, or the existence of non-terpene alkenes/esters in the essential oil, increases its antioxidant effect.

Keywords: *C. schoenanthus*, *C. Citratus, A. judaica, A. herba alba, P. chloranthus, P. scoparius*, essential oil, antioxidant activity.

INTRODUCTION

Essential oils (EOs) are complex mixtures that can contain 20–60 compounds in different concentrations, and are characterized by two or three main components with higher concentrations compared to others present in lower concentrations. In general, the major compounds that are present in EOs are responsible for the biological activities (Budel et al., 2018), Antioxidant properties of essential oils from many plants have also been of great interest to the food processing industry, since their possible use as natural additives has emerged from a growing tendency to replace synthetic antioxidants with natural ones (khadri et al., 2008; Djeridane et al., 2006).

The medicinal plants contain various types of antioxidants, mostly polyphenols and flavonoids which exhibit high antioxidant activity (Gohari et al., 2011). As part of evaluation of the biological effectiveness of the essential oil from the medicinal plants, this paper presented a study of the antioxidant activity associated with the chemical composition of essential oils isolated from *Cymbopogon schoenanthus* (Poaceae), *Cymbopogon Citratus* (Poaceae), *Artemisia judaica* (Asteraceae), *Artemisia herba alba* (Asteraceae), *Pituranthos chloranthus* (*Apiaceae*), *Pituranthos scoparius* (*Apiaceae*).

MATERIALS AND METHODS

Plant material and extraction of EOs: The aerial parts of each plant were collected as follows: *C. schoenanthus and P. chloranthus* were collected from two Southern regions of Algeria, the first is Ghardaïa (weddi Cebceb, on April, 2014) and the second is Illizi (weddi tasset, on April, 2014). *A. herba alba* and *P. scoparius* were collected from weddi Cebceb in Ghardaïa (Algeria), on April 2014. *C. Citratus* were collected from Djanet in Illizi (Algeria), on December 2011. And concerning *A. judaica* were collected from weddi Tasset in Illizi (Algeria), on April 2014. Extraction of the EOs was carried out by hydrodistillation in a modified Clevenger-type apparatus for 3 h, The oil collected from each plant was dehydrated with Na_2SO_4 and stored in sealed glass vials at 4°C, prior to analyses.

Essential oil chromatographic analysis: The EOs were injected on a gas chromatography (Hewlett-packar computerized system, Agilent model 6890 GC coupled to a Agilent 5973 N mass selective detector , and equipped with an Agilent technologies capillary HP-5MS column (30 m, 0,25 mm I.d, 0.25µm thickness), temperature program and conditions were described by Hellale et al., (2016).

Antioxidant activity determinations: Three methods have been applied for the antioxidant activity of extracted EOs in this study: the 1,1-diphenyl- 2-picrylhydrazyl radical ($DPPH^{\cdot}$) assay, the 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\cdot+}$) assay and the ferric reducing antioxidant power (FRAP) assay. All chemicals, antioxidant activity essays and calculation of Trolox equivalent antioxidant capacity (TEAC) were described by Hellali et al., (2016).

Statistical analysis: Correlation analysis of antioxidant activity versus the chemical composition of EOs were carried out using the correlation and regression program in Excel 2007 program.

RESULTS
The compounds identified by GC-MS of the EOs extracted and their relative proportions are listed in the Table 1.

Table 1: Chemical composition of the studied essential oils

		Taux %							
	Compound	**Cyc**	**CyIl**	**CyGh**	**Ps**	**PcGh**	**PcIl**	**Arh**	**ArJ**
1	cis-Salvene	-	-	-	-	-	-	**0.31**	-
2	β-thujene	-	-	-	-	**1.55**	-	-	-
3	α-thujene	-	-	-	0.29	-	1.50	-	-
4	**α-Pinene**	-	-	-	**10.53**	**12.95**	**14.24**	-	0.41
5	Unknown	-	-	-	-	-	-	-	0.35
6	Camphene	-	-	-	0.11	-	-	1.24	-
7	Sabinene	-	-	-	**11.97**	5.17	-	0.94	-
8	β-Pinene	-	-	-	-	3.23	3.80	-	-
9	β-Myrcene	5.52	-	-	0.65	1.01	-	-	-
10	α-Phellandrene	-	-	1.48	**17.50**	**21.96**	1.22	-	-
11	2-Carene	-	4.923	-	-	-	-	-	-
12	**4-Carene**	-	-	**19.76**	-	0.19	-	-	-
13	O-Cymene	-	-	-	-	-	1.90	-	-
14	p-Cymene	-	-	-	-	3.51	-	-	0.84
16	Limonene	-	1.413	7.39	**36.90**	**31.55**	3.85	-	-
17	Eucalyptol	-	-	-	-	-	-	4.11	-
18	Geraniol ester	-	-	-	-	-	-	0.61	-
19	cis-b-Ocimene	-	0.014	-	-	-	-	-	-
20	trans-b-Ocimene	-	0.151	1.79	-	-	-	-	-
21	trans-Ocimene	-	-	-	-	-	3.33	-	-
22	γ-terpinene	-	-	-	1.57	0.96	-	-	-
23	Terpinolene	-	-	-	0.17	-	-	-	-
24	α-terpinolene	0.49	-	-	-	-	-	-	-
25	**α-Thuyone**	-	-	1.41	3.14	-	-	**73.33**	-
26	**β-Thuyone**	-	-	-	0.44	-	-	**11.96**	-
27	**cis-p-menth-2-en-1-ol**	-	-	**29.21**	-	-	-	-	-
28	**(4E,6E)-2-Methyl-2,4,6-octatriene**	-	-	-	-	-	-	-	**5.32**
29	Camphor	-	-	-	0.32	-	-	5.92	-
30	**trans-p-menth-2-en-1-ol**	-	-	**15.62**	-	-	-	-	-
31	Cyclohexaneacetaldehyde, 2-Methylene	-	-	-	-	-	-	-	1.08
32	trans-sabinenehydrate	-	-	-	-	-	-	0.97	-
33	D-fenchone	-	0.037	-	-	-	-	-	-
34	1-terpineol	-	0.429	-	-	-	-	-	-
35	4-terpineol	-		-	0.93	0.88	-	-	-
36	α-terpineol	-	1.447	1,71	-	-	-	-	-
37	cis piperitol	-	0.106	**5.81**	-	-	-	-	-
38	trans piperitol	-	0.029	**5.05**	-	-	-	-	-

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39	**Neral**	**37.06**	-	-	-	-	-	-	-
40	**Piperitone**	-	63.35	-	-	-	-	-	**79.04**
41	Geraniol	0.54	-	-	-	-	-	-	-
42	**Geranial**	**54.91**	-	-	-	-	-	-	-
43	α-Cubebene	-	-	-	0.10	-	-	-	-
44	O-Methyleugenol	-	-	-	-	0.79	-	-	-
45	β-elemene	-	0.616	-	-	-	-	-	-
46	β-caryophyllene	-	0.060	-	-	-	-	-	-
47	Calarene	-	0.094	-	-	-	-	-	-
48	α-caryophyllene	-	0.032	-	-	-	-	-	-
49	Germacrene-D	-	0.210	-	0.92	-	-	0.62	1.41
50	β-selinene	-	0.269	-	-	-	-	-	-
51	α-selinene	-	0.242	-	-	-	-	-	-
52	α-muurolene	-	0.562	-	-	-	-	-	-
53	β-Cubebene	-	-	-	-	1.15	1.19	-	-
54	Unknown	-	-	-	0.23	-	-	-	-
55	Bicyclogermacrene	-	-	-	0.27	-	-	-	0.77
56	Davana ether (isomer 1)	-	-	-		-	-	-	0.40
57	Davana ether (isomer 2)	-		-	-	-	-	-	1.18
58	γ-cadinene	-	0.345	-	-	-	-	-	-
59	δ-cadinene	-	0.946	-	0.76	-	-	-	-
60	**Myristicin**	-	-	-		**12.58**	**65.15**	-	0.95
61	Davana ether	-	-	-	-	-	-	-	0.56
62	Elemol	-	6.915	3.48	-	-	-	-	
63	Spathulenol	-	-	-	-	-	1.49	-	0.47
64	Davanone	-	-	-	-	-	-	-	**7.23**
65	Selina-6-en-4-ol	-	-	1.82	-	-	-	-	-
66	Apiol	-	-	-	-	0.97	1.18	-	-
67	α-Cadinol	-	-	-	0.44	-	-	-	-
68	10-epi-γ-eudesmol	-	1.266	-	-	-	-	-	-
69	β-eudesmol	-	9.305	1.39	-	-	1.15	-	-
70	α-eudesmol	-	1.875	-	-	-	-	-	-
71	Globulol	-	-	1.89	-	-	-	-	-
72	Butylidene phthalide	-	-	-	0.18	-	-	-	-
73	2-Isopropenyl-4a,8-dimethyl-1,2,3, 4,4a,5,6,7-octahydronaphthalene	-	-	2.19	-	-	-	-	-
74	(Z)-Ligustilide	-	-	-	**12.26**	1.74	-	-	-
75	3-Butyl-2-benzofuran-1(3H)-one	-	-	-	0.13		-	-	-
	Monoterpene hydrocarbons (%)	**6.01**	6.501	30.42	**79.88**	**81.89**	29.8	**2.18**	**1.25**
	Oxygenated monoterpenes (%)	**92.51**	65.398	53.76	4.83	0.88	**0**	96.3	**79.04**
	Sesquiterpene hydrocarbons (%)	**0**	3.376	2.19	2.05	1.15	**1.19**	0.62	**2.18**
	Oxygenated sesquiterpenes (%)	**0**	19.361	13.63	0.44	0	**2.64**	0	**9.84**
	Composes oxygenes non terpeniques (%)	**0**	**0**	**0**	12.57	**16.08**	**66.3**	**0**	**0.95**
	Other compounds (%)	**0**	**0**	**0**	**0**	**0**	**0**	0.92	**6.4**

Cyc: *C. Citratus,* **CyIl**: *C. schoenanthus* from Illizi, **CyGh**: *C. schoenanthus* from Ghardaïa, **Ps** *: P. scoparius* , **PcGh:** *P. chloranthus* from Ghardaïa, **PcIl**: *P. chloranthus* from Illizi , **Arh** *: A. herba alba* , **ArJ** *: A. judaica,*

Results from the antioxidant activities assays of extracted EOs are summarized in Table 2.

Table 2. Antioxidant properties against DPPH, ABTS and FRAP assays of the studied EOs

Essential oil	Harvest area	DPPH assay IC_{50} (mg/ml)	ABTS assay IC_{50} (mg/ml)	FRAP assay TEAC (µmoles / g)
C. schoenathus	Illizi	44.219±3.78	18.91±0.17	3.33± 0.3
	Ghardaïa	21.68± 5.1	15.16±4.42	2.94± 0.6
C. citrates	Illizi	15.584±0.72	53.74	6.64± 2.3
A. judaica	Illizi	**3.63±0.17**	**2.09±0.17**	**86.28± 3.2**
A. herba alba	Ghardaïa	11.478±0.66	6.36±1.43	17.02± 0.7
P. chloranthus	Illizi	8.24±1.11	6.10±1.37	24 .27± 2.2
	Ghardaïa	10.747±3.24	5.9±1.72	25.96± 3.9
P. scoparius	Ghardaïa	5.812±0.88	7.47±2.64	49.18± 3.8

In order to correlate the antioxidant activity with the chemical composition of the extracted essential oils, and to identify compounds in the EOs potentially associated with antioxidant activities, the coefficients of correlation " R^2 " between the abundances of the compounds and the antioxidant activities were calculated and determined by Excel 2007. The results are summarized in Table 3.

Table 3. Correlation coefficients between antioxidant activity and chemical classes that exist in extracted EOs

	Correlation coefficient "R^2"		
Grouped componets	**DPPH assay**	**FRAP assay**	**ABTS assay**
Monoterpene hydrocarbons	0,000	0,001	0,032
Oxygenated monoterpenes	0,009	0,011	0,001
Monoterpenes cyclic ether	0,013	0,019	5E-05
Monoterpene ketones	0,052	0,078	0,214
Monoterpene Alcohols	0,104	0,118	0,066
Monoterpene Aldehydes	0,052	0,083	0,149
Sesquiterpene hydrocarbons	0,392	0,375	0,293
Oxygenated sesquiterpenes	0,042	0,017	2E-06
Sesquiterpenes cyclic ether	**0,678**	**0,709**	**0,840**
Sesquiterpènes Alcohols	0,304	0,249	0,153
Phenylpropene	0,002	0,000	0,002
Furan cycle compounds	**0,711**	**0,682**	**0,845**
Alkenes and esters non-terpenics	**0,658**	**0,682**	**0,859**
Hyterocycles	0,622	0,655	0,326

DISCUSSION AND CONCLUSION

The chemical composition of essential oils obtained from *C. schoenanthus* of Ghardaïa and of Illizi was different. The *C. schoenanthus* EOs of Illizi, for instance, was characterized by the high content of piperitone (Hellali et al., 2016), whereas the major compounds found in *C. schoenanthus* EO of Ghardaïa are cis-p-menth-2-en-1-ol (29.21%), trans-p-menth-2-en-1-ol (15.62 %), 4-Carene (19.76%), cis piperitol (5.81%) and trans piperitol (5.05 %).

P. chloranthus EO of Illizi is a chemotype characterized by absence of oxygenated monoterpenes content and high myristicin content (65.15 %). The second *P. chloranthus* chemotype was found in Ghardaïa region, which differs from the first at the presence of high content of monoterpene hydrocarbons (81.89%), Limonene (31.55 %), α-Phellandrene (21.96%) and *α*-pinene (12.95%) were the main compounds in this chemotype. Limonene, α-Phellandrene and *α*-pinene they also the major compounds in *Pituranthos scoparius EO of* Ghardaïa (36.90% and 17.50% and 10. 53 respectively), in addition to the presence of a significant percentage of **Sabinène 11.97%** in *Pituranthos scoparius* EO. Hellali et al., (2017) have shown that for the *A. judaica* EO, the oxygenated monoterpenes had the most important proportions (79.04%), which represents the proportion of piperitone. The oxygenated monoterpenes have been found 96.29% in *A. herba alba* EO. α-thuyone (73.33%) and β-thuyone (11.96%) were detected as major compounds in *A. herba alba* EO. Finally, *C. citratus* was mainly constituted by oxygenated monoterpenes (92,51%), the results showed that Néral (37.06%) and Géranial (54.91%) were the two chemical compounds, taking part of the major compounds of *C. citratus* EO.

Antioxidant activities of studied essential oils were analyzed using free radical scavenging (DPPH and ABTS) and reducing power (FRAP) assays; the results are presented in Table 2. In the DPPH assay, the radical scavenging activity of *A. judaica essential* oil was stronger than *P. scoparius essential oil* and followed by the *P. chloranthus* essential oil of Illizi, *P. chloranthus* essential oil of Ghardaïa, *A. herba alba*, *C. citratus*, *C. schoenanthus* of Ghardaïa and *C. schoenanthus* of Illizi. In the ABTS assay, the most potent radical scavenging activity was also found in *A. judaica*, which was followed by *P. chloranthus* essential oil *of Ghardaïa*. The results obtained by the method of FRAP confirm the important antioxidant potential of *A. judaica* essential oil. Antioxidant activities of essential oils from aromatic plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them (Politeo et al., 2006). Some study indicate that the antioxidative effectiveness of essential oil depends on the content of phenolic compounds and the reaction activity of the phenol towards the chain-carrying peroxyl radicals and on the stability of the phenoxyl radical formed in the reaction (Ćavar et al., 2012). The antioxidant activity may be ascribed to the presence of the several chemical components. Monoterpenes found in these essential oils may act as radical scavenging agents. It seems to be a general trend that the essential oils, which contain monoterpene hydrocarbons, oxygenated monoterpenes and/or sesquiterpenes, have high antioxidative properties (khadri et al., 2008). The statistical analyzes allowed us to highlight a possible correlation between the antioxidant activity and the chemical composition of the essential oils. Through these results of Table 3, we can be noted that there are a strong correlations between the antioxidant capacity of an EOs and cyclic ether sesquiterpene compounds, furane ring compound and Alkenes and esters non-terpenics with correlation coefficients 0.840, 0.45, 0.859 (in ABTS assay) respectively. This result suggests that the antioxidant capacity of essential oils is not related to the presence of some phenolic compounds or to the major compounds, it can be due to other minor ingredients in the EOs might contribute to bioactivity through synergistic effects.

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FULL PAPER

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF THE ESSENTIAL OILS FROM TOW CHEMOTYPES OF *CYMBOPOGON SCHOENANTHUS* (L.) SPRENG. GROWING IN SOUTHERN ALGERIA.

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Abstract
The chemical composition of the essential oils of tow populations of *Cymbopogon schoenanthus* collected in Ghardaïa and Illizi (Southern Algeria), were identified by (GC-MS), this study allowed us to identify the presence of two chemotypes of *C. schoenanthus* essential oil, the first one was characterized by the high content of piperitone, and the second group was characterized by other compounds. The screening of antimicrobial activity of oils was individually evaluated against representatives of gram-positive, gram-negative bacteria and fungi, using the agar diffusion method, whereas, the antioxidant activity of the essential oils was evaluated using the DPPH, ABTS and FRAP assay. The results of in vitro testing of the antimicrobial activities of *C. schoenanthus* essential oil of Ghardaïa region proved the existence of strong activity against all the tested strains, specially the growth of *E. faecium, S. aureus and S. agalactiae*. However, the same species growing in Illizi region shown less power in the inhibition of microorganisms. The essential oil obtained from *C. schoenanthus* growing in Illizi is markedly rich in non-phenolic constituents; due to this fact the essential oil is relatively weak antioxidant, which the same remark in the essential oil extracted from Ghardaïa.

Key words: *C. schoenanthus*, essential oil, chemotype, antimicrobial activity, antioxidant activity.

INTRODUCTION

Cymbopogon genus is a member of the family of Poaceae (Gramineae) which are herbs known worldwide for their high essential oil content. They are widely distributed across all continents where they are used for various purposes (Avoseh et al., 2015). *Cymbopogon schoenanthus* L. belongs to this family, it is widely distributed from the tropical region through North Africa and Asia. In Algeria it grows as wild plant in dry areas and valleys. It is widely spread in the Algerian Sahara. *C. schoenanthus* is an aromatic and medicinal plant rich in essential oil, this plant is widely used in traditional medicine in the sahara of Algeria specially in Tassili n'ajjer. The traditional use of this species was varied from one region to another, for example in Algeria, it was used as: aches, toothaches, myalgia, anuria in Ghardaïa area and as an strengthening in Beni Abbes, but it was used to cooling and to treat anuria in Ouargla (Maiza et al., 1993). In addition to this Hammiche et al., (2006) reported that this plant is used as traditional medicine to treat digestive diseases (flatulence, urinary decrease), analeptic drink for the new mother after childbirth, bad breath, gumboils and urinary incontinence. *In vitro C. schoenanthus* essential oil showed different activities for example Ketoh et al., 2004; Koba et al., 2004 were reported that this plant has sedative, digestive and aromatic properties, with a strong and characteristic aroma, with some reports on its insecticidal activity.

MATERIALS AND METHODS

Plant material: The aerial parts of *C. schoenanthus* were collected from weddi Cebceb in Ghardaïa (Algeria), on April, 2014.

Essential oil extraction: The fresh aerial parts of *C. schoenanthus* were subjected to hydrodistillation in a modified Clevenger-type apparatus for 3 h. The essential oil obtained was dried over anhydrous sodium sulfate and stored in sealed glass vials at 4 to 6°C prior to analyses.

Essential oil chromatographic analysis: 0.2 µL of sample was injected on a gas chromatography (Hewlett-packar computerized system, Agilent model 6890 GC coupled to a Agilent 5973 N mass selective detector , and equipped with an Agilent technologies capillary HP-5MS column (30 m, 0,25 mm I.d, 0.25µm thickness), a split/splitless injector used in the split mode (200:1), using Helium (20 mL/min). The initial temperature of the column was 40°C which was heated gradually to 325°C with a 2°C /min. High purity helium was used as carrier gas at 36 cm/s. The quadrupole, source and transfer line temperatures were maintained at 150, 230 and 280°C, respectively. A solvent delay of 3 min. Identification of components was assigned by matching their mass spectra of peaks with those obtained from authentic samples.

Antioxidant activity determinations: The difference in their chemical composition of extracted *C. schoenanthus* essential oil between the two regions (Ghardaïa and Illizi), urged us to study the antioxidant activity *of C. schoenanthus* essential oil again in the same conditions as cited by Hellali et al. (2016), in order to evaluate and compare the capacity antioxidant of this herb. Three methods have been applied for the antioxidant assessment of the *C. schoenanthus* essential oil in this study: the 1,1-diphenyl- 2-picrylhydrazyl radical ($DPPH^{\cdot}$) assay (Brand-Williams et al.,1995), the 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\cdot+}$) assay (Miller et al.,1993) and the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). All measurements were performed in duplicate.

Antimicrobial screening: The antimicrobial activities were determined by using the drop agar diffusion method (Lopes et al., 2008). The microorganisms tested were the fungi *Candida albicans ATCC 10231*, and the bacteria *Escherichia coli ATCC 8739, Salmonella typhimurium ATCC 14028, Staphylococus aureus ATCC 6538, Enterococcus feacium ATCC 19434, and Streptocoque B (Streptococcus agalactiae).*

RESULTS

Chemical composition of essential oil: The aerial parts of *C. schoenanthus* essential oils from Ghardaïa area, were analyzed by GC–MS. Fifteen compounds were identified showing high amounts in monoterpenes (84,18%), with mainly oxygenated monoterpene (53,76%) (Table 1). The main components identified were cis-p-menth-2-en-1-ol (29.21%), trans-p-menth-2-en-1-ol (15.62 %), 4-Carene (19.76%), cis piperitol (5.81%) and trans piperitol (5.05 %).

The results completed by literature search, the comparison show a significant difference in the content of the volatile oil of the aerial parts of this species collected in Illizi area which characterized by the presence remarkable of piperiton in their composition (Hellali et al., 2016).

Antioxidant activities: In this piper we report the antioxidant activity of *C. schoenanthus* essential oil growing in Ghardaïa area (Table 2), In our previous studies, the antioxidant investigation of the *C. schoenanthus* essential oil from Illizi region, were previously evaluated using various antioxidant tests (DPPH, ABTS and FRAP assay).

The results of Table 2 indicated that the *C. schoenanthus* essential oil from Ghardaïa region strongly transformed the ABTS radical into its reduced form. It has an IC_{50} value of 15.16 mg/ml. These measurements are compared with our previously results, its showed that the essential oil obtained from *C. schoenanthus* growing in Illizi is markedly rich in non-phenolic constituents, due to this fact the essential oil is relatively weak antioxidant, which the same remark in the essential oil extracted from Ghardaïa.

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Table 1. Chemical composition of essential oil of *C. schoenanthus*

N	Tr	Component	Percentage (%)
1	19.76	4-Carene	**19.76**
2	13.33	α-Phellandrene	1.48
3	14.787	Limonene	7.39
4	15.601	trans-β-Ocimene	1.79
5	19.620	α-Thujone	1.41
6	**20.911**	**cis-p-menth-2-en-1-ol**	**29.21**
7	**22.106**	**trans-p-menth-2-en-1-ol**	**15.62**
8	25.439	α-terpineol	1,71
9	25.706	cis piperitol	**5.81**
10	26.654	trans piperitol	**5.05**
11	47.673	Elemol	3.48
12	51.392	Selina-6-en-4-ol	1.82
13	53.111	β-eudesmol	1.39
14	53.478	Globulol	1.89
15	55.568	2-Isopropenyl-4a,8-dimethyl -1,2,3, 4,4a,5,6,7-octahydronaphthalene	2.19
-	-	monoterpenes hydrocarbons	30,42
-	-	Oxygenated monoterpenes	53,76
-	-	sesquiterpenes hydrocarbons	2,19
-	-	Oxygenated sesquiterpènes	13,63

Table 2. Antioxidant activity of *C. schoenanthus* essential oil expressed in TEAC and IC50.

Variables	**DPPH**	**ABTS**	**FRAP**
TEAC (µmol/g)	2.105	14.577	2.94±0.6
IC_{50} (mg/mL)	21.68±5.1	15.16±4.42	-

Averages ± Standard Deviation were obtained from two different experiments.

Antimicrobial activity: The results of *in vitro* testing of the antimicrobial activities of *C. schoenanthus* essential oil from Ghardaïa region are presented in the Table 3, we used antibiotic ampicillin and antifungal nystatin as positives probe. The qualitative test was to determine the presence or absence of inhibition zones around the disks.

The results obtained proved the existence of strong activity against all the tested strains, specially the growth of *E. faecium, S. aureus and S. agalactiae.* The results obtained with same species growing in Illizi region (Hellali et al., 2016) shown less power in the inhibition of microorganisms, and that antimicrobial activities have been mainly explained through the high proportion of piperitone.

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Table 3. Antimicrobial activity of *C. schoenanthus* essential oil.

Name of bacterial strain	Inhibition diameter (mm)*		
	Ampicillin/ Nystatin 10µg/100µg	***C. schoenanthus Of Ghardaïa***	***C. schoenanthus Of Illizi* (Hellali et al., 2016)**
Escherichia coli ATCC 8739	12±1	25±1.4	15±1.4
Salmonella typhimurium ATCC 14028	17,33±1.5	16,5±0.7	10.5 ±0.7
Staphylococcus aureus ATCC 6538	44.3±0.5	34±2.8	19.5 ±0.7
Enterococcus faecium ATCC 19434	45.3±1.5	41±1.4	21±1.4
Streptocoque B (Streptococcus agalactiae)	34.3±0.5	31±1.4	12.75 ±0.3
Candida albicans ATCC 10231	42±1	21±1.4	12±1.4

*Including disc diameter of 6 mm, Averages ± Standard Deviation were obtained from two different experiments.

DISCUSSION AND CONCLUSION

To the best of the study knowledge, this is the first study providing data on chemical composition of the essential oil of *C. schoenanthus* from Ghardaïa especially and evaluating the influence of the climatic and geographic condition on their chemical composition. As a conclusion we can say that according to the chemical composition and geographical origin, two chemotypes were defined for *C. schoenanthus* for the first time in southern Algeria: the type of Illizi area (piperitone) and and the type of Ghardaïa area (cis-p-menth-2-en-1-ol, trans-p-menth-2-en-1-ol, cis piperitol and trans piperitol).

The essential oil of Ghardaïa showed an activity antioxidant more important than the Illizi origin. We can explain this result by the ability of some terpenes (such us the monoterpene alcohols) to give up a hydrogen atom or more to the free radicals, but on the other hand, these compounds give another compounds are more stable. For example: we can obtain the piperitone by the dehydrogenation of trans or cis pipiritol. But generally, C. *schoenanthus* essential oil can be considered as a relatively weak antioxidant. The antibacterial of this oil was studied *in vitro* on five bacterial strains and one fungal strain, the results obtained proved the existence of strong activity against all the tested strains. *C. schoenanthus* essential oil of Ghardaïa region was characterized by the presence of cis-p-menth-2-en-1-ol, trans-p-menth-2-en-1-ol, cis piperitol and trans piperitol, this components are alcohols monoterpene. *C. schoenanthus* essential oil of Illizi region (Hellali et al., 2016) shown an important activity antimicrobienne due to the presence of high proportion of monoterpenes specially the high content of piperitone, and we supposed that the piperitone, cis-p-menth-2-en-1-ol, trans-p-menth-2-en-1-ol, cis piperitol and trans piperitol, all these compounds have the same cyclic intermediate in the biosynthesis in *C. schoenanthus*, and the presence of hydroxyl function in place of the ketone function increases the inhibition efficiency of the essential oil of *C. schoenanthus* collected from Ghardaïa region Against the microorganisms tested.

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FULL PAPER

ASSESSMENT OF CHOLINESTERASE AND TYROSINASE ENZYMES INHIBITORY, PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF *MENTHA SPICATA* L.

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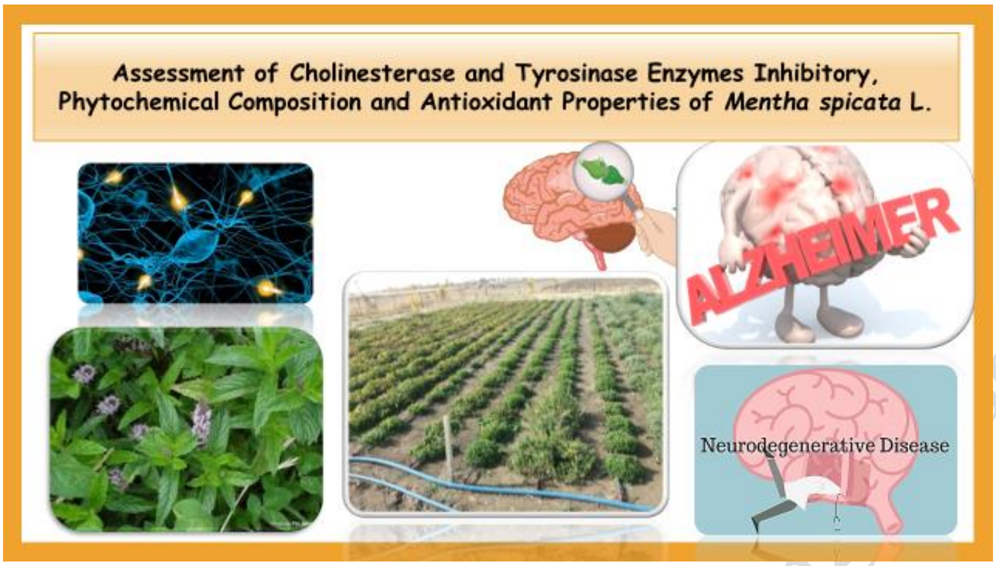
Abstract

Because oxidative damage is one of the major factor contributing to neurodegeneration, combination of antioxidant activity and enzyme inhibition potential of the herbal extracts is closely related to combat neurodegenerative diseases and disorders efficiently. Spearmint (*Mentha spicata* L., Lamiaceae), an edible aromatic plant, are of important food sources used as flavoring, tea infusions and spicing in the world. Besides its importance in food, beverage, cosmetic and confectionary industries, there has been huge ethnobotanical information on the curative properties of *M. spicata* L. against common diseases. Even though biological properties of spearmint have been documented by previous scientific reports, one less known biological activity of spearmint is its neuroprotective activity. **Material and Methods:** In this concern, neuroprotective potentials, antioxidant capacities, mineral contents and total polyphenolic compositions of aqueous and methanol extracts of the leaves from *M. spicata* L. were aimed to examine in the current research. Neuroprotective potentials of the extracts were determined against inhibition of AChE, BChE, and tyrosinase enzymes, which are associated with pathogenesis of Alzheimer's disease. *In vitro* assays including DPPH, ABTS, FRAP, and CUPRAC were carried out to reveal antioxidant capacities of the extracts. The total phenolic and total flavonoid contents were analyzed spectrophotometrically. Mineral contents (K, Ca, Mg, Fe, Zn, Cu, Mn, Cd, and Pb) in spearmint leaves were also determined by Atomic Absorption Spectrometry. **Results:** A wide variation was observed in the mineral contents of the plant that Fe was found the major mineral with the values of 1471.41±12.28 mg/kg, whilst Cu and Pb were determined as minor minerals. With regard to total polyphenolic components, the methanol extract possessed higher phenols and flavonoids contents (3.158±0.52 mg/g extract as GAE and 1.31±0.08 mg/g extract as QE, respectively). A significant correlation was found between total polyphenolic compositions and antioxidant capacities of the spearmint leaves. Correlation analysis indicated that the methanol extract, which possessed higher total phenols and flavonoids components than the water extract, was also found the more efficient in terms of antioxidant capacities and neuroprotective potentials. Both of the extracts demonstrated powerful inhibition on BChE, whereas they showed moderate inhibition on AChE and tyrosinase enzymes with the values ranging from 24.06±0.12% to 57.68±0.18%. **Conclusions:** Consequently, our results indicate that *M. spicata* L., a natural antioxidant and neuroprotective agent, can provide considerable benefits against oxidative stress-related diseases.

Keywords: *Mentha spicata* L.; Alzheimer's Disease; neuroprotection; antioxidant; polyphenolic; mineral content

Graphical Abstract



1. INTRODUCTION

Spearmint (*Mentha spicata* L., Lamiaceae), an edible aromatic plant, are of important food sources used as flavoring, tea infusions and spicing in the world. The plant is indigenous to northern England and is cultivated in areas with climate ranging from tropical to temperate, such as America, Europe, China, South Africa and Brazil. Nowadays, spearmint is widely grown throughout all regions in the world such as America, Europe, China, South Africa and Brazil. Since ancient times, both fresh and dried spearmint plants have been practiced as medical and aromatic plants in western and eastern cultures (Fitsiou et al., 2016). Spearmint has been used broadly in cosmetic and soap, as well as toothpaste, breath freshener and antiseptic mouth rinse. It is widely used in food preservation and imparts food taste and aroma, confectionery, and chewing gum industries. Additionally, it has also been used in the treatment of several ailments in Turkish folk medicine. In term of medical uses, spearmint is considered as an herbal medicine in folkloric remedies for treating of colds and flu, respiratory tract problems, gastralgia, hemorrhoids, and stomachache (Brahmi et al., 2017; Kee et al., 2017).

Because oxidative damage is one of the major factor contributing to neurodegeneration, combination of antioxidant activity and enzyme inhibition potential of the herbal extracts is closely related to combat neurodegenerative diseases and disorders efficiently. The formation of Reactive Oxygen Species (ROS), such as superoxide ion (O_2), hydroxyl radical (OH) and Hydrogen peroxide (H_2O_2), have often been reported to induce DNA damage, protein carboxylation, and lipid peroxidation, causing a variety of chronic health disturbances and diseases, including cancer, ageing, Parkinson's disease, Alzheimer's disease, and cardiovascular diseases. Recent research indicates that several herbal plants can offer alternative sources of dietary ingredients to promote human health and might open promising opportunities for the treatment of a wide range of troublesome diseases and infections (Sun et al., 2016; Sahoo et al., 2018) . Although, there are so many methods for combatting neurodegenerative diseases and disorders, they cannot always provide effective treatments and mediations. Hence, an extensive research on developing new treatment strategies against these disorders are still needed to cure nowadays. Since ancient times, natural products (NPs), originated from natural sources such as plants, have been used for the cure and treatment of many diseases in Anatolian folk medicine ‘herbal therapies’ (Gezici and Sekeroglu, 2019a; Awasthi et al., 2016; Godyń, et al, 2016).

So far, we have investigated various of medicinal plants using in vitro antioxidant, anticancer, antiproliferative, anticholinesterase, etc. experiments, which aimed to contribute to the finding of new herbal products for prevention and treatment of cancer and neurodegenerative diseases (Gezici, 2019;

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Gezici and Sekeroglu, 2019a; Gezici and Sekeroglu, 2019b; Sekeroglu et al., 2018a; Sekeroglu et al., 2018b; Gezici, 2018; Senol et al., 2018; Gundogdu et al., 2018; Karik et al., 2018; Belkhodja et al., 2017; Gezici et al., 2017; Sekeroglu et al., 2017; Akgunlu et al., 2016; Orhan et al., 2013; Sekeroglu et al., 2012; Orhan et al., 2012, etc.). Taking our previous researches on medicinal plants and plant-derived natural products, the current study was undertaken to examine total polyphenolic and mineral contents, *in vitro* antioxidant capacities, neuroprotective and enzyme inhibitory activities of the extracts from leaves of *Mentha spicata* L. Besides its importance in food, beverage, cosmetic and confectionary industries, there has been huge ethnobotanical information on the curative properties of *M. spicata* L. against common diseases. As far as our knowledge, no detailed studies on combined biological activities have been performed with extracts spearmint growing under special conditions. Hence, this study may help us to find new potential sources as natural antioxidant and neuroprotective agents.

2. MATERIAL AND METHODS

2.1. Collection of Plant Material: The plant was growing by Prof. Dr. Murat Tuncturk at Medicinal and Aromatic Plants Garden, Van Yuzuncu Yil University, and the voucher specimen was deposited in the herbarium Van Yuzuncu Yil University, Van-Turkey. Taxonomic classification of the plant was given in the Table 1.

Table 1. Taxonomic classification of the plant and plant sample in the Medicinal and Aromatic Plants Garden

Kingdom:	*Plantae*
Subkingdom:	*Tracheobionta*
Division:	*Magnoliophyta*
Class:	*Magnoliopsida*
Subclass:	*Asteridae*
Order:	*Lamiales*
Family:	*Lamiaceae*
Genus:	*Mentha*
Species:	*spicata L.*

2.2. Extract Preparation: To prepare crude extracts, air dried samples (50 g) of the aerial parts of the plant were individually extracted with 250 ml methanol (70%) and distilled water for 2 days at the room temperature, as described in our previous research (Gezici and Sekeroglu, 2019b). The extracts yields (w/w%) are given in the Figure 1. Extraction yields of the methanol and water extracts of the aerial parts of the plant were determined as 21.370% and 11.517% (w/w), respectively.

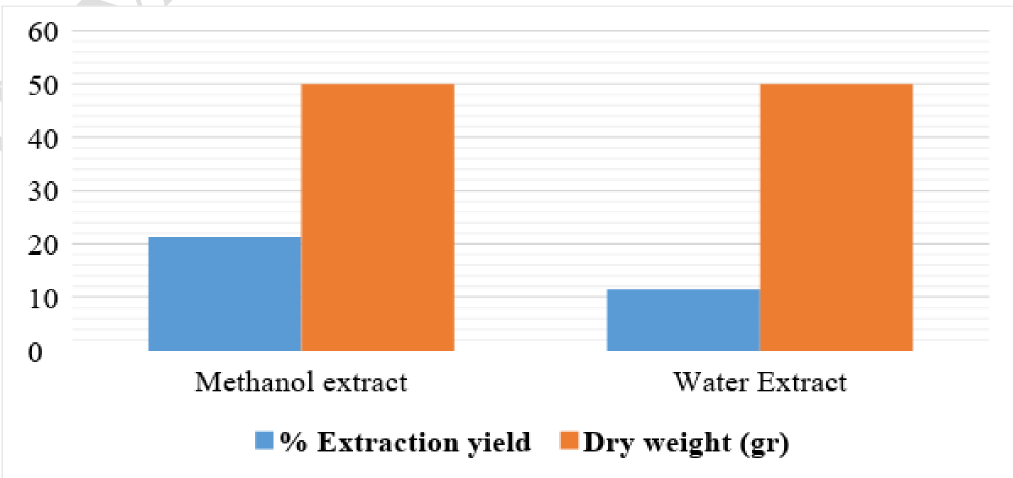

Fig. 1. Extraction yield (w/w) of the plant

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Determination of Total Polyphenolic Contents: Phenolic compounds in total were determined in accordance with slightly modified Folin-Ciocalteau's method (Singleton and Rossi, 1965; Gezici and Sekeroglu, 2019). Absorption was measured at 760 nm at a using a 96-well microplate reader (VersaMax Molecular Devices, USA). Total flavonoid content of the extracts was calculated by aluminum chloride colorimetric method (Woisky and Salatino, 1998; Gezici and Sekeroglu, 2019b). A number of dilutions of quercetin were obtained to prepare a calibration curve. Absorbance of the reaction mixtures was measured at wavelength of 415 nm with a using a 96-well microplate reader (VersaMax Molecular Devices, USA). The total phenol and flavonoid contents of the extracts were expressed as gallic acid and quercetin equivalents (mg g-1 extract), respectively.

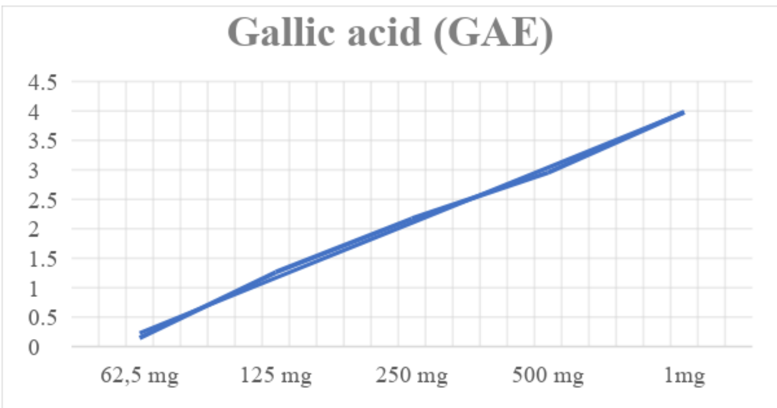
2.3. Determination of Mineral Contents: The presence of mineral elements such as potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), cadmium (Cd), and lead (Pb) in the aerial parts of the plant were determined by Atomic Absorption Spectrometry (AAS, Perkin-Elmer 2280) (Sekeroglu et al., 2017).

2.4. Antioxidant Activity Assays: Since oxidative damage is one of the major factors contributing to both cancer and neurodegeneration, *In vitro* methods including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and cupric ion reducing capacity (CUPRAC) were performed to reveal antioxidant activity of the extracts (Gezici et al., 2017; Sekeroglu et al., 2017; Gundogdu et al., 2018; Gezici and Sekeroglu, 2019b).

2.5. Enzyme Inhibition Assays: Neuroprotective activities of the extracts on AChE, BChE, and tyrosinase were evaluated in the current study. AChE and BChE inhibitory activity of the samples was measured by slightly modified spectrophotometric method of Ellman et al. (1961). Electric eel AChE (EC 3.1.1.Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1. Sigma, St. Louis, 7 MO, USA) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction. 5,5′-Dithio- bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the anticholinesterase activity. All reagents, conditions and calculations were same as described in our previous publication (Senol et al., 2018; Gezici and Sekeroglu, 2019b). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. The experiments were done in quarduplicate. Galanthamine hydrobromide (Sigma, St. Louis, MO, USA) was used as the reference drug. Inhibition of tyrosinase (EC 1.14.1.8.1, 30 U, mushroom tyrosinase, Sigma) was determined using the modified dopachrome method with L-DOPA as substrate (Sekeroglu et al., 2012).

3. RESULTS AND DISCUSSION

Total polyphenolic compositions of the extracts were identified spectrophotometrically in the current research. With regard to total polyphenolic components, the methanol extract possessed higher phenols and flavonoids contents (3.158±0.52 mg/g extract as GAE and 1.31±0.08 mg/g extract as QE, respectively). Gallic acid and quercetin equivalent as commercial standards for total phenolic phenolic and flavonoid contents were shown in the Figure 2.



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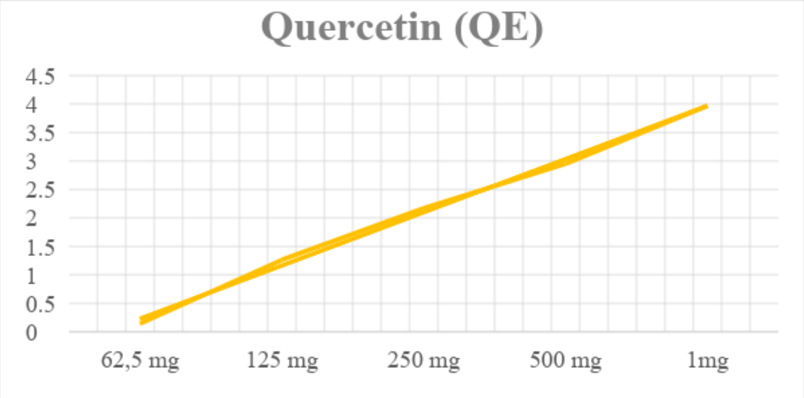

Fig. 2. Gallic acid and Quercetin Equivalent

K, Ca, Mg, Fe, Zn, Cu, Mn, Cd, Pb mineral contents of the plant were also determined in the presented research. A wide variation was observed in the mineral contents of the plant and the mineral values were given as mean±Sd in the Table 2.

Table 2. Mineral contents (mg/kg) of *M. spicata* L.

K (g/kg)	**Ca (g/kg)**	**Mg (g/kg)**	**Fe (mg/kg)**	**Zn (mg/kg)**
14,84±0,95	15,20±0,83	6,64±0,40	1471,31±12,28	19,53±1,08
Cu (mg/kg)	**Mn (mg/kg)**	**Cd (mg/kg)**	**Pb (mg/kg)**	
6,69±072	110,46±1.74	0,045±0,05	0,024±0,01	

Antioxidant activity of the extracts obtained from the aerial parts of the plant were evaluated by using radical scavenging against DPPH and ABTS radicals, and ion reducing antioxidant power on FRAP and CUPRAC. Regarding of antioxidant assays, both methanol and water extracts obtained from leaves of *M. spicata* L. exhibited excellent scavenging activities on DPPH, FRAP, ABTS, and CUPRAC, comparing the standard antioxidants. A significant correlation was found between total polyphenolic compositions and antioxidant capacities of the spearmint leaves. Correlation analysis indicated that the methanol extract, which possessed higher total phenols and flavonoids components than the water extract, was also found the more efficient in terms of antioxidant capacities and neuroprotective potentials.

Neuroprotective activity of the extracts was assessed through enzyme inhibition assays on cholinesterase enzymes. The spearmint leaves were extracted with water and methanol solvents, and subjected to enzyme inhibitory assays on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase which are closely related to pathogenesis of neurodegenerative disease. According to the results, both of the extracts demonstrated powerful inhibition on BChE, whereas they showed moderate inhibition on AChE and tyrosinase enzymes with the values ranging from 24.06±0.12% to 57.68±0.18%.

4. CONCLUSION

Consequently, a strong correlation was observed between the phytochemical and mineral constituents of the plant and its biological properties. In the light of the findings, our data indicate that *M. spicata* L., a natural antioxidant and neuroprotective agent, can provide considerable benefits against oxidative stress-related diseases. Moreover, it appears to be a natural source having promising inhibitory molecules, are worth to conduct further *in vivo* investigations that is under further investigation by our group.

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FULL PAPER

DETERMINATION OF VOLATILE AND SEMI-VOLATILE OIL CONTENT OF *ISATIS TINCTORIA* L. VIA SPME-GC-MS

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Abstract
Isatis tinctoria L. is a species of the genus Brassicaceae and generally distributed in Central Asia. The plant is also used for medical purposes as well as being used as a colorant. The aim of this study is to determine and characterize the volatile oil components found in the leaves and flowers of *Isatis tinctoria* via the SPME-GC-MS method. The plant material was collected from the Ardıçlı Village (Konya Province) and preserved under suitable conditions. 65 µm PDMS/DVB and 85 µm polyacrylate fibers were used to determine the volatile and semi volatile oil composition of the samples. Analyzes were performed using the Shimadzu QP2010 ULTRA FID GC–MS system. According to the data obtained from the analysis of *Isatis tinctoria*, in leaf content 148 compounds and in flower content 156 compounds were detected respectively. According to the analysis, with 65 µm PDMS/DVB fiber, the most amount of components in the leaf Benzeneethanol (4.67%) and 5-Methylundecane (3.93%), the most common components in flower, 4-Octadecylmorpholine (18.68%), 4-Amino-N-(3-morpholin-4-ylpropyl)-1,2,5-oxadiazole-3-carboxamide (11.91%). With 85 µm polyacrylate fiber, the most amount of components in the leaves, benzene, 2,4-diisocyanato-1-methyl (33.41%) and pyrrole-3-carbonitrile, 5-formyl-2,4-dimethyl (14.47%), in the flower content 2,2,3,3,4,4,4-Heptafluoro-N-(2-morpholin-4-yl-ethyl)-butyramide (19.67%) and 4-Octadecylmorpholine (18.07%), respectively. This study is the first study in terms of characterization and comparison of volatile and semi volatile oil components by SPME-GC-MS analysis from leaves and flowers of *Isatis tinctoria* L. species from Turkey.

Keywords: Çivitotu, solid phase micro-extraction, Turkey.

INTRODUCTION

One of the rare colors used as dyestuff in natural dyeing is blue. *Isatis tinctoria* L. (çivit otu, Brassicaceae) is one of the primary sources of blue color in nature. Also, it is recommended for the treatment of wounds, ulcers and tumors, hemorrhoids, snake bites and various inflammatory diseases. For this reason, *I. tinctoria* has a long and well documented history as an indigo dye plant and a medicinal plant (Mohn, 2009). In recent years, a large number of phytochemical, biological and pharmacological studies have been conducted. More than 100 secondary metabolites have been described in *I. tinctoria*. These are like isatin, tryptanthrin, deoxyvasicinone, isaindigotone, isaindigotidione, quinazolines, indolinone, benzodiazepine, the indigo dyes indigo and indirubin, or their natural precursors. *I. tinctoria*, a rich source for glucosinolates represented by glucoraphanin, progoitrine and glucobrassicine compounds, has been reported to contain compounds such as aromatic and aliphatic carboxylic acids (Honda et al., 1980; Goetz and Schraudolf, 1983; Lockwood and Belkhiri, 1991; Hartleb and Seifert, 1994, 1995; Wu et al., 1997a,b; Zhu, 1998; Fréchard et al., 2001).

The species was used as a medical plant in both Europe and East Asia. However, Hamburger (2002) reported that *Isatis tinctoria* is a plant that has been neglected in Europe today but could be returned to the European phytotherapy list with continue sytematic investigations. Heinemann (2004) and Recio et al. (2006a,b) reported that lipophilic *Isatis tinctoria* extracts have anti-inflammatory activity in skin erythema, acute and chronic inflammation, contact allergy and rheumatoid arthritis. Although several approaches can be pursued in the assessment of a plant’s pharmacological potential, depending on the preparation mode, the composition of an extract can vary greatly. Therefore, in the evaluation of the volatile and semi-volatile profile of *Isatis*, we directly analyzed the flowers and leaves of plant pulverized by drying with SPME-GC-MS.

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Solid phase micro extraction (SPME) is a simple, fast and economical extraction method that has gained popularity in foods and especially in the determination of volatile aroma profile in recent years. SPME has been successfully used to identify aroma compounds with different functional groups (Koufogianni, 2006). The aim of this study is to determine the volatile content of wild *Isatis tinctoria* and to put the economic importance of the identified components for this species of our town.

MATERIAL AND METHODS

Material: The *Isatis tinctoria* samples were collected from its natural habitat. The powdered samples (3gr) sealed in a 10 ml vial. The SPME fibres preferred for analysis were 65 µm PDMS/DVB and 85 µm polyacrylate. The SPME apparatus was directly injected into the upper space of the vial to adsorb volatile substances and then directly injected into the Shimadzu QP2010 ULTRA GC-MS apparatus using a Restek Rxi-5 MS capillary column.

GC-MS Analysis: The volatile components separated for 15 min by inserting the SPME fibre into a GC injector (the injector temperature was 250 °C). The extracts from SPME procedure analysed on a Shimadzu QP2010 ULTRA FID GC–MS system. A Restek Rxi-5 MS column (30 m×0.25 mm id, film thickness 0.25 µm) used with helium as a carrier gas. The GC oven temperature was programmed to hold at 40 °C for 3 min and then to increase to 240 °C at 5 °C/min, finally holding at 240 °C for 3 min. The column flow rate was 1.8 mL/min. The ion source temperature was 200 °C, and the interface temperature set at 250 °C.

Evaluation of data: After the GC–MS analysis, each sample was demonstrated with GC–MS chromatograms. Chromatograms of all samples were subjected to noise reduction prior to peak area integration, and then the peak areas of compounds in the chromatogram were integrated. Compounds were identified by comparison with three libraries, W9N11, SWGDR4G4 and SWGDR4G5. Identification of components in the sample was based on the retention time (RT). The relative percentage of the volatile oil constituents was calculated from peak areas. All analyses were carried out three times.

RESULT AND DISCUSSION

Totally, 148 component identified from the leaves and 156 compound identified from the flowers during our analyses. As a result of all analyzes with flowers and leaves, some common components were detected. These are 1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl; Dimethyl sulfone; 5,9-Undecadien-2-one, 6,10-dimethyl; 3-Methylbutanoic acid; 2-Methylbutanoic acid; Benzaldehyde; 3,5-Octadien-2-One; Naphthalene; Tetradecane; Benzene, 2,4-diisocyanato-1-methyl; Dihydroactinidioide; 4-Octadecylmorpholine; Dimethyltetradecylamine, Phytone, 2-Ethylhexanol and Phytane (Table 1). With 85 µm polyacrylate fiber, the Benzen, 2,4-diizosiyanato-1-metil compound is 33.41% on the leaves and 10.81% on the flower. With 65 µm PDMS/DVB fiber, the Benzen, 2,4-diizosiyanato-1-metil compound is 0.81% on the leaves and 0.31% on the flower. Allyl isothio cyanate, which is an essential component in the leaves of species, was identified by 65 µm PDMS/DVB fiber. These compounds are responsible for preventing the proliferation of cancer cells and promoting apoptosis or programmed cell death of tumor cells. The compounds such as isothiocyanate and D-limonene were reported to be related to cancer. Isothiocyanates are chacteristic flavour compounds in *Brassica*, and the cancer chemo-protective attibutes are recently responsible for their growing interest (Condursa et al., 2006; Hashimoto et al., 2019).

The other common compound is 4-Octadecylmorpholine. This compound is the highest in the flower of species, and it has been previously reported to possess antibacterial and antifungal potential. 4-Sec-Butyl-2,6-di-tert-butylphenol, an industrial chemical, is known as DTBSBP. DTBSBP may be used as an antioxidant in mineral/vegetable oils to prevent oil thickening and the oxidation process, which can lead to sediment formation. This compound has a value of 4.18% (65 µm PDMS/DVB) and 5.17% (85 µm polyacrylate) in the flowers of the species and 0.27% in the leaves (85 µm polyacrylate fiber). Parallel to these pharmacological investigations, advanced phytochemical and analytical studies of the plant have been carried out (Hamburger 2002). Condurso et al. (2006) were reported that leaf volatile in *I. tinctoria* were analyzed by 50/30 mm DVB/CAR/PDMS fiber of SPME/GC-MS, and they were

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characterized by acids, alcohols, aldehydes, ethers, furans, hydrocarbons, isothiocyanates, ketons, nitriles, terpenes and sesquiterpens. Furthermore, these components were detected by Verzera et al. (2010). These data are consistent with our results.

Table 1. Volatile oil and semi-volatile oil of flowers and leaves from *Isatis tinctoria*

Flowers 65 µm PDMS/DVB Volatile compounds	**Area %**	**Flowers 85 µm polyacrylate Volatile compounds**	**Area %**	**Leaves 65 µm PDMS/DVB Volatile compounds**	**Area %**	**Leaves 85 µm polyacrylate Volatile compounds**	**Area %**
Hexanal	0.24	3-Methylbutanoic acid	0.06	Butyric acid	0.18	3-Methylbutanoic acid	0.58
Hexamethylcyclotrisiloxane	0.94	2-Methylbutanoic acid	0.33	Hexanal	0.61	2-Methylbutanoic acid	1.29
3-Methylbutanoic acid	0.52	Oxime-, methoxy-phenyl	0.13	4-Methyl-1-pentanol	1.21	Valeric acid	0.24
2-Hexenal	0.20	Dimethyl sulfone	0.04	3-Methylbutanoic acid	1.17	Dimethyl sulfone	0.11
3-Hexen-1-ol	0.17	Benzaldehyde	0.08	2-Hexenal	0.67	Benzaldehyde	0.25
Ethylbenzene	0.55	Glycerin	0.04	3-Hexen-1-ol	1.12	Glyserol	0.07
2-Methylbutanoic acid	0.25	Hexanoic acid	0.98	2-Methylbutanoic acid	2.06	Hexanoic acid	0.55
p-Xylene	0.25	Benzene, 1,3-dichloro	0.07	4-Methyloctane	0.43	Benzene, 1,3-dichloro	0.20
Styrene	0.29	2-Ethylhexanol	0.40	Allyl isothiocyanate	0.12	4-Methyldecane	0.14
Xylose	0.30	Benzyl alcohol	0.28	Valeric acid	0.56	2-Ethylhexanol	0.16
4-Heptenal	0.15	Acetophenone	0.07	Dimethyl sulfone	0.33	1,5-Heptadien-4-one, 3,3,6-trimethyl	0.57
Oxime-, methoxy-phenyl	1.13	3,5-Octadien-2-one	0.12	Methyl hexanoate	1.29	Tridecane	0.36
Dimethyl sulfone	0.18	Nonanal	0.05	alpha-Pinene	0.11	3,5-Octadien-2-One	0.40
Benzaldehyde	0.79	3-Acetyl-2,6-heptanedione	0.04	4-Methylpentanoic acid	0.11	2,6-Dimethylcyclohexanol	0.31
Nonane, 2-methyl	0.33	Benzeneethanol	0.95	Benzaldehyde	1.08	Propan-2-yl tetradecyl sulfite	0.04
Heptanoic acid	1.07	Naphthalene	0.17	4-Methylnonane	0.26	5-Butylnonane	0.03
2-Pentylfuran	0.28	1-Tridecene	0.34	Nonane, 2-methyl	0.68	3-Methylpyrazole	0.07
Decane	0.35	1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl	0.09	Nonane, 2,5-dimethyl	1.8	2,4,4-Trimethylhexane	0.05
Octanal	0.12	2-Ethoxyethyl acrylate	0.25	octenol	0.12	Naphthalene	0.29
Cyclotetrasiloxane, octamethyl	0.74	Anethole	0.10	6-Methyl-5-hepten-2-one	1.20	1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl	0.10
1,4-Dichlorobenzene	0.83	Decanoic acid, methyl ester	0.21	Butyl acetate	1.06	Dodecane	0.12
2,4-Heptadienal	0.42	2-Methyl-5-nitro-3-hexanol	1.4	Hexanoic acid	0.85	2-Methyldecane	0.12
5-Methyldecane	0.47	**Benzene, 2,4-diisocyanato-1-methyl**	**10.81**	Ethyl hexanoate	2.43	Dodecane, 4-methyl	0.22
Nonane, 2,5-dimethyl	0.56	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	0.16	1,4-Dichlorobenzene	2.88	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl	0.75
4-Methyldecane	1.5	1,3-Benzenediamine, 4-methyl	0.67	Undecane	2.06	3,7-Dimethyldecane	0.03
D-Limonene	0.49	myristyl alcohol	0.35	4-Methyldecane	3.53	4,6-Dimethyldodecane	0.13
2-Ethylhexanol	2.25	Decanoic acid, ethyl ester	0.38	l-Limonene	0.47	Tetradecane	0.21
1,5-Heptadien-4-one, 3,3,6-trimethyl	0.73	4-Pyrrolidinopyridine	2.27	Eucalyptol	0.58	2-Ethoxyethyl acrylate	0.38
Benzeneacetaldehyde	0.18	**Pyrrole-3-carbonitrile, 5-formyl-2,4-dimethyl**	**4.81**	2-Ethylhexanol	0.37	4-Propylbenzaldehyde	0.49
Undecane, 4,7-dimethyl	1.80	.alpha.-Ionone	0.09	1,5-Heptadien-4-one, 3,3,6-trimethyl	1.23	Phytane	0.21
3,5-octadien-2-one	1,4	2-Heptanol, 6-methyl	0.06	2-Bromo-4-methylpentane	0.27	Undecane, 4,7-dimethyl	0.08
Dodecane, 5-methyl	0.10	5,9-Undecadien-2-one, 6,10-dimethyl	0.17	**5-Methylundecane**	**3.93**	4-Methyltridecane	0.04
Undecane	0.41	Cetyl alcohol	0.10	5-(2-Methylpropyl)nonane	1.23	5-Methyltetradecane	0.12
2,3,6,7-Tetramethyloctane	1.44	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	0.39	**3,5-Octadien-2-one**	**3.15**	2,6,11-Trimethyldodecane	0.20
Nonanal	0.55	Tetradecane	0.30	7-Methyl-1-undecene	0.11	**Benzene, 2,4-diisocyanato-1-methyl**	**33.41**
3-Acetyl-2,6-heptanedione	0.77	pentadecane	0.06	Methyl 6-methylheptanoate	0.71	1,3-Benzenediamine, 4-methyl	0.67
4,6-Dimethyldodecane	1.65	N,N-Dimethyldodecylamine	0.31	2,3,6,7-Tetramethyloctane	0.51	4-Pyrrolidinopyridine	6.63
4-Methylene-1,3-dioxolane	0.14	2,4-Di-tert-butyl-1-methoxybenzene	0.05	Octane, 6-ethyl-2-methyl	2.52	**Pyrrole-3-carbonitrile, 5-formyl-2,4-dimethyl**	**14.47**
3,7-Dimethyldecane	0.12	Dicyclohexyl ketone	0.15	2,6-Dimethylcyclohexanol	1.03	5,9-Undecadien-2-one, 6,10-dimethyl	0.65
6-Methylundecane	0.13	Dodecanoic acid, methyl ester	0.57	2,6,10-Trimethyldodecane	0.89	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	0.11
Decamethylcyclopentasiloxane	0.95	Dihydroactinidioide	0.35	**Benzeneethanol**	**4.67**	beta-Ionone	1.90
Isododecane	0.64	Megastigmatrienone 2	0.19	Methyl octanoate	0.40	Octadecane	0.14
2,6-Octadiene, 2,6-dimethyl	0.13	Ethyl laurate	0.85	Isododecane	1.23	**Dihydroactinidioide**	**5.01**
2,4-Dimethylundecane	0.38	Hexadecane	0.27	Ethyl palmitate	1.78	Methanone, diphenyl	0.34
2,5-Dimethylundecane	1.32	Hexadecanal	0.15	4a,5-Dimethyl-6,7,8,8a-tetrahydro-5H-benzo[d][1,3]dioxin-4-one	0.40	Decanoic acid, decyl ester	0.56
Naphthalene	0.85	Methanone, diphenyl	0.40	2,5-Dimethylundecane	1.94	4-Sec-Butyl-2,6-di-tert-butylphenol	0.27
alpha-Terpineol	0.21	**2H-1,3-Oxazine-2-thione, tetrahydro-4,4,5,6-tetramethyl**	**5.55**	Naphthalene	1.19	4-Octadecylmorpholine	2.52
1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl	0.32	**4-Sec-Butyl-2,6-di-tert-butylphenol**	**5.07**	1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl	1.57	Dimethyltetradecylamine	0.49
Dodecane	0.69	**4-Octadecylmorpholine**	**18.07**	Dodecane	1.44	Citronellol	0.13
Tetradecane	2.1	Naphthalene, 2,6-bis(1-methylethyl)	0.12	Hexadecane	1.03	Phytone	2.46
Dodecane, 4-methyl	0.52	Phytane	0.11	2,4-Dimethylundecane	0.44	**5H-1,4-Dioxepin, 2,3-dihydro**	**5.79**
1H-Pyrrole-2,5-dione, 3-ethyl-4-	0.25	Dimethyltetradecylamine	2.44	Dodecane, 4-methyl	0.84	**Dimethyl palmitamine**	**4.21**

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methyl							
2,6-Dimethylundecane	0.37	Benzoic acid, 2-ethylhexyl ester	0.72	Tetradecane	1.3	4-(Hexyloxy)-1-butanol	0.18
3,3-Dimethyloctane	0.14	Methyl myristate	0.23	2,6-Dimethylundecane	0.50	1,3,5-Triazine-2,4(1H,3H)-dione, 6-(2-methylphenylamino)	3.19
2,4-Dimethyldodecane	0.80	Dibutyl adipate	0.13	4,6-Dimethyldodecane	1.76	Laurinsaeure, 4-Octylester	0.39
N-Formyl-DL-phenylalanine	0.19	Ethyl nonadecanoate	0.18	N-Formyl-DL-phenylalanine	0.18	**2,5-Dimethyl-4-hydroxy-3-hexanone**	**4.58**
Phytane	1.57	2,3,7-trimethyloctanal	0.18	Phytane	2.25		
Estragole	0.31	Phytol	0.37	3-Methyl-5-propylnonane	0.82		
pentadecane	0.76	Phytone	0.66	Tridecane	0.19		
Tridecane	0.30	**2,2,3,3,4,4,4-Heptafluoro-N-(2-morpholin-4-yl-ethyl)-butyramide**	**19.67**	2,3,7-Trimethyldecane	0.62		
2,3,7-Trimethyldecane	0.43	Nonadecane	0.33	decanoic acid, methyl ester	1.96		
Butanoic acid, 2-ethylhexyl ester	0.26	**1-Hexadecanaminium, N,N,N-trimethyl-, bromide**	**11.47**	Dodecamethylcyclohexasiloxane	0.47		
2,6,11-Trimethyldodecane	0.85	Methyl palmitate	0.11	2,6,11-Trimethyldodecane	0.21		
Dodecamethylcyclohexasiloxane	0.63	Dodecyl propan-2-yl sulfite	0.21	Benzene, 2,4-diisocyanato-1-methyl	0.81		
Sulfurous acid, pentyl tridecyl ester	0.10	13-.Alpha.-Methylandrosta	0.12	2-Methyltridecane	0.14		
Benzene, 2,4-diisocyanato-1-methyl	0.30	Eicosane	0.28	Decanoic acid	0.26		
10-Methylnonadecane	0.13	1,3,5-Triazine-2,4(1H,3H)-dione, 6-(2-methylphenylamino)	1.4	1-Oxaspiro[4.5]decan-2-one, 3-methylene	0.32		
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	0.20	Heneicosane	0.27	Decanoic acid, ethyl ester	1.71		
Cadinol	0.26	Dodecanoic acid, isooctyl ester	0.28	6,10-Dimethylundecan-2-one	0.24		
Dodecanal	0.43	Tetracosane	0.19	Pentamethylbenzaldehyde	0.20		
Heptadecane	0.21	Methoxyacetic acid, 4-tridecyl ester	0.07	Nonadecane	0.18		
5,9-Undecadien-2-one, 6,10-dimethyl	0.55			.alpha.-Ionone	0.19		
Nonadecane	0.09			5,9-Undecadien-2-one, 6,10-dimethyl	0.40		
beta-Ionone	1.12			4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	1.08		
N,N-Dimethyldodecylamine	0.19			Methyl 10-methylundecanoate	1.45		
bis(2-Ethylhexyl) ether	0.09			Eicosane	0.36		
Dihydroactinidioide	1.98			Pentadecane	0.28		
Hexadecane	0.54			Dicyclohexyl ketone	0.31		
Isopropyl dodecanoate	0.10			Dodecanoic acid, methyl ester	2.58		
Decanoic acid, decyl ester	4.51			Dihydroactinidioide	0.90		
4-Sec-Butyl-2,6-di-tert-butylphenol	**4.18**			Lauric acid	0.71		
4-Octadecylmorpholine	**18.68**			Megastigmatrienone	0.75		
Dimethyltetradecylamine	1.51			Ethyl laurate	2.66		
Benzoic acid, 2-ethylhexyl ester	0.49			Decanoic acid, decyl ester	0.20		
Octadecane	0.17			Dimethyltetradecylamine	0.27		
Hexadecanal	0.18			Methyl myristate	0.17		
Phytone	1.00			Ethyl tetradecanoate	0.20		
4-Amino-N-(3-morpholin-4-ylpropyl)-1,2,5-oxadiazole-3-carboxamide	**11.91**			Phytone	1.46		
Hexadecanyldimethylamine	**6.32**			4-Octadecylmorpholine	1.12		
Eicosane	0.71			hexadecanyldimethylamine	2.12		
Hexacosane	0.14			Docosane	0.16		
laurinsaeure, 4-octylester	0.72			Laurinsaeure, 4-Octylester	2.16		
2-Linoleoylglycerol	0.24						
Linoleoyl chloride	0.67						
1,3-Di(trans-9-octadecenoyl)glycerol	0.56						

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CONCLUSION

In conclusion, the volatile oil and semi-volatile oil contents of *Isatis tinctoria* have been examined for the first time by SPME/GC-MS analysis. When considering the substances contained in *I. tinctoria*, we can say that the species has an antifungal, antibacterial, therapeutic effect. Although the volatile and semi-volatile oils such as Anethole, Limonene, Cetyl alcohol, Decamethylcyclopentasiloxane, Terpinol, Estragole, Valeric acid, Glycerol, Citronellol, Butyric acid, Eucalyptol, Ethyl palmitate and Cadinol are found at low levels in leaves and flowers, these compounds are flavoring, aroma enhancer, food additive, It is used for perfume, cosmetics, pharmacological and medical purposes.

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FULL PAPER

IN VITRO SUSCEPTIBILITY TESTING OF TEA TREE OIL ACNE CREAM IN COMBINATION WITH SALICYLIC ACID AND NICOTINAMIDE

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Abstract: Acne vulgaris, is a commonly occurring skin disease caused by *Staphylococcus aureus* and *Propionibacterium acnes* (Gollnick *et al.*, 2008). Tea Tree Oil (TTO) is derived mainly from the Australian native plant *Melaleuca alternifolia* (Maiden & Betche) Cheel, belongs to family Myrtaceae. The oil is very much effective in treating a broad range of infectious conditions, especially fungal skin conditions, as well as warts, acne, and vaginal yeast infection (Edris, 2007). The present study is focused to evaluate the *in-vitro* susceptibility and effectiveness of tea tree oil acne cream against acne culprits; *Staphylococcus aureus* and *Propionibacterium acnes.* **Materials and Methods:** Tea tree oil acne cream is a compounding and dispensing product of the *Nigehban Pharmaceuticals (Pvt.) Ltd.* Lahore Pakistan. The bacterial samples were collected from the skin portion of forehead and cheeks of the patients from outdoor of Mayo Hospital Lahore. The formulation was tested for its susceptibility by microwell dilution method on 15 samples of *S. aureus* and five samples of *P. acnes*. After finding the susceptibility, minimum inhibitory concentration of the cream was estimated to establish its effectiveness, by 96 well plate method. SPSS Ver. 21 was employed for data analysis obtained from experiments. All the results were presented in terms of ± SEM. For analysis of variance, two-way ANOVA was applied with post-hoc Bonferroni test. $p<0.05$ was considered as statistically significant.

Key words: tea tree oil, acne, salicylic acid and nicotinamide.

RESULTS

Antimicrobial assay of tea tree oil acne cream against *S. Aureus*

Table 1. Antimicrobial assay of tea tree oil acne cream against *S. aureus* by microwell dilution assay

Sr. No.	Antimicrobial assay of tea tree oil acne cream against *S. aureus*					
	1:5	1:10	1:20	1:50	1:100	1:1000
SMP 1	12.4±0.22	12.2±0.22	8.26±0.17*	12.16±0.17	8.3±0.25*	2.46±0.27*
SMP 2	12.5±0.35	4.26±0.22*	12.9±0.22	12.5±0.22	4.43±0.32*	3.36±0.32*
SMP 3	12.6±0.17	1.43±0.20*	9.53±0.17*	8.93±0.17*	4.26±0.17*	3.36±0.25*
SMP 4	13.3±0.26	3.36±0.32*	12.4±0.32*	9.83±0.32*	3.5±0.26*	1.43±0.26*
SMP 5	12.9±0.18	8.26±0.22*	8.93±0.22*	13.3±0.26	1.5±0.29*	9.53±0.22*
SMP 6	13.4±0.26	2.46±0.34*	9.3±0.26*	8.6±0.31*	3.36±0.32*	4.26±0.228
SMP 7	8.26±0.22	8.93±0.22	9.53±0.22	9.33±0.26	12.4±0.32	9.83±0.32
SMP 8	13.3±0.26	12.5±0.33	13.4±0.26	12.9±0.18	12.6±0.22	13.3±0.26
SMP 9	14.2±0.18	3.5±0.26*	12.6±0.22	13.5±0.29	2.46±0.34*	3.5±0.26*
SMP 10	13.5±0.30	14.2±0.18	13.3±0.26	12.9±0.14	8.9±O.23*	8.5±0.32*
SMP 11	9.53±0.22	9.3±0.26	14.2±0.18*	13.3±0.26*	9.33±0.28	8.26±0.22
SMP 12	13.5±0.29	8.26±0.22*	9.83±0.32*	13.4±0.26	12.1±0.18	8.1±0.18*
SMP 13	12.1±0.18	13.4±0.26	12.9±0.22	8.1±0.18*	9.53±0.22*	9.3±0.26*
SMP 14	8.1±0.18	9.5±0.18	14.2±0.18*	9.83±0.32	10.1±0.18*	8.4±0.26
SMP 15	9.83±0.32	8.1±0.18	10.1±0.18	2.43±0.32*	4.2±0.18 β	3.3±0.26*
Vancomycin	13.67±0.50	13.67±0.50	13.67±0.50	13.67±0.50	13.67±0.50	13.67±0.50

All the results are presented as ± SEM. For statistical analysis two-way ANOVA with Bonferroni test was applied. Multiple comparisons were done using $p<0.05$. *represents comparison among 1:5 with all other dilutions.

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Antimicrobial assay of tea tree oil acne cream against *P. acnes*

Table 2: Antimicrobial assay of tea tree oil acne cream against *P. acnes* by microwell dilution assay

Sr. No.	Antimicrobial assay of tea tree oil acne cream against *P. acnes*					
	1:5	**1:10**	**1:20**	**1:50**	**1:100**	**1:1000**
Sample 1	2.46±0.34	9.6±0.26*	8.26±0.23*	8.6±0.32*	3.5±0.26	4.26±0.23*
Sample 2	8.23±0.23	9.3±0.26	8.53±0.26	9.1±0.18	8.2±0.18	8.2±0.26
Sample 3	4.26±0.23	13.3±0.26*	2.6±0.18*	3.3±0.18	10.13±0.14*	2.6±0.38*
Sample 4	1.2±0.15	1.1±0.22	1.6±0.15	1.2±0.22	1.3±0.22	1.6±0.15
Sample 5	No effect	No effect	No effect	No effect	No effect	No effect
Vancomycin	13.67±0.71	13.67±0.71	13.67±0.71	13.67±0.71	13.67±0.71	13.67±0.71

All the results are presented as ± SEM. For statistical analysis two-way ANOVA with Bonferroni test was applied. Multiple comparisons were done using $p<0.05$. *represents comparison among 1:5 with all other dilutions.

Minimum Inhibitory Concentration (MIC) of tea tree oil acne cream against *S. Aureus*

Table 3. Minimum Inhibitory Concentration (MIC) of tea tree oil acne cream against *S.aureus*

No of obs.	Conc. (mm)	Bacteria + Compound			Compound	Minimum Inhibitory Concentration			Avg.
1	12.5	0.132	0.127	0.149	0.058	0.074[a]	0.069[b]	0.091	0.078
2	6.25	0.399	0.553	0.479	0.089	0.31	0.464	0.39	0.388
3	3.125	0.465	0.424	0.341	0.075	0.39	0.349	0.266	0.335
4	1.87	0.440	0.507	0.483	0.068	0.342	0.356	0.273	0.323
5	0.935	0.452	0.486	0.491	0.061	0.379	0.446	0.422	0.415
6	0.467	0.569	0.486	0.471	0.058	0.511	0.428	0.418	0.452
7	0.233	0.488	0.492	0.450	0.062	0.426	0.367	0.388	0.393
8	0.116	0.512	0.448	0.478	0.056	0.455	0.391	0.422	0.422
9	0.05	0.553	0.443	0.459	0.057	0.496	0.386	0.402	0.428
10	0.02	0.459	0.457	0.469	0.070	0.389	0.387	0.399	0.391

a = shows MIC at 12.5 conc.
b = shows MIC at 6.25 conc.

Minimum Inhibitory Concentration (MIC) of tea tree oil acne cream against *P. Acnes*

Table 7. Minimum Inhibitory Concentration (MIC) of tea tree oil acne cream against *P. acnes*

No of obs.	Conc. (mm)	Bacteria + Compound			Compound	Minimum Inhibitory Concentration			Avg.
1	12.5	0.359	0.090	0.081	0.058	0.301	0.032	0.023[a]	0.118
2	6.25	0.626	0.155	0.096	0.089	0.537	0.066	0.007[b]	0.203
3	3.125	0.444	0.273	0.148	0.075	0.369	0.198	0.073[c]	0.2130
4	1.87	0.535	0.307	0.352	0.068	0.467	0.239	0.284	0.33
5	0.935	0.635	0.461	0.365	0.061	0.574	0.4	0.304	0.426
6	0.467	0.560	0.380	0.312	0.058	0.502	0.322	0.254	0.359
7	0.233	0.527	0.511	0.388	0.062	0.465	0.449	0.326	0.413
8	0.116	0.568	0.462	0.472	0.056	0.512	0.406	0.416	0.4446
9	0.05	0.536	0.521	0.423	0.057	0.479	0.464	0.366	0.436
10	0.02	0.384	0.517	0.142	0.070	0.314	0.447	0.072[d]	0.277

a = shows MIC at 12.5 conc.
b = shows MIC at 6.25 conc.
c = shows MIC at 3.125 conc.
d = shows MIC at 0.02 conc.

DISCUSSION/ CONCLUSION

A wide variety of essential oils are known to possess antimicrobial properties, and in many cases this activity is due to the presence of active monoterpene constituents (Delaquis *et al.*, 2002). Similarly, in

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the present study we employed the essential oil of *Melaleuca alternifolia* to establish its efficacy in the form of a topical preparation in combination with salicylic acid and nicotinamide. The formulations showed promising effects against these both bacteria strains. The results suggest that the tea tree oil acne cream is effective in a narrow dilution range and shows potent anti-acne effect. The results of the MIC reveal that the formulation has profound action on the bacterial strains. Tea tree oil (5%) had a significant effect in ameliorating the patient's acne by reducing the inflamed and non-inflamed lesions (Carson, 2006). It was reported, that exposing these organisms to MIC and MBC of tea tree oil, inhibited respiration and increased the permeability of bacterial cytoplasmic membrane by uptake of propidium iodide. In the case of *S. aureus*, tea tree oil also causes potassium ion leakage (Trombetta *et al.*, 2005). The tea tree oil acne cream was prepared with salicylic acid and nicotinamide. Salicylic acid is a mild keratolytic and anti-inflammatory agent. (Jacquet, 1988). Nicotinamide is reported to be effective as antibiotic, and provides potent anti-inflammatory action without the risk of inducing bacterial resistance (Shalita, 1995). The ingredients will not only improve the product's susceptibility against the strains but will also show patient compliance.

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FULL PAPER

HEADSPACE SOLID PHASE MICROEXTRACTION (HS-SPME) ANALYSIS OF *SIDERITIS OZTURKII* AYTAC & AKSOY

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Abstract

The genus *Sideritis* (Lamiaceae) is represented by more than 150 bushy species and annual or perennial plants. Many studies have been conducted to elucidate the chemical composition and pharmacological activities of plants of the genus. *Sideritis ozturkii* Aytac & Aksoy is an endemic plant to Turkey and used as herbal tea and folk medicine in central Anatolia. The purpose of this study was the detection of the volatile oil components of *S. ozturkii* by solid phase microextraction/gas chromatography/mass spectrometry (SPME-GC-MS) technique. Dried and powdered samples were used directly. Two SPME fibres, 50/30 µm DVB/CAR/PDMS and 85 µm Carboxen/PDMS were preferred for analysis. SPME procedure was analysed on a Shimadzu QP2010 ULTRA FID GC–MS system. The volatile oil compositions were identified in *Sideritis ozturkii* samples by SPME-GC/MS analysis using two different fibres. The major constituents of dried leaves detected with 50/30 µm DVB/CAR/PDMS fiber were octanoic acid (11.70 %) and nonanoic acid methyl ester (16.68 %) for 85 µm Carboxen/PDMS fiber, respectively. On the other hand, the major constituents of flowers were detected with both fibres were hexanoic acid (24.32 %- 26.34 %) and hexanal (18.67 % -16.68 %) respectively. The comparative volatile oil composition of *S.ozturkii* leaves and flowers were revealed via the SPME method for the first time with this study.

Keywords: Paşa çayı, Solid-phase microextraction, Turkey.

INTRODUCTION

Sideritis (L.) is located within the Lamiaceae family. There are more than 160 species of the genus in all over the world. *Sideritis* is represented by 53 taxa, 40 of which are endemic, in Turkey (Duman, 2012). Endemism rate is 73%. *Sideritis* spp., known as Adaçayı (Sage) and Dağçayı (Ironwort), are widely used in Turkey. Although *Sideritis* species which are widely used as herbal tea in the folk have not been studied extensively in terms of the components which they contain, there are some studies (Topçu et al., 2001; Kırımer et al., 2004; Gonzalez-Burgos et al., 2011). The chemical components of the plant extracts of the species *Sideritis* are flavonoids, terpenoids, lignans, coumarins and iridoids (Gonzalez-Burgos et al., 2011; Koutsaviti et al., 2013; Çarikçi et al., 2018). Moreover, it was reported that some components of the plant have anti-inflammatory, antirheumatic, antistress, analgesic, antioxidant, antispasmodic, antibacterial, digestive, diuretic, and antimicrobial activities (Gonzalez-Burgos et al., 2011; Koutsaviti et al., 2013; Topçu et al., 2001; Topçu et al., 2002; Goren et al., 2002; Tunalier et al., 2004; Fraga 2012).

Sideritis ozturkii, which is an endemic species to our country, is known as Dağçayı. In Central Anatolia, it is used as herbal tea and spices. There is a limited number of studies related to this species. There are some studies including anti-inflammatory and antinociceptive effect s as well as phenolic content and antioxidants associated with antimicrobial activities of *S. ozturkii* (Küpeli et al., 2007; Sagdic et al., 2008). SPME is an increasingly common method for rapidly ejecting volatile compounds from plants (Zhu et al., 2013). SPME is a pre-concentration technology based on direct sample analysis without the use of any solvent and preventing the production of artefacts. Conjunction with gas chromatography (GC)–mass spectrometry (MS) analysis, it has been successfully applied for profiling the metabolomic pattern of environmental, food, forensic and pharmacological samples, and as a powerful technique for the extraction of urinary potential biomarkers of various diseases, such as diabetes, cancer and autism syndrome (Gonçalves et al., 2012; Cozzolino et al., 2014).

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In this study, solid phase microextraction/gas chromatography/mass spectrometry (SPME-GC-MS) technique was used for profiling the volatile oils of dried leaves and flowers from Turkish endemic sage species.

MATERIAL AND METHODS

Material: The plant material was collected from Konya-Derebucak province in 2016. The collected plant material washed and dried without sunlight. Dried and powdered leaf and flower samples were used directly. The ground samples (3gr) sealed in a 10 ml vial. Two SPME fibres, 50/30 μm DVB/CAR/PDMS and 85 μm Carboxen/PDMS were used for analysis. The SPME apparatus was directly injected into the upper space of the vial to adsorb volatile substances and then directly injected into the Shimadzu QP2010 ULTRA GC-MS apparatus using a Restek Rxi-5 MS capillary column.

HS-SPME-GC/MS Analysis: The volatile components were separated for about 15 minutes with different SPME fibres attached to a GC injector (the temperature of the injector was 250 °C). The plant material analysed on a Shimadzu QP2010 ULTRA FID GC–MS system. A Restek Rxi-5 MS column (30 m×0.25 mm id, film thickness 0.25 μm) used with helium as a carrier gas. The GC oven temperature was programmed to hold at 40 °C for 3 min and then to increase to 240 °C at 5 °C/min, finally holding at 240 °C for 3 min. The column flow rate was 1.8 mL/min. The ion source temperature was 200 °C, and the interface temperature set at 250 °C.

Evaluation of data: After the analysis, each sample was demonstrated with GC–MS chromatograms. Chromatograms of all samples were exposed to noise reduction before peak area integration, and then the peak areas of compounds in the chromatogram were combined. Compounds were identified by comparison with three libraries, W9N11, SWGDR4G4 and SWGDR4G5. Compounds were mostly found in the W9N11 library and specified with other libraries. Identification was performed based on the retention time (RT) of the components in the sample. The relative percentage of the volatile oil constituents was calculated from peak areas. All analyses were carried out triplicate, and all data are means of three independent analysis.

RESULTS AND DISCUSSION

The results obtained from the analysis of *Sideritis ozturkii* leave and flower by two different SPME fibres are shown in **Table 1.** Totally, 172 component identified from the leaves and 133 compound identified from the flowers during our analyses. However, we preferred to give 45 main components and their quantities in leaf and flower content are given in the table, respectively. When the content of the leaf was evaluated, it was found that octanoic acid, methyl ester (11,7- 9,73 %) and nonanoic acid, methyl ester (11,25- 16,68 %) ratios were high in both fibres. These compounds are also found in floral content but less in quantity. The most common found components in the flower content are hexanoic acid (24,32- 26,34 %) and hexanal (18,67- 14,29 %). 9 components are common to both leaf and flower content and were detected with both fibres. These are; nonanoic acid, methyl ester, Cyclopentasiloxane,decamethyl-, hexanoic acid, nonanal, octanoic acid, cyclohexasiloxane, dodecamethyl-,1-octanol, heptanoic acid, nonanoic acid respectively. The phenolic composition, essential oils and flavonoids of *Sideritis* species were studied by many researchers before (Gergis et al., 1989; Gomez-Serranillos et al., 1998; Tabanca et al., 2001; Aboutabl et al., 2002; Kilic et al., 2003; Tunalier et al., 2004; Gabrieli et al., 2005). According to the previous study, it was reported that the main components of *S. ozturkii* essential oils were α-pinene and β-pinene via GC/MS analysis in three samples (Kırımer et al., 2001). In our analyses, α-pinene and β-pinene were present but in minor amounts. In this study, the analyses were performed after extraction, but in our study, the sample was used directly without any procedure. One another study, anti-inflammatory and antinociceptive effects of the *Sideritis ozturkii* acetone extract were investigated, and it was reported that the extract has anti-inflammatory and antinociceptive activity (Küpeli et al., 2007). According to the analyses, the components in the content have different effects. For example, octanoic acid and nonanoic acid methyl ester used as a flavouring agent and is present in many plants. On the other hand, hexanal which is one of the most compounds in flower is potentially useful as a natural extract that prevents fruit spoilage (Sharkey, 2016).

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Table 1. The most common compounds identified in *S. ozturkii* leaf and flowers via SPME method

		Fibres			
		50/30 µm DVB/CAR/PDMS	**85 µm Carboxen/PDMS**	**50/30 µm DVB/CAR/PDMS**	**85 µm Carboxen/PDMS**
		Leaf		**Flower**	
	Compound Name	**% area**	**% area**	**% area**	**% area**
1.	Octanoic acid, methyl ester	11,7	9,73	0,76	-
2.	Nonanoic acid, methyl ester	11,25	16,68	0,86	0,86
3.	Cyclopentasiloxane,decamethyl-	4,52	1,4	1,75	1,16
4.	Hexanoic acid	4,3	5,1	24,32	26,34
5.	Nonanal	4	3,46	3,88	2,79
6.	Octanoic acid	3,89	5,7	0,93	0,73
7.	Benzene, 1-methoxy-4-(1-propenyl)-	3,78	-	1,62	5,66
8.	Cyclohexasiloxane,dodecamethyl-	3,54	9,73	2,54	5,08
9.	1-octanol	2,99	1,85	1,83	0,94
10.	Heptanoic acid, methyl ester	2,74		-	-
11.	Octanal	2,51	1,04	-	1,67
12.	Hexanoic acid, methyl ester	2,5	-	0,66	0,30
13.	1,2 Benzenedi carboxylic acid, diethyl ester	2,07	-	-	-
14.	1,2-Benzenedicarboxylic acid, dinonyl ester	2,05	-	-	-
15.	Pyridine, 2-methyl-4,6-dipropyl-	1,8	-	-	-
16.	1-heptanol	1,75	-	0,73	-
17.	Diethyl Phthalate	1,69	-	-	-
18.	Cyclotrisiloxane, octamethyl-	1,55	-	-	-
19.	Hexanal	1,47	-	18,67	14,29
20.	6-methyl-5-hepten-2-one	1,44	-	-	-
21.	Heptanoic acid	1,38	1,06	1,51	1,54
22.	Nonanoic acid	1,28	2,08	0,69	0,80
23.	Benzene, methyl(1-metylethyl)-	1,04	-	-	-
24.	Anethole	-	7,7	-	-
25.	2-furancarboxaldehyde	-	4,58	0,47	2,20
26.	Estragole	-	1,59	0,66	0,51
27.	Cycloheptasiloxane,tet-radecamethyl	-	1,27	0,48	0,91
28.	Decanoic acid, methyl ester	-	1,07	-	-
29.	Decanal	-	1,02	0,63	0,70
30.	3-Methyl-hepta-1,6-dien-3-ol	-	-	-	2,71
31.	Pentanoic acid	0,86	-	1,70	2,07
32.	5-penthyl-2(5H)-furanone	-	-	-	1,73
33.	2-Octenal	-	-	3,70	2,56
34.	Dodecanal	-	-	0,60	1,28
35.	1-Decanol, 2-hexyl-	-	-	-	1,20
36.	Pentanoic acid, 2-methyl-, anhdride	-	-	-	1,15
37.	Furan, 2-penthyl-	-	-	6,21	-
38.	Butyric acid, 2,2-dimethyl, vinyl ester	-	-	2,58	-
39.	D-Limonene	-	-	1,95	0,48
40.	3-Octen-2-one	-	-	1,59	-
41.	2-Heptanone	-	-	1,52	-
42.	2-Heptenal	-	-	1,41	-
43.	Heptanal	-	0,23	1,20	0,25
44.	1-Pentanol	0,16	-	1,13	0,18
45.	1-Octen-3-ol	0,18	-	1,07	-

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CONCLUSION

In this study, the endemic *Sideritis ozturkii* species from Turkey was analysed via HS-SPME-GC/MS method. According to the findings of this study, the leaf and flowers of *S. ozturkii* had a broad, volatile oil content. It is essential to identify the biological effects of a plant and determine the content for the determination of active ingredients. If the plant is an endemic species, its content is much more valuable. This study is a pioneering study, and our research will continue to determine the active phytochemicals and their effects in the content.

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FULL PAPER

FROM A TRADITIONAL REMEDY TO MODERN THERAPY; *IN VIVO* ANTIHEMORRHOIDAL STUDY OF *Malva sylvestris* L.

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Abstract
It is estimated that about 50% of the world population is suffering from haemorrhoids, which is known as the most common anorectal disease. Patients have been trying to cope with the hemorrhoids by utilizing both herbal and chemical preperations. Leaves of *Malva sylvestris*, which is known as mallow in English, Ebegümeci' in Turkish, belongs to Malvaceae family, have been utilized as a folk medicine to treat haemorrhoids. The studies demonstrated that the leaves have potent anti- inflammatory, antioxidant, anti- complementary, anti-cancer and skin tissue integrity activities. In this study, *in vivo* antihemorrhoidal activity and supporting *in vitro* activities of *M. sylvestris* leaf extract were examined in order to find a scientific base for a folkloric usage. **Material and Methods:** Hemorrhoid model was induced by applying croton oil externally in anal part of the rats. After the occurrence of hemorrhoids *M. sylvestris* leaf (50%) extract and a commercial herbal product (Pilex®) as a positive control were implemented externally in different rat groups. 7 days after the treatment, the animals were anesthetized in order to collect a large blood sample via intracardiac bleed and the serums were obtained to determine the Tumor Necrosis Factor alpha (TNF-α), Vascular Endothelial Growth Factor (VEGF) and Superoxide Dismutase (SOD) levels. Sacrified rats were weighed and 2 cm long anorectal tissues were cut and weighed in a sequence to calculate the Anorectal Coefficients (ARC) for each rat. Moreover, the excised tissues were fixed in formaldehyde (10%) for histopathological evaluations. **Results:** According to the ARC scores, biochemical and histopathological results, the leaf extract of *M. sylvestris* showed significant biological activity compared to the controls. The value of ARC was 1.86±0.09 of the plant extract, while serum TNF-α and VEGF levels were 163.22±11.15 ng/L and 246.37±12.64 ng/L, respectively. SOD inhibition of the plant extract was 91.58±6.17%. **Conclusion / Discussion:** The data demonstrated that the antihemorrhoidal activity is directly proportional to the effect on capillary permeability. The effectiveness of the traditional usage has been verified by scientific studies.

Keywords: hemorrhoid, antihemorrhoidal, *in vivo*, croton oil, mallow

INTRODUCTION

Hemorrhoids term is originated from the Greek words "haema (blood)" and "rhoos (flow)". Hippocrates (BC 460) used to describe it as a vascular bleeding in the anus (Leff, 1987). There are limited epidemiological studies on the prevalence of hemorrhoid disease (Lohsiriwat et al., 2012; Everhart et al., 2008). Since the patients suffers from hemmorhoids, refrain from consulting physicians and do consent from the operations, they try to treat themselves with alternative, mostly natural products. Even if a curative preparation is demanded not only in Turkey but also in all over the world, the number of effective drugs derived from natural products is limited. To heal early stages of hemorrhoids, herbals are quite effective, but they are not sufficient to demonstrate the desired performance in more severe stages. The most popular plants/extracts used against hemorrhoids are *Ruscus aculeatus* (butcher's broom), *Aesculus hippocastanum* (horse chestnut), and *Hammamelis virginiana* (witch hazel) (Abascal et al., 2005). Although there are several plants that have

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ethnomedicinal usage for hemorrhoids, pharmacological efficacy of many of them, particularly *Malva sylvestris,* have not been proven scientifically. In traditional medicine, leaves and flowers of the plant are used for the treatment of hemorrhoids, inflammatory and respiratory problems, dermatological and urologic symptoms and gastrointestinal diseases such as heartburn, diarrhea/constipation, and stomachache (Gasparetto et al., 2012). Additionally, experimental studies on leaves revealed that they have an anti- inflammatory, antioxidant, anti- complementary, anticancer effects and improving of damaged skin activity (Baytop, T., 1999; Gasparetto et al., 2012).

The aim of this study is to evaluate antihemorrhoidal activity of the traditionally used *M. sylvestris* leaf extract based on ethnobotanical usage via *in vivo* experimental method.

MATERIALS AND METHODS

Chemicals: All the chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA), and solvents were obtained from Merck at HPLC grade. Croton oil was purchased Alfa Aesar®. Rat Tumor necrosis factor-α (TNF-α) ELISA Kit (YL Biont), Rat Vascular Endothelial Growth Factor (VEGF) ELISA Kit (YL Biont) and Superoxide Dismutase (SOD) Assay Kit 500 test (Sigma Aldrich) were used for biochemical tests.

Plant material and preparation of the extract: The whole flowering cultured plant material was collected during August-September 2017 at Beysehir-KONYA region in Turkey. The leaves of plants were dried in shade and then grounded to powder. Powdered samples (500 g) were extracted with methanol (6×1500 mL) by maceration for 6 days. Filtered methanolic extracts were collected and evaporated until dryness.

Animals: The application for the ethics committee with protocol number 176 was approved by Kobay Experimental Animals Laboratory Local Ethics Committee on 28 April 2016. Healthy adult male Wistar albino rats (2 months old and 180±20 g weight) were used for this study. The rats were maintained at Kobay Experimental Animals Laboratory at 23-25 °C, under standard experimental conditions, at 50% relative humidity and 12 h light/dark cycle. The animals were fed with standard rodent chow and water *ad libitum*.

Induction of hemorrhoid: Hemorrhoid was induced by croton oil solution (deionized distilled water, pyridine and 6% croton oil in diethyl ether) in rats (Nishiki et al., 1988; Azeemuddin et al., 2014; Dey et al., 2016). According to literature cotton swabs (0.4 cm diameter) soaked in 200 µl of croton oil solution were inserted into the anus and ano-rectal section, 1.5 cm from anal opening) of all the studied animals and kept for exactly 30 seconds. Hemorrhoid induction was repeated once a day for the following three days. The presence of edema was denoted at the end of the third day after the induction.

Treatment of hemorrhoid: After the observation of edema at the anus, treatment was started. The dose of Pilex cream was chosen as reference control according to the previous literture (Azeemuddin et al., 2014).

Cold cream was preferred as a base cream. The aqueous phase containing *M. silvestris* extract dissolved in water, was mixed with an oily phase at a temperature of 70-80 °C. The cream was applied to the animals twice a day for 7 days (Gurel et al., 2013).

Table 1. Animal experimental groups

Animal Groups	**Applications**
Control	Rectal 500 µL croton oil solution,
Vehicle control (base cream)	Rectal 500 µL croton oil solution, 2x7days, externally 0.5 mL base cream
Reference control (Pilex® cream)	Rectal 500 µL croton oil solution, 2x7days, externally 0.5 mL pilex cream
***Malva sylvestris* (50%) extract**	Rectal 500 µL croton oil solution, 2x7days, externally 0.5 mL *M. sylvestris* cream

On the day 8th, 5 mL blood samples were collected with intracardiac technique from rats under anesthesia for evaluation of the biochemical parameters. After the animals were sacrificed, both animals and their isolated rectoanal tissues (2 cm in length) were weighed.

Anorectal coefficient (ARC): On order to calculate the Anorectal Coefficient (ARC), animals and their isolated recto-anal tissues (2 cm in length) were weighed. ARC was calculated by the following formula: ARC= Anorectal tissue weight (miligram) / Animal weight (gram).

Biochemical parameters: Rat Tumor Necrosis Factor-α (TNF-α) ELISA Kit (YL Biont), Rat Vascular Endothelial Growth Factor (VEGF) ELISA Kit (YL Biont), Superoxide Dismutase (SOD) Assay Kit 500 test (Sigma Aldrich) were used for evaluating serum TNF-α and VEGF levels in order to interpret the inflammation degree. SOD is associated with the antioxidant capacity.

Histomorphometric Analysis: The anorectal tissues were fixed in 10% formaldehyde at room temperature. After washing the tissues, they were dehydrated in a graded series of ethanol and cleared in xylene solution. Then the tissues were embedded in paraffin by using a tissue processor with vacuum (Leica, Germany). Thin sections of 3-5 micrometre thickness were obtained with a microtome and subsequently stained with Haematoxylin-Eosin. A histomorphometric examination was carried out in order to define the edema, necrosis, inflammation, congestion, haemorrhage, thickened mucosa, degenerative changes and fibrosis in lesion areas (Dey et al., 2016). Histomorphometric parameters are shown in Table 2. Sections referring to lesion areas were evaluated under the bright field microscope (Leica DMB6 B (Wetzlar, Germany) and attached digital camera Leica DFC7000 T (Wetzlar, Germany) using an image processing software (Leica, LAS Germany). Average scores per group were evaluated by two different researchers.

Table 2. Histomorphometric score parameters are indicated.

Parameter	**Lesion Degree in Microscopic Area (%)**		**Histomorphometric Score**
***Edema,** ***Necrosis,** ***Inflammation,** ***Congestion/Thickened,** ***Mucosa Haemorrhage,** ***Degenerative Changes** ***Fibrosis**	No lesion (0%)	= -	0
	Minimal (5-10%)	=+	1
	Mild (10-25%)	=++	2
	Moderate (25-50%)	=+++	3
	Severe (more than 50%)	=++++	4

Immunohistochemical Analysis: Paraffin sections were labelled with myeloperoxidase using indirect immune peroxidase method. Anti-Myeloperoxidase Antibody (Boster Bio USA, PB9057, 1%) was used for immunohistochemical analysis. Myeloperoxidase immune reactivity in sections were scored in lesion areas and scaled in a range from – (0%), + (<10%), ++ (10-50%), +++ (>50%) (Krawisz et al., 1984; Sakai et al., 2002). Circumference of veins and stromal cells were taken into consideration in the analysis.

Statistical Analysis: Experiment and control groups are independent variables, whereas histomorphometric and immunohistochemical scoring parameters are dependent variables. The statistical analysis was performed in SPSS 25.0 statistical software and all data were evaluated for the normal distribution Shapiro-Wilk test. Then the total score values were analysed by Kruskal-Wallis non-parametrical analysis followed by Bonferonni Correction. Values were evaluated under 95% confidence interval and $p<0.05$ was considered significant in all groups.

RESULTS

Anorectal coefficient (ARC) values: As a result, Among the treated groups, the lowest ARC value was observed in *M. sylvestris* cream administered group (Table 3).

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Table 3. ARC values of (Mean ± SD) experimental groups

Animal Groups	**ARC (Mean±SD)**
Control	3.50±0.03
Vehicle control (base cream)	3.43±0.05
Reference control (Pilex® cream)	2.26±0.04***
***Malva sylvestris* (50%) extract**	1.86±0.09***
*:p<0.05; **:p<0.01; ***:p<0.001; SD: Standart error mean	

Serum TNF-α, VEGF and SOD levels: Serum TNF-*α* and VEGF levels of the plant showed a significant difference compared to negative control (Table 4). All the detected TNF-*α* and VEGF serum levels of mallow were less than the reference control group Pilex®. SOD inhibition of mallow was greater than the reference control group Pilex®.

Table 4. Serum TNF-*α*, VEGF and SOD experimental groups

Animal Groups	**TNF ALPHA (Mean ± SD)(ng/L)**	**VEGF (Mean ± SD)(ng/L)**	**SOD (Mean % inhibition ±SD)**
Control	217.30±1.35	400.20±6.39	84.16±1.73
Vehicle control (base cream)	192.80±3.65***	319.40±6.70***	86.74±1.88
Reference control (Pilex® cream)	186.50±1.42***	282.20±6.55***	89.51±1.30
***M. sylvestris* (50%) extract**	163.22±11.15***	246.37±12.64***	91.58±6.17*
*:p<0.05; **:p<0.01; ***:p<0.001; SD: Standart error mean			

Histological analysis: Hemorrhoid induced control and experimental groups exhibited severe inflammation, edema and congestion in histological sections. Histomorphometric scores of lesions excised from rats receiving *M. sylvestris* cream were evaluated histomorphometrically and the recovery of the rectoanal area was found significantly better than that of the control group (p=0.032) (Fig. 1A). *The M. sylvestris* treated group presented similar histomorphometric healing scores with base cream and Pilex cream groups. Although scores belonging to Pilex cream treated group were lower than those of the control group, the difference was not statistically significant (Fig. 2).

Myeloperoxidase (MPO) Immunohistochemical Analysis: The myeloperoxidase immunoreactivity of inflammatory cells in control and experiment groups showed no statistically significant differences in immunohistochemical analysis (Fig. 1B, Fig. 3).

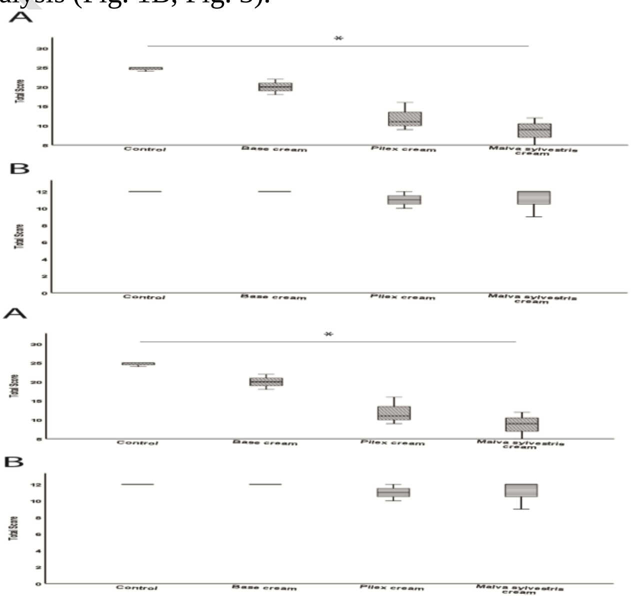

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Figure 1. (A) Minimum, maximum and median values referring to experiment (topical administration) and control groups are depicted as box-plot graphs. Total score of topical administered *M. sylvestris* group was significantly less than that of the control group (*)p<0.05. (B) Minimum, maximum and median values referring to myeloperoxidase labelling in experiment (topical administration) and control groups are depicted as box-plot graphs. Note that the scores remained similar in both groups.

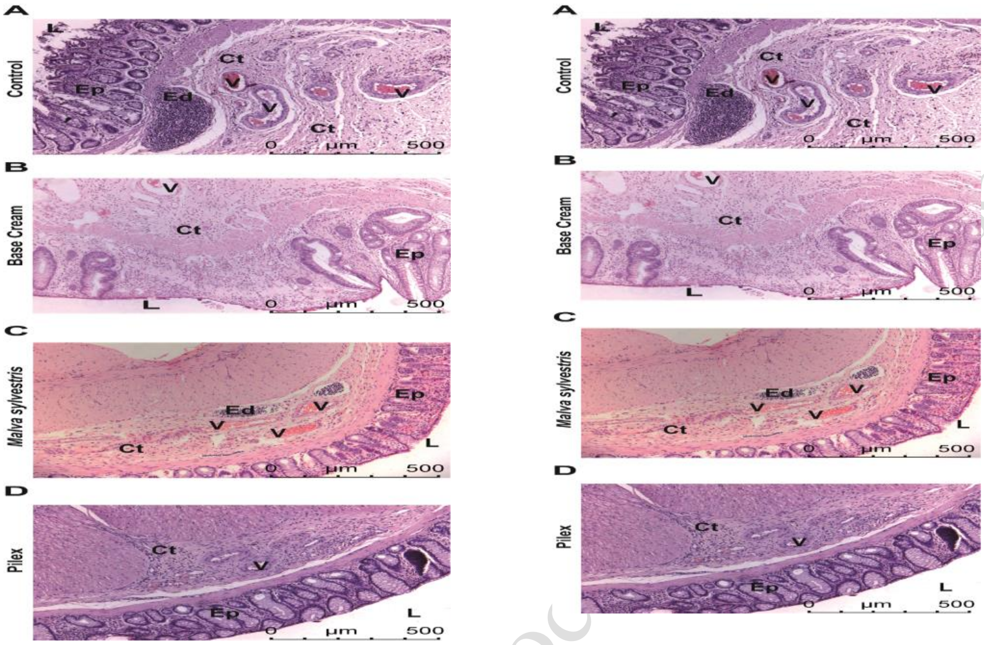

Figure 2. Photomicrographs of histological sections referring to topical applications are demonstrated. Edema and congestive vessels in mucosal and submucosal connective tissue are apparently observed in control group (A) and epithelial loss is clearly seen in vehicle control (base cream) administered group (B). Note the inflammatory cell infiltration in A, B C, and a lesser amount in D. ***Ep: Epithelium, Ct: Connective tissue, V: Vessel, Ed: Edema, L: Lumen, Haematoxylin-Eosin 100x***

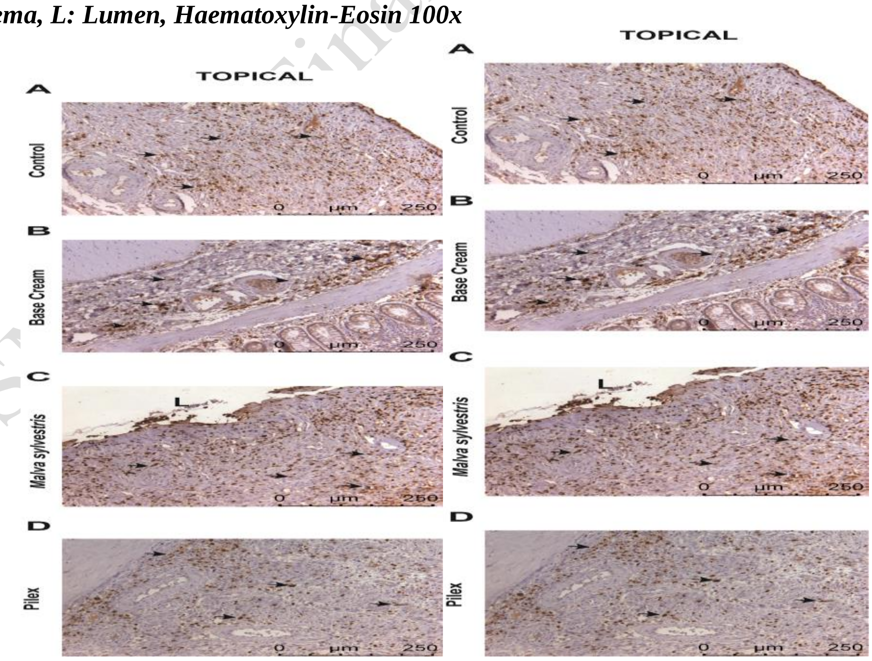

Figure 3. Photomicrographs of myeloperoxidase labelled sections obtained from topical administered groups are demonstrated. Inflammatory cells (arrow) showing positive immune reactivity with myeloperoxidase are marked brown. Inflammatory cells labelled with immune peroxidase are obviously seen in mucosal and submucosal connective tissue in the control (A) and the vehicle control (base cream) administered groups (B). Inflammatory cells labelled with immune peroxidase are lessen in C, however the appearance of the inflammatory cells is observed close to D (reference control). ***L: Lumen, 200x.***

DISCUSSION AND CONCLUSION

The effects of the *M. sylvestris* methanolic extract were examined by *in vivo* studies. According to ARC scores, TNF-*α* and VEGF levels, SOD enzyme inhibition and histopathological-immunohistochemical results, *M. sylvestris* methanolic extract exhibited greater biological activity than the control and vehicle control groups, close to reference control group. TNF-*α*, which is an indicator of inflammation, was significantly lower in the Malva treatment group, which is compared to all the control groups (Yarijani et al., 2018). VEGF, which regulates vascular permeability in mucosal tissue, was detected at low levels in examined rat serum in Malva extract treated group compared to the controls including reference (Bahramsoltani et al., 2014). SOD enzyme inhibition, which is related to lowering of oxidation level in cells, closely related to many pharmacological activities, was noteworthy in Malva extract applied group compared to the control groups (Saad et al., 2016). Although it was not statistically significant, the total histomorphometric scores of the lesions were lower than the control group. Edema and congestive vessels in mucosal and submucosal connective tissue were apparently observed in control group. Unlike the results obtained with hematoxylin staining, MPO immunohistochemical analysis results have not seen a significant activity. Considering the parameters in the serum, the results of MPO immunohistochemical analysis can be neglected. The effectiveness of the traditional usage has been proven on the scientific platform. It was found that the mallow, rich in mucilage and flavonoids, which contributed to healing of hemorrhoidal edema, vasoconstriction of the vessels and inflammation. It is thought that the effective mechanism might be caused by major phytochemicals flavonoids in the Malva methanolic extract, which show effects on anti-inflammatory pathway (Gasparetto et al., 2012). Further phytochemical studies were needed to clarify the actual antihemorrhidal mechanism/s. The various pharmaceutical formulations containing plant extracts is an important factor considering its suitability for commercial applications in pharmacy and cosmetics. Our future goal is to prepare novel antihemorrhoidal formulations.

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FULL PAPER

RADICAL SCAVENGING AND ANTI-BACTERIAL ACTIVITIES OF METHANOLIC EXTRACT FROM ALGERIAN FIR (*ABIES NUMIDICA*)

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Abstract: The objective of this study was the extraction of secondary metabolites from algerian fir leaves (AFL) and the evaluation of the anti-oxidant and the anti-bacterial activities of the crude extract. The air-dried powdred leaves were extracted with methanol solvent using Soxhlet apparatus. The extract was evaporated under reduced pressure by rotary vacum -evaporator type Buchi R-215 at 40°C. DPPH and ABTS assays were used to evaluate the anti-oxidant activity. The results showed that methanolic extract of algerian fir leaves by DPPH method can reduce radicals (IC_{50}=18.08±0.21) lower than ABTS (IC_{50}=23.51±0.54). The Anti-bacterial activity was also tested using the disc diffusion method against five bacterial strains; *Escherichia coli*, *Pseudomonas aeruginosa*, *Morganella morgani*, *Staphylococcus aureus* and *Bacillus subtillus*. The methanolic extract exhibited no anti-bacterial activity against Gram negative bacteria ; *E. coli*, *P. aeruginosa* as well as *M. morganii*. However, it represented a moderate activity against Gram positive; *S. aureus* and *B. subtillus*.

Keywords : Algerian fir, leaves, methanolic extract, DPPH assay, ABTS assay, anti-bacterial activity.

INTRODUCTION

Secondary metabolites or phytochemicals are aromatic, non-nutritive compounds; they are an abundant and diverse group of molecules in plant kingdom. They have an important role for plant in the defense reaction to protect against abiotic stresses and biotic factors (Mazid*et al* ; 2011), for animal because they have a great ability to scavange free radicals (Ali khani ; 2012). There is an increasing suggestions by considerable evidence that the free radicals induce oxidative damage to biomolecules (lipids, proteins and DNA) (Leonard *et al* ; 2003, Medini *et al* ; 2014) caused a degenerative diseases as cancer (Momina *et al* ; 2017), cardiovascular diseases and alzheimer (Brieger *et al* ; 2012). Also for food spoilage caused by several bacterial strains, nowadays, there is a marked interest in the food industry toward the development and manufactures of functional products. For that, there is a huge deal of attention to search and discover a new molecules or products from natural resources (Khoddam*et al*, 2013) that have a beneficial effect comparing with synthetic one. Algeria by its geographical position represents a high biodiversity in plant kingdom major of them are endemic and a few are known about them for exemple : algerian fir (*Abies numidica*) (Quezel ; 1964). *A. numidica* is a conifer belonging to pinaceae family, an endemic tree solely to Algeria; it covers only an area of 2300 ha, found only in Babor's mounts, in the north of Sétif. It is very branchy tree, needles arranged around twigs (Bennadja *et al* ; 2012). It used in traditional medicine as an anti-inflammatory, to treat cataplasm and respiratory system problems. (Ait-kaki *et al* ; 2013). The goal of this research is to investigate the radical scavenging ability of crude methanolic extract extracted from Algerian fir needles; furthermore to evaluate its anti-bacterial activity.

MATERIAL AND METHODS

Collection and preparation of the plant: Leaves of Algerian fir were collected from Constantine, they were harvested randomly from branches during the month of September 2018, after which they were dried for 15 days in the dark at room temperature. Then the sample were powdered using manual grinder IKIa 10 type and kept in the dry, dark place until they were used.

Extraction of secondary metabolites : Secondary metabolites are extracted as the method of Ramadan *et al* ; 2003 with slight modifications, with methanol using soxhlet apparatus for 2 hours. The extract was evaporated under reduced pressure by rotary vacum evaporator type Buchi-R15 type at 40°C.

Biological activities:

***Anti-oxidant activities**

-DPPH radical scavenging assay: The DPPH free radical scavenging assay was tested as Blois, 1958 and Tel G *et al,* 2012 methods with some modifications. The different dilutions of the methanolic extract were prepared in methanol. 40µl of various sample concentrations was added to 160 µl of methanolic DPPH solution in 96-well microplate. Methanol with DPPH reagent mixture was used as control. After 30 min of incubation in the dark at room temperature, the absorbance of each solution was determined at 517 nm using a microplate reader. BHT, BHA were used as standards. Reduction of DPPH radical in percent (R %) was calculated in following way:

% Inhibition= $[A_{blank} – A_{sample}/A_{blank}] * 100$

Where : A_{blank} : is the absorbance of control reaction.

A_{sample} : is the absorbance of test sample.

Tests were carried out in triplicates. The IC_{50} is the inhibition percent represents the half of free radicals (50%), was calculated from the graph of DPPH radical scavenging effect percent against extract concentration.

-ABTS$^{·+}$ radical scavenging assay (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid): This anti-radical activity was measured using a cation decolorisation assay as described by Re *et al,* 1999. Briefly, ABTS·+ solution was prepared by mixing of ABTS$^{·+}$ solution (7 mM) with potassium persulfate solution (2.45 mM). The mixture was left in the dark at room temperature for 16 h before use. This solution was diluted and was adjusted to obtain an absorbance of 0,70 ±0,02 at 734 nm. 160µl of ABTS$^{·+}$ was added to 40µl of sample at different concentrations. The mixture was incubated in the dark for 10 min at room temperature. The blank solution was methanol, ABTS$^{·+}$ solution without methanolic extract was used as control. The antioxidant standards were BHT and BHA. The experiment was performed in triplicate. The inhibition percent was calculated the same as described in DPPH radical assay.

***Anti-bacterial activity**

The anti-bacterial assay was carried out according to the Biondi *et al*, 1993 method with minor modifications, using disc diffusion, against 5 human pathogenic bacteria strains including Gram positive ; *Bacillus subtillis*, *Staphyloccocus aureus* and gram negative ; *Escherichia coli*, *Pseudomonas aeruginosa* as well as *Morganella morganii*. The bacterial strains were first cultured on nutrient agar and were incubated at 37°C for 24 hours. Bacterial suspensions were spread on the surface of Mueller-Hinton agar plates. The sterile filter papers discs (6mm in diameter) were soaked with 10µl of methanol extract for the following concentrations (mg/ml) (100, 50, 25, 12.5, 6.25, 3.125, 1.562 W/V in DMSO). The discs were placed on the inoculated agar medium, kept at 4°C for 1h, and incubated at 37°C for 24h. The anti-bacterial activity was assessed by measuring the inhibition zone surrounding the discs. Each experiment was done in triplicate.

RESULTS

Anti-oxidants activities: The anti-oxidant activities of methanolic extract were tested using DPPH and ABTS assays. The results in **Table 1** showed that the methanolic extract obtained from extraction by soxhlet apparatus has a moderate DPPH and ABTS anti-oxidant activities versa to BHA and BHT standards.

Anti-bacterial activity : Results in **Table 2** revealed that methanolic extract has a mild inhibition zones for Gram (+ve) bacterial strains however, it exhibited no activity against gram(-ve) bacteria.

DISCUSSION AND CONCLUSION

Free radicals or reactive oxygene species are the products of normal cells metaboloism, represent the essential part of aerobic life (Shettar *et al* ; 2015), but the imbalance between these molecules and body system defense, or the excess of them lead to many diseases (Sasikumar ; 2014). For that a lot of works have been done to look for new products from natural substances that have the ability to

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scavenge free radicals and stop this chain reaction, in addition this group of products is well known for its ability to inhibit and suppress the growth of several microbial strains (Nascimento *et al* ; 2000). Natural anti-oxidants have an important function in the enhancement of body system defense and help to prevent many diseases as cancer and diabetes (Kenjal *et al* : 2007). Duo to the complex strucrure of secondary metabolites, the examination of its anti-oxidant activities cannot be tested by a single assay, herein, in this study two methods were used to evalaute the anti oxidant ability of crude methanolic extract from *A. numidica* leaves, DPPH and ABTS assays. DPPH is a stable free radical which produce a violet solution in methanol, changed to yellow color when it is reduced in the presence of an anti-oxidant molecules (Chang *et al* ; 2002). In the present study anti-oxidant activity of *A.numidica* leaves was evaluated using the crude mathanol extract obtained by soxhlet extraction and was compared with standards (BHA, BHT). Results obtained from chemical assay tested showed that standards had higher scavenging activity than the extract, in comparison with results reported by Ghadbane *et al* ; 2016 revealed that DPPH anti-oxidant assay of methanol extract by maceration showed a potential radical scavenging assay. ABTS assay is a decolorization method in the presence of an anti-oxidant agents. Results obtained also showed that standards have a potential anti-oxidant ability compared with methanolic extract. These results might be duo to the extraction by soxhlet that degrade and destroy molecules or phytochemicals by heat (thermal degradation) (Martino *et al* ; 2006).

Results for anti-bacterial activity showed that methanolic extract have an inhibition zone against Gram (+ve) bacterial strains however it exhibited no anti-bacterial effect against Gram (-ve) bacterial strains tested that was resistant to methanolic extract, this result is coinciding with the statement given by Ghadbane *et al* ; 2016. *E.coli* and *P. aeruginosa* well known to be multi-resistant strains. Gram (+ve) bacteria were sensitive to the spectrum-broad of phenolic compounds. This is an indication that these group of products (secondary metabolites) has different mode of action or that the metabolism of some micro-organisms are better able to stop the effect of these molecules or adapt to it. Also plant extract is less effective on gram (-ve) bacteria might due to the degradation of phenolic compounds by heat of soxhlet.

In conclusion, the crude extract showed a moderate DPPH and ABTS anti-oxidant activities duo to the presence of secondary metabolites, further it exhibit an inhibition activity against Gram (+ve) bacteria: *S.aureus* and *B. subtillus* which suggested that have a potential role in food spoilage. The results should be encouraged in the future by *in vivo* studies in order in integrate this extract of plant in pharmaceutical, cosmetic and diet.

Table 1. Antioxidant activities of methanolic extract extracted from *A.numidica* leaves

Extract (0,25µg/µl)	**IC_{50} (µg/µl) DPPH assay***	**IC_{50} (µg/µl) ABTS assay***
Methanolic extract	18,08±0,21	23,51±0,54
BHA	15,74±0,47	7,54±0,67
BHT	6,55±0,59	1,55±0,26

*Values were expressed as means±SD. Of three parallel measurements, ($p<0.05$). SD: Standard deviation, BHT: butyl hydroxyltoluene, BHA: Butyl hydroxyl anisole, DPPH: 2,2-diphenyl-picrylhydrazyl, ABTS: 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid, IC_{50}: Half inhibitory concentration.

Table 2. Anti-bacterial activity of methanolic extract of *A. numidica* leaves

Inhibition zones				
Gram (-ve) bacteria			**Gram (+ive) bacteria**	
E. coli ATCC 25922	*P. aeruginosa* ATCC 27853	*M. morgani* ATCC 25830	*S. aureus* ATCC 43300	*B. subtillus* ATCC 6633
0,0±0,0	0,0±0,0	0,0±0,0	8,33±0,57	7,66±0,57

Data are means of three replicates (n = 3) ± standard error

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FULL PAPER

ETHNOMEDICINAL AND AROMATIC AND AROMATIC SALT TOLERANT PLANTS USE VALUE IN KHEWRA SALT MINE, PUNJAB, PAKISTAN

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Abstract: It is the principal quantitative investigation of halophytic vegetation with their customary therapeutic and fragrant uses in Khewra salt mine, Punjab, Pakistan. In this investigation, the reliance of nearby individuals on halophytes gives pragmatic proof about conventional social insurance frameworks. An aggregate of 151 respondents including 13 customary healers were talked with utilizing quick evaluation strategy. The reported information was mapped and examined quantitatively and in GIS too. The mineral portrayal of the dirt was completed utilizing X-beam diffraction (XRD) strategy. Ethno-restorative information was gathered for 38 salt tolerant plant species having a place with 23 families. Results showed the Fabaceae as much of the time referred to plant family (5 species) and Acacia nilotica (L.) Delile as most referred to plant species. Herbs (half) were the prevailing living thing and leaf (37%) was the generally utilized plant section (24 reports). The general significance of the halophytes and homogeneity of the respondents' learning was dictated by utilizing source agreement factor (ICF) and use esteem (UV). The most noteworthy ICF (0.62) was found for gastrointestinal illness class. Relative recurrence of reference and UV ranges from 0.08 to 0.3 and 0.22 to 0.48 separately. The XRD examination demonstrated the copper sulfide nature of soil which is the pointer of halophytes dispersion in the investigation territory. Lion's share of the halophytes has been seen to have solid pharmacological and sweet-smelling proof. It is the main recorded provide details regarding halophytes utilized for therapeutic purposes in this locale. The discoveries of this study will be valuable for the safeguarding of Ethnomedicinal and sweet-smelling legacy and for future pharmacological, sweet-smelling and phytochemical screening of salt tolerant plants to discover their potential for normal medication improvement.
Keywords: Halophytes, Medicinal, aromatic, GIS, X-ray diffraction, Khewra Salt Mines, Phytochemicals, Pakistan.

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INTRODUCTION

Ethnobotanical appraisals of therapeutic plants give information about their customary uses, protection, indigenous networks, and vital for the natural medications improvement (Heinrich and Gibbons, 2001; Vitalini et al., 2013). These reviews are pivotal to illuminate the essential plant species, principally to grow new rough medications (Cox, 2000; Leonti et al., 2002). Indigenous learning has been hold by the customary social orders taken as principal to their wellbeing and social premiums (Cotton, 1996; Getahun, 1976). In addition, Ethnomedicinal and fragrant examinations bolster the network in sociocultural and financial setting and vital for the preservation of worldwide legacy with respect to plants (Sanz-Biset et al., 2009).

The therapeutic plants are as yet perceived as fundamentally medicinal services in immature networks in view of their more viability and social inclinations while in created nations it give elective for human services (Svarstad and Dhillion, 2000). The evaluated report demonstrated the unwavering quality of 70-80% individuals on the ethnobotanical and ethno-restorative employments of therapeutic plants world generally (Muthu et al., 2006). A harsh gauge revealed 35-000 to 75,000 restorative plants giving the premise to social insurance frameworks around the world (Farnsworth and Soejarto, 1991). Natural pharmaceuticals assume huge job in fix of number of human afflictions (Rehecho et al., 2011). The ethnobotanical examines have increased huge consideration among scientific networks concerning therapeutic plants use (Tripathi et al., 2017). Various Ethnomedicinal and sweet-smelling reviews have been changed over into preservation and medicinal services programs (Quamar and

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Bera, 2014). The methods for plants use are differing in different networks far and wide (Abebe et al., 2003; Kassaye et al., 2006).

Numerous medications accessible in market are engineered analogs of disconnected mixes from plants (Amjad et al., 2017). Customary phytotherapies are still prevalently polished in numerous country networks passed on from one age to next inspite the assortment of clinical operators created by the pharmaceutical businesses (Abbasi et al., 2010). The broad social esteem considers and their methodical documentation have been put on high need to feature the pharmaceutical capability of plants and to investigate their new properties in the previous decades. The customary investigations of plants assume vital job in cultivating, nutraceutical and pharmaceutical enterprises (Cox, 2000; Idrisi et al., 2010). The cooperations of number of synthetic constituents in restorative plants cause amicability and collaboration in human body and recuperate the body tissues logically. The medication ventures have created prescriptions utilizing confined substance mixes from restorative plants which deliver physiological reaction (Shinwari and Gilani, 2003). So as to find out the remedial employments of plant based medications, it is important to lead the pharmacological and pharmacognostic examines and to investigate their utilizations (Ahmad et al., 2009)

Pakistan has assortment of atmosphere zones and topographic locales and is supplied with decent variety of therapeutic plants (Yaseen et al., 2015). The parochial information and home grown use mirror the diverse common assets present in various parts of the nation. In Pakistan, around 400-600 therapeutic plants has been accounted for having use in customary medicinal services rehearses (Ahmad et al., 2014; Shinwari and Qaisar, 2011). Qureshi et al., (2007) uncovered that 84% masses of Pakistan depends upon standard drugs for the fix of various issue. Past review portrayed that around 60,000 customary healers in remote territories use restorative plants against a few infirmities (Rehman et al., 2017). The indigenous home grown meds depend on the properties of restorative plants (Bartam, 1995). Conventional Yunani restorative framework is drilled famously among the provincial populace. Yunani therapeutic framework was embraced and archived by Muslim researchers in the glorious time of Islamic human advancement and they rehearsed it for a long time (Khan et al., 2012). In Pakistan, indigenous information is diminishing step by step because of quick land corruption, present day culture presentation, urban improvement, and war impacts yet in rustic networks the customary learning about restorative plants makes due because of its change from one age to people to come (Figueiredo et al., 1993; Kayani et al., 2015; Manandhar, 1995; Tabuti et al., 2003). Different Ethnomedicinal and sweet-smelling contemplates led in Pakistan revealed the long time routine with regards to therapeutic plants a few infections including extraordinary number of halophytes against (Adnan et al., 2010; Akhtar et al., 2013; Khan et al., 2013; Qasim et al., 2010; Shah and Hussain, 2012; Shinwari, 2010).

In excess of 400 types of halophytes are accounted for from Pakistan (Khan and Qaiser, 2006). The saltiness of soil is taken as limitation for the development of plants as about 6% of the world aggregate land region is salt affected (Khan et al., 2011). The 6.30 million hectares of Pakistan's aggregate land is salt influenced and 0.45 hectares of this aggregate saline land is available in Punjab. The broad review gave the wide record on the monetary uses and circulation of halophytes in Pakistan with rich decent variety of halophytes from Punjab because of differing biological living spaces (Khan and Qaiser, 2006). Ethnobotanical contemplates have been done wherever scale in the Punjab domain (Arshad et al., 2011; Ikram et al., 2014; Mahmood et al., 2013; Parvaiz, 2014; Qureshi et al., 2009; Sardar et al., 2015) which furthermore demonstrates the significant number of halophytes use in indigenous social orders. Halophytes are accounted for as underutilized source (Lieth and Hamdy, 1999) which verify their centrality for subsistence and their job in nearby social legacy. On the opposite side, the rising enthusiasm for halophytes demonstrates their gigantic potential (Khan et al., 2009).

Sadly, the halophytic greenery of a few sections of Punjab territory, for example, Khewra is unexplored with respect to the ethnobotanical investigations of restorative plant species. Khewra salt mine is situated in the Khewra city and is among the 5 salt mines of Pakistan and it is the Pakistan's old and biggest salt mine. In this angle, the present overview can be taken as the primary review in the zone as before the Khewra salt mine has not been considered for ethno-therapeutic examination. The

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investigation region has particular geology with incredible assorted variety of halophytes. The neighborhood individuals of Khewra have been utilizing salt tolerant plants with long time experienced strategies and amount for treating different sicknesses since ages. The elderly individuals want to utilize restorative plants against a few illnesses and have adequate learning with respect to Ethnomedicinal and sweet-smelling rehearses. There is absence of essential conveniences, instruction and human services offices for the local individuals; in this way, they depend on the use of restorative halophytic vegetation in customary route as an essential wellspring of drug and as their domesticated animals.

The GIS and vegetation system together with PC based robotized field mapping methodology have been key gadgets for vegetation mapping (Ismail, 2010). On the opposite side, X-beam diffraction (XRD) is a valuable nondestructive strategy for the portrayal of crystalline materials (Bunaciu et al., 2015) which is utilized to abuse minerals recognizable proof in the dirt of study region. Halophytes got little consideration notwithstanding of having gigantic potential as they have capacity to blossom with poor soil conditions. The re-development of worry in the utilization of halophytes as restorative plants in social insurance rehearses has been energized because of the symptoms and increasing expense of engineered drugs. Besides, the conventional learning in the investigation region is draining because of deforestation and numbness with respect to ethnobotanical documentation before. Remembering every one of these issues, it was felt important to record the indigenous information with respect to salt tolerant plant species. The reason for the present study is the request and documentation of the assorted variety and ethno-therapeutic information of halophytes to depict the status of current ethnobotanical data among associates of novel ages, to get to the component of traditional getting the hang of using quantitative records, to find the association between soil minerals and halophytic vegetation by performing XRD, and to make the vegetation layout GIS to portray the varying scattering of halophytes in the examination locale.

MATERIALS AND METHODS

Geo-ethnographical diagram: In Pakistan, Khewra salt mine is the subdivision of Jhelum locale in region Punjab. The study was conducted from March to October 2018 regarding plant collection and data compilation. It lies 102 km far from the Jhelum. It is situated at 32° 38' N and at 73° 00' E. The underground piece of salt mine spread on the region of 110 km2. It is the Pakistan's first and world's second biggest salt mine. It lies in the mountains of salt scope of Punjab. The half of salt is separated utilizing old Room and Pillar mining techniques while half of salt is left as columns to hold mountains. There is a distinction in temperature and mugginess because of the blasts and ignition motors. The nearby individuals use Punjabi dialect chiefly in discourse. The investigation zone incorporates sub-muggy zone, uneven and subtropical semi-bone-dry zone.

The region of Khewra salt mine has expansive number of minerals and have rich assorted variety of salt tolerant plants. Khewra salt dig is the site for travelers. Out of 19 stories, 6th one is intended for sightseers in whom salt made mosque, saline solution lakes, and Minar-e-Pakistan is available. The nearby individuals have adequate wellbeing offices as number of private facilities and single government healing facility however the vast majority of the general population still depend on standard medications due to ordinary tradition that the development people support to use indigenous standard techniques for the treatment of various issue instead of later remedial system. Agribusiness is the essential wellspring of benefit for inhabitants as half of the total tenants of the examination region are associated with agriculture. The money related conditions of the examination locale can end up being better unequivocally by engaging the advancement of salt tolerant therapeutic plants.

Ethnobotanical examination: The field work was directed between February 2017 and October 2018 after conventional methodologies. Ethno-therapeutic information was gathered utilizing distinctive methods like semi-organized discourses, open-finished meetings and field understandings (Martin, 1995). A fast evaluation approach and survey was utilized to record the therapeutic employments of halophytes. This strategy is viewed as successful in ethnobotanical examinations since it go about as an extension between formal overviews and network support. The survey contained data on the demography of nearby respondents and in addition on their sexual orientation, instruction, experience

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and age. In addition, it incorporated the plant nearby name, society formulas, readiness techniques, parts utilized, usage mode, and the illnesses treated with halophytic therapeutic plants.

One fifty-one individuals from network including customary healers, agriculturists and ladies took an interest in this review. These respondents included13 conventional wellbeing experts, 83 men, and 68 ladies. A Punjabi talking individual was locked in as interpreter to record data precisely that would be lost amid meetings and translation. The approval of salt tolerant plant for restorative use was made just when at least two sources concurred to a similar use.

Recognizable proof and safeguarding of Halophytes: Five examples of each salt tolerant plant species was gathered from study zone. The open writing and Flora of Pakistan (http://www.efloras.org) was utilized to recognize the gathered halophytes. The halophytes were additionally contrasted and herbarium examples in Herbarium of Pakistan (ISL), Quaid-I-Azam University Islamabad for their recognizable proof. The Plant List (www.theplantlist.org) and Medicinal plant names administrations (www.kew.org/mpns) were counseled for ordered and organic names verification. The salt tolerant therapeutic plants were mounted on standard herbarium sheets subsequent to squeezing, drying, and treatment with mercuric chloride and ethanol. The voucher numbers were appointed to gathered plant examples and put in the Pakistan Herbarium (ISL), Quaid-I-Azam University Islamabad.

Information examination: Various quantitative files, for example, relative recurrence of reference (RFC), use esteem (UV) and witness agreement factor (ICF) was connected on ethno-restorative information to break down it quantitatively.

Source Consensus Factor (ICF): The ICF was connected on ethnobotanical information utilizing given recipe (Logan, 1986; Tabuti et al., 2003; Teklehaymanot, 2009):

$ICF = (Nur – Nt)/(Nur – 1)$

Where Nur demonstrates the utilization reports in number for a distinct disease class and Nt shows the taxa in number for the infirmity classification. ICF depict the agreement of data on therapeutic plant species use for an explicit issue (Canales et al., 2005). The estimation of ICF esteem fluctuates from 0 to 1. The most noteworthy ICF evaluation demonstrates the fix of number of different illnesses utilizing taxa educated by vast number of respondents. Then again, low ICF appraisal demonstrates the fix of couple of various illnesses utilizing taxa arbitrarily gathered from respondents having absence of information about plant species use (Abu-Irmaileh and Afifi, 2003; Gazzaneo et al., 2005; Kloutsos et al., 2001; Teklehaymanot, 2009).

Use esteem (UV): The UV was connected in ethnobotanical information as appeared in writing utilizing standard strategy (Phillips et al., 1994; Šavikin et al., 2013):

$UV = U/n$

Where U demonstrates the utilization reports with aggregate number for a notice plant animal varieties while 'n' demonstrates the aggregate number of respondents addressed for a notice plant animal varieties. In the event that UV is near 1, the esteem will be high with the sign of many use reports and plant species essentialness among respondents. In the event that UV is near zero, the esteem will be low with the sign of notice plant species with few use reports.

Relative Frequency Citation (RFC): The RFC was connected on ethno-restorative information which shows the nearby essentialness of therapeutic halophytes utilizing following equation (Tardío and Pardo-de-Santayana, 2008; Vitalini et al., 2013):

$RFC = FC/N \ (0 < RFC < 1)$

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Where FC shows number of sources who reported the use of species and N is the total number of observers who appreciated the examination. High RFC regard shows the obvious nature of a plant creature assortments among the respondents. In case respondents see the plant species as supportive, the RFC regard will be 1 for notice plant species while if no respondent give information about the plant species use, the RFC will be zero for notice plant species (Medeiros et al., 2011).

Geographic data framework mapping: In geographic examination, the method for showing information on the guide is a pivotal advance. A GIS is an electronic database intended for spatial information the executives. The halophytes were gathered amid field review utilizing GPS. The information inspecting and mapping is made through GIS. The choice in the GIS database for the structure of fundamental organizer and subfolder was chosen. The recorded halophytes later on were moved into Arc GIS programming 10.5 for creating halophytes mapping framework.

X-Ray Diffraction: X-beam diffraction is utilized for the distinguishing proof of crystalline strong and to decide precious stone introduction and other basic parameters (Bish et al., 2014). It gives data as pinnacles delivered by productive impedance from a cross section planes in test utilizing monochromatic light emission beams dissipated at various edges. So as to perform XRD, salt example from study region is crush utilizing mechanical pounding to go through 45 μm strainer. The example in powder shape is cautiously arranged to stay away from spottiness, changes in force, and to acquire molecule size and introduction. The unreasonable pounding results in grid mutilation. The got outcomes were broke down utilizing Origin and High Score software's.

Moral endorsement/Intellectual property assention: The present investigation was directed with the full assent of members. Formed Prior taught consent' (PIC) was gotten from the close-by systems which consolidate the benefit of an indigenous system to give or hold its consent to the proposed research adventure that may impact the grounds, resources, ordinary learning and conventions that they by and large have, include or for the most part use. The verbal understanding was additionally made that this review will be utilized just to illuminate the network and Khewra, Punjab on the salt tolerant plants and their restorative uses and recorded information won't be utilized for business purposes. The morals board of Quaid-I-Azam University, Islamabad allowed moral endorsement for the examination**.**

RESULTS AND DISCUSSION

Socio-statistic data: One fifty-one respondents including laypeople, cultivators and customary healers between the 18-80 years of ages took part in the study. Regarding sexual orientation, 55% of the respondents were men and 45.03% were ladies. The more respondents were guys because of the impediments on females in the investigation territory. The ladies were not taking into account discussion with obscure guys or outsiders. The female respondents have been seen to have critical learning about the home grown arrangements utilizing halophytes. The provincial ladies have noteworthy job in the conveyance of customary social insurance framework because of her culinary exercises and home garden development additionally (D'Avigdor et al., 2014).

The inconstancy in respondent's age has critical effect on the customary restorative learning of salt tolerant plants in Khewra. Based on age, respondents were partitioned into 6 noteworthy companions. The 73% of the witnesses were over 40 years, while 27% were beneath the 40 years. This suggests the customary therapeutic learning of salt tolerant plants is declining step by step among the youthful age in the investigation region. A few different examinations have detailed that the decrease in customary information among youthful age isn't restricted to think about zone or halophytes, yet in different indigenous populaces or provincial networks the conventional learning is declining step by step among young people (Giday et al., 2009; Hong et al., 2015; Rehman et al., 2017). Most of the respondents (28.47%) were uneducated. The unskilled sources shared increasingly conventional information of therapeutic halophytes when contrasted with instructed witnesses (24.50%). This may be because of the urbanization and utilization of allopathic meds. Comparable discoveries were additionally announced by Umair et al., (2017), Kadir et al., (2012) and Hayta et al., (2014). The conventional healers are the caretakers of the therapeutic information in the investigation region. This demonstrates

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laypeople can treat minor sicknesses, for example, skin issue, fever, and migraine while treatment of significant illnesses, for example, Hepatitis, fruitlessness, and diabetes are as yet limited to cultivator and customary healers. The dominant part of customary healers was noted to have 5-10 years' training (2.64%). **[Table 1]**

Diversity of halophytes and their Life form: This investigation establishes thirty-eight salt tolerant plant species dispersed among twenty-three families which is the sign of rich assorted variety of halophytes. The herbal names of halophytes with their family name, neighborhood names, part(s) utilized, society formulas, strategy for usage, living thing, development shape and remedial uses alongside quantitative information examination are displayed in Table 2. Fabaceae contributed the most taxa with five species while different families have species under five in number (Figure 2). Fabaceae is prevailing family in light of the fact that a large portion of the individuals from this family are developed for sustenance and the nearby individuals utilized them for the fix of different diseases. Fabaceae is among the transcendent families on the planet (Ali, 2008). Different examinations additionally demonstrate the comparative outcomes in term of overwhelming family (Cheikhyoussef et al., 2011; Mahwasane et al., 2013; Qayum et al., 2016; Wagh and Jain, 2015). Most of the prevailing halophytes were gathered from wild (80%) trailed by developed (20%) (Figure 3). A few ethnobotanical examinations additionally revealed the restorative proficiency of wild plant species (Hong et el., 2015; Soukand et al., 2017). The WHO announced that 80% of Asian populace relies upon wild therapeutic plant species because of their simple access, adequacy and social recognition. The examination dependent on remedial utilizations demonstrated numerous restorative employments of single species. The developing worry in the present period about restoratively critical plant species is their over-misuse and intemperate utilization (Anon, 2009).

The prevailing halophytes were enduring plants (65%) trailed by yearly (31%) and biennial plants (2%). The explanation for the most perpetual plants is the flighty and uncommon precipitation in the dry and semi-bone-dry locales (Qasim et al., 2010). The recorded overwhelming living thing was herbs (half) (Figure 4). The purposes behind herbs high rate are their simple openness in assortment of living spaces as in fields, woodland territories, rocks and prairies (Ayyanar and Ignacimuthu, 2005; Sanz-Biset et al., 2009; Uniyal et al., 2006) and their adequacy in the fix of a few sicknesses because of the nearness of dynamic pharmacological constituents and straightforwardness in gathering accumulation (Adnan et al., 2012). Another reason is the straightforwardness in home grown arrangements (Arshad et al., 2011; Lulekal et al., 2013) and have a noteworthy part in keeping up processing and digestion (George and Nimmi, 2011). Herbaceous living thing is the regular broad environmental wonders around the world (Jan et al., 2011). Jan et al., (2017) affirm the discoveries of this examination. In the examination of the organic range of halophytic vegetation following Raunkiaer (Akman and Ketenoglu, 1992), it was discovered that 47% of the halophytes were phanerophytes. The strength of phanerophytes over other living things gives the thought regarding their better reaction to the topographic difference.

[Table 2]

[Figure 2]

[Figure 3]

[Figure 4]

The present examination moreover selected the most-a significant part of the time refered to halophytes with something close to 55 references or progressively (Table 2). The regularly alluded to halophytes are utilized by the broad number of adjacent individuals especially development people because of their simple openness and normal presence. The expansive number of respondents was likewise found to have beliefin the viability of usage of halophytes customarily in light of the fact that they support to use the home grown arrangements over manufactured medications in view of different reactions and their surprising expense.

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Plant parts used and methods of utilization: In this examination, nearby individuals use a few sections of therapeutic halophytes in rough home grown medication arrangements; consequently, different society formulas were recorded. For the most part leaves (37%) was the real plant part utilized by neighborhood networks for a few infections because of simplicity in culling (Kadir et al., 2012), their most elevated corrective powder and high auxiliary metabolites (Verpoorte, 1998; Verpoorte and Memelink, 2002) (Figure 5). In various examinations at various spots, ethnic peoples were found to exhibit practically identical results if there ought to emerge an event of plant parts used for the arranging of home developed medications as in the present examination (Ahmed et al., 2015; Dolatkhahi et al., 2014; Giday et al., 2009; González et al., 2010; Mahishi et al., 2005; Mowobi et al., 2016; Parvaiz, 2014; Srithi et al., 2009). The products of the soil of the plants after leaves are fundamental for the treatment of different sicknesses since they contain high dynamic substance of bioactive mixes (Moore and Thomas, 1994). Other plant parts detailed for treatment of a few illnesses are given in Table 2. **[Figure 5]**

Mode of administration and utilization: The strategy utilized for medication planning and organization differ as indicated by the illness type. In this review, 80 home grown arrangements are recorded. Greater part of the plans (59%) were conveyed orally in ailments classifications. In couple of diseases, the details utilizing salt tolerant therapeutic plants (41%) were topically directed (Figure 6). Liquor, water and couple of added substances like salt, oil and nectar were utilized regularly in the cures readiness. The vast majority of the cures arrangements included blending of various plant parts or distinctive plant species while the utilization of single plant or a solitary piece of the plant was less experienced in the enquired territory. The oral course was likewise recorded as the significant method of organization in prior examinations (Brandão et al., 2012; Chekole et al., 2015; Kadir et al., 2012; Mood, 2008; Nadembega et al., 2011; Poonam and Singh, 2009). The oral organization of prescription is constantly powerful for inside medical issues (Shinwari et al., 2010).

The most utilized arrangement technique was glue (25%) trailed by decoction (24%) (Figure 7). These discoveries demonstrate that for the most part individuals in the examination territory want to utilize glue of different plant parts either through oral or topical course of organization to mend different afflictions. Customary healers of the investigation region educated about the numerous arrangements with different plants parts because of high viability for relieving infections. Decoction is set up by bubbling diverse parts of the plants until the required volume of water is gotten. Warming or bubbling velocity up different natural responses which deliver a few valuable and dynamic mixes (Chen et al., 2008). Indigenous individuals favored to use crisp plant parts particularly to get their concentrate and squeeze by decoction or imbuement (extraction of flavors or synthetic mixes by suspending the plant parts in a dissolvable, for example, water or liquor after some time) because of restorative adequacy and don't store and dry diverse parts of the plant. Sweet fixings, for example, sugar and nectar were included the home grown medications to lessen their severe taste particularly for kids (Balangcod and Balangcod, 2011). It was watched additionally that the respondents utilize different portion of medications relying upon the malady and age.

[Figure 6]

[Figure 7]

Quantitative analysis

Relative frequency of citation (RFC): The estimation of RFC portrays the successive utilization of halophytes with therapeutic esteem and the essentialness of that plant species with reference to respondents. In the present investigation, the most astounding RFC esteem alludes to the need and use of plant species announced by respondents. The estimation of RFC fluctuates from 0.22 to 0.48. The therapeutic halophytes having most noteworthy RFC were Acacia nilotica (L.) Delile and Abelmoschus moschatus Medik. Acacia nilotica (L.) has the most elevated RFC esteem as a result of its therapeutic regard yet it was also observed that people moreover use it for fuel, timber and advancement purposes; along these lines, it is an exceptional plant of the district. Most refered to plant species are most normal and valuable with respect to restorative purposes among the masses (Samoisy,

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et al., 2015). Other imperative halophyte was Abelmoschus moschatus Medik. with high RFC esteem because of its simple openness to respondents and straightforwardness in development in fields and home patio nurseries. The halophytes with minimum RFC values were Podocarpus latifolius (Thunb.) R.Br. ex Mirb. Furthermore, Verbascum thapsus L. The ethno-restorative investigations directed in other place additionally announced some plant species with most astounding RFC values like the present examination (Kankara et al., 2015). The plant species with high RFC esteem ought to be exposed to organic, phramcological and phytochemical thinks about (Mukherjee et al., 2012).

Use value (UV): UV list has been utilized for the quantitative examination of information and to affirm the general criticalness of species or family implied for populace (Vendruscolo and Mentz, 2006). The overall significance of salt tolerant plant species utilized by neighborhood individuals for drugs was validated by assessing the information utilizing use esteem. In this investigation, UV esteem ranges from 0.08 to 0.3. High use esteems demonstrate the huge number of reports for plants by respondents while plants with less use esteem show the less use reports. The halophytes with most astounding UV were Podocarpus latifolius (Thunb.) R.Br. ex Mirb. (0.3) and Pentatropis nivalis (J.F.Gmel.) D.V. Field and J.R.I. Wood (0.28). The most elevated UV of these halophytes demonstrates their basic presence and the customary restorative essentialness of these halophytes by respondents against a few issue. The over and again revealed plant species are seen as more naturally dynamic than other plant species (Logan, 1986). The halophytes having minimum UV are given in Table 2. The previous reports show that the plant species in the present investigation with minimum UV are likewise compelling as these halophytes can be utilized for the human pharmaceuticals advancement (Holling et al., 2012; Macuja et al., 2015; Oliveira et al., 2015). Plant species with high use esteem should prepared further to acquire significant mixes for the advancement of characteristic medications (Mahmood et al., 2012).

Informant Consensus Factor (ICF): The ICF is a fair execution for the elaboration of utilization repeat of therapeutic halophytes for various suffering arrangements. The present examination delineated 12 essential disturbance classes reliant on estimations of ICF. The ICF estimations of halophytes move from 0 to 0.62 (Table 3). The most astonishing ICF regard (0.62) was represented gastrointestinal dissipates. The more inescapable disease class was GIT issue in the examination domain due to the low-quality sustenance permit by teenagers and adolescents who need to eat drive-through nourishment than cautiously collected dishes in home. Equivalent revelations were moreover filed by a couple of various examinations coordinated in Pakistan and in various countries (Logan, 1986; Rokaya et al., 2010; Teklehaymanot, 2009). The second most shocking ICF regard (0.38) was represented on edge disarranges in the examination zone. These disclosures reveal the manner in which that the respondents living in salt mine work barely and exhaustingly accumulate things from the forested areas for cooking as they live in rough zone or with the agriculture occupation, lock in fields as they have poor money related conditions. The smallest ICF regard (0.00) was found for immunizing agent and urinary issue. The need consistency about the usage of salt tolerant therapeutic plants was seen among respondents and spoke to the base ICF values. Scarcest ICF regard exhibits that respondents have no simultaneousness on the use of assembled and referenced therapeutic halophytes. Assortments in relationship of helpful plants for easing sicknesses show the social example in the examination zone.

[Table 3]

GIS mapping of halophytic vegetation: The database configuration has a noteworthy effect to plant mapping as it decides the highlights and information association. The GIS essential introduce is the relationship between's highlights traits and spatial information. The computerized guide delineating the halophytic vegetation of Khewra salt mine was delivered utilizing GIS (Figure 8). The vegetation mapping has been utilized in the present investigation to discover the conveyance of halophytes. The vegetation mapping utilizing GIS these days is turning into a critical instrument so as to make a database including a spatial area and property table data of the plants utilizing both subjective and quantitative accumulation procedures. The utilization of GIS in vegetation mapping was likewise introduced different examinations (Monto et al., 2005;Guisan and Thuiller, 2005).

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[Figure 8]

X-Ray Diffraction: The XRD technique is extensively used in characterization of rock-based minerals (Wu *et al.,* 2017). In the current study, this technique was used to determine the nature of salt sample and to understand the relationship between salt type and distribution of halophytes in study area. The XRD analysis of salt sample using High Score software demonstrates that the salt nature of study area is copper sulfide. It contains copper sulfide mineral Cu2S which is chalcocite. This technique was performed for structural studies using salt sample in powder form. The XRD pattern shows that salt of study area is crystalline in nature and reported as cubic crystal Cu2S (a=5.6286 A, b=5.6286 A, c=5.6286 A), with spacer group Fm3m and its density was calculated as 5.93 g/cm^3. Copper sulfide nature of soil in relation with plants attained interest in recent years for many reasons. Copper sulfide is chemically composed of copper and sulfide. The 'CU' and 'S' both are important element for plant growth, plant sterility and photosynthesis and they also help in maintaining soil PH.

On the other hand, sulfide inhibits the rate of plant growth in several ways as it is toxic for plant roots, cause deficiency of essential elements in plant roots and also results in sulfur deficiency in plants (Wu *et al.,* 2017). The copper sulfide based salty soil as compare to loamy soil has small particle size than loamy soil which affects the PH of soil and flow of water and minerals in soil. The halophytes distribution in the study area revealed that they have the ability to adopt such type of soil conditions. The local floras composition could be altered by global environmental changes therefore it should revise regularly and knowledge about indigenous use of medicinal plants, soil and environ The XRD method is widely utilized in portrayal of shake based minerals (Wu et al., 2017). In the present investigation, this procedure was utilized to decide the idea of salt example and to comprehend the connection between salt kind and appropriation of halophytes in study zone. The XRD examination of salt example utilizing High Score programming exhibits that the salt idea of study region is copper sulfide. It contains copper sulfide mineral Cu2S which is chalcocite. This method was performed for basic examinations utilizing salt example in powder frame. The XRD design demonstrates that salt of study region is crystalline in nature and announced as cubic gem Cu2S (a=5.6286 A, b=5.6286 A, c=5.6286 A), with spacer aggregate Fm3m and its thickness was determined as 5.93 g/cm3. Copper sulfide nature of soil in connection with plants accomplished enthusiasm for ongoing years for some reasons. Copper sulfide is synthetically made out of copper and sulfide. The 'CU' and 'S' both are vital component for plant development, plant sterility and photosynthesis and they additionally help in keeping up soil PH.

Then again, sulfide represses the rate of plant development in a few different ways as it is poisonous for plant roots, cause inadequacy of basic components in plant establishes and furthermore results in sulfur lack in plants (Wu et al., 2017). The copper sulfide based salty soil as contrast with loamy soil has little molecule estimate than loamy soil which influences the PH of soil and stream of water and minerals in soil. The halophytes dispersion in the investigation territory uncovered that they can embrace such sort of soil conditions. The nearby verdures structure could be modified by worldwide natural changes along these lines it should overhaul routinely and information about indigenous utilization of therapeutic plants, soil and condition ought to be reported legitimately. ment should be documented properly.

[Figure 9]

Phytochemical constituents of Medicinal halophytes: The pharmacological confirmation of any plant species dependent on ethno-therapeutic information assume critical job for the fuse of customary natural meds into current human services framework. In the present examination, the vast majority of the salt tolerant plant species have been seen to have a solid pharmacological movement while couple of halophytes demonstrate some pharmacological action. The reported restorative halophytes with their dynamic phytochemicals have been given in Table 2. These phytochemical mixes are connected with a few pharmacological exercises utilized for the treatment of different ailments in everywhere throughout the world (Verpoorte et al., 2002). The nearness of gylcosides, phytohormones, saponins, alkaloids and flavonoids in plant species are in charge of their pharmacological exercises (Khan et al., 2012). Podocarpus latifolius is an essential salt tolerant plant species which have solid ethno-therapeutic confirmations however have no pharmacological record. This halophyte is suggested for

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cutting edge exploratory pharmacological strategies for future investigations. Phytochemical thinks about give pharmacological proof to the confirmation of Ethnomedicinal and fragrant employments of plants species. The past writing announced the recognized phytochemical constituents of revealed salt tolerant plant species. It is broke down that these pharmacological exercises can be favored in future because of co-dismal circumstance and moral pondering and can handled further for more subtleties. In the ebb and flow examination, the salt tolerant restorative plants not revealed in past research ought to be surveyed for pharmacological investigation which can help in the revelation of new medications.

CONCLUSIONS

The present investigation reported the conventional restorative practices of halophytes, mapped the halophytic vegetation utilizing GIS and portrayed the dirt minerals of Khewra salt mine utilizing X-beam diffraction examination. This investigation is embraced to record the indigenous restorative information about halophytes for future confirmation utilizing phytochemicals and phramcological exercises. Thirty-eight salt tolerant restorative plants having a place with twenty-three families were reported. Fabaceae was the much of the time refered to plant family. Lion's share of the salt tolerant plants were herbs and leaf was the most well-known utilized plant part. This review uncovers the novel data that the investigation region is enriched with rich decent variety of halophytes which are utilized as nourishment and additionally for home grown details for the treatment of different afflictions. The discoveries show the insufficient wellbeing administrations for provincial populace by the legislature of Pakistan, along these lines, lion's share of the neighborhood individuals utilize conventional restorative information which additionally include the huge number of halophytes. The outcomes help the phytochemistry analysts for the screening of therapeutic salt tolerant plants for medication disclosure in future with progressively worldwide intrigue. The reconciliation of characteristic prescriptions in the essential human services for neighborhood individuals is approved by the logical bio-movement validation of the therapeutic plants. This exhaustive investigation is of exceedingly importance since it is first archived ethno-therapeutic write about salt tolerant plants of Khewra salt mine. The further research on preservation systems ought to be directed for the natural medications reasonable advancement since it will give the better financial conditions in the investigation region.

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Table 1. Demographics of informants in Khewra (Punjab)

S. NO.	Variable	No. of persons	Percentage
1.	Informant category -THPs	13	8.60
	Local People	138	91.39
2.	Gender : F/M	68	45.03
		83	54.96
3.	Age	06	3.97
		11	7.28
		25	16.55
		27	17.88
		49	32.45
		33	21.85
4.	Educational background	43	28.47
		12	7.94
		04	2.64
		29	19.20
		15	9.93
		11	7.28
		37	24.50
5.	Experience of the traditional health practitioners	02	1.32
		02	1.32
		04	2.64
		03	1.98
		02	1.32

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Table 3. ICF value of halophytic medicinal plants used against various diseases

GIT diseases	72	189.47	28	73.68	0.62
Respiratory diseases	29	76.32	24	63.16	0.18
Sexual disorders	19	50	14	36.84	0.28
Urinary disorders	17	44.74	17	44.74	0.00
Muscle and skeletal disorders	40	105.26	26	68.42	0.36
Nervous disorders	41	107.89	26	68.42	0.38
Glandular disorders	31	81.58	25	65.79	0.2
Cardiovascular disorders	35	92.11	27	71.05	0.24
Ear, nose and eye disorders	17	44.74	13	34.21	0.25
Nail, skin and hair disorders	18	47.37	23	60.53	0.29
Body energizers	6	15.79	5	13.16	0.2
Antidote	10	26.32	10	26.32	0.00

*ICF = Informant Consensus Factor

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Table 2. List of halophytes and their medicinal used by the local people of Khewra salt mine, Punjab, Pakistan

Family Plant Names/ Voucher Specimen No	**Common name**	**Flowering season**	**Habit**	**Status**	**Life form**	**Growth form**	**Part used**	**Mode of preparation**	**Mode of administration**	**Phytochemicals**	**Ethno-medicinal uses**	**FC***	**UV***	**RFC***	**Herbal Recipes**
Aizoaceae *Trianthema portulacastrum* L. ***ISL- 97***	Itsit	Sep-Oct	Herb	Wild	Therophyte	Annual	Whole Plant	Decoction , Paste	Oral	Ecdysterone, Akaloids (Trianthemine, Punarnavine), Saponins, Tannins, Trianthenol, Steroids, Flavonoids, Anthraquinones, Tri-Terpenoids, Leptorumol, Nicotinic acid, Ascorbic acid, Beta cyanin, Cardiac Glycosides [72,73]	****Respiratory disorders** , Headache , Liver disorders , **Arthritis** , Heart problems , Tuberculosis , Obesity , Hypersomnia	57	0.14	0.38	3 spoon paste of whole plant is mixed with 1 spoon of *Piper nigrum* powder; take it twice in day for respiratory diseases Decoction of plant is used to treat arthritis
Amaranthaceae *Achyranthes aspera* L. ISL- 175	Putkanda	Sep-Aug	Herb	Wild	Chamaephyte	Annual	Root, Leaf	Infusion, Paste	Oral, Topical	Alkaloids (betaine), Carbohydrates,Tannins, Saponin, Flavonoids, Proteins, Amino acids, Tannins, Phenols, Steroids, Glycosides, Ecdysterone, β-sitosterol, Hydroxyecdysone, Pentatriaontane, Hexatriacontane, Tritriacontane, Quercetin[74,75]	**Abdominal pain** , **Scorpion sting** , Headache , Heart problems , Pneumonia , *Tuberculosis* ,*Kidney stones*, *Obesity* , *Skin diseases*, *Constipation* (DS/ I), *Fever*	59	0.19	0.39	Roots are placed in hot boiled water overnight then used after straining for abdominal pain The fresh leaves paste is applied on scorpion bite
Amaranthus viridis L. ISL- 586	Cholai	Aug-Sep	Herb	Wild	Therophyte	Annual	Leaf	Decoction, Paste	Oral	Rutin, Quercetin, 24-ethyl-22-dehydrolathosterol, Dehydrolathosterol, Amasterol (24-methylene-20-hydroxycholesta- 5,7-dien-3β-ol)[76]	**Diuretic** , Diabetes , , Depression , **Influenza** , Skin diseases , Snake bite (CS/E)	63	0.11	0.42	1 teaspoon of leaf paste is taken with milk once in day which is diuretic The leaves decoction is given twice a day to treat influenza
Spinacia oleracea L. ISL- 111	Palak	Jun-Oct	Herb	Cultivated	Therophyte	Annual	Leaf	Cooked, Raw	Oral	Coumerin, Anthocyanins, Emodins, Phlobatannins, Spinacetin, Jaceidin, Violaxanthin, β-carotene, Folic acid, Oxalic acid, Diacylglycerol, Sulfoquinovosyl diacylglycerol, beta-basrubrins, 4'pentahydroxi-6-methoxiflavone[77,78,79]	Digestive disorders , , Skin diseases , Influenza , Diabetes , **Liver inflammation** , Urethral ulcer , **Obesity** , Diuretic	65	0.14	0.43	Leaves are eaten in raw form to treat liver inflammation Leaves are cut into pieces and boiled with *Trigonellafoenum-graecum*, *Menthapiperita*and *Coriandrumsativum* leaves and then add 1 spoon salt and fruit paste of *Capsicum annuum*; take half plate in twice a day to treat

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															obesity
Suaeda vermiculata **Forssk. Ex J. F. Gmel.** ISL- 399	Laani	Mar-Sep	Shrub	Wild	Nanophanerophyte	Perennial	Leaf	Powder, Paste	Oral, Topical	Alkaloid, Coumarins, Flavonoids (Genistein), Saponins, Sterols, Terpenes, Tannins Triterpenoids, Polyphenols[80,81,82]	Constipation , **Skin diseases** , Stomach ache , Dysentery , Hepatitis , Nausea , Insomnia , **Cholera**	49	0.16	0.32	Dried leaves powder is mixed with sugar and 1 spoon is taken thrice in day Leaves paste is applied on skin topically to treat skin rashes and wounds
Apocynaceae *Nerium oleander* L. ***ISL- 155***	Kaneri	Mar-Sep	Shrub	Wild	Phanerophyte	Perennial	Leaf	Paste, Juice	Topical	Rhamnose, Arabinose, Galactose, Cardenolides, Neriucoumaric, Isoneriucoumaric acids, Kaneroside, Neriumoside, Proceragenin, Neridienone A, Beta-neriursate, D-sarmentose, D-diginose, Neridiginoside, Nerizoside, Neritaloside, Odoroside, Isoricinoleic acid, Folinerin[83,84]	Arthritis , **Wound healing** (MS/E), **Skin diseases** , Tuberculosis , Malaria , Insomnia , Diabetes , Meningitis , Blood purifier , Scorpion sting , Body swellings (MS/E)	53	0.21	0.35	Leaf paste is applied on wounds for healing Leaf juice with rose water is applied topically for skin diseases
Arecaceae *Phoenix sylvestris*L. ***ISL- 185***	Khajoor	Mar-Apr	Tree	Cultivated	Phanerophyte	Perennial	Fruit	Paste	Oral	β-sitosterol, β-amyrin, Quercetin, Quercitrin, Alkaloids, Flavonoids, Phenols, Tannins, Saponins[85,86]	Obesity , **Fever** , **Brain tonic** , Heart tonic , Sexual problems (RS/I), Epilepsy , Cold , Dysentery , Aphrodisiac (RS/I)	71	0.13	0.47	1 teaspoon of fruit paste is given with ½ teaspoon of vinegar to cure fever Fruit paste is given with warm milk which is brain tonic
Asclepiadaceae *Calotropisprocera* (Aiton) W.T.Aiton ISL- 58	Aak	Throughout year	Shrub	Wild	Nanophanerophyte	Perennial	Latex, Flower, Leaf	Raw	Oral, Topical	Alkaloids, Terpenoids, Flavonoids, Tannins, Saponins, Sterols (b-sitosterol, Stigmasterol, Multiflorenol) Cardiac glycosides, Terpinoids, Cardenolides[87,88]	**Piles** , Diabetes , Obesity , Jaundice , Malaria , Tuberculosis , Toothache (DS/E), Hysteria , **Snake bite** , Wound healing (MS/E), Teeth problems (DS/E), Stomach disorders	65	0.18	0.43	Latex is applied on the wounds of piles to get relieve. Fresh flowers and leaves are eaten in raw form for snakebite
Pentatropis nivalis (J.F.Gmel.) D.V.Field&J.R.I.Wood ***ISL- 177***	-	June-Oct	Climber	Wild	Phanerophyte	Perennial	Root	Paste, Juice	Oral	Glycoside, Steroids, Terpenoids, Phenolic, Saponins, Flavonol glycosides, Flavonol sulphates, Flavonol disulphates[89,90]	Dysentery , Hepatitis , **Piles** , Wound healing (MS/E), **Obesity** , Lung disorders , Eczema (MS/E), Skin allergy , Earache (SO/E), Leucorrhoea (RS/I), Nail disorders (MS/E)	39	0.28	0.26	One teaspoon of roots paste is used to treat piles Root juice is taken in the night before sleeping to treat obesity

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Asteraceae *Carthamus oxyacantha* M. Bieb. ISL- 137	Pohli	Apr-July	Herb	Wild	Therophyte	Annual	Seed	Powder, Oil	Oral, Topical	Flavonoids, Cardiac glycosides, Triterpenoids, Tannins, Carbohydrates, Phenols, Palmitic acid, Stearic acid, Oleic acid, Linoleic acid, Carthamidin, Serotonin[91,92,93]	**Fever** , Skin allergy , Influenza , Impotence (RS/I), Arthritis , Hepatitis , Dog bite (CS/E), Itching , **Skin rashes** , Conjunctivitis (SO/I), Heart disorders (MS/E)	41	0.27	0.27	Dried seeds are ground to make powder which is taken after boiling in water once in day to treat fever Seeds oil is applied topically on skin to treat itching and rashes
Brassicaceae *Malcolmia africana* (L.) R.Br. ISL- 359	Kambar	Mar-July	Herb	Wild	Therophyte	Annual	Leaf, Flower	Decoction, Extract	Topical	Oleic acid, Linoleic acid, Linolenic acid, Erucic acid, Flavonoids, Phenols[94]	, **Skin allergy** , Lung cancer , **Dog bite**	49	0.08	0.32	Leaves are boiled to obtain juice of leaves which is applied thrice in day to treat dog bite Crushed flowers are placed in hot water to obtain extract which is used topically to treat skin allergy
Capparaceae ***Capparis decidua*** **(Forssk.) Edgew.** ***ISL- 470***	Karineh	Mar-Sep	Shrub	Wild	Phanerophyte	Perennial	Fruit, Stem	Raw, Paste	Oral, Topical	Alkaloids (Capparine, Cappariline, Capparinine), Terpenoids (Lupine), Glycosides, Sterol, Methyl isothiocyanate, β-Carotene, Ascorbic acid, Phthalic acid[95]	Digestive disorders , **Blood purifier** , Arthritis , Aphrodisiac (RS/I), Impotence (RS/I), Tuberculosis , Stomach ulcer , **Teeth disorders** (DS/E), Arthritis , Alzheimer's disease	53	0.19	0.35	Fruit is eaten in raw form for digestive disorders and blood purification The twig paste is applied on teeth for shining
Cucurbitaceae *Citrullus colocynthis* (L.) Schrad. ISL- 351	Tumma	June-Sep	Herb	Wild	Therophyte	Annual	Fruit, Root	Decoction	Oral	Colocynthin, Colocynthetin, Albuminiods, Starch, Tannins, Saponins, Proteins, Reducing sugars, Alkaloids, Glycosides, Diterpenes, Carotenoids, Anthraquinones, Phlobatannins, Flavanoids[96,97]	**Constipation** (DS/ I), , **Earache** (SO/I), Haemorrhoids , Dysentery , Liver diseases	55	0.11	0.36	Fresh fruit pieces are boiled in water with sugar to make paste which is used for constipation Root decoction is used to get relieve from earache
Cyperaceae *Cyperus rotundus* L. ISL- 89	Delluca	May-Oct	Shrub	Wild	Nanophanerophyte	Perennial	Root	Powder, Paste	Oral	Flavonoids, Coumarin, Tannins, Polyphenols, Sterols, Carbohydrates, Glycosides, Anthraquinone, Anthocyanin, Saponins, Alkaloids[98,99]	**Diuretic** , Diabetes , **Digestive disorders** , Skin diseases , Nausea , Cold , Cough , Dysentery , Stomach ache (DS/ I), Oedema , Headache	46	0.24	0.3	Root powder is taken with milk once in day which is diuretic 1 teaspoon of root paste is used with rose water to cure digestive disorders

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Euphorbiaceae *Ricinus communis* L. ***ISL- 193***	Harnoli	Mar-Sep	Herb	Wild	Phanerophyte	Perennial	Leaf, Root	Paste	Topical	Chlorogenic acid, Decanamine, Ellagic acid, Linoleic acid, Oleic acid, Palmitic acid, Palmitoleic acid, Stigmasterol, Tartaric acid, Tannins, Arachidic acid, Glycine, Ricinine, Tryptophan[100]	**Wound healing** (MS/E), Snake bite (CS/E), **Arthritis** , Constipation (DS/ I), Skin diseases , Fever , Diuretic , Digestive disorders , Liver diseases ,*Haemorrhoids*	67	0.15	0.44	Leaves are crushed and heated to make poultice for healing wounds Young root paste is applied on joints to treat arthritis
Fabaceae *Acacia modesta* Wall. ISL-103	Phulai	Nov- Mar	Tree	Wild	Phanerophyte	Perennial	Bark, Stem	Decoction, Powder, Raw	Oral, Topical	Flavonoids (Quercetin, Kaempherol) Alkaloids, Terpenoids (Lupeol, Betulin, α-amyrin), Tannins, Linoleic acid, Oleic acid, Cyclitols (Pinitol)[101]	****Digestive disorders** , Obesity **, Teeth disorders** (DS/E), Kidney pain , Gastric disorders , Backache , Epilepsy , Sexual infertility (RS/I), Jaundice , Skin dryness , Skin allergy (MS/ E), Skin boils(MS/ E)	67	0.18	0.44	Bark is grinded to make powder which is used for digestive disorders Bark is boiled in water to make decoction which is used to reduce obesity Fresh stem is used for cleansing teeth
****Acacia nilotica* (L.) Delile** **ISL- 241**	Kikar	May-Oct	Tree	Wild	Mesophanerophyte	Perennial	Bark, Leaf	Decoction, Infusion	Oral, Topical	Alkaloids, Saponin, Proteins, Amino Acids, Anthraquinone, Tannin, Flavonoids, Fixed oils, Fats, Cardiac Glycosides, Leucocyanidin, Polyphenols (dicatechin, Quercetin, Gallic acid), Amino acids (Histidine, Methionine, Valine, Cystine)[102,103]	Mouth sores , **Teeth and gum disorders** (DS/E), Blood purifier , **Dysentery** , Diabetes , Gonorrhoea (RS/I), Diabetes , Asthma , Aphrodisiac (RS/I), Liver diseases , Arthritis , Eye diseases (SO/I)	**73**	0.16	0.48	Bark is soaked in tepid water for few hours; use this water for mouth sores and teeth and gum disorder Fresh leaves are boiled in water which is orally used for straining twice in day for dysentery
Medicago polymorpha L. **ISL- 146**	Maina	Mar-May	Herb	Wild	Therophyte	Perennial	Leaf	Juice, Raw	Oral	Triterpene saponins, echinocystic acid, Isoflavones[104,105]	Skin dryness , Diuretic , **Stomach ache** (DS/ I), **Alzheimer's disease** , Skin boils	57	0.09	0.38	One spoon of leaf juice is taken for stomach ache Leaves are eaten in raw form to treat Alzheimer's disease
Melilotusindicus (L.) All. ISL- 484	Sainji	Mar-Aug	Herb	Wild	Therophyte	Annual	Leaf, Seed	Decoction, Raw	Oral	Flavonoids (Catechin, Epicatechin, Taxifolin), Tannins, Xanthoprotein, Starch, Cystine, Sterols, Triterpenoids, Reducing sugars, Saponins, Alkaloids [106,107]	, **Abdominal pain** , Arthritis , Pneumonia , Skin diseases , **Dysentery** , Obesity , Skin cancer	44	0.18	0.29	Leaves are boiled in water which is used twice in a day after straining for and abdominal pain Seeds are taken in raw form with *Piper nigrum* to treat dysentery
***Prosopis juliflora* (Sw.) DC.** ISL- 76	Pahari kikar	Mar-June	Shrub	Wild	Mesophanerophyte	Perennial	Bark, Fruit	Decoction	Oral	Flavonoids, Alkaloids, Saponins, Phenols, Tannins, Phlobatanins, Triterpenes, Cteroids, cardiac glycoside[108]	**Asthma** , Dysentery , Pneumonia , Influenza (RS/I), , Improves memory , Fever , Bladder tonic ,	53	0.19	0.35	Bark is boiled in water for 15 minutes which is used twice in a day to treat asthma

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											Paralysis , Testis swellings (RS/I)				A tea is prepared from the dried pods which is useful for
Malvaceae ***Abelmoschus moschatus* Medik.** ISL- 272	Junglibhindi	Apr-Sep	Herb	Wild	Therophyte	Perennial	Leaf, Seed	Paste, Oil	Topical	Resins, Farnesol, Dodecyl acetate, beta-Sistosterol, Anthocyanins (cyanidin - 3 - sambubioside ,cyanidin - 3 – glucoside), Linoleic acid, Carbohydrates, Phenols, Flavonoids, Terpenoids, Tannins, Saponins[109,110]	**Headache** , Wound healing (MS/E), Hypertension , Skin diseases , Obesity , Mouth sores , , Hepatitis , Piles	72	0.13	0.48	Leaf paste is applied on wounds for healing Seeds oil is applied on forehead to get relieve from headache
Meliaceae *Melia azedarach* L. ***ISL- 289***	Dharek	Apr-June	Tree	Wild	Phanerophyte	Annual	Leaf, Fruit	Infusion, Powder	Oral, Topical	Terpenoids (β-pinene, a-terpinene), Limonoid (Kulinone, Fraxinellone), Steroid , Flavonoids (Quercetin), Saponin, Anthraquinones, Alkaloids, Phytosterols (Stigmasterol, Campesterol), β-carotene, Tocopherol, Organic acids (Linoleic acid, Linolenic acid, Oleic acid, Benzoic acid, Vanillic acid)[111]	**Itching** , **Piles** , Migraine , Diabetes , Malaria , *Tuberculosis*, *Earache*(SO/I), *Skin diseases*, *Blood purifier* ,*Wound healing*(MS/E), *Depression* , *Constipation*(DS/ I), Sciatica	70	0.19	0.46	Leaves are crushed and placed in tepid water; apply this water topically to get relieve from itching Dried fruits are crushed to make powder and taken once in day for piles
Myrtaceae *Eucalyptus camaldulensis* Dehnh. ***ISL- 427***	Lhacesufaida	Sep-Nov	Tree	Cultivated	Macrophanerophyte	Perennial	Leaf	Decoction, Oil	Oral	Tannins, Sterols, Triterpenes, Saponins, Flavonoids, Phenolic compounds, Anthraquinones, Alkaloids[112,113]	**Influenza** , ***Pneumonia***, *Liver diseases*, *Brain tonic*, *Cough*, *Sedative*, *Sexual infertility*(RS/I), *Epilepsy*	63	0.13	0.42	Five leaves are boiled in water which is taken after staining for influenza twice a day One drop of leaves oil is taken twice in a day to treat pneumonia
Eucalyptus globulus Labill. ***ISL- 367***	Sufaida	Mar-Aug	Tree	Cultivated	Phanerophyte	Perennial	Leaf	Decoction, Paste	Oral	Tannins, Saponins, Alkaloids, Phenols, Glycosides, Steroids, Terpenes, Camphene, Caproic acid, Citral, Eudesmol, Glutamic acid, Dextrin, Homoserine, Chrysanthemin, Chrysin, Cyanin, Cynaidin, Oleanolic acid, Masinic acid[114,115]	Digestive disorders , **Diabetes** , **Cough** , Cold , Tuberculosis , Constipation (DS/ I), Brain tonic , Jaundice , Kidney stones , Piles , Backache , Paralysis , Ringworm	63	0.21	0.42	Leaves decoction is used for cough Leaves paste is placed in hot water overnight to obtain extract; used it after straining twice in a day for diabetes

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Nitrariaceae *Peganum harmala* L. ***ISL- 141***	Hermal	Apr-Oct	Herb	Wild	Chamaephyte	Annual	Seed, Leaf	Raw, Smoke	Oral, Inhale	Flavonoids, Alkaloids (Harmine, Harmaline, Vasicine, Vasicinone, Tetrahydroharmine), Saponins, Tannins, Terpenoids , Steroids , Anthraquinons[116,117]	**Abdominal pain** , Liver diseases , Malaria , Cataract (SO/E), Digestive disorders , **Hypertension** , Eye weakness (SO/I), Narcotic , Stomach ulcer , Sciatica	58	0.17	0.38	Seeds are taken with water for abdominal pain Burnt leaves are smoke for reducing hypertension
Poaceae *Arundo donax* L. ***ISL- 445***	Nari	June-Oct	Herb	Wild	Microphanerophyte	Perennial	Root, Leaf	Decoction, Paste	Oral , Topical	Carbohydrates (Rhamnose, Mannose, Arabinose, Xylose, Galactose), Lignin, Alkaloids (Tryptamine, Bufotenidine, Gramine, Arundamine), n-Eicosanoic acid, B-Sitosterol, α-Tocopherol[118]	Haematuria , **Constipation** (DS/ I), **Migraine** , Eye sight (SO/E), Blood cancer , Epilepsy , Female infertility (RS/I), Liver problems	52	0.15	0.34	Root paste is applied on forehead to treat migraine Leaves decoction is take once in a day to treat constipation
***Cynodon dactylon* (L.) Pers.** ISL- 101	Khabbalghass	Throughout year	Herb	Wild/ Cultivated	Sub-FruticoseChamaephyte	Perennial	Leaf, Stem	Decoction, Paste, Raw	Topical	Terpenoids, , Alkaloids, Flavonoids (Apigenin, Luteolin, Orientin, Vitexin), Carotenoids (Neoxanthin, Violaxanthin), Phenolic phytotoxins (Ferulic, Syringic, Paracoumaric, Vanillic), Phytosterols, Glycosides, Saponins, Palmitic acid, Linoleic acid[119,120]	**Wound healing** (MS/E), **Eczema** (MS/E), Skin diseases , **Depression** , Earache (SO/I), Headache , Arthritis , Urethra inflammation (US/ I), Constipation (DS/ I), Diabetes , Stomach ache (DS/ I), Eye infections (SO/E)	69	0.17	0.46	Leaf paste is applied on wounds for healing Aerial parts are boiled in water which is applied on skin to treat eczema The walk with barren feet on this plant give relieve from depression
Hordeum vulgare L. ISL- 309	Joo	Sep-Feb	Herb	Cultivated	Therophyte	Annual	Seed	Decoction, Raw	Oral	Phenolic acids (Ferulic acid, Vanillic acid, Syringic acid, p-coumaric acid), Flavonoids (Flavanols, Anthocyanins, Proanthocyanidins) Lignans (Pinoresinol, , Syringaresinol, Lariciresinol), Tocopherols, Phytosterol (Sistostanol , Campestanol), Folates, Ubiquinones[121,122]	**Kidney disorders** , **Diabetes** , Malaria , Blood purifier	49	0.08	0.32	Seeds are boiled in water and taken for kidney pain Roasted seeds are taken with milk twice in a day to treat diabetes

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Podocarpaceae *Podocarpus latifolius* (Thunb.) R.Br. ex Mirb. ***ISL- 38***	-	Mar-May	Tree	Wild	Mesophanerophyte	Perennial	Bark, Leaf	Decoction, Paste	Oral, Topical	Not known	Backache , **Constipation** (DS/ I), Asthma , High Blood pressure , Blood cancer , Bone fracture , Cough , **Wound healing** (MS/E), Skin boils (MS/ E), Ringworm	33	0.3	0.22	1 spoon of bark decoction is taken twice in a day to treat constipation Leaves paste is applied on wounds for healing
Rhamnaceae *Ziziphus mauritiana* Lam. ***ISL- 158***	Bairi	Sep	Tree	Wild/ Cultivated	Phanerophyte	Perennial	Bark, Fruit, Leaf	Decoction, Raw, Powder	Oral	Alkaloids (Berberine, Quercetin, Kaempferol, Sitosterol), Flavonoids, Glycosides, Phenols, Lignins, Tannins, Sterols, Saponins, Ceryl alcohol, Holosides, Mucilages, , Triterpenoids, Carotene, Thiamine, Riboflavin, Niacin, Citric Acid, Ascorbic Acid, Fluoride, Pectin, Malic acid, Oxalic acid [123,124]	**Blood Purifier** , **Obesity** , Skin diseases , Digestive disorders , Diabetes , Dysentery , Kidney stones, Depression , Brain cancer	71	0.13	0.47	Fresh leaves are boiled in water to make decoction; add honey in it. Take two times in day for blood purification Fruit is eaten in raw form to reduce obesity Bark powder is used with water once in a day for dysentery
Ziziphusnummularia (Burm.f.) Wight &Arn. ***ISL- 279***	Jungli Bair	Mar-Aug	Shrub	Wild	Phanerophyte	Perennial	Leaf	Paste, Infusion	Oral, Topical	Alkaloids, Glycosides (Nummularogenin), Saponin (Dammarane), Steroids Triterpinoids, Flavonoids, Oil Protein, Tannins, Phenolic compounds[125,126,127]	**Shining Hairs** (MS/E), Dog bite (CS/E), Fever , Female infertility (RS/I), Nausea , Epilepsy , **Skin diseases** , Toothache (DS/E), Flatulence , Leucorrhoea (RS/I),*Amenorrhea(RS /I)*	67	0.16	0.44	Fresh leaves are crushed and place in tepid water, after straining used it for hair washing for shining hairs Leaf paste is placed in hot water for 1 hour then take orally for skin diseases

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Salvadoraceae *Salvadora oleoides* Decne. ***ISL- 60***	Peelu	Mar-June	Tree	Wild	Microphanerophyte	Perennial	Fruit, Root	Paste, Raw	Oral, Topical	Carbohydrates, Glycosides, Sterols (Beta-sitosterol), Steroids, Triterpenes, Proteins, Amino acids, Mucilage, Tannins, Flavonoids, Dihydroisocoumarin, n-hexadecanoic acid, Octacosane, Nonacosane, Octadecene, Heptacosane, Pentacosane, Squalene[128,129]	**Digestive disorders** , Blood purifier , **Periodontitis** (DS/E), Asthma , Arthritis , Dengue fever , Obesity , Abortive (RS/I), Night blindness (SO/I), Eczema (MS/E)	71	0.14	0.47	Fruit paste is used to treat digestive disorder Root is chewed to treat periodontitis
Scrophulariaceae *Verbascum thapsus* L. ***ISL- 176***	Geddarta mbakoo	May-Sep	Herb	Wild	Therophyte	Biennial	Leaf, Flower	Juice, Powder	Oral, Inhale	Iridoid glycosides, Phenylethyl glycoside, Sesquiterpenes, Diterpene, Biflavonoid, Triterpene saponins, Polysaccharides, Phenolic acids, Phytosterols (β-sitosterol, Ergosterol peroxide)[130,131]	Liver diseases , **Tuberculosis** , Scorpion sting (CS/E), Diarrhoea , , Diuretic , **Asthma**	35	0.2	0.23	Flowers juice is taken with tuberculosis Leaves powder is smoke to treat asthma
Solanaceae ***Solanum i ncanum* L.** ***ISL- 402***	Peele mokre	Mar-May	Shrub	Wild	Nanophanerophyte	Perennial	Leaf, Seed	Decoction, Powder	Oral, Inhale	Alkaloid, Steroid, Resin, Glycosides, Flavonoid, Saponin, Tannins, Triterpens, Cardicglycosides[132,133]	**Heart diseases** , Fever , **Narcotic**, Blood purifier , Digestive problems , Skin diseases , Cold , Rheumatism , Depression , Sexual tonic (RS/I), Kidney stones , Abdominal worms	45	0.27	0.3	Leaves are boiled in water which is used after straining twice a day for heart diseases Seeds powder with tobacco is smoke which is narcotic
Solanumv vrginianum L. ISL- 262	Kindair	Throughout year	Herb	Wild	Chamaephyte	Perennial	Fruit, Seed	Powder, Raw, Smoke	Oral, Topical	Alkaloid (Solasodine), Solasonine, Solamargine, Solanocarpine, beta – Solamargine, Solanocarpidine, Isochlorogenic, Neochronogenic, Chronogenic, Caffeic acids, Sterol (Carpesterol)[134]	, Malaria , **Toothache** (DS/E), Asthma , Skin diseases , Liver diseases , Wound healing (MS/E), Alzheimer's disease , **Leprosy** , Influenza , Fever , Nausea , Rheumatism	55	0.24	0.36	Dried fruit is crushed to make powder which is taken twice in a day for gastric problem Seeds are burnt and smoke is topically used to treat leprosy Fruit is chewed to treat toothache
Withania coagulans (Stocks) Dunal ISL- 183	Khamjira	Jan-Apr	Herb	Wild	Phanerophyte	Perennial	Seed, Fruit	Decoction, Paste	Oral, Topical	Alkaloids, Steroids (Dihydrostigmasterol), Phenolic compounds, Tannins, Saponins, Carbohydrates (D-galactose, D-arabinose), Proteins, Amino acids, Organic acids, Withanolides, Linoleic	**Diabetes** , Diuretic , Improves memory , Hypertension , Hypersomnia , Heart diseases , **Wound healing** (MS/E)	55	0.13	0.36	Seeds are boiled in hot water which is taken with water after straining to treat diabetes Fruit paste is applied topically on wound for healing

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										acid[135,136]					
Withaniasomnifera (L.) Dunal ISL- 85	Aksun	Throughout year	Shrub	Wild/ Cultivated	Phanerophyte	Perennial	Leaf, Root	Decoction, Powder	Oral	Withanolides, Alkaloids (Withanine, Withasomnine, Somniferinine, Pseudotropine, Cuscohygrine, Scopoletin, Tropanol), Alcohols (Withaniol, Somnirol, Somnitol), Saponins, Cysteine, Chlorogenic acid[137,138,139]	**Rheumatism** , Aphrodisiac (RS/I), Diabetes , **Kidney stones** , Constipation , Improves memory , Scorpion sting , Hypertension , Fever	59	0.14	0.39	Fresh leaves are boiled in water; used this one cup of decoction once in a day for rheumatism Root powder is given with milk to remove kidney stones
Zygophyllaceae *Tribulus terrestris* L. ***ISL- 655***	Bhaakra	July-Sep	Herb	Wild	Chamaephyte	Annual	Root, Fruit	Powder, Paste	Oral	**Saponins (**Furostanol**,** Spirostanol) Flavonoids , Glycosides, Alkaloid (Harmane, Norharmane, Tribulusterine), Tannins, Terrestriamide, Hecogenin, Aurantiamide acetate, Xanthosine, Fatty acid ester, Ferulic acid, Vanillin, Sterols (β-sitosterol, Stigmasterols)[140,141]	**Rheumatism** , **Diuretic** , Aphrodisiac (RS/I), Impotence (RS/I), Tuberculosis , Blood purifier , Headache , Piles , Migraine , Depression	66	0.15	0.44	Dried plant without root is crushed to make powder which is taken for rheumatism for ten days Fruit paste is taken with milk which is diuretic

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FULL PAPER

THE ROLE AND USE OF OLIVE OIL AND OLIVE LEAVES AS A FUNCTIONAL FOOD

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Absract: The main feature of the Mediterranean diet is the pharmaceutical and nutraceutical effects of plant-derived foodstuffs and beverages include mainly fruits, vegetables, herbs and spices used in human nutrition. These phenylpropanoid-rich foods and beverages are important as pharmanutrients due to their antioxidant, anti-inflammatory, antimutagenic, cardioprotective, antihypersensitive and antitumoral activities. Olive (Olea europaea L.) is a characteristic plant of the Mediterranean region. The olive plant has been an important source of nutrition and medicine for centuries. Due to the recognition of its high dietetic nutritional value, olive oil and olive leaves have been used for ancient times. The leaves, fruits and fruit oil of the olive tree contain significant amounts of bioactive and phenolic compounds. Oleuropein, hydroxytyrosol, catechin, chlorogenic acids, p-coumaric acid, caffeic acid, 3-hydroxcinnamic acid are some of the important compounds. The amount of these substances varies according to factors such as tree age, fruit load, cultivar, harvest time, biotic and abiotic stress conditions, processing, and storage status of the crop. Nutritional and health benefits of olive and olive products stand out it as a functional food.

Keywords: *Olea europaea* L., Olive oil, olive leaves, polyphenol, functional food.

INTRODUCTION

Olive and olive oil has been a very important nutrient for human diet especially in the Mediterranean region for ancient times. Besides human nutrition, olive oil is used in health and cosmetics sectors as well. The homeland of the olive tree is Eastern Mediterranean basin and then it has spreaded to Europe from westwards beyond Turkey. Turkey is one of the important producing countries of olive because of its geographical location.

The main fatty component of olive oil, which has an important role in the mediterranean diet, is one of the majör promoting health factors. It plays a key role in the prevention of cardiovascular and metabolic diseases, inflammatory and autoimmune diseases as well as breast and colon cancer (La Lastra et al., 2001). Olive leaves are used for medical purposes locally as a public medicine. Olive leaves are rich in polyphenols such as oleuropein, routine, verbacoside, apigenin-7-glucoside and luteolin-7-glucoside (Bilgiç and Uğur, 2015). The concentration of polyphenolic compounds in the olive leaves may vary depending on the age of the olive tree, the environment and climate. (Çetinkaya and Kulak, 2016).

Phenolic Compounds in Olive Oil and Leaves: While 99% of the olive oil composition is composed of triglycerides, which are the main components, the other components are pigments, phenolic compounds and tocopherols which provide typical flavor, aroma, color, appearance and stability characteristics (Nergiz ve Engez, 2000; Vinha et al., 2005).
Toxopherol and phenolic substances are among the components of olive oil and they have high antioxidant properties. Although the oxidation stability and shelf life of olive oil vary depending on many factors, the degree of maturation of the olive fruit is the most important factor in the olive oil. As the degree of maturation increases, the amount of α-tocopherol and the total phenolic substance, which constitute the antioxidant properties of olive oil, decreases (Baccouri et al., 2007). There are also studies indicating that the amount of α-tocopherol increases according to the form (Gutierrez et

al., 1999), while β-tocopherol and b-tocopherol levels fluctuate due to maturation (Dağdelen, 2008). Hydroxytyrosol and tyrosol concentrations increase proportionally with increasing fruit maturity and decrease in total phenolic content as maturation increases and decreases in bitterness (Âşık and Özkan, 2010).

In olive oils oleuropein and 4-hydroxyphenyl acetic acid are the phenolic compounds present in the largest amount. Oleuropein produced from secondary metabolism of terpenes consists of hydroxytyrosol, elenoic acid and glucose molecule (Ötleş and Özyurt, 2012). Total phenolic content of olive oils differ according to varieties. In Turkish cultivars oil from Nizip yağlık cultivar has got highest total phenol, contrast Halhalı cultivar were least among 21 varietes (Temiz and Temur 2017). Besides this oleuropein, the routine, the ferulic acid, the hydroxyphenyl carboxylic acid, luteolin, apigenin, Cinnamic acid, 4-Hydroxyphenyl acetic acid, The 3-4-hydroxybenzoic acid, the caffeic acid, verbaktoz, the chlorogenic acid, p-coumaric acid, the syringic acid, tyrosol and vanic acid content vary according to cultivar origine, and age (Temiz and Temur 2017).

Since ancient times the olive leaf has been used in the treatment of many diseases because of its beneficial effects on health (Özçimen et al., 2010). In a study, it was reported that olive leaf extracts were traded as olive-leaf extracts in enterprises and as a supportive product for immune system in the medical goal (Köçkar et al., 2010). In a study on wound healing, olive leaf extract was found to be more effective than conventional wound dressing (Samancıoğlu, 2013).

The extract from the olive leaf used in the herbal treatment of diabetes has been found to increase the expression of receptors and transport proteins that provide glucose entry into the cell. Olive leaf extract showed antidiabetic effect by decreasing glucose and glycolized hemoglobin levels, decreased oxidative stress caused by diabetes, increased protective effect of oxidative stress on cells by increasing total antioxidant level, increased enzymatic and non-enzymatic defense mechanisms of cells, showed a positive effect (Shamsulddin, 2017).

Oleuropein, the phenolic component of the olive leaf against lung damage, caused an increase in the antioxidant status in the lung due to dose, and attenuated the pathological findings due to oxidative stress (Önalan, 2018; Danahaliloğlu et al., 2018). Oleuropein has an antitumor effect and has been shown to increase cell proliferation in human hepatoma cell.

Usage Areas of Olive Oil and Olive Leaves: The olive plant (*Olea europaea* L.) has been an important source of nutrition and medicine for centuries. Olive oil and olive leaves are widely used in food, health, food, and some industrial areas.
Olive oil is rich in fatty acids, oleic acid and the natural antioxidant content of olive oil makes olive oil an important food in the control of problems such as cardiovascular diseases, colesterol, high blood pressure and diabetes (Aşık ve Özkan 2010).

The extract obtained from olive leaf shows antimicrobial effect by increasing the shelf life and microbiological quality due to its positive effect against spoilage in food products due to oleuropein content (Gökmen et al., 2016). When added to the diets, oleuropein increases the oleic, linoleic acid values; it changes the lipid profile, increases the amount and quality of the fat, has positive effects on hormone levels and against hot stress (Gökmen et al., 2016; Dalkılıç, 2018). Compared to butylated hydroxytoluene, a synthetic antioxidant of olive leaf infusion; It has a significant antioxidant effect. (Elgin et al., 2012)

CONCLUSION

Olives, olive products and olive oil used in the Mediterranean diet are becoming increasingly important in both the nutrition and the pharmaceutical industry due to the bioactive substances it contains. The leaves, fruits and fruit oil of the olive tree contain significant amounts of bioactive and phenolic compounds

After the harvesting of olives, a significant number of bioproducts are formed, and olive leaves are the major part of this. The number of studies on the use of olive leaf in all areas should be increased.

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FULL PAPER

AS A GOOD SOURCE OF MELATONIN: GRAPE AND GRAPE PRODUCTS

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Abstract: Melatonin is known to be naturally found in a wide range of mammals and birds mainly responsible for the circadian rhythm of the body. It was then identified in plants. Studies look for melatonin in higher plants have been focused on the physiological roles in plants and also beneficial health effects for humans as a bioactive plant based compound. Recent studies have been indicated that melatonin in the edible plants has health-promoting effects on human health due to its anti-inflammatory, antioxidant and anticancer features as well as the therapeutic effect on cardiovascular diseases. Melatonin has been identified and quantified in different plants such as grapes, walnuts, sweet cherries, tomatoes and cucumbers. In this context grape and grape products provide a wide range of natural products as pharmanutritional effects. However, melatonin content may alterable according to cultivars, ecological conditions and some agrochemical treatments. Grape and grape products are represented to be good melatonin sources. In this review, we focus on melatonin content of grape and grape products and its effects on human health.

Keywords: *Vitis vinifera* L., circadian rhythm, foodstuffs, phytochemicals

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is an important pleiotropic molecule with multiple physiological and cellular actions in animals and plants. Melatonin (MT) was first isolated from pineal gland of cattle (Lerner et al., 1958). For a long time thereafter, melatonin was considered exclusively as an animal neurohormone (Reiter, 1991). Since the first report of melatonin in plants in 1995 and named phytomelatonin in 2004 (Bonomini et al., 2018). It has been identified in different plants in a wide range of concentrations from picograms to micrograms per gram of tissue (Tan et al., 2012). The discover improved the interest in investigating the content and the potential beneficial effects of phytomelatonin in plants, particularly in edible plants. MT has become more important recently both in plants and in human who utilizes plants for nutritional and health purposes. MT, the often called “hormone of darkness” plays a decisive role in the regulation of circadian and seasonal rhythms. MT is beneficial for the immunological system, enhancing resistance to infection and diseases, presenting inhibitory activity on some cancer forms and inducing beneficial effects on neuronal disorders (Posmyk and Janas, 2009). Several characteristics of MT such as its direct, non-receptor-mediated free radical scavenging activity – distinguish it from a classic hormone (Hardeland, 2008).

Given the potent health effects of MT, many foods have been tested in the past decades and MT was identified and quantified in edible plants. In crop plants, the highest values of phytomelatonin have been shown in the fruits of apple, cherry, tomatoes, peppers, grapes, coffee beans, mustard and almond seeds but it has also been quantified in cereals such as oat, corn, rice and barley (Bonomini et al., 2018).
Grapes are also good dietary sources of melatonin. In 2006, it was first reported that melatonin exists in grapes (Iriti et al., 2006). In this review, we discuss the melatonin content in grapes and grape products from previous studies and potential health effects for human.

Melatonin Content in Grapes and Grape Products: Considerable researches has been done on melatonin contain of grapes similar to some other plants. Table 1 summarizes the berry melatonin content of grape cutivars reported in previous studies.

Table 1. Melatonin content in grape berries (ng/g)

Cultivar	**Tissue**			**Ecology**	**References**
	Skin	**Must/Flesh**	**Seed**		
Nebbiolo	0.965				
Croatina	0.870				
Barbera	0.633				
Sangiovese	0.332			Italy	Iriti et al. (2006)
Marzemino	0.031				
Cabernet Sauvignon	0.422				
Merlot	0.264				
Cabernet Franc	0.005				
Malbec	1.20				
Cabernet Sauvignon	0.80			Argentina	Stege et al. (2010)
Chardonnay	0.60				
Merlot	9.3-17.5			Italy	Vitalini et al. (2011a); Vitalini et al. (2011b)
Malbec	9-159			Argentina	Boccalandro et al. (2011)
Malbec	120-160			Argentina	Gomez et al. (2012)
Malbec	440			Argentina	Gomez et al. (2013)
Merlot			3.5-10	Italy	Vitalini et al. (2011a); Vitalini et al., (2011b)
Merlot		0.2-3.9			Vitalini et al. (2011a); Vitalini et al. (2011b)
Merlot		100.000-150.000*		Canada	Murch et al. (2010)
Albana Sangiovese		1.2-1.5*		Italy	Mercolini et al. (2012)

*: Whole berry

The concentration of melatonin varied greatly among the eight different grape (*Vitis vinifera* L.) cultivars skin with the same degree of maturation, being the highest in Nebbiolo and Croatina (Iriti et al., 2006). The finding of this compound in grape skin suggests that the well-established pharmaconutritional properties of this fruit were due not only to the presence of polyphenolic nutraceuticals, such as resveratrol, anthocyanins, and proanthocyanidins, but also to the powerful antioxidant activity of melatonin. Thereafter, there has been continued research on grape and wine chemistry, with a focus on melatonin. Melatonin has not only been found in the berries of grape (*Vitis vinifera* L.) cultivars, but also in most grape products, e.g. wine, grape juice and grape vinegar. The finding of melatonin, in opens an interesting perspective associated with the plethora of health benefits related to the moderate consumption of red wine (Meng et al. 2017).

The differences in reported melatonin levels can, in part, be explained by many endogenous and external factors, such as the genetic traits of the cultivar, the berry tissue/grapevine organ analyzed, the phenological stage, pathogen infections and phytosanitary treatments, agro-meteorological conditions and environmental stresses, and the vintage (Meng et al. 2017). The melatonin contents in the different tissues of a grape berry change with the phenological stage. The highest melatonin content was observed in the skin during pre-veraison, which then decreased by 47% during veraison; whereas, the transition from pre-veraison to veraison increased the melatonin content by 63% in the seed and by 95% in the flesh (Vitalini et al. 2011b). A study by Murch et al., (2010), during veraison, the melatonin concentration in the seed was significantly higher than that measured in the skin and flesh. In whole berries, the concentration of melatonin was highest in wine grapes harvested during the early stages of veraison when the seed is developing.

In addition to wine, grape-related foodstuffs also include grape juice, raisins, vinegar. Currently, there are limited studies on the melatonin content of grape products, with the exception of wine. Table 2 summarizes the determined melatonin contentof grape products in previous studies (Meng et al. 2017).

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Table 2. Melatonin content in grape products (ng/mL)

Grape products	Ecology	Content	References
Sangiovese red wines	Italy	0.4	Mercolini et al. 2008
Trebbiano white wines		0.5	
Malbec red wine	Argentina	0.16	
Cabernet Sauvignon red wine		0.24	Stege et al. (2010)
Chardonnay white wine		0.32	
Groppello wine	Italy	4.1	Vitalini et al. (2011a)
Merlot wine		8.1	
Pressed wines (Sauvignon, Merlot, Syrah, Tempranillo, and Tintilla de Rota)	Spain	74-322	Rodriguez-Naranjo, et al. (2011a)
Racked wines (Sauvignon, Merlot, Syrah, Tempranillo, and Tintilla de Rota)	Spain	250-423	Rodriguez-Naranjo, et al. (2011b)
Monovarietal red wines (Groppello DOC, Melag DOC, Nebbiolo IGT, Terre di Rubinoro DOCG, and Syrah IGT)	Italy	0.14-0.62	Vitalini et al. (2013)
Polyvarietal red wines (Placido Rizzotto IGT and La Segreta IGT)	Italy	0.05-0.31	Vitalini et al. (2013)
White wine (Chaudelune-Vin de glace DOC)	Italy	0.18	Vitalini et al. (2013)
Dessert wines (Recioto di Soave DOCG, Santelmo-in Santo DOC, Marsala DOC, Passito di Pantelleria DOC, Moscato di Pantelleria DOC, and Zibibbo IGT)	Italy	0-0.31	Vitalini et al. (2013)
Modena balsamic vinegars	Italy	0.11-0.13	Vitalini et al. (2013)
Grape juice Italy	Italy	0.5	Mercolini et al. (2012)

Health Benefits of Melatonin in Grapes and Grape Products: As the biological roles of melatonin were widely studied, the recognized therapeutical effects and the health benefits of melatonin could cover a broad range. Melatonin could regulate human physiological rhythm, alleviate related disorders like jet lag and insomnia, scavenge free radical species, enhance the immune system, show anti-aging and anti-inflammatory effects and perform anticancer activities, exhibit neuroprotective effects, facilitate the control of chronic diseases, such as cardiovascular diseases, diabetes and obesity, regulate the mood, sexual maturation and body temperature (Meng et al. 2017). It has been proved that the melatonin concentration in human serum could significantly increase after the consumption of melatonin containing food.

Insomnia is a serious worldwide health threat, affecting nearly onethird of the general population. Melatonin has been reported to improve sleep efficiency and it was found that eating melatonin-rich foods could assist sleep.

Since the secretion of endogenous melatonin decreases after childhood, increasing dietary consumption could be a good option. The studies showed that intake of the food rich in melatonin may gain health impacts by increasing circulating melatonin (Oba et al., 2008; Gonzalez-Flores et al., 2012; Sae-Teaw et al., 2013). In a study conducted with young, middle-aged and elderly participants (20 ± 10 year-old, 45 ± 10 year-old and 75 ± 10 year-old, respectively), the total antioxidant capacity were reported to significantly increase in the three groups of individuals after the intake of the experimental juice of grape (*Vitis vinifera* cv. Tempranillo), 200 mL twice a day (as the lunch and dinner desserts) for 5 days (Gonzalez-Flores et al., 2012) as well as the urinary 6-sulfatoxymelatonin, a major metabolite of melatonin commonly used as a biomarker indicating its bioavailability (Schernhammer et al., 2009). Recently, a study found that the cardioprotective effects of consuming red wine are closely related to the melatonin content (Lamont et al. 2011). The presence of melatonin in red wine contributes to the cardioprotective effect of chronic and moderate consumption of red wine against lethal ischemia-reperfusion injuries. In addition, melatonin-rich wines protect against oxidative stress related to the central nervous system and influence the generation of DNA oxidation catabolites linked to mutagenesis (Meng et al., 2017).

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CONCLUSIONS

The intake of melatonin containing foods could significantly increase the melatonin concentration in human serum, indicating melatonin could provide beneficial effects on health through foods. Therefore, the consumption of foods rich in melatonin provides health benefits. Grapes and grape products are good dietary sources of melatonin. Therefore, those foods containing melatonin are now popular and regarded as promising nutraceuticals. In the future, the content of melatonin is worth testing and evaluating in more foods to find new natural sources of melatonin, and the mechanisms of action are needed to be investigated more comprehensively.

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FULL PAPER

ANTIOXIDANT ACTIVITY OF THE FRUIT EXTRACTS OF *WITHIANIA SOMNIFERA* AND *SOLANUM NIGRUM*

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Abstract: Antioxidants are known for the prevention and treatment of oxidative stress related disorders, such as diabetes and cardiovascular diseases. Synthetic antioxidants have considerable amount of toxicity, therefore there is an interest in developing herbal drugs. Medicinal herbs possess many phytochemicals which are efficient scavengers of free radicals. The present study was conducted to assess the antioxidant activity of two medicinal herbs, *Withiania somnifera* and *Solanum nigrum* from the family Solanaceae. The dried berries (10g each) were extracted in alcohol, separately. Total phenolic content was determined by Folin- Ciocalteau Method and antioxidant activity was measured by DPPH (1,1-Diphenyl-2-picryl hydrazyl) radical scavenging activity and β-carotene linoleate model. The total phenolic content and antioxidant activity of *Solanum nigrum* was considerably higher than *Withiania somnifera*. However, both herbal extracts showed good amount of total phenolic content and antioxidant activity. The alcoholic extract of *Withiania* somnifera shows more than 50% DPPH radical scavenging activity at 200µg/ml while the ethanol extract of *Solanum nigrum* showed above 80% DPPH radical scavenging activity at the same concentration.In the β-carotene linoleate model also *Solanum nigrum* extract showed higher antioxidant activity as compared to *Withiania somnifera*.

Keywords: Antioxidants, total phenolic content, medicinal herbs, fruit extracts, *Withiania somnifera*, *Solanum nigrum.*

INTRODUCTION

Free radicals are capable of oxidising bio-molecules like proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, atherosclerosis, arthritis etc. [1] Antioxidants are the compounds which inhibits the attack of free radicals and thus reduce the risk of these disorders [2] .These days, much attention is given on the use of natural antioxidants to protect the human body against degenerative diseases. Prior and Cao, [3] reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals. Keeping this in view, the present study has been conducted to evaluate the comparative antioxidant properties of the berries of *Withiania somnifera* and *Solanum nigrum.*

Withania somnifera, also known as **Ashwagandha**, **Indian ginseng**, is a plant in *Solanaceae* family. It has cardioprotective activity [4] and anti-inflammatory activity [5]. In Ayurveda ashwaganda is considered a rasayana herb, which works on a nonspecific basis to increase health and longevity. The roots and berries of the plant are used in herbal medicine. The berries are used as a substitute for rennet, to coagulate milk in cheese making. Withanolides have been reported from *Withiania somnifera* [6,7]

Solanum nigrum L. is an herbal plant indigenous to Asia. It has been used in traditional Oriental medicines for treating various kinds of tumors and is believed to have various biological activities [8]. It produces black coloured fruits; fruits of some strains are eaten in pies and are also used for making preserves.

Previous investigations have shown that extracts of *Solanum nigrum* suppressed the oxidant-mediated DNA sugar damage [9].Aqueous extract of *Solanum nigrum* inhibits growth of cervical carcinoma (U14) via modulating immune response of tumor bearing mice and inducing apoptosis of tumor cells [10].

Fruits of *Solanum nigrum* contain glucose, fructose and beta-carotene (15 to 20%). [11].Two quercetin gycosides [12] were reported from *Solanum nigrum.*

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MATERIALS AND METHODS

Chemicals, Reagents and Biologicals: 1,1- Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Fluka Chemika (Switzerland), Folin- Ciocalteau reagent was obtained from Sisco Research Laboratories, Mumbai. All other chemicals were of analytical grade.

Instruments: Shimadzu UV-VIS Spectrophotometer (1240) was used for all spectrophotometric studies. Buchi rotavapor was used for vacuum evaporation and Remi R24 centrifuge was used for centrifugation. Remi Cyclomixer (CM-101 DX) was used for rapid mixing.

Measurement of total phenolic content: We used the method of Saucier et. al [13] with slight modification, and the results are expressed directly in absorbance units at 765 nm. In each analysis, 1.58 ml of water was mixed with 20 µl of sample solution. Then 100 µl of Folin-Ciocalteau (FC) reagent was added to the solutions and mixed again. After 2 min, 300 µL of a 20% sodium carbonate solution was added. The solutions were left at room temperature for 2 h. Then the absorbance was determined at 765 nm. The amount of light absorbed is proportional to the amount of oxidizable material present, that is, phenolic compounds. Gallic acid was used as a standard for the calibration curve. The total phenolic content is reported as gallic acid equivalents (µg) using the following linear equation based on the calibration curve:

$$\mathbf{A = 0.0011x + 0.0025} \qquad \mathbf{R^2 = 0.9995}$$

Where **A** is the absorbance and **x** is the gallic acid equivalents (µg)

DPPH Radical Scavenging Activity: DPPH radical scavenging activity was evaluated according to the method described by Nagai et al. [14]. The assay mixture contained 0.3 ml of 1.0 mM DPPH radical solution, 2.4 ml of 99% ethanol, and 0.3 ml of test sample solution of different concentration. The solution was rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid was used as positive control while reaction mixture (DPPH radical solution) minus extract solution was taken as control. The percent (%) radical scavenging was calculated by the following equation.

$$\text{\% radical scavenging} = \left[\frac{Ac - As}{Ac} \right] \times 100$$

Where $\mathbf{A_c}$ = Absorbance of control at 517 nm and $\mathbf{A_s}$= Absorbance of sample at 517 nm.

β- Carotene- linoleic acid assay: In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [15]. A stock solution of β-carotene was dissolved in 1 ml of chloroform (HPLC grade); 25 µl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml of distilled water saturated with oxygen (30 min 100 ml/min) were added with vigorous shaking. 2500 µl of this reaction mixture were dispensed in to test tubes and 350 µl portions of the test samples (at a concentration of 25µg/ml for plant extracts & 20 µM for compounds) were added and the emulsion system was incubated for up to 48 h at room temperature.

The same procedure was repeated with the synthetic antioxidant, butylated hydroxytoluene (BHT) as positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and blank.

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RESULTS AND DISCUSSION

As shown in Table 1, percent yield from the ethanol extracts of the fruits of *Solanum nigrum* and *Withiania somnifera* was 11.41 % and 8.28%, respectively. Total phenol content in the berries of *Solanum nigrum* was 106.48 µg of GAE/ µg of dry weight and 59.27 µg of GAE/µg of dry weight in the fruits of *Withiania somnifera.* The total phenolic content of *Solanum nigrum* berries is much higher as compared to *Withiania somnifera*.

Figure 1 shows the DPPH radical scavenging activity of *Solanum nigrum* and *Withiania somnifera* fruit extracts. At a concentration as low as 50 µg/ml, extracts of *Solanum nigrum* fruits showed 62.93% DPPH radical scavenging activity while the extracts of *Withiania somnifera* fruits showed above 50% DPPH radical scavenging activity at considerably higher concentration of 200 µg/ml. At higher concentration of 400 µg/ml both the fruit extracts of *Solanum nigrum* and *Withiania somnifera* showed good DPPH radical scavenging activity. Thus DPPH radical scavenging activity is concentration dependent and the fruit extracts of *Solanum nigrum* have better potential to be utilised as nutraceuticals.

Figure 2 shows the antioxidant activity of the fruit extracts of *Withiania somnifera* and *Solanum nigrum* on β-carotene-linoleate model where BHT is used as standard. The extracts of the berries of *Solanum nigrum* again showed exceptional antioxidant potential and its activity at 25µg/ml is even higher than synthetic antioxidant BHT. Withiania somnifera showed good antioxidant potential, nearly similar to *Solanum nigrum* at slightly higher concentration of 400 µg/ml. This might be due to the fact that *Withiania somnifera* has lesser amount of total phenols as tested through Folin- Ciocalteau method.

The higher antioxidant activity of the berries of *Solanum nigrum* could be attributable to the presence of β-carotene [11], phenolic acids and phenol glycosides [16] in addition to other radical scavenging compounds.

CONCLUSION

Withiania somnifera and *Solanum nigrum* are well known medicinal herbs and used in traditional system of medicine [11]. Our study on the antioxidant properties of the fruit extracts of these herbs concluded that their berries have excellent potential to be used as natural antioxidants. Further studies can be done to identify and characterise specific compounds responsible for antioxidant activities, many of them are already identified [16].Since the berries of *Solanum nigru*m are edible in small amount they can be used as food supplements. Further studies on their toxicity can be done for proper utilisation of these promising herbs especially *Solanum nigrum*.

TABLE AND FIGURES

Table 1: Yield and total phenolic content of the ethanol extracts of the fruits of *Withiania somnifera a*nd *Solanum nigrum*.

Family	Herbs Studied	Part Used	Yield (%)	Total phenolic content (µg of GAE/µg of dry weight)
Solanaceae	*Solanum nigrum*	Edible fruits	11.41	106.48 + 0.09
	Withiania somnifera	Fruits	8.28	59.27 + 0.12

* Values are expressed as Mean + S.E.M., n= 4.

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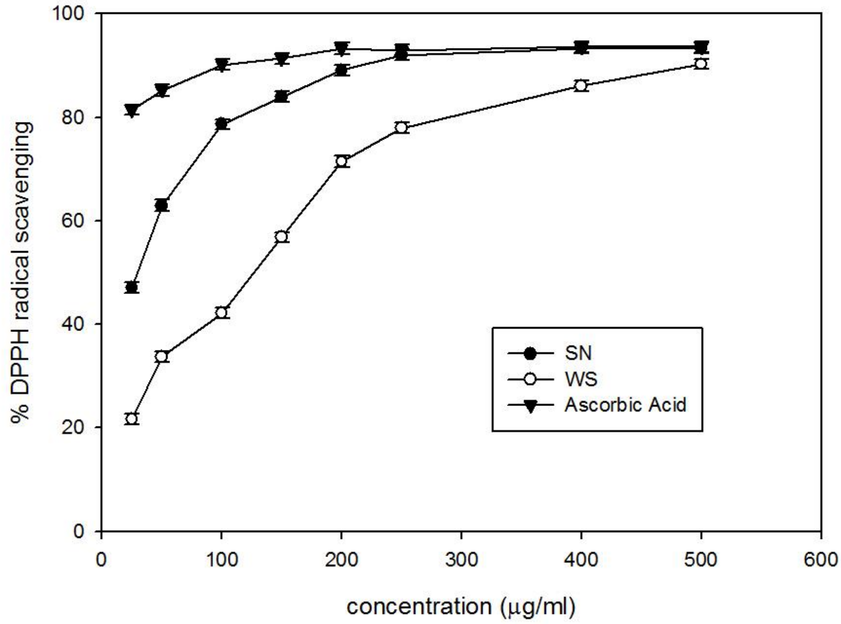

Figure 1: DPPH radical scavenging of the fruit extracts of *Withiania somnifera* and *Solanum nigrum*.Ascorbic acid is used as positive control.Values are expressed as Mean ± S.D.,n=3. SN=*Solanum nigrum* ;WS=*Withiania somnifera.*

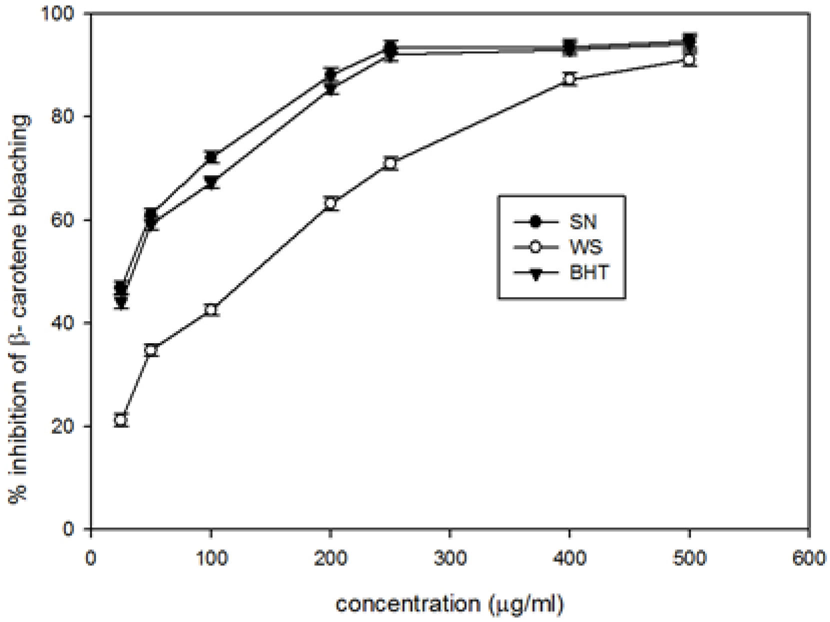

Figure 2: Antioxidant activity of the fruit extracts of *Withiania somnifera* and *Solanum nigrum* as determined by β- carotene linoleate model. BHT is used as a standard. Values are expressed as mean ± S.D., n=3. SN= *Solanum nigrum*; WS=*Withania somnifera.*

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FULL PAPER

CHEMICAL ANALYSIS OF WILD PLANT *INULA HELENIUM SUBSP. PSEUDOHELENIUM* GRIERSON GROWING IN VAN LAKE AROUND.

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Abstract: The genus *Inula* belongs to the *Compositae* (*Asteraceae*) family and comprises 27 species growing wild in Turkey. *Inula helenium* L. *subsp. pseudohelenium* is of the commonly consumed wild edible plants as food and medicinal purposes inhabitants in Eastern Anatolia. It has been used in traditional Anatolia medicine for abdominal distension and pain, acute enteritis and bacillary dysentery. 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 *Inula helenium subsp. pseudohelenium* Grierson was investigated.In laboratory analysis, dry matter, total ash, % N, crude protein, pH and crude fiber were examined as nutritional value. Useful minerals (Na, Mg, K, Ca, P, S, Mn, Fe, Cu and Zn) and heavy metals (Cd, Co and Pb) that hazardous elements for livings were also determined. Results in this research showed that *Inula helenium subsp. pseudohelenium* Grierson contains high value of macro elements such as sodium (1.07 mg/g), potassium (19.16 mg/g), magnesium (2.12 mg/g) and calcium (14.17 mg/g). Also, it has maximum micro-elements such as iron (246.24 mg/kg), manganese (52.98 mg/kg, cupper (40.27 mg/kg and zinc (26.84 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: Mineral content, Wild plant, *Inula helenium* L, East Anatolia

INTRODUCTION

The genus Inula L. belongs to the family Compositae (Asteraceae) and comprises about 120 species, distributed in World. Elecampane is an aromatic perennial originating from Central Asia, both wild and cultivate make in Europe, Asia Minor, Japan and North America for a long time (Sulborska, 2007). According to current records the genus *Inula* L. is represented by 32 taxa belongs to 27 species (4 subspcies and 1 variates), of which 8 endemics and 1 rare distributed. The rate of endemism of *Inula* species is 28.5% in Turkey (Guner et al., 2012). Elecampane (*Inula helenum* L.) is a plant is commonly found in streams, lake side, forest and shrub areas in 1000-2560 m altitudes above sea level in Eastern Anatolia/Turkey (Eren, 2014). *Inula helenium* is a perennial with rhizome and thick root. The stem is grooved and straight and up to two-metre high (Blinska and Buchwald, 2015). *Inula helenium* is an important herb traditionally used in the treatment in Turkey, China, Japan and Europe. Recent studies have demonstrated that the root extracts possess diuretic, cholagogue, antihelminthic, anti-tumor, antimicrobial and insecticidal activities (Konishi et al., 2002; Stojakowska et al., 2006; Huo et al., 2008). Helenine substances, due to their restraining bacterial flora development properties, can be applied in skin and mucous membrane disinfection (Blinska and Buchwald, 2015).

Until today, there are no reports on the chemical composition of the *Inula helenium subsp. pseudohelenium* Grierson species. Therefore, the aim of this study was to determine the nutritional values and mineral compositions of *Inula helenium subsp. pseudohelenium* Grierson species which grew as wild plants in the Van region of Eastern Anatolia, Turkey.

MATERIAL AND METHODS

Some chemical compositions of *Inula helenium subsp. pseudohelenium* Grierson which located in natural flora at around Van Lake in Eastern Anatolia Region, were determined. The plants were collected from natural flora in 2013 and botanical identifications were made by the Department of Biology, Science Faculty, Van Yuzuncu Yil University according to 'Flora of Turkey' [8] Some information for *Inula helenium subsp. pseudohelenium* Grierson species are given in Table 1.

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Tablo1. Some introductory information of *Inula helenium subsp. pseudohelenium* species

Plants Scientific Name	Family	Local Name	Used Parts	Use	Col. No. - Locality
***Inula helenium subsp. pseudohelenium* Grierson**	*Asteracea (Compositae)*	Andız otu	Underground parts (Rhizome and Root)	asthma, influenza, fever, cancer, tuberculosis, indigestion, wounds healing	F9229-B9

B9: Van, Gevas, north slopes of the Alacabuk mountain, east of Altınsac church situation, step, 09.vii.2001, 2800 m.

Cleaned plants from foreign materials were separated to parts and washed with deionized water, dried in room temperature, ground, packaged in plastic bags and kept in desiccators until analysis. Electric Muffle Furnace set at 550 ºC were used for determination of total ash content (inorganic matter). Kjeldahl apparatus and method were used to find the nitrogen content of the samples (underground parts). After total nitrogen content determination, crude protein contents were calculated by formula. pH values were determined by pH-meter in the plant samples. Crude fiber analyses were accomplished by AOAC (Association of Analytical Chemists) method (AOAC 2000). Mineral compositions of the samples were determined as follows; dried plant samples were ashed in a furnace by nitric (AR) and hydrochloric acid (AOAC). Afterwards, distilled water (50 ml) was added to samples in a volumetric flask. All the analyses were repeated three times and standard materials were used for chemical analyses. Atomic Absorption Spectrometry was used for determination of mineral contents. Phosphorus (P) was determined by molybdate-vanadate and sulphur (S) was observed according to the Mitchell (1992) method in conjugation with a UV-Visible spectrophotometer (Shimadzu UV-1201 V; Shimadzu, Kyoto, Japan). The average data obtained from chemical analyses have been shown in Table 2 with their standard deviations.

RESULTS AND DISCUSSION

In this study, nutrient content of *Inula helenium subsp. pseudohelenium* Grierson species that are important for human health for the prevention and control of diseases were investigated and some properties such as the values of dry matter, total ash, total nitrogen, crude protein, pH and crude fiber contents are given in Table 2 and mineral compositions in Table 3. The values are given as mean ± SD.

Table 2. The average chemical composition values of *Inula helenium subsp. pseudohelenium* Grierson

Parameters	Inula helenium subsp. pseudohelenium
Dry matter (%)	22.11 ± 0.33
Total ash (%)	8.40 ± 0.50
Total nitrogen (%)	1.60 ± 0.03
Crude protein (%)	9.99 ± 0.19
pH	5.92 ± 0.71
Crude fiber (%)	36.65 ± 1.89

The dry matter, total ash, total nitrogen, crude protein, pH and crude fiber contents of *Inula helenium subsp. pseudohelenium* situated in Van flora were determined as 22.11 %, 8.40 %, 1.60 %, 9.99 %, 5.92 and 36.65 %, respectively. In previous studies total ash, total nitrogen, crude protein, pH and crude fiber content of some medicinal and edible plants were found in intervals of 6.43-22.32%, 0.20-3.47%, 1.30-21.69 %, 3.50-7.44 and 0.90-36.68 % respectively (Yıldırım et al., 2001; Tuncturk et al., 2017a; Tuncturk et al., 2017b). We conclude from this study that obtained values are compatible with the previous scientific reports on medicinal and aromatic plants. Lack of sodium causes negative effects such as forget fullness and low blood pressure and the daily sodium need per person is of 5-15 g. It is known that hypertension is triggered by excessive consumption. Potassium have role in starch synthesis and regulate to the osmotic pressure of the plant. The World Health Organization (WHO) declared that a minimum daily potassium requirement is 3.51 g/kg of a person for the protection against heart disease and the regulation of blood pressure (Saltan and Canbay, 2015). Therefore, it is

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required to be limited and careful use of plant materials containing high levels of sodium and potassium. Magnesium is involved in hundreds of enzyme reactions in the body. It performs an array of biological functions as activation of muscles and nerves, digestion of proteins, carbohydrates, fats, building block for RNA and DNA synthesis (Maiti et al., 2016) in adults daily consumption, but its amount must be up to 300-420 mg (Saltan and Canbay, 2015). In this study, Na, Mg, K, Ca, P and S contents were determined as 1.07, 2.12, 19.16, 14.17, 1.34 and 1.76 g/kg from *Inula helenium subsp. pseudohelenium* species, respectively. Sodium (Na) values of medicinal and edible plants were found between 0.21 and 63.32 g/kg; magnesium (Mg) concentrations varied from 1.17 to 86.43 g/kg (Akgunlu, 2012) . Potassium (K) content changed between 5.57-20.33 g/kg in wild vegetables (Tuncturk et al., 2017a; Tuncturk and Tuncturk, 2017). Calcium (Ca) concentrations were found in a wide range from 0.18 to 21.89 g/kg (Tuncturk et al., 2017a; Saltan and Canbay, 2012); phosphorous (P) contents varied from 1.14-69.13 g/kg (Tuncturk et al., 2017a; Akgunlu, 2012; Tuncturk et al., 2017b); sulphur (S) concentrations were reported in the range of 1.12-3.27 (Tuncturk et al., 2017a; Tuncturk and Tuncturk, 2017). Nutrition element rates of the plants are affected from a number of factors such as plant genetic structure, growing conditions, soil characteristics, water availability, growing seasons etc.

In the present study, we also determined some micronutrients and heavy metal contents. As seen in Table 3, the concentrations of Mn, Fe, Cu and Zn in studied samples were determined as 52.98, 246.24, 40.27 and 26.84 mg/kg, respectively. Different micronutrients, although required in minor quantities are essential for good health of mankind and animals. The deficiency of these elements causes abnormalities leading to infection of diseases (Maiti et al., 2016). It is known that heavy metals such as Cu, are necessary as proteins and enzymes (as catalytic and structural components) as a cofactor for plants to grow up (Altıntıg et al., 2014), but its excess intake might cause health problems. 2.5 mg of daily Cupper intake can meet the daily requirement of adults.

Zinc is required for normal growth, a healthy immune system function, for new protein synthesis, DNA synthesis and cell division as well for different chemical reactions (Maiti et al., 2016). Therefore, Zn an essential element for nearly all living cells, is recommended to be consumed at the amount of 15 mg a day (Ulger and Coskun, 2003). Cu and Zn critical concentrations could be different for perennial and annual plants. Bowen (1966) and Allaway (1968) stated that agricultural products could contain 4-15 mg/kg Cu and 15-200 mg/kg Zn (Esetlili et al., 2014). Manganese is acts as a coenzyme in a very small amount in our body and facilitates various metabolic processes. For some medicinal plants species manganese content was determined between 7.0 and 315.0 mg/kg, iron contents between 59.0 and 1317.0 mg/kg, zinc (Zn) values between 1.0-79.0 mg/kg and cupper contents were reported between 1.8 and 19.3 mg/kg (Esetlili et al., 2014). In this study, determined concentrations of Mn, Fe and Zn except for Cu are in accordance with previous studies.

Table 3. Mean values of mineral compositions of *Inula helenium subsp. pseudohelenium.*

Minerals and heavy metals	**Inula helenium subsp. pseudohelenium**
Na (g/kg)	1.07 ± 0.06
Mg (g/kg)	2.12 ± 0,02
K (g/kg)	19.16 ± 0.43
Ca (g/kg)	14.17 ± 0.04
P (g/kg)	1.34 ± 0.02
S (g/kg)	1.76 ± 0.13
Mn (mg/kg)	52.98 ± 1.69
Fe (mg/kg)	246.24 ± 2.31
Cu (mg/kg)	40.27 ± 0.74
Zn (mg/kg)	26.84 ± 1.46
Cd (mg/kg)	0.18 ± 0,04
Co (mg/kg)	0.92 ± 0,08
Pb (mg/kg)	0.19 ± 0,07

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In the current study; analyzed plant samples had limited and trace levels of cadmium (Cd), cobalt (Co) and (Pb) as 0.18, 0.92 and 1.19 mg/kg, respectively. These metals known as heavy metals and their hazardous effects on living organisms in certain quantities were reported. Cd concentrations were reported between 0.02-0.71 mg/kg for medicinal plants and wild vegetables (Tuncturk et al., 2017a; Esetlili et al., 2014). Co and Pb concentrations of some medicinal and edible plants were stated in the ranges of 0.04-1.69 mg/kg (Akgunlu, 2012) and 0.15-2.59 mg/kg (Esetlili et al., 2014; Saltan and Canbay, 2015; Tuncturk et al., 2017a) in previous studies. In this respect heavy metal contents are found similar with previous studies findings.

CONCLUSION

Inula helenium, like many other plants from the *Asteraceae* family, has multiple applications in therapeutics. It is attributable to the presence of biologically active compounds contained in excretions of secretory tissues situated in different partsof plants. *Inula helenium subsp. pseudohelenium* which has well known positive effects on human health are determined to be rich in minerals. Essential elements are important for human health for the prevention and control of diseases. As a result, it is noteworthy that heavy metals such as Co, Pb and Cd could be harmful to health according to international references. The accumulation of toxic elements in the human body will cause to adverse health effects. This study showed that are not pose any risk to human health of heavy metal concentrations of investigated plant. Many of the plants used for medical purposes are collected from nature and marketed. The effects of these crops on human health should be investigated and these plants need to use caution.

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FULL PAPER

THE CHEMICAL COMPOSITION OF *DELPHINIUM SZOWITSIANUM* BOISS. SPECIES SPREADING IN VAN REGION

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Abstract: The family Ranunculaceae, which includes the Delphinium species, has 59 genera and 2500 species worldwide. In Turkey, it has 18 genus and 216 species and its endemism rate (23.5%) is quite a high family. *Delphinium szowitsianum* Boiss. is a perennial herbaceous species. It has been spread in the Eastern Anatolia Region of our country and it is known as "zarif hezaren". In the study, it is aimed to determine some and nutrients mineral substance contents of *Delphinium szowitsianum* Boiss which is naturally grown around the Van lake in the Eastern Anatolia Region. In plant samples, some nutrients and mineral contents such as total ash, crude protein, pH, crude cellulose and N, Na, Mg, K, Ca, P, S, Mn, Fe, Cu, Zn, Cd, Co have been investigated. As a result of the research; it has been determined as crude protein ratio 4.60%, pH 5.99, total nitrogen content 0.74 %, crude ash content 6.31% and crude cellulose content 28.20%. In addition, while some mineral substance contents were determined as K 5.31 g/kg, Ca 16.37 g/kg, P 1.07 g/kg, Mn 35.41 mg/kg, Fe 459.69 mg/kg and Zn 32.23 mg/kg, heavy metal contents were determined as Cr 0.49 mg/kg, Cd 0.11 mg/kg, Co 1.35 mg/kg and Pb 0.44 mg/kg.

Key Words: *Delphinium szowitsianum* Boiss, Eastern Anatolia, Heavy Metal, Medicinal Plants

INTRODUCTION

The Ranunculaceae (Delphinieae) family is represented by approximately 59 genera and 2500 identified species with a worldwide distribution (Antoń and Kamińska 2015). The largest genera in this family are Ranunculus (600 species)(Hörandl and Emadzade 2012), Delphinium, Thalictrum, Clematis and Aconitum (Cai et al. 2009). Delphinium is a genus, which has approximately 300 species in the family of Ranunculaceae (Lin et al. 2014; Koparal and Bostancıoğlu 2016). In Turkey, Ranunculacea which has about 18 genera and 216 species, it is quite a high family with an endemism rate (23.5%) (Yaprak, Körüklü, and Ketenoğlu 2011; Özçelik and Korkmaztürk 2013). Delphiniums are the plant that grow for the flashy spikes of colorful summer flowers in magnificent blue, pink, white and purple shades. It is popular in cutting gardens and cottage-style gardens. Delphiniums, despite some difficulties, is the favorite of many gardeners in the landscape. They prefer moisture and cool summers, and their development in hot and dry weather is poor. Also, these plants do not like sudden wind or rain.

The genus Delphinium is a rich source of biologically productive compounds, often containing more complex structures with diterpenoid and norditerpenoid alkaloids (Benn and Jacyno 1983; Ahmad et al. 2017). Recently a number of alkaloids have been reported from Delphinium species (Atta ur and Choudhary 1999). The chemical components and molecular mechanisms underlying the biological activity of *D. szowitsianum* Boiss are largely unknown. Studies conducted to date show that the secondary metabolites and essential oil components of this species have been investigated. However, it was determined that almost no research was carried out on the chemical components and other plant values of the species. Therefore, in this study, it was aimed at elucidating chemical composition and heavy metal contents of *D. szowitsianum* Boiss.

MATERIAL AND METHOD

Some chemical compositions of *Delphinium szowitsianum* Boiss. which located in natural flora at around Van Lake in Eastern Anatolia Region, were determined. The plants were collected from natural flora in 2013 and botanical identifications were made by the Department of Biology, Faculty of

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Science, Van Yuzuncu Yil University according to “Flora of Turkey” (Davis 1965). Some information for *Delphinium szowitsianum* Boiss. species are given in Table 1.

Tablo1. Some introductory information of ***Delphinium szowitsianum* Boiss.** species.

Plants’ Scientific Name	Family	Local Name	Used Parts	Locality	Col. No.
Delphinium szowitsianum Boiss.	Ranunculaceae	Bit otu, mezevek, Zarif hezaren	Aerial part and seeds	Bitlis, Tatvan, Alacabük Mountain west slopes, Cow Cave, step, altitude 2600 m	F 9784

The plants were purged for purity, separated into used parts and washed with deionized water, dried at room temperature, milled, placed in plastic bags and held in desiccators until analyzed. In order to determine the total ash content (inorganic material), the Electric Furnace was set to 550 ^{o}C. Kjeldahl apparatus and method were used to find the nitrogen content of the samples (Kjeldahl 1883).

After determination of total nitrogen content, crude protein contents were calculated by formula. pH values of plant samples were determined by pH-meter. Crude fiber analysis was performed by AOAC method (AOAC 2000). The samples mineral compositions were determined as follows; samples of dried plants were ashed in an oven with nitric (AR) and hydrochloric acid. Subsequently, distilled water (50 ml) was added to the samples in a volumetric flask. Standard materials were used for chemical analysis and all analyzes were repeated three times. Atomic Absorption Spectrometer was used to determine mineral contents. Phosphorus (P) was determined by molybdate-vanadate and sulphur (S) was observed according to the Atomic Absorption Spectrometry was used to determine the mineral content. Phosphorus (P) was determined according to the molybdate-vanadata procedure and sulfur (S) was observed according to the method reported by Mitchell (1992) that conjugated with a UV-Visible spectrophotometer (Shimadzu UV-1201 V; Shimadzu, Kyoto, Japan). The mean data obtained from chemical analyses have been shown in Table 2 with their standard deviations.

RESULTS AND DISCUSSION

In this study, total ash, total nitrogen, crude protein, pH and crude fiber content of Delphinium szowitsianum Boiss species which are important for human health in the prevention and control of diseases were being investigated and the average values were given in Table 2, and also, the means of some important minerals is given in Table 3. The all values are given as mean ± SD.

Table 2. The average chemical composition values of *Delphinium szowitsianum* Boiss.

Parameters	***Means*** ± Sd
Total ash (%)	6.31 ± 0.31
N (%)	0.74 ± 0.04
Crude protein (%)	4.60 ± 0.22
pH	5.99 ± 0.32
Crude Fiber (%)	28.20 ± 2.05

Total ash, total nitrogen, crude protein, pH and crude fiber ratios of *D. szowitsianum* Boiss situated in Van flora were determined as 6.31%, 0.74%, 4.60%, 5.99 and 28.20%, respectively. In previous studies, total ash, total nitrogen, crude protein, pH and crude fiber content of some medicinal and edible plants were found in intervals of 1.36-8.46 %, 6.43-8.54 % 1.30-21.69 %, 5.41-7.44 % and 36.19 46.33 % (Eryiğit, Tunctürk, and Tunctürk 2019; Dias et al. 2013; Maiti et al. 2016) respectively. We conclude that the results of this study are consistent with the results of previous studies.

In the study, macro elements such as Sodium (Na), magnesium (Mg), Potassium (K), Calcium (Ca), phosphorous (P), sulphur (S) and Manganese (Mn) contents were determined as 0.44, 1.94, 5.31, 16.37, 1.07, 1.00 and 35.41 g/kg from *D. szowitsianum* Boiss species, respectively. Mineral concentration values of some other medicinal and wild plants obtained from previous studies are summarized for above minerals here: Na, Mg, K, Ca, P and S contents were reported in ranges of

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0.21-63.32 g/kg, 1.17-86.43 g/kg, 245.78-557.91 g/kg, 0.03-777.52 g/kg, 34.92 to 69.13g/kg and 12.34-108.01 g/kg, respectively (Akgunlu 2012; Turan et al. 2003; Holland et al. 1997; Demir 2006; Koca, Özkutlu, and Sekeroglu 2009). Considering previous studies on the macro element concentrations of some medicinal and wild plants, our findings are consistent with the results of the researchers. It is well known that the mineral composition of plants is greatly influenced by many factors such as plant characteristics, soil properties, water availability in the soil. Mineral element ratios of plants are affected by many factors such as plant genetic structure, growing conditions, soil properties, water availability, growing season, etc. Therefore, substantial variability is expected in the mineral compositions of plants in different parts of plants.

Table 3. Mean values of mineral compositions of *Delphinium szowitsianum* Boiss.

Minerals	***Means*** ± Sd	Minerals	***Means*** ± Sd
Na (g/kg)	0.44 ± 0.004	**Fe (mg/kg)**	459.69 ± 7.02
Mg (g/kg)	1.94 ± 0.06	**Cu (mg/kg)**	10.59 ± 0.49
K (g/kg)	5.31 ± 0.06	**Zn (mg/kg)**	32.23 ± 9.45
Ca (g/kg)	16.37 ± 0.14	**Cr (mg/kg)**	0.49 ± 0.04
P (g/kg)	1.07 ± 0.12	**Cd (mg/kg)**	0.11 ± 0.03
S (g/kg)	1.00 ± 0.16	**Co (mg/kg)**	1.35 ± 0.09
Mn (mg/kg)	35.41 ± 0.97	**Pb (mg/kg)**	0.44 ± 0.11

In the present study, some micronutrients and heavy metal concentrations were observed. In Table 3, the concentrations of Fe, Cu and Zn in studied samples were determined as 459.69, 10.59 and 32.23 mg/kg, respectively. Micronutrients, albeit in low amounts, are essential nutrients for human and animal health. Lack of these elements can lead to infections that are the cause of many diseases. Maiti et al. (2016) reported that Fe, Cu and Zn concentrations of the traditionally used 44 herbal plant species were 98.28-3972.55, 4.17-33.88 and 9.49-216.31 mg/kg, respectively.

The analyzed plant samples were found to contain limited and trace levels of chromium (Cr), cadmium (Cd), cobalt (Co) and lead (Pb) 0.49, 0.11, 1.35 and 0.44 mg/kg, respectively. In a number of previous studies, Cr, Cd and Co contents of some medicinal and wild plants were determined between 0.10-425.0 mg/kg (Esetlili et al. 2014), 0.007-0.47 mg/kg (Canbay and Saltan 2015) and 0.05-1.35 mg/(Łozak et al. 2002)(Łozak et al. 2002)(Łozak et al. 2002)kg (Łozak et al. 2002), respectively.

CONCLUSION

One way to discover new and more effective drugs is to explore natural products from medicinal plants. Medicinal plants are a very often valid source for researchers looking for bioactive substances that may be potentially useful against many diseases. Although it is a toxic plant as mentioned above, because of its beneficial aspects, it is still used in public health and in many other fields, and since it has not been studied on mineral and heavy metal contents, we found it suitable to work on *D. szowitsianum* Boiss. Our results provide scientific evidence for ethnobotanical demands and are a preliminary step for future research.

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FULL PAPER

NEGATIVE DATA BUT NO WORRIES

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Abstract: There have been numerous studies using both individual supplements and multivitamins, and the results are mostly negative; evidence shows lack of benefit. However, omega-3 supplements, vitamin and other supplement sales soar without any stop. Fish oil sales increased 10-fold in 15 years, whereas vitamin D use simply increased fourfold. Meanwhile, 22,071 men who receive beta-carotene or placebo for 12 years in Physicians Health Study, shows no benefit for cardiovascular disease risk and neoplasms or on overall mortality. 2nd Physicians Health Study, the Women's Antioxidant Cardiovascular Study, VIDA and VITAL studies also shows no benefit for Vitamin C, D and E again and again. Then why nearly everybody still use vitamins and supplements? Unaware or not concerned? Do expectations and believes have any role in it? This review will summarize all the published surveys about these questions to find the reason behind the global non-stopping non-declining vitamin and supplement trend.

Keywords: supplements, vitamins, evidence-based results, surveys, health behavior

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INTRODUCTION

Micronutrient deficiencies, identification, treatment and prevention is one of the biggest achievement in health sciences in last century. The well-known example is the treatment of scurvy, the disease characterized by weakness, easy bruising and hemorrhages, bleeding gums, only with vitamin C via citrus fruit, is may also be the first clinical trial in this kind (Baron 2009). Human health as well as livestock animal health is refined by continuous nutritional science experiments and similar findings, while today, attention in micro-nutrients has shifted recently from prevention of classic deficiencies to promotion of overall health and longevity using supplemental vitamins and minerals (Jenkins et al. 2018). The obsession with vitamins and dietary supplements continues to fuel a multi–billion-dollar industry. In the United States alone, the supplement market was almost $30 billion in 2015. The global dietary supplement market is projected to reach $278 billion by 2024 (Walton-Shirley 2019). The United States' National Health and Nutrition Examination Survey data (1999 to 2012) with 37,958 adults, shows that up to 52% of the population taking supplements; multivitamins were taken by 31% of the population, vitamin D by 19%, calcium by 14%, and vitamin C by 12% (Kantor et al. 2016). While for Europe, The European Prospective Investigation into Cancer and Nutrition (EPIC) data with 36,034 men and women indicated a wide range of supplement use, while with a strong north Europe south Europe difference such as, Denmark: 51% versus Greece: 2.0% (Skeie et al. 2009).

WHAT BENEFITS EXPECTED?

Primary intention of consuming dietary supplements is to enhance the intake of essential nutritional components but seems to be for variety of reasons. The reasons motivating the public to use supplements was investigated by The United States' National Health and Nutrition Examination Survey data (2007 to 2010) from adults (≥20 years; n = 11956). The most commonly reported reason is "improve" (45%) or "maintain" (33%) overall health. Women and men have different needs. Calcium supplements are used by women for "bone health" (36%), where men state that they use supplements for "heart health or to lower cholesterol" (18%). Older adults are more specific about supplemental use. Adults over 60 years are more likely stating they use supplements for site-specific reasons like heart, bone and joint, and eye health. Health care providers also may recommend the products, nearly ¼ of consumers report usage based on these recommendations. Most frequently reported type of supplements are multivitamin and mineral products, calcium and ω-3 or fish oil supplements are followers (Bailey et al. 2013).

Market review reports shows a rising alertness towards calorie reduction among gym users, so which is expected to promote the application movement in sports nutrition industry. Furthermore, big pharma companies is expected to have a significant impact near future too (GrandView Report 2018).

WHAT REPORTS SAYS?

If anyone search US National Library of Medicine website pubmed.com for "dietary supplements", over 82000 results will return, increasing nearly every year steady state markedly starting with year 1997. Basic research and observational studies suggest these supplements "appeal to hopes and there is biologic plausibility", researchers already said (Haslam and Prasad 2018). Supplementation with folic acid/prenatal vitamins is precribed for pregnant, while vitamin B12, vitamin D, and/or calcium is prescribed very often for older adults. High-risk groups, such as those with malabsorption syndromes, restricted eating patterns, osteoporosis, pernicious anemia, and age-related macular degeneration, proton-pump inhibitor users and also metformin users also may benefit from dietary supplements, and may be prescribed by doctors if any necessity seen (Fairfield 2019).

Although, if necessary, have to use approach, which was as stated well before for the treatment of scurvy, does not seem to work for general population to be more healthy approach. 1996 study (Hennekens et al. 1996), which enrolled 22,071 males, 40 to 84 years of age with randomized, double-blind, placebo-controlled trial of beta carotene for 12 years, as observational studies suggest that people who consume more fruits and vegetables containing beta carotene have somewhat lower risks of cancer and cardiovascular disease, shows that there were no differences in the overall incidence, early or late timing of malignant neoplasms, cancer, deaths from cancer, or any form of cardiovascular disease, stroke or myocardial infarction, or in overall mortality. 1999 study (Lee et al. 1999) with women participants also found same results, no difference for overall incidence of cancer or cardiovascular disease. The Women's Antioxidant Cardiovascular Study (Cook et al. 2007) try to investigate the combined effect of antioxidants on the outcome of myocardial infarction, stroke, coronary revascularization, or CVD death among 8171 female with history of any CVD history or above normal risk factor, but, the study with 9 year follow up shows no overall effects of antioxidants on cardiovascular events among women at high risk for CVD. The Physicians' Health Study II Randomized Controlled Trial, with 8 year follow up, with only male subjects also shows no supplementation reduce the risk of major cardiovascular events (Sesso et al. 2008).

To prevent and treat bone-related disorders, also for immunity, supplemental vitamin D and omega 3 fatty acids have been prescribed for a long time for nearly everyone and viewed in recent years as a potential strategy for preventing cancer and cardiovascular disease; while a very recent studies called VITAL (Manson et al. 2019a; Manson et al. 2019b), try to figure out whether supplementation with n−3 fatty acids and vit D has such effects in general populations as they are associated with reduced risks of cardiovascular disease and cancer in several observational studies. These US National Institutes of Health funded VITAL studies with over 25000 participants with a median follow up 5 years, shows that supplementation with n−3 fatty acids or vit D did not result in a lower incidence of major cardiovascular events or cancer than placebo too. No excess risks of bleeding, hypercalcemia or other serious adverse events were identified. Monthly high-dose vitamin D supplementation study VIDA (Scragg et al. 2017), in which vit D supplementation was given to participants with vitamin D deficiency (baseline 25-hydroxyvitamin D levels <20 ng/ml), study has also similar results, with no prevention for CVD.

PUBLIC OPINION?

Except benefits that are expected, as a one-way belief to use them; it is important if consumers will cease this products if no benefit has seen, which may be called more reasonable consumer activity. A 2001 article researched the views of consumers both users and nonusers of supplements based on results of multiple national opinion surveys. Report shows that dietary supplements taken regularly as a part of their routine health regimen, while many consumers have a strong connection to and belief about the potential health benefits of supplements that it is report as "they would continue to take them even if they were shown to be ineffective in scientifically conducted clinical studies", while 25% of consumers said that they would cease only (Blendon et al. 2001).

A more recent study with similar aspect, investigating the awareness of the number of people using dietary supplements (n=1579 respondent), shows that more than 40% of consumers use supplements "to feel better" (Blendon et al. 2013). Only 25.4% of supplement consumers responded that they would cease their use of a supplement if public health authorities stated that it was ineffective, which is the same as that reported in a 2001 above, reflects that there is no change in public opinion on supplements.

Another US nutrition survey (Dickinson et al. 2015) also shows the consumer action and expectations as study publishes the percent of survey respondents who agreed or disagreed with each of statements about the role of multivitamins and other dietary supplements in helping to improve dietary intake and health. 87 % of the participants agree or strongly agree that, supplements can help people meet nutrient needs that cannot be met through food alone, while, 80 % of the participants of the same study agree or strongly agree that supplements should not be used to replace healthful dietary and lifestyle habits. The consumer attitude and will seems to be all about the consumers understand of the role of dietary supplements.

CONCLUSION

A global non-stopping non-declining vitamin and supplement trend is already on and large majority of consumers already uses multivitamins and other dietary supplements. Policy makers' and governmental bodies' observation on the subject should continue on, while more studies need how to approach to consumer about their understand of the subject, and how to make it more logical/rational.

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FULL PAPER

VACCINIUM VITIS-IDAEA L., PICKED IN BULGARIA INDICATE *IN VITRO* ANTITUMOR ACTIVITY ON HUMAN CERVICAL AND BREAST CANCER CELLS

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Objective: Cancer is a socially significant disease. Along with efforts to understand the complex genetic/epigenetic factors that trigger a carcinogenesis, it is also necessary to analyze the potential natural active substances that may delay or even stop neoplastic transformation. Promising candidates are Bulgarian cranberries from high mountain plant populations, which are rich in phenolics and anthocyanins and have proven beneficial effects on human body. The present study aims to evaluate *in vitro*, antitumor activities of total extracts and purified fractions of *Vaccinium vitis-idaea L.*, picked in Bulgaria on human cervical (HeLa) and breast (MCF7) cancer cell lines, as well as to examine some of the mechanisms underlying them.

Materials and methods: Four methanol extracts and respective number purified fractions (B- nonanthocyanin / C- anthocyanins) of Bulgarian lingonberry were used. Antitumor effect was established by Trypan Blue method and MTT cell viability assay. Assessment of apoptotic activity was performed using DNA fragmentation method.

Results: The results from MTT analyses showed that B- nonanthocyanin fractions of Bulgarian lingonberry have well expressed inhibitory effect on survival of tested tumor cells. The observed effect dependent of the dose administered and were stronger in relation with the high-mountain populations and HeLa cell line. The integrity of the extracted DNA from treated survival cells indicates possible apoptosis mechanisms under the action of biologically active ingredients from lingonberries.

Conclusion: Evaluation of antitumor activities of Bulgarian lingonberries using modern molecular methods, could contribute to establish the natural substances useful for human health in general and modern oncology.

Key Words: *Vaccinium vitis-idaea L*, antitumor activity, HeLa, MCF7

INTRODUCTION

Cancer is a serious problem for modern medicine in relation with increasing incidence rate and less effective prevention and therapy. One alternative approach in cancer treatment is to analyze the antitumor activity of natural products known and used for therapy in traditional medicine. At present, several natural chemotherapeutic agents have been effectively used in oncology practice, such as paclitaxel (*Taxus brevifolia*), vinblastine and vincristine (*Catharanthus roseus*), artemisinin (*Artemisia annua*) (Shoeb M., 2006). For some of the active substances in plants, there is evidence that they induce apoptosis in different types of cancer cells. An example in this regard is Thaliblastine (an original Bulgarian medical product *Th. Aquilegifolium*) and Hyperatomarin (*H. annulatum*) with proven inhibitory effect on chronic myeloid leukemia cell lines (Nikolov S et al., 2007).

Since ages, wild berries have been widely used based on its proven antiseptic properties, antioxidant and antimicrobial activities (Singh I et al., 2016). *Vaccinium vitis-idaea L.* (family *Ericaceae*, genus *Vaccinium*), also known as lingonberries refer to a group of functional foods. Nowadays a lot of drugs for the treatment of urogenital infections (Jepson RG et al, 2012), cardiovascular and eyes disorders include berries as an active substants (Puupponen-Pimia R., at al., 2005). Lingonberries are rich in bioactive compounds such as anthocyanins, polyphenols, tannins, vitamins (A, C, E, B, PP etc.) and minerals (Battino M et al., 2009).

A review of literature data shows that there is a strong interest and actively studies the antitumor potential of various active fractions of berries (Hossain MM et al., 2012, Etienne-Selloum et al., 2013). To our knowledge, at present, no data exist concerning the antitumor activity of the Bulgarian lingonberries, except a single publication of Nikolaeva-Glomb and a team (Nikolaeva - Glomb et al., 2013) evaluated antiviral potential of various wild berries, including lingonberries.

In this regard the present study aims to investigate the *in vitro* antitumor potential of total extracts and purified fractions of Bulgarian lingonberries, through analysis of viability of human cervical (HeLa) and breast (MCF7) cancer cell lines after extract exposure, as well as to examine morphological changes of treated cancer cells underlying apoptosis progress.

MATERIALS AND METHODS

Plant Material

For the purposes of the analyses four plant populations of wild lingonberries growing at different altitudes in the regions of mountains - Balkan (Stara Planina) and Rhodope were selected. The ripe berries were collected at the time period when they are typically harvested for commercial purposes. Bushes were randomly selected within the populations, on the precondition that the minimum distance between the studied plants was 10 m. The previous data show variation in the chemical composition and content of biologically active substances in the *Vaccinium vitis-idaea L.* inhabiting various geographic regions and growing under specific soil conditions (Dincheva I., et al., 2016). In connection with this approach fruit from wild populations *Vaccinium vitis-idaea L.* with habitat in the Balkan (Vasiliov - GPS: 42°52.753'N 24°28.968'E; altitude: 1360 m and Beclemeto - GPS: 42 46.460'N 24 37.000'E altitude: 1470 m) and the Rhodope Mountains (Perelik - GPS: 41°36.400'N 24°35.805'E; altitude: 1970 m and Gela - GPS: 41°37.967'N 24°33.183'E altitude: 1780 m) in Bulgaria were collected in 2018. All samples were freeze-dried, ground and stored at – 80◦C prior to extraction.

Sample descriptions

Tv, Tb, Tp and Tg – total extracts from *Vaccinium vitis-idaea L*, picked in the regions of mountains: Balkan - locations Vasiliov (Tv) and Beclemeto (Tb) and Rhodope - locations Perelik (Tp) and Gela (Tg) respectively. Cv, Cb, Cp and Cg - the -anthocyanin and Bv, Bb, Bp and Bg the – non – anthocyanin (polyphenol) fruits fractions from lingonberries corresponding to the total extracts from the above described habitats.

Plant Extraction

Briefly, freeze-dried berries were grounded into a powder. Then extraction solution consisted of 80% solvent A [AcN-MeOH (80:20 v/v)] and 20% solvent B [0.1 % aqueous HCOOH] were added. The samples were sonicated (10 min), and centrifuged (12000 rpm, 5 min, 4°C). The filter cake was reextracted two times with extraction solution. The supernatants were combined and dried in vacuum-concentrator. The extracts were fractionated by Solid Phase Extraction (SPE) using a Giga tubes 2 g/12 ml, C18-E units (Strata, Phenomenex®). The columns were activated with 0.1% (v/v) formic acid in AcN then followed by EtOAc and 0.1% (v/v) HCOOH in water. Water-soluble compounds (fraction 'A') - sugars, organic and amino acids were removed with 2 volumes (2 x 12 ml) 0.1% (v/v) formic acid in water. The non-anthocyanin components (phenolic acids, flavonols, and condensed tannins - fractions 'B') were eluted from the columns with 2 volumes (2 x 12 ml) EtOAc. Finally the anthocyanins (fractions 'C') were be subsequently eluted with 2 volumes (2 x 12 ml) 0.1% (v/v) HCOOH in AcN. Fractions 'A' were not retained. Fractions 'B' and 'C' were dried using a vacuum-concentrator.

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Cell lines and cultivation
Human Breast (MCF7) and cervical (HeLa) cancer cell lines were included in the study. HeLa cell line is obtained from a patient with cervical cancer. The line has been widely used for *in vitro* analyzes for over fifty years. MCF7 is isolated from pleural effusion of a 69-year old Caucasian woman with metastatic breast cancer. The cell lines ware supplied by the American Type Culture Collection (ATCC).

Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco), 1% sodium pyruvate (Eurobio) and 1% MEM Non-Essential Amino Acids (Gibco) and were incubated at 37ºC in a humidified atmosphere with 5% CO_2. The cell lines were kept free from fungal or bacterial contamination.

Trypan blue exclusion assay
Trypan blue exclusion assay was carried out to calculate percentage of cell lines MCF7/HeLa viability. An aliquot of 50µl cell suspension was mixed with an equal volume of 0.4% trypan blue solution. Viable (unstained) and nonviable (dark blue stained) cells were counted under inverted light microscope. Percent viability was calculated by the formula:

Viability (%) = (Live cell count/Total cell count) x 100

MTT assay
In vitro cell viability was evaluated through MTT (3-(4,5-dimethylthiazol-2-yl)-2-5 diphenyl tetrazolium bromide) assay. Indicative for antitumor activity is the IC50 value (half maximal inhibitory concentration) – the concentration of active compound needed to reduce the cell viability to 50%. Cells were seeded into 96-well tissue culture plates ($1x10^5$ per well) in a final volume of 100 µl. After incubation for 24 h in complete cell culture medium, for the next 24 h cells were starved in serum-free medium, supplemented with 0.1% BSA. Subsequently cells were treated with different concentrations of tested extract (from 0.5 to 1000 µg berry/ml medium) for 48h/72h using cultivating medium as a solvent. Wells with serum-free medium were used as negative controls. During the last 3 h of the incubation an aliquot of 10 µl MTT per well was added (stock solution of 5 mg/ml MTT was used). After incubation, the medium was removed and the formazan complex was dissolved with 100 µl/well 10% SDS in 0.01M HCI. The absorbance was subsequently measured at 570 nm using ELISA microplate reader. The MTT test was repeated at least twice and each concentration had three repeats.

The percentage of cell viability after extract exposure of the above-mentioned concentrations was determined using the following formula:

Viability (%) = (Experiment value/Control value) × 100q, and the 50% inhibitory concentration (IC50) was determined.

MTT analysis was also applied to establishment of cell viability alteration with time after treatment - for 48 and 72 h.

Morphological observation of tumor cells
MCF7 and HeLa cells were plated into 12-well plates, cultivated and treated for 48 and 72 h with the same concentrations, as described above. Morphological changes were observed using inverted light microscope. The morphological observation analysis was synchronized with MTT cell viability assay.

DNA fragmentation analysis
Cells were plated into 6-well culture plates ($1x10^5$ per well). After treatment with total extract, fractions 'C' and 'B' from lingonberries for 24h, 48 h and 72 h the cells were detached with 0.05% trypsin-0.53 mM EDTA, incubated for 15 min at 37ºC and collected. After centrifugation for 5 min at 125G the supernatant was discarded and 200µl PBS pH 7.2 was added to the cell pellet. DNA was isolated using DNeasy Blood & Tissue Kit (Qiagen). An aliquot of 2.5 µg genomic DNA was analysed on 1.5% agarose gel in 1xTBE, at 80 V and 50 mA for 1 h. The samples were visualized under UV after gel staining with ethidium bromide.

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RESULTS

Cell Viability

Using Trypan blue exclusion test percentage cell lines viability was assessed. The results showed that the percentage viability of MCF7 cell line was 95, 02% ± 2, 31(SD) and HeLa was 96, 04% ± 2, 51(SD), which is suitable to perform the following tests.

The antitumor effect of lingonberries total extracts (Tv, Tb, Tp and Tg), fractions ‘C’ (Cv, Cb, Cp and Cg) and ‘B’ (Bv, Bb, Bp and Bg) were examined through MTT cell viability assay. Untreated tumor cells were used as vehicle controls and their viability was accepted as 100%. The screening was performed at multiple concentrations ranging from 5 to 1000 µg/ml. The range of concentrations (5, 10, 20, 32, 100, 320, 1000 µg B/ml) for MTT was selected according to previous data for nontoxic properties and an effect (antiviral) of total extract and/or fractions from wild berries on viability of human cell lines (Nikolaeva-Glomb et al., 2013; Boivin et al., 2007).

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- MTT assay on total methanol extracts and C – anthocyanin fruits fractions of *Vaccinium vitis-idaea L.* picked in Bulgaria on human breast and cervical cancer cell lines.

The results showed lack of effect on treated tumor cells and a high survival rate (100% and over) (increased compared to untreated controls) in treatment for both 48 and 72 hours at all concentrations tested (5, 10, 20, 32, 100, 320, 1000 µg Berry/ml media) and for all four total extracts ((Tv, Tb, Tp and Tg) and also for C – anthocyanin fruits fractions of lingonberry picked in Bulgaria on the cells from MCF7 and HeLa lines.

-
- MTT assay on B non – anthocyanin fruits fractions of lingonberry on MCF7 and HeLa cell lines.

The here obtained data indicated that B – non anthocyanins fractions from lingonberries picked in Bulgaria had a marked dose and time dependent effect on viability of breast and cervical cell lines. In the range of lower concentrations (up to 5 µg B/ml medium) the viability of tumor cells is comparable to this in the control and with the increase of concentration a steady decrease in cancer cells viability was observed (Figure 1). Fifty percent (IC50) reduction of tumor cell viability was observed at a concentrations rate 100 - 320µg B /ml medium for HeLa cells and 320- 1000µg B /ml medium for MCF7 cells treated for 48 and 72 hours with B - phenolic fractions from the representatives of different populations raised in Bulgaria.

The most significant anti-tumor effect was determined in B fractions from lingonberries from higher mountains populations.

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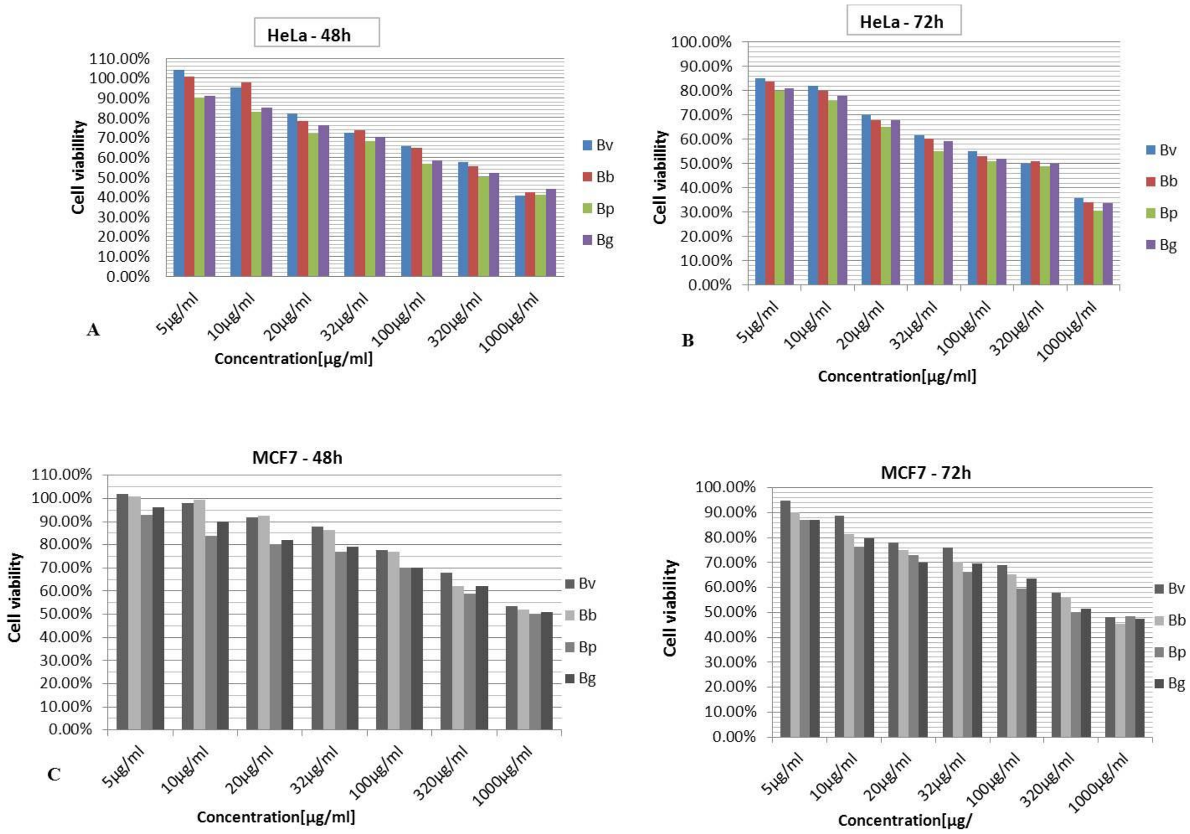

Figure 1. MTT assay of HeLa (A/B) and MCF7 (C/D) cell lines treated for 48 h (A/C) and 72 h (B/D) with increasing range of concentrations of B – non anthocyanins fractions from *Vaccinium Vitis-idaea L* picked in the regions of mountains: Balkan - locations Vasiliov (Bv) and Beclemeto (Bb) and Rhodope - locations Perelik (Bp) and Gela (Bg).

The statistical analysis according to GraphPad Prism indicated considerable significant differences between control and treated groups with p-values of less than 0.05.

DNA fragmentation analysis

Cells from HeLa and MCF7 lines were treated with 100 µg/ml of B non – anthocyanin fruits fractions of *Vaccinium Vitis-idaea L.* (Vasiliov – Bv, Beclemeto – Bb, Perelik – Bp and Gela – Bg) for 24 h, 48 h and 72 h (Figure 2). The results showed presence of DNA fragmentation both in breast and cervical cancer cells after 24 h treatment with B non – anthocyanin fruits fractions. Slight increase in the level of DNA fragmentation in both cancer cell lines with extension of duration treatment (48 h and 72 h) was indicated.

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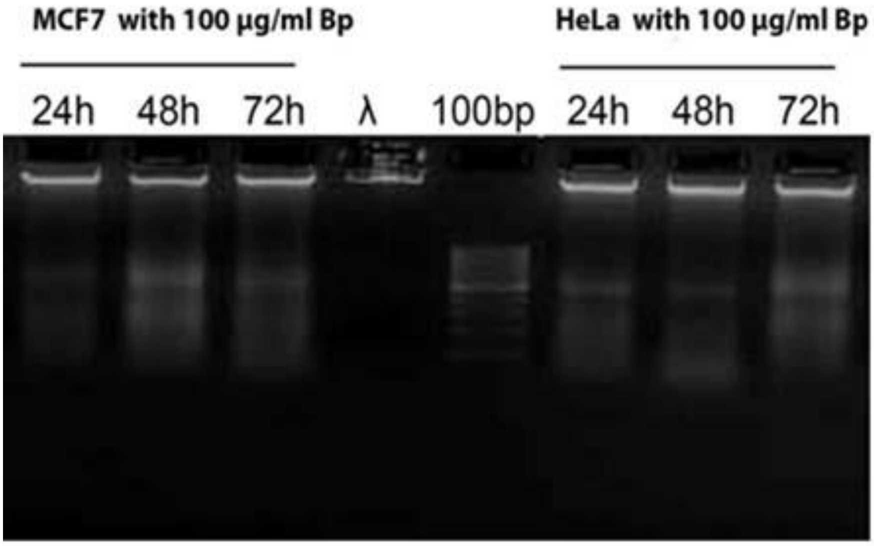

Figure 2. DNA fragmentation analysis in 1.5% agarose gel electrophoresis: DNA from MCF7 and HeLa cells treated with and Bp fraction (100µg/ml) for 24h, 48h, 72h.

Morphological Alterations

Morphological changes of treated MCF7 and HeLa cancer cells were observed under inverted light microscope. Extract treated cells became round and shrank in comparison with untreated control cells with normal shape (Figure 3). Evident reduction of viable cells which were monolayer adherent and increased number of floating dead cells after treatment with concentrations above 320 µg/ml ware observed. The number of tumor cells detached from the monolayer increase in extract concentration.

Control 320 µg/ml 1000 µg/ml

Figure 3. Morphological changes of HeLa cells after treatment for 72 hours with 1000 µg/ml, 320 µg/ml of *Vaccinium vitis-idaea L.* - Bp fraction compared to untreated control.

DISCUSSION

At present plant products are intensively studied as a potential source of active components for cancer therapy. From 1940 to 2010, 48.6% of the 175 small molecules approved in oncology treatment were natural- or natural derived products (Newman DJ and Cragg GM, 2012). The biological activity of the medical plants depends of the chemical composition. Phytochemicals, especially phenolic in berries, are suggested to be the major bioactive compounds responsible for their health benefits. Phenolic extracts of plant materials are always a mixture of different classes such compounds that are soluble in the solvent system used (Es-Safi N., 2012; Quideau S et al., 2011) *Vaccinium* berries have been shown to contain high levels of the flavanoid compounds that contains a common molecular structure - tricyclic C6-C3-C6 "flavanoid skeleton" (Prior et al., 1998; Dincheva I and Badjakov I., 2016). The high phenolic rate in *Vaccinium vitis-idaea L.* and the data on their antimutagenic, anti-inflammatory and antimicrobial activities presumes a high antitumor potential of this medicinal plant (Heinonen, 2007; Battino et al., 2009).

To our knowledge, the present study reported the first data on the antitumor effect of Bulgarian *Vaccinium vitis-idaea L.* The investigations were carried out on breast adenocarcinoma MCF7 and HeLa cervical cancer cell lines through MTT assay for cell viability in a wide range of extract concentrations. A profound reduction in tumor cell viability was found after treatment with B non – anthocyanin fractions. The effect was dose and time dependent resulting in decrease in cell viability of treated cancer cells. The non – anthocyanin fraction had stronger inhibitory effect on viability of cervical compared to the breast cancer cells. Here we found that IC50 of B non – anthocyanin fruits fractions from different plant populations varies in the range of concentrations for HeLa and MCF7 cells treated for 48 and 72 hours and were lowest for lingonberries growing at high altitudes.

By the moment data from a scientific reports represent that berry fruits such as blueberries, strawberries, raspberries and lingonberries, inhibits the growth of human oral (KB, CAL-27), breast (MCF-7), colon (HT-29, HCT116), prostate (LNCaP) tumor cell lines and may influence on multiple stages of carcinogenesis (Masoudi M., and Saiedi, M., 2017; Seeram, N.P., et al., 2004, Kizhakkayil J et al., 2010). In accordance with our results other authors also detected increasing inhibition of cell proliferation rate after treatment with increasing concentration of berry extract in a wide spectrum of cell lines tested (Jurikova T et al., Dhandapani KM et al., 2007, Hossain MM et al. 2012, He X et al., 2006; Kondo M et al., 2006; Neto CC, 2007, Etienne-Selloum, N et al, 2013).

The variations in IC50 values between publications are probably due both to the different drug sensitivity of studied tumor cell lines, diversity in chemical composition and environmental conditions of plants from different geographical areas and populations. Higher amounts of total phenolics were detected in samples harvested from localities exposed to the sun in comparison with berries grown in shadow. It has been noticed that at altitude higher than 1500 m, higher amounts of total phenolics in the lingonberry was observed (Dincheva I et al. 2016).

Some phytochemicals, contained in fruits of the Vaccinium genus, are expected to affect cancer-related processes. Phenolics, proanthocyanidins and flavonoids, presented in lingonberries and other Vaccinium berries, show some promising effects toward limiting processes involved in tumor invasion, apoptosis and metastasis (Etienne-Selloum, N et al, 2013, Lee HP et al., 2012, Walter A et al., 2010, Singh N et al., 2012, Kizhakkayil J et al., 2010). Apoptosis (programmed cell death) is initiated in response to damages in hereditary material and represents a series of genetically controlled events, resulting in elimination of damaged cells. The process is associated with activation of cellular endonucleases, which digest cellular DNA to well-differentiated fragments that can be visualized through gel electrophoresis (Arden N and Betenbaugh MJ, 2004). Our results showed induction of apoptosis after treatment with B non – anthocyanin fruits fractions of *Vaccinium vitis-idaea L.* both in breast and cervical cancer cells. This observation is in accordance with the data from the MTT assay and by increasing the duration of treatment with polyphenol fractions, DNA fragmentation level in tumor cells slightly increased. This is consistent with the observations that DNA fragmentation occurs in the later stages of the apoptosis (Johnson et al., 2000). Similar data for induction of apoptosis are also obtained in the analysis of the effect of V. oxycoccos fruits on breast cancer cells. Masoudi M and Saiedi M (Masoudi M and Saiedi M, 2017) found that V. oxycoccos fruits were able to suppress the proliferation of human MCF7 cells and attributed to the initiation of apoptosis and the G1 phase arrest.

In conclusion, B non – anthocyanin fruits fractions of Bulgarian *Vaccinium vitis-idaea L.* has a marked dose-dependent inhibitory effect on viability of breast and cervical cancer cells. In the mechanisms underlying the antitumor effect of *Vaccinium vitis-idaea L.*, apoptotic processes are involved. Morphological changes and DNA fragmentation were observed as markers for apoptosis process in tumor cells after treatment. The obtained results are the first showing an antitumor activity of the Bulgarian *Vaccinium vitis-idaea L* in human cancer cells and are indicative for further more detailed investigations concerning detailed screening for the active compounds determining the antitumor activity of the plant.

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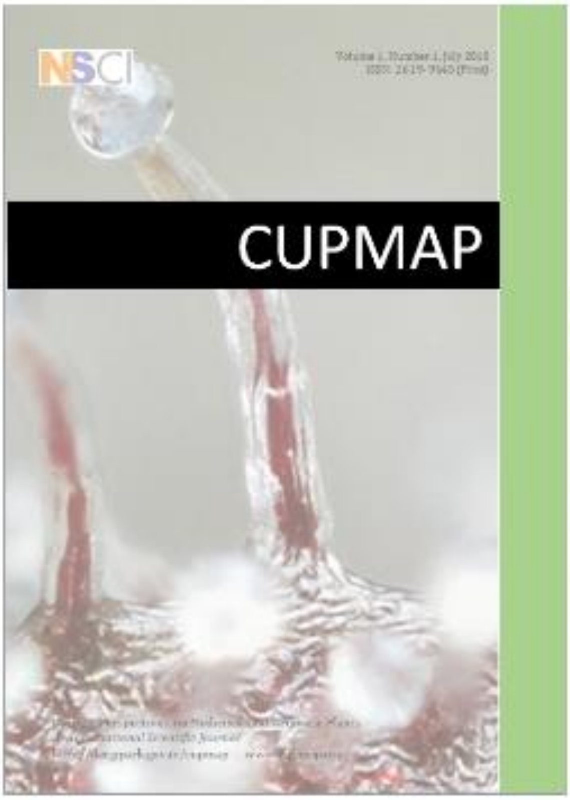

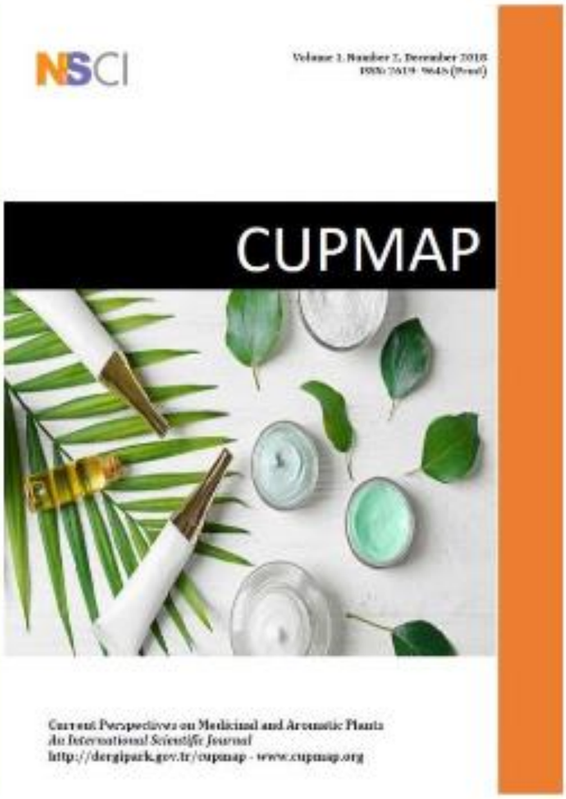

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