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Supplement Part - A

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ABSTRACT 1

Antidiabetic and radioprotective effects of green tea extract in gamma-irradiated diabetic rats

Mai Raslan, Mohamed Nasr, Saleh shour, Sohair Mahmoud and Khalid El-Nesr

ABSTRACT

Background: Diabetic individuals exposed to gamma irradiation during their work or pollution, are considered complicated cases to be saved due to their high exposure to free radicals. The promising anti-diabetic and radio-protective activities of green tea leaves alcoholic extract (GTE) were investigated in our study.

Materials and Methods: This work aimed to evaluate the role of green tea extract (GTE) at a dose of 200 mg/kg B.W in improving the toxic activity of Alloxan (ALX) and ALX accompanied with gamma radiation in albino rats after GTE administration for 21 days. The biochemical and histopathological changes in liver, kidney and pancreas were investigated.

Results: Results revealed the ability of GTE to reduce significantly the increment in blood glucose level in diabetic group and diabetic irradiated groups with 3 and 5 Gy by 53.3%, 42.3%, and 46.4%, respectively. Treatment with GTE showed improvement in urea level by 60.6%, 39.2%, and 45.3%, and in creatinine level by 54.8%, 38.5%, and 43.6% in case of the diabetic group and diabetic irradiated group with 3 and 5 Gy, respectively.

Discussion and Conclusions: The histopathological findings were confirmed with those obtained by biochemical results for all experimental groups as compared to diabetic and diabetic irradiated groups. GTE was identified using LC/DAD/ESI-MS showing thirty-one compounds including caffeine, phenolic acid derivatives, catechins, procyanidins and flavonoids. The study concluded that GTE can be used as a promising hypoglycemic and radio-protective agent after being administrated for 3 weeks in diabetic and diabetic irradiated rats.

Keywords: Diabetes, Camellia sinensis, LC/MS, Gamma radiation, Histopathological observations.

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ABSTRACT 2

Hypoglycemic activity and phyto-constituents from *Ficus platypoda* (Miq.) A. Cunn.

Naglaa I. Afifi, Sally A. El-Awdan, Nemat Z. Yassin, Mona H. Hetta, El-Sayeda A. El-Kashoury

ABSTRACT

Background: *Ficus platypoda* (Miq.) A. Cunn is known as desert fig or rock fig related to family Moraceae, there are only few reports showing its phyto-constituents and biological activities. The purpose of this research was isolation of phytochemicals and testing the hypoglycemic activity of its ethanolic extract as well as other biological studies as antimicrobial and antioxidants.

Materials and Methods: Chemical structures of isolated compounds were established using different spectral data as NMR, IR and MS. *In vivo* hypoglycemic effect was tested by measuring the blood glucose level. The antibacterial and antifungal activities were determined by disc agar diffusion method using Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis*), Gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Neisseria gonorrhoeae*) as well as fungal species (*Aspergillus flavus* and *Candida albicans*). The antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and using ascorbic acid as a standard.

Results: This study led to isolation and characterization of ten compounds of which one phenolic compound was described for the first time; 4(3'-Acetoxy-2'-hydroxypropoxy)-3-hydroxybenzoic acid (**1**), α amyrin (**2**), β -amyrin (**3**), β -sitosterol (**4**), α -spinasterol (**5**), quercetin (**6**), β -sitosterol-O- β -D-glucopyranoside (**7**), kaempferol 3-O- β -D-glucopyranoside (**8**), quercitrin (**9**), and rutin (**10**). The acute toxicity study revealed that the extract was safe up to 2 g/kg. The oral hypoglycemic activity in diabetic rats using alloxan revealed a significant decrease in blood glucose level at 200, 400 mg/kg/day (107.9 ± 5.817 , 64.11 ± 4.358 mg/dl), when compared with the hypoglycemic drug; gliclazide (110.8 ± 7.240 mg/dl). The ethanolic extract showed a moderate antibacterial activity with no anti-fungal activity against all tested species. It also showed antioxidant activity ($EC_{50} = 232.6$ μ g/ml) compared to ascorbic acid as a positive control ($EC_{50} = 21.12$ μ g/ml).

Discussion and Conclusions: Results suggest that *Ficus platypoda* may possess further potential for chemical and biological studies leading to possible discovery of potent anti-hyperglycemic natural source.

Keywords: *Ficus platypoda*, Moraceae, 4(3'- Acetoxy-2'-hydroxypropoxy)-3-hydroxybenzoic acid, Hypoglycemic, Antimicrobial, Antioxidant.

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ABSTRACT 3

Synthesis and characterization of silk protein and sericin nanoflowers

Gökşen Dilşat Durbilmez and Ufuk Koca Çalışkan

ABSTRACT

Background: The silk protein obtained from *Bombyx mori* cocoons consists of two proteins called fibroin and sericin. Fibroin is used in biomedical applications because of its mechanical properties and controllable biodegradability, while sericin is used especially in skin, hair and nail cosmetics. Sericin improves skin elasticity as well as anti-wrinkle and anti-aging effects. In addition to these, organic-inorganic hybrid nanoflowers from the flower-like hybrid nanoparticle class, which is a synthesis product, have gained importance in the biotechnology sector in recent years due to their simple synthesis, high efficiency and ability to stabilize enzyme and increase enzyme activity.

Materials and Methods: In this study, we synthesized copper (Cu) and Zinc (Zn) nanoflowers at various pH and concentration using silk total protein and sericins purchased commercially. PBS, CuSO₄ and Zn (CH₃CO₂)₂ metal ions were also employed as inorganic components. For the Cu nanoflowers synthesis of the protein-inorganic hybrid nanoflowers, 333 µl of aqueous CuSO₄ solution (120 mM) added to 10 mM of PBS, (pH 6-7,4-8) containing protein with varied concentrations, followed by incubation at 30°C for 3 days. The final products, which are called hybrid nanoflowers were dried and they were characterized using SEM. For the Zn nanoflowers synthesis of the protein-inorganic hybrid nanoflowers, 0.016 g of protein was dissolved in 20 mL of PBS buffer. Moreover, 1.6 mL of zinc acetate solution with the concentration of 0.05 g/mL was added into the flask. After being stirred for 3h—12h-1d protein /Zn (CH₃CO₂)₂ hybrid particles were separated by centrifugation. The final products were dried at 30°C and the hybrids were characterized using SEM.

Results: As a result of preliminary studies, Cu and Zn hybrid nanoflowers synthesized at pH between 7 and 8 and their optimal SEM images were obtained in different concentrations. The sphere shaped snow ball or flower pollen like images were characterized.

Discussion and Conclusions: The preliminary results of Cu and ZN hybrid nanoflowers will be presented at the congress. The characterziation of hybrid nanoflowers will be continued with different chromatographic methods and by changing parameters such as duration and concentration to obtain optimized products in order to use in cosmeceutical preparations.

Keywords: Silk Protein, Sericin, Nanoflowers, Biotechnology

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ABSTRACT 4

Evaluation of some plant derived natural ingredients against Sars-Cov-2: an *in silico* approach

Athira M. Menon, Bhanupriya Dhabhai and Tikam Chand Dakal

ABSTRACT

Background: The novel coronavirus, SARS-CoV-2, has emerged as a highly infectious virus from the Wuhan city of China and has caused serious health crisis across the globe. The infectivity of the SARS-CoV-2 is unimaginable higher as compared to all previously known corona viruses and other pathogenic microorganisms and the reasons are largely unknown as yet. The disease has complex etiology and infected patients display complex patho-physiological complications such as unleashed cytokine production which is the main cause of multi-organ failure in aged and immuno-compromised patients. Researchers are rushing to discover vaccines and drugs for the treatment of COVID-19; however, all such vaccines and drugs are either under development stage or under clinical trials. In this study, we have assessed the pharmacological potential of 100 antiviral compounds derived from plants. Our focus is to screen compounds that can potentially inhibit attachment of the spike protein of SARS-CoV-2 with the receptors present on human cells. Besides this, we also screened compounds that can significantly modulate immune system towards successful management of the diseased patients.

Materials and Methods: We retrieved names of 100 potential plant-derived antiviral compounds from our literature survey. The essential formats such as SMILES format, sdf, mol2, pdb files were retrieved/ obtained from different online databases. We employed some bioinformatics tools and *in silico* approaches such as SWISS ADME (www.swissadme.ch), PASS (<http://www.pharmaexpert.ru/passonline/>), SwissTarget Prediction (<http://www.swisstargetprediction.ch/>), and SEA search server (<http://sea.bkslab.org/>) to ascertain the physico-chemical, pharmacological, biological activity of all compounds. The gene expression induced by the compounds was analyzed by DIEGP-pred (<http://www.way2drug.com/GE/>). In order to investigate whether the screened compounds could potential block the receptor binding sites of the SARS-CoV-2 spike protein or could modulate host-immune system, we used bioinformatics based molecular docking employing SwissDock (www.swissdock.ch) and other tools. All structural analyses were done using UCSF Chimera.

Results: We used different bioinformatics tools and *in silico* approaches to screen compounds with potential antioxidant, anticoagulant, anti-inflammatory and immuno-suppressive properties that could be specifically beneficial in inhibiting SARS-CoV-2 infection & modulating immune response in COVID-19 patients. Out of 100 phytochemical compounds analyzed, 20 compounds were found to be fit in all the criteria and were further used for docking with Receptor Binding Domain of spike protein of SARS-CoV-2. Although almost every one of them showed binding with RBD of the spike protein, two phytochemicals namely, orientin and apigenin were found to interact precisely with RBM (Receptor Binding Motif) via amino acid sequences that are mainly involved in RBM-ACE2 interaction. Besides this, we found 35 compounds that upregulate 5 genes (CD14, CCL2, CD83, CD86 and PRDX2) implicated in boosting immune response.

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These compounds can be mixed in different combinations to prepare different immunity booster formulation for diseased COVID-19 patients.

Discussion and Conclusions: Our study demonstrated that the antiviral compounds screened in the study can be grouped in two categories: 1) compounds that block SARS-CoV-2 interaction with host cell ACE2 receptors and prevent infection, multiplication and pathogenesis, and 2) compounds that could modulate and boost the immune system of the diseased patients to avoid patho-physiological outcomes, such as cytokine storm. Compounds of former category hold immense potential in the development of a herbal medicine against SARS-CoV-2; while compounds of latter category can be used for preparation of powered medicinal formulation, such as “Kadha”- an Indian Ayurvedic Natural Immunity Booster. We believe that our study could prove be a robust therapeutic solution for treatment of COVID-19 patients using plant derived antiviral phytochemicals compounds and the medicinal formulations made from them.

Keywords: SARS-CoV-2, COVID-19, Phytochemicals, Phytochemistry, *In silico* Analyses, Bioinformatics, Kadha, Ayurvedic Herbal Formulations

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ABSTRACT 5

Development of naturaceutics topical formulations with extracted microemulsioned active ingredients from flora of Costa Rica

Valeria Jiménez Gutiérrez, German Madrigal Redondo and María Fernanda Rojas Salas

ABSTRACT

Background: Colloidal systems are one of the most important issues for the development of new pharmaceutical forms, as well as for achieving the bioavailability and stability of pharmaceutical products, cosmetics and nutritional supplements. One of the main fields of study in this type of systems is microemulsions, due to the increase in the demand for cosmetic formulations with greater stability, in addition to the need for the integration or combination of several active ingredients. The species *Bactris gasipaes* whose fruit is known as pejibaye and *Rubus adenotrichusen* which is a berry known as blackberry, are two species normally used in Costa Rica for human diet. In previous studies, both the seed and the mesocarp of pejibaye and blackberry were found to contain components with antioxidant, anti-aging, anti-wrinkle, moisturizing and emollient properties, thus being very useful as feedstock for the preparation of cosmetic products and medicines. The objective of the present study was the development of a topical formulation with microemulsified active ingredients extracted from the flora found in Costa Rica.

Materials and Methods: The concentration range of the microemulsion components is obtained and a mixture of isopropyl myristate, cocamide and/or cocamidopropyl betaine, ethanol (95%) and USP purified water is used for the base system. The microemulsions were prepared and, once formed, were characterized and then selected those that maintained their monophasic macroscopic characteristics. Next, the vehicles were developed to incorporate the microemulsions. Finally, each prepared formulation was subjected to physicochemical tests in addition to the determination of sun protection factor (SPF) applying Colipa and Manzur methods, critical wavelength and UVA/UVB ratio.

Results and Conclusions: A pharmaceutical natural topical formulation of gel microemulsion was prepared with the following characteristics: average viscosity of 955557 cp, pH 4.6, specific gravity 0.98883 and extensibility of 19.10 cm². In addition to this, it was the formulation with the best sun protection factor having a value of 6.1991 using the Manzur method and 6.9309 using the *in vitro* Colipa method, which greatly contributes to the prevention of skin diseases such as cancer. A formulation of pleasant appearance, easy preparation, thermodynamic stability, high solubilization power and good skin permeability was achieved, in addition to aiming to be an environmentally friendly product.

Keywords: Microemulsions, pharmaceutical formulation, gel, antioxidant

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ABSTRACT 6

Domestication and breeding of endemic medicinal and aromatic plants in Israel

Nativ Dudai

ABSTRACT

There are many traditional medicinal and aromatic plants (MAP) endemic to the Middle East. Today, the fact is that the modern industry demands uniform and high quality raw materials, along with the decline of people that still collect plants from the wild and that many of the wild species are protected, lead to domestication and cultivation of various species. On the other hand, there is a significant increase of the openness of the market to innovations, such as new crops and novel products. The domestication and development of new crops need a process of breeding. During the domestication process, we select from the wild germplasm genetic types that can stand and survive the intensive conditions of the commercial cultivation, but on the other hand have the quality and the uniformity that are required by the consumer. It is clear that for an effective selection process, high variation and biodiversity of the source germplasm are essential. In the case of MAP the secondary metabolites content and composition are also very important, so using an in house well-equipped chemical analytical laboratory is a big advantage. Ultimately, the aim is breeding elite varieties that have an optimal combination of traits that cater to the grower and the consumer as well. For this reason we have to collect a plethora of genetic material, representative of the biodiversity in the dispersed area, while also increase the variations using various breeding methods. A first step of an effective domestication and breeding process of wild plants requires a survey of their dispersion according to existing distribution databases in order to be able to represent the natural variation. Seed or vegetative propagation material of the target crops are systematically collected as representatives of their natural diversity and are grown in experimental fields in the NeveYa'ar Research Center under intensive conditions – irrigation, fertilization and several harvests per year, such practices as we wish to use in the future commercial cultivation. Selection of new cultivars is somewhat different if the plant is annual or perennial and depends on the propagation methods as well. With annual species, propagation is usually done by seeds, but with perennial species it could be done also by cuttings. In breeding of a new cultivar, single plants with high performances (yield and quality) are selected from as many sources as possible. These single plants are tested in the same soil and climate conditions that will later be the setting for commercial production. In perennial species, if there is a possibility of using vegetative propagation, the selection time could be shortened. The selected plants can be tested in plots after they are propagated by cuttings, as is necessary – before commercialization. This method has been used for Oregano, Thyme, Sage and others. Cross-breeding can be conducted through artificial crossing when the selection process alone does not produce a cultivar that has all the characteristics that are needed for commercial production. The aspects listed above are of a genetic basis and are important, but to that there is an addition of environmental factors, agro-technical methods and practices which can have a cardinal effect on the yield and quality,

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including the content and composition of bioactive compounds. Therefore, once a stable cultivar is achieved, a meticulous study is required on the plant's physiology, the effects of environmental factors on yield and secondary metabolites composition which are essential for the optimization of agro-technical methods and practices used in order to produce a sustainable, newly introduced novel crop.

Keywords: Domestication, Endemic Medicinal Plants

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ABSTRACT 7

Formulation of Greek flora derived innovative dietary supplements aiming to improve human health-span

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ABSTRACT

Background: The dramatic increase in average life expectancy is inevitably connected with the development of new strategies, aiming to promote and maintain well-being across the age spectrum, through the employment of innovative solutions and ideas for better quality of life. This work aims to the development of novel food supplements based on natural extracts/substances, specifically targeted to promote healthy aging.

Materials and Methods: For this purpose, a variety of extracts/substances derived from the Greek and Mediterranean Flora and their potential combinations, were tested in order to evaluate their ability to delay senescence in human cells and reduce the manifestation of aging hallmarks. In more detail, we assessed a powerful set of molecular biomarkers of aging, including the telomere length, proteasome content and activities, global DNA methylation and the levels of carbonylated proteins during senescence progression of treated and control cell cultures.

Results: The results of the *in vitro* evaluation defined the formulation of new dietary supplements, which are currently further tested *in vivo* in healthy volunteers.

Discussion and Conclusions: The clinical trial is expected to provide the new products with credible scientific evidence in order not only to validate existing health claims about the active components, but also to create new ones.

Keywords: aging, biomarkers, natural compound, Greek flora, health span, wellness

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ABSTRACT 8

Marine magnesium and minerals in burn and wound care: implications for skin health

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ABSTRACT

Background: Burn injuries are a major cause of morbidity and mortality worldwide. Despite advances in therapeutic strategies for the management of patients with severe burns, the sequelae are patho-physiologically profound, up to the systemic and metabolic levels. Management of patients with a severe burn injury is a long-term, complex process, with treatment dependent on the degree and location of the burn and total body surface area (TBSA) affected. In adverse conditions with limited resources, efficient triage, stabilisation, and rapid transfer to a specialised intensive care burn centre is necessary to provide optimal outcomes. This initial lag time and the form of primary treatment initiated, from injury to specialist care, is crucial for the burn patient. This study aims to investigate the efficacy of a novel visco-elastic burn dressing with a proprietary bio-stimulatory marine mineral complex (MXC) as a primary care treatment and to initiate a healthy healing process prior to specialist care.

Materials and Methods: A new versatile emergency burn dressing saturated in a >90% translucent water-based, sterile, oil-free gel and carrying a unique bio-stimulatory marine mineral complex (MXC) was developed. This dressing was tested using LabSkin as a burn model platform. LabSkin a novel cellular 3D-dermal organotypic full thickness human skin equivalent, incorporating fully-differentiated dermal and epidermal components that functionally models skin. Cell and molecular analysis was carried out by *in vitro* Real-Time Cellular Analysis (RTCA), thermal analysis, and focused transcriptomic array profiling for quantitative gene expression analysis, interrogating both wound healing and fibrosis/scarring molecular pathways. *In vivo* analysis was also performed to assess the bio-mechanical and physiological effects of this novel dressing on human skin.

Results: This hybrid emergency burn dressing (EBD) with MXC was hypoallergenic, and improved the barrier function of skin resulting in increased hydration up to 24 hours. It was demonstrated to effectively initiate cooling upon application, limiting the continuous burn effect and preventing local tissue from damage and necrosis. xCELLigence RTCA on primary human dermal cells (keratinocyte, fibroblast and micro-vascular endothelial) demonstrated improved cellular function with respect to tensegrity, migration, proliferation and cell-cell contact (barrier formation). Quantitative gene profiling supported the physiological and cellular function finding. A beneficial *quid pro quo* regulation of genes involved in wound healing and fibrosis formation was observed at 24 and 48 hour time points.

Discussion and Conclusion: Utilisation of this EBD+MXC as a primary treatment is an effective and easily applicable treatment in cases of burn injury, proving both a cooling and hydrating environment for the wound. The magnesium rich marine mineral complex regulates inflammation

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and promotes healing in preparation for specialised secondary burn wound management. Moreover, MXC promotes a healthy remodelling phenotype that may potentially mitigate scarring. Based on our findings, this EBD+MXC is ideal for use in all pre-hospital and resource limited environments.

Keywords: Burn injuries, patho-physiological , marine mineral complex , In vivo analysis , Skin wound treatment

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ABSTRACT 9

Nutraceuticals and healthspan

Janayra Maris Texiera

ABSTRACT

With the increase in life expectancy, there is also an increase in concerns about improving health and quality of life linked to a healthy lifestyle, which includes food. Nutraceuticals are gaining more and more prominence as they contain active ingredients from food phytochemicals, with numerous health benefits. They act directly on the key factors modulating gene expression, inflammation, and oxidative stress, and therefore they can even prevent or delay several diseases. Currently, the new generation of nutraceuticals focuses on disease prevention, especially neurodegenerative diseases, including depression, and promoting longevity, through bone and muscle improvement and prevention of chronic non-communicable diseases, for example. These non-essential compounds include curcumin, eugenol, morin, anethole, thymoquinone, carnosol, kaempferol, phytic acid, quercetin, and isoprenes. Vitamins are also gaining more and more space in this field, especially vitamin E, of which research indicates that its benefits go beyond those already known. These compounds act in the reduction of inflammatory cytokines and oxidative damage, in cell protection, including neurons, in the interruption of apoptotic DNA fragmentation, in the regulation of proteins, especially those involved in the transcription of genes that encode antioxidant enzymes, in the stimulation of mitochondrial biogenesis, in improving mitochondrial function, neuronal activities control and in the formation of synapses. Even obtaining equal results and in most cases, superior to synthetic drugs. At the bone level, they also act in the regulation of cellular homeostasis, in the proliferation and apoptosis, maintenance, and production of components of the cellular matrix and in the production of hormones. As for the muscles, they can also act in the regulation and proliferation of satellite cells, in cell differentiation and viability. In the metabolic control, they can act in the modulation of hormones such as insulin, leptin, and ghrelin, in the inflammation control and in the gene expression regulation. Therefore, the use of nutraceuticals can promote a better quality of life, especially if consumed before the installation of the pathology.

Keywords: Phytochemicals; modulation; longevity; neurodegenerative disease

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ABSTRACT 10

Effect of temperature and day length on morphology and volatile oil composition of *Origanum ramonense* *Danin*

Reena Sharma, Gilad Oren and NativDudai

ABSTRACT

Background: *Origanum* is a genus of herbaceous perennial plants mainly distributed in the Mediterranean, Euro-Siberian, and Iran-Siberian regions. *Origanum ramonense* Danin is a rare desert plant of *Lamiaceae* family. It reaches 30-80 cm in height, exhibits an oblique rhizome, erect flower-bearing stalks and is endemic to Israel, belonging to *Campanulaticalix* section. The major volatile organic compounds of the essential oil of *O. ramonense* oil were α -terpineol, 1,8-cineole, terpinen-4-ol, *cis*-sabinene hydrate, γ -terpinene and eugenol. α -terpineol was reported as the predominant component of the essential oil of *O. ramonense*. This compound has various biological applications as an antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive and antinociceptive agent. The essential oil of *O. ramonense* showed antibacterial activity and can be used against urinary tract infections. In our studies we found that *O. ramonense* also exhibited high levels of rosmarinic acid. This plant is less explored but its volatile oil composition and high content of rosmarinic acid makes it a potential phytotherapy agent. **Materials and Methods:** A plant was grown in an 8 liter pot and placed in a Phytotron. Few cuttings were then transferred into 0.5 liter pots and kept in 8 different treatments. Four temperature treatments were used (12-16 °C, 16-22 °C, 22-28 °C and 28-34 °C). The plants were also exposed to either a short-day treatment of 12 h (SD) or a long-day treatment of 19 h (LD) with 1 plant in each treatment. Morphological differences among the plants grown under different temperature and day length treatments were examined. The leaves were separated from the stem and the volatiles were extracted using MTBE (Methyl tert-butyl ether) as extraction solvent and analyzed using GC-MS analysis. The quantification of rosmarinic acid in the plant under different environmental conditions was performed using HPLC chromatographic technique. **Results:** With the increase in the temperature the average leaf size was diminished, which can either be related to the plant's requirement for less respiration in order to prevent the loss of water or due to the deficient activity of certain metabolites. Plants exposed to lower temperature were also larger than those of warm temperature. The content of α -terpineol was most abundant in low temperature and short day treatment whereas at high temperature treatment it becomes reduced to half. The relative amount of α -terpineol was also influenced by day length at low temperature treatment; longer day length reduced the content of α -terpineol from 32.6% to 27.7%. The percentage of 1,8-cineole and *trans*-sabinene hydrate also showed a significant decrease in higher temperature treatment. **Discussion and Conclusions:** According to the results, temperature treatments had large influence on the chemical composition of volatile oil although difference in day length has less effect on the volatile composition. Also the results showed substantial influence of

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temperature and day length on the morphology of the plant. The findings obtained from the study can be further utilized for the cultivation conditions and also for strengthening the production of rosmarinic acid in *O. ramonense*.

Keywords:

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ABSTRACT 11

Environmental factors affecting morphology, flowering and essential oil of *Origanum dayi* post

Gilad Oren, Reena Sharma and Nativ Dudai

ABSTRACT

Background: *Origanum dayi* post. is a species of herbaceous perennials sub-shrubs in the genus *Origanum* of the family Lamiaceae which belongs to the campanulaticalyx section. It is a desert plant, endemic to the northern Negev area and Southern Hebron mountain regions in Israel. *Origanum dayi*'s volatiles composition is unique due to the fact that it resembles the composition of the Tea Tree (*Melaleuca alternifolia*) more than that of Oregano (*Origanum vulgare*) - the main components are α -Terpineol, 1,8-Cineole and Terpinen-4-ol, of which 1,8-Cineole has been associated with suppression of growth in human leukemia Molt 4B and HL-60 cell lines due to induction of apoptosis by this compound. Studies have also been made on the plant extract and show strong evidence of anti-tumor activity in HepG2 human hepato-carcinoma cells, excelling over a number of other select desert plants. The plant has also shown high levels of antioxidant activity and total phenols, along with Rosmarinic acid. There is a small number of publications about the plant, but its abundance in Essential Oil (EO) and the composition of it show a high potential as a phytotherapy agent, mainly as an antimicrobial, anti-inflammatory and respiratory medicine. Previous studies on members of the *Origanum* genus showed the effects of environmental factors on the plant's morphology, induction of flowering and the essential oil content and composition.

Materials and Methods: 32 pots were divided into 8 different treatments of temperature and photoperiod inside a Phytotron. Each pot had 1 plantlet of *Origanum dayi* collected as a cutting from the same plant to create a homogenized sample. Each 4 pots were grown in a different room in the Phytotron under fixed temperature and length of day - Winter (12-16 C°), Autumn (16-22C°), Fall (22-28 C°) and Summer (28-34 C°), while day length was divided into Long (17-7h) and Short (12-12h) days. Morphological differences were examined during a period of 6 months and data was collected, including induction of flowers. Samples were taken to be extracted in MTBE and analyzed through GC-MS analysis, along with antioxidant activity, total phenols and content of Rosmarinic acid. Finally, at the end of the experiment, the plants were weighed and hydro-distilled using a Clevenger apparatus. Essential oil quantity was recorded and composition was analyzed through GC-MS analysis. **Results:** Differences were visible in very early stages of growth, as induction of flowering appeared after a few weeks in treatments under warm temperatures and long days. Cold temperatures did not help induction of flowering, yet gained mass through juvenile growth. Short days also served as inhibition on flowering, though warm temperature plants grown in a short day treatment did flower eventually. Colder treatments showed higher mass at the final stages of growth, but plants grown in warmer temperatures had a higher percentage of Essential oil after distillation. The composition of essential oil drastically changed between the different treatments, as plants grown in cold temperatures showed high

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levels of α -Terpineol and Terpinen-4-ol while the amount of 1,8-Cineole was low. With the rise in temperatures the abundance of 1,8-Cineole of the total EO composition grew while α -Terpineol and Terpinen-4-ol diminished in a linear fashion. All 3 compounds constituted for around 60% of the total essential oil. **Discussion and Conclusions:** The results show dramatic effects of temperature and day length on the plant's morphology, induction of flowering and the essential oil content and composition. Such results can explain the dramatic seasonal differences of these parameters and also shed light on a future agro- technique or harvesting regime of the plant to optimize its yield and quality. It can also shed light on correct practices done on other plants when a certain composition can be retrieved using a correct growing method and scheduled harvest. The plant's potential as an anti-carcinogenic agent should be further investigated, as are all of the rest of its therapeutic abilities.

Keywords: Morphology, Flowering and Essential Oil , *Origanum dayi* Post

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ABSTRACT 12

Development of *Salvia dominica* as a novel crop with great potential as a phytocosmetic and therapeutic plant

Gilad Oren, Hadas Ben-Gera and Nativ Dudai

ABSTRACT

Background: Omega-3 fatty acids play an important role in the human diet and in human physiology, though mammals cannot synthesize the essential omega-3 fatty acid α -linolenic acid (ALA) and can only obtain it through diet. Omega-3 fatty acids are either obtained through a marine organism source (mainly fatty fish) or a plant source. Compared to mariculture, agriculture is still a much more stable form of production, and so a plant source for omega-3 fatty acids can be seen as a better, more sustainable origin and also cater to the rise of the vegan diet across the world. Today, plant omega-3 fatty acids are obtained from the likes of walnut oil, linseed oil, chia seeds and clary sage seeds. Clary sage seeds (*Salvia sclarea*) have shown to have an abundance of the omega-3 fatty acid ALA in its most stable form. Clary sage flowers also contain the diterpene sclareol, which is of high value for the fragrance industry. It has also shown potential as an anti-carcinogenic agent in a few studies. Sclareol mainly accumulates in essential oil-producing trichomes that densely cover flower calices. Therefore, clary sage serves as a source for both omega-3 fatty acids and sclareol. *Salvia dominica*, a perennial shrub of the family Lamiaceae, is endemic to the eastern shores of the Mediterranean, and is literally unknown to the phytotherapeutic and phytocosmetic world. It grows up to a meter at height and its white bloom appears between mid-February and late May. These flowers, much like those of the clary sage, contain an abundance of sclareol in its essential oil, as is the monoterpene linalool acetate, the acetate ester of linalool, a sought after compound in the cosmetics industry. The seeds later produced inside those flowers contain plant oils rich in the omega-3 fatty acid ALA. In this research, we obtained seeds of *Salvia dominica* collected throughout Israel, from desertous plains to northern Mediterranean woodlands and grown them in the fields of Ne've Yaar to analyze their differences in time of flowering, essential oil content and chemical composition in the flowers, omega-3 fatty acids content in seeds and overall yield to determine the differences in populations, divide the chemo-geographically and to further push the plant towards domestication and cultivation as a new source of the sclareol and plant based omega-3 fatty acids.

Materials and Methods: *Salvia dominica* seeds were obtained from the Israel Plant Gene bank in Volcani Center (Agricultural Research Organization) that were previously collected and documented from different regions across Israel. Twenty four populations were sampled and sown in germination trays. Fourteen plantlets were then planted in the field in winter to allow in a randomized order. Growth and the appearance of flowers were documented. When plants reached full bloom, flowers were taken to be extracted through MTBE and analyzed using GC-MS. When seeds have reached full maturation, they were collected and analyzed to determine fatty acids composition. Finally, after all data was collected, including survival rate of different populations, the plants were harvested to determine yield.

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Results: Documentation of growth stages has shown that populations appeared to have different induction to flowering as some flowered earlier than others. Survival rates also differed. Sclareol and linalool acetate content in essential oil collected from the different plants have shown that the content may vary between populations and that it is possible to pick the population most abundant with that compound. Seeds also showed variations in content of fatty acids, specifically omega-3 fatty acids. The final yield of each population varied due to size of plant material harvested. **Discussion:** This research has shown that there is a variation in multiple factors within the populations of *Salvia dominica* in Israel. Some populations have shown stronger resilience to change, different time of flowering and vegetative growth. More importantly, the populations have shown a geo-chemical variation in volatile composition, including sclareol and linalool acetate. Variations in omega-3 fatty acids quantity also allowed to choose a preferable population which can be further developed towards a domesticated source of plant based omega-3 fatty acids, sclareol and linalool acetate.

Keywords: Development of *Slavia dominica* , phytochemistry, experimental success

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ABSTRACT 13

Analysis of phytochemical diversity of *Asteriscus graveolens* population in Israel: Towards cultivation as a phytotherapy plant

Hadas Ben-Gera, Keren Bensimon Mashraki, Reena Sharma, Alona Shachter, David Chaimovitch, Itay Gonda, Nadav Nitzan and Nativ Dudai

ABSTRACT

Background: *Asteriscus graveolens* (Forssk.) Less., belonging to the *Asteraceae* family is an aromatic desert shrub which holds medicinal potential. This species is endemic to the Mediterranean region, dispersed throughout south-western Algeria and south-eastern Morocco. It is also found in Jordan, Egypt and Israel. In Israel the *A. graveolens* species is distributed throughout the southern part of the country, a desert area, which includes the Arava, the Negev, the Judean desert and the Dead Sea basin regions. The volatile composition of *A. graveolens* had been reported to contain a high percentage of oxygenated mono- and sesquiterpenes, including the sesquiterpene lactones and asteriscunolides, which are known to have therapeutic activities. The plant is used in traditional medicine to treat fever, vomiting, bronchitis, gastrointestinal pain and as an anti-inflammatory agent. Local inhabitants of the regions where it grows use it for tea due to its aroma that resembles that of peaches. Several current studies have reported antimicrobial and anti-fungal activities along with pharmacological properties attributed to the essential oil and extracts of *A. graveolens*. Methyl *tert*-butyl ether (MTBE) extraction of the plant, containing a asteriscunolides fraction, was tested for anticancer activity. Considering the plant's plethora of therapeutic and antimicrobial activities, it serves as a promising plant candidate for future agricultural and pharmacological inquiries. **Materials and Methods:** *A. graveolens* wild plants were collected from eleven locations, which represent the natural distribution of *A. graveolens* in Israel. The volatile profiles of MTBE extraction from leaves and flowers were analyzed using gas chromatography–mass spectrometry (GC-MS). Following that, *k*-Means clustering analysis and MANOVA analysis were utilized to explore the chemical diversity (chemotypes) within the *A. graveolens* population. All analyses were performed in JMP 14 Pro (SAS Institute, Inc.) at 5% significance level. Further inquiries were made on a chosen population in order to compare the chemical profile between three production methods: solvent-extraction (methyl *tert*-butyl ether), hydro-distillation of the essential oil and headspace solid-phase micro extraction (SPME).

Results: The majority of volatiles were characterized as oxygenated mono- and sesquiterpenes in all production methods. The leaves were dominated by three asteriscunolide isomers, *cis*-chrysanthenyl acetate and intermedeol. The flowers were dominated by bisabolone, 6-hydroxybisabol-2-en-1-one, *cis*-chrysanthenyl acetate, epi- α -cadinol and germacrene-D. Detection of asteriscunolides isomers, which were previously reported as anticancer molecules, was exclusive to solvent extraction. Based upon these findings, that method was chosen for the analyses of the chemical survey of the wild population. *k*-Means clustering analysis of this data divided the *A. graveolens* population into four clusters which are significantly different in their volatiles composition. This was further demonstrated by MANOVA analysis. A chemically diverse geographic distribution of *A. graveolens* populations growing in Israel was found with unique varieties growing in the Dead Sea basin and the Arava region.

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Discussion and Conclusion: This research has demonstrated how varied the populations of *A. graveolens* growing in Israel are, divided chemo-geographically based on volatiles composition. It is also clear that the method of production had significant impact on that composition. These findings emphasize the importance of choosing the appropriate germplasm source in order to exploit *A. graveolens* plants as a natural source for therapeutic compounds such as asteriscunolides and also the exact production method to use. As an example for chemo-geographic variations, according to this research, *A. graveolens* plants which originated in the Dead Sea basin might contain higher percentages of asteriscunolide isomers compared with those originated in the Arava or the Negev. These findings on chemical variations can be exploited for cultivation controlled conditions of plants containing desirable chemical compositions.

Keywords: *Asteriscus graveolens* population in Israel, *Asteriscus graveolens*, Israel, germplasm source, cultivation controlled conditions of plants

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ABSTRACT 14

Comparison of the anti-fungal and antibacterial effect of the essential oil and ethanolic extract of the *Zingiber officinale* rhizome (ginger) cultivated to standardize a hydroponic medicinal cultivation in San Carlos Zone, Costa Rica

German Madrigal

ABSTRACT

Background: Ginger is a medicinal plant native to India. Its potential use in cosmetics, medicines and natural products has been reported, however depending on the conditions of cultivation the medicinal components of the different parts of the plant not only changes in its concentration, but in its composition, this modifies its medicinal action. The investigation was undertaken to characterize the chemical composition of the essential oil obtained from the rhizomes of *Zingiber officinale* cultivated in the San Carlos area, Costa Rica, and to compare its antifungal and antibacterial effect in order to standardize future crops of the plant. Hydroponic form and validate its pharmacological effect and, or Cosmetic a posteriori.

Materials and Methods: The rhizomes of the plant were used and the active ingredients were extracted by ethanolic extraction with Soxhlet and steam distillation. The analysis was performed by a qualitative phytochemical profile for the ethanolic extract, and the composition of the essential oil was studied by means of Gas Chromatography coupled to a mass detector (GC-MS). In addition, microbiological tests were performed by plate diffusion for ethanolic extracts and essential oil with strains of bacteria and fungi common in cutaneous pathologies. By means of a scanning electron microscope.

Results: The presence of flavonoids, alkaloids, saponins, tannins and triterpenes in the ethanolic extract was qualitatively determined. In the characterization of the essential oil by GC-MS, geranial (27.42%), neral (20.11%), 1,8-cineol (13.35%), camphene (4.65%) and E-geraniol (%).

Discussion and Conclusions: The results obtained were compared with composition reported in the literature. It was clear that our composition of our sample was quite different with that reported in other studies. This allows to predict an antimicrobial behavior different from most of the traditional essential oils of this rhizome. It was determined that the essential oil inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Candida albicans*, *Enterococcus faecalis*, but not against *Pseudomonas aeruginosa*, the ethanolic extract showed no antifungal or antibacterial activity, in addition it was demonstrated that the oil essential has a stronger anti-*Candida Albicans* effect than inhibition halo at the same concentration of essential oil and that its mechanism of action included destruction of the membrane by pore formation and an osmotic shock.

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Keywords: *Zingiber officinale*, essential oil, gas chromatography, natural product, antimicrobial

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ABSTRACT 15

Chronobiological usage approach of vitamins and minerals

Neda Taner

ABSTRACT

Every day, our bodies produce skin, muscle, and bone. A body itself churns out rich red blood that carries nutrients and oxygen to remote outposts, and it sends nerve signals skipping along thousands of miles of brain and body pathways. It also formulates chemical messengers that shuttle from one organ to another, issuing the instructions that help sustain our life. But to do all this, our bodies require some raw materials. These include at least 30 vitamins, minerals, and dietary components that our bodies need but cannot manufacture on its own in sufficient amounts. Vitamins and minerals are considered essential nutrients because acting in concert, they perform hundreds of roles in the body. Physicians are frequently confronted with patients complaining of fatigue, tiredness, and low energy levels. In the absence of underlying disease, these symptoms could be caused by a lack of vitamins and minerals. In particular, the B complex vitamins are essential for mitochondrial function and a lack of just one of these vitamins may compromise an entire sequence of biochemical reactions necessary for transforming food into physiological energy. Vitamins and minerals are often called micronutrients because a body needs only tiny amounts of them. Yet failing to get even those small quantities virtually guarantees disease. Deficiencies in vitamin A and/or iron are prevalent among reproductive-aged women, infants, and children in developing countries, with therapeutic doses required for treatment. The reproductive-aged women or those who are pregnant must ensure adequate intakes of folic acid, iron, calcium, and iodine, which might require supplementation. Post-menopausal women and elderly men could benefit from a combination of low-dose calcium and vitamin D for bone health. The use of a multivitamin supplement with low levels of essential vitamins and minerals could be linked to reductions in the incidence of cancer and cataracts among men. Inadequate intake of micronutrients, or increased needs, impairs health and increases susceptibility to infection, but may also result in tiredness, lack of energy, and poor concentration. The immune system undergoes many changes over the life course developing and maturing during childhood, potentially achieving peak function in early adulthood, and gradually declining in most people in older age. A common factor throughout life is the need for an adequate supply of micronutrients, which play key roles in supporting immune function. Multiple micronutrient deficiencies are common throughout the world, with the likelihood increasing with age. Various micronutrients are essential for immunocompetence, particularly vitamins A, C, D, E, B2, B6, and B12, folic acid, iron, selenium, and zinc. Dietary supplements are widely used and offer the potential to improve health if appropriately targeted to those in need. Inadequate nutrition and micronutrient deficiencies are prevalent conditions that adversely affect global health. Although improvements in diet quality are essential to address these issues, dietary supplements and/or food fortification could help meet requirements for individuals at risk of deficiencies.

Keywords: Minerals, Vitamins, Chronobiological

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ABSTRACT 16

Development of plant-based oral care products and testing their antimicrobial efficiency against planktonic and biofilm-enclosed cariogenic *Streptococcus mutans* bacteria

Efstathios Giaouris and Paraskevi Aggelopoulou

ABSTRACT

Background: Dental caries is the most common infectious disease affecting human, being more prevalent in the developed world mainly due to the high sugar consumption. This is provoked by bacteria forming biofilm on the tooth surface and producing acid, dissolving that way the hard tissues of the teeth, ultimately resulting in their decay. *Streptococcus mutans* is included in the leading microorganisms which, together with other anaerobes, play major role in the establishment of the early biofilm community. Oral hygiene throughout life is important to avoid the progressive build-up of dental plaque and the subsequent appearance of periodontal problems.

Materials and Methods: In this work, two oral care products (*i.e.*, mouthwash and toothpaste) were developed based on natural ingredients of plant origin, including essential oils of basil and mountain tea, together with extracts of St John's wort and solidago (Bucovia™). The minimum inhibitory and bactericidal concentrations (*i.e.*, MICs, MBCs) of the final formulations, as well as of their individual components, were determined against a *S. mutans* carious dentine isolate, by applying the broth microdilution and agar spot assays. The mouthwash was also tested for its efficiency against streptococcal biofilm formation by calculating its minimum biofilm inhibitory concentration (*i.e.*, MBIC). To do this, *S. mutans* bacteria were left to form biofilm on a model surface (*i.e.*, 96-well polystyrene microtiter plate) anaerobically incubated at 37°C for 48 h, in a laboratory broth supplemented with 1% w/v sucrose, and in the presence of ten different mouthwash concentrations. The accumulated biofilm biomasses were quantified at each concentration through staining with crystal violet and absorbance measurements (at 590 nm). The biofilm eradicating activity of the mouthwash was finally tested against already preformed (48 h) *S. mutans* biofilm, by dislodging the remaining viable sessile bacteria (following a 15-min exposure) and enumerating them by agar plating.

Results: The results revealed that the mouthwash and toothpaste presented MICs equal to 0.63% and 0.31%, respectively. Those values were also identical to the respective MBCs. The application of the mouthwash at just its MIC was sufficient to completely inhibit biofilm formation by *S. mutans*, which was otherwise capable of developing a robust biofilm on the abiotic surface.

Discussion and Conclusions: Once developed, that biofilm seemed to contain rich amount of extracellular material protecting the cells, being thus impossible to be eradicated even by applying the mouthwash at 100%, highlighting the great recalcitrance of biofilm-embedded bacteria.

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Keywords: oral care products, *Streptococcus mutans*, biofilm, antimicrobial, phytochemicals, essential oils

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ABSTRACT 17

Dynamism in ethnobotanical knowledge of the BHIL tribe of Rajasthan

Manjit Singh and Anita Jain

ABSTRACT

Background: Ethnobotany is the branch of botany that deals with the study of use of plants by the ethnic communities /tribals for food, medicine and all other purposes. Indian subcontinent has been bestowed with remarkably rich plant diversity coupled with the extraordinary multiethnic cultures making an ideal subject for conducting the ethnobotanical investigations and has tremendous potential for value additions. The concept of Ethnobotanical Knowledge (EBK) is linked to that of Traditional Ecological Knowledge (TEK), which is a contribution to the conservation of biocultural diversity, from an integrative perspective. It is usually assumed that the EBK regards to culturally homogeneous contexts, with a long experience of the human group in its environment, where knowledge and beliefs transmitted from generation to generation, orally and in shared practices. In traditional societies, the people who consume, produce, therefore, the relationship between production and consumption is a direct link. Also, the EBK is adaptative, because it allows different adjustments of the human group to changes in their surroundings, consequently TBK is neither conservative nor static but dynamic and innovative, that is, it evolves. Dynamism in TK can be notice in every phase of life of people in their day-to-day affairs such as survival techniques, livelihood, faith and agricultural practices of the Tribal people. According to Census of India (2011), in Rajasthan, Bhil tribe comprised 44% of the total tribal population of the state. Bhils are predominantly forest dwellers, totally dependent on the forest. The vital ethnobotanical knowledge trail down in the tribe from one generation to the next generation since ancient times but due to numerous reasons, transfer of ethnobotanical knowledge has ceased. In fact, the knowledge is left only in traces among the older generation of Bhil and the younger generation has negligible knowledge regarding the utilisation of plants and other life forms dwelling in their surroundings. The string of interdependence of people and their surrounding life form is steadily slackening which is the major concern raised in this study.

Materials and Methods: Ethno-botanical survey, screening of literature and use of ethnobotanical tools like Relative Frequency of Citation (RFC) ,Relative Importance Index (RI) and Cultural Importance Index (CI) have been taken into account for the study.

Results: A vast number of plants are used by the Bhils and the ethnobotanical knowledge stored in the treasure of tribal people is yet not explored fully. The Tribal Bhil has been transformed into rural population which has shown drastic dynamism in EBK.

Discussion and Conclusions: Bhil have faced a major setback in the form of loss of biodiversity in their vicinity as well as a extreme social transformation which has led to dynamism of ethnobotanical knowledge. It raises the questions that if due to huge force of dynamism Bhils themselves continue to expunge their knowledge, then how traditional ethnobotanical knowledge (TBK) would help mankind and emphasis on the idea that maintaining cultural transformation is the need of the hour to save their existence and aid mankind

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Keywords: Ethnobotany, ethnobotanical knowledge, Bhil, tribe, dynamism, traditional botanical knowledge

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ABSTRACT 18

Effect of sample amount and decoction time on total polyphenols, antioxidant capacity and element content of sage's decoction herbal teas

Cennet Yaman and UfukKoca-Caliskan

ABSTRACT

Background: Herbal tea consumption has increased recently because of its high antioxidant activity and natural therapeutic potentials for many diseases. Sage is an important medicinal plant that was consumed due to phytotherapy feature all around the world.

Materials and Methods: The present work was carried out to identify and compare the antioxidant activity, total bioactive and element contents which may be found in herbal teas from different quantity (2 g and 3 g) and decoction time (5 min and 10 min) of the popular herbs sage. Antioxidant activity was evaluated using DPPH and H₂O₂ radical scavenging activity. Known antioxidant compounds such as total phenolics, flavonoids and flavanols were also determined. The element contents that are important for human health have been analyzed.

Results: The most potent antioxidant activities and highest total bioactive contents were found in herbal teas prepared at 10 min decoction time and 3 g quantity of sage. But, element concentrations of herbal teas differed according to applications.

Discussion and Conclusions: The resulting data were evaluated in terms of human health.

Keywords: sage, decoction, herbal tea, antioxidant activity, minerals

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ABSTRACT 19

Ellagitannins as potential antihypertensive agents: *in vitro*, *in silico* and *meta* analysed data as evidence

Deming Looi, Sonia Phang and Uma Palanisamy

ABSTRACT

Hypertension is defined as the persistence of elevated blood pressure in the circulation system. The renin-angiotensin-aldosterone system is a major modulator of blood pressure. Among the risk factors of cardiovascular disease, hypertension is the most preventable and treatable, with drugs such as ACE inhibitors. Many ACE inhibitors are known to have undesirable side effects and hence, natural alternatives are being sought. Dietary polyphenols, particularly ellagitannins, are derived from plant products and are known to exhibit a variety of bioactivities. Geraniin, an ellagitannin has been shown to have antihypertensive activity in animal experiments. It is speculated that the metabolites of geraniin are responsible for its ACE inhibitory activity. We have performed *in vitro* ACE inhibition and *in silico* studies with geraniin and its metabolites (ellagic acid, urolithins). Our studies confirm that ellagic acid exhibited a similar inhibitory potential to ACE as the positive control captopril.

Keywords: antihypertensive, Bioactives, Dietary polyphenols

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ABSTRACT 20

Families' approach to practice of phytotherapeutics for children in Turkey

Ayşe Akkus and Ufuk Koca-Caliskan

ABSTRACT

Use of phytotherapeutics have been increasing in pediatric patients recently. Herbal treatments are easy to reach, mostly inexpensive and natural. In this regard, it is important for health care professionals to know the educational status, beliefs, social, economic and health status and habits of the public preferring and using phytotherapeutics in order to provide necessary, efficient and effective service to their patients. In terms of pharmacodynamics, most drugs have the similar effects in all patients groups. However, the required dosage and potential side effects vary by age. Therefore, parents' approaches on this issue exhibit that they mostly prefer herbal products. Still, level of their knowledge differs in terms of dosage, duration of use and safe supply. Risky situations requiring medical treatment can be seen for children with improper use of phytotherapeutics. There are just a few studies on using herbal treatment methods in children. In order to obtain information about the approach of families on practice of herbal products, a questionnaire consisting of descriptive characteristics of parents and their approach to usage of phytotherapeutics was created by scanning the literature. This questionnaire was applied face-to-face in 'Pediatric Outpatient Clinics' of general hospitals around Ankara-Turkey. The study revealed information regarding the delay or abandonment of modern medical treatments with herbal products. Information collected with this research will help healthcare providers in increasing the quality and effectiveness of the healthcare service and also help families increasing their awareness about the phytotherapeutics. Most of the families use herbal products in order to prevent their children from illness like flu like symptoms, colic and strengthen immune system. The most common reason why people prefer herbal treatment is that acquaintances benefit from them.

Keywords: Phytotherapeutic, pediatrics, phytotherapy for children, phytotherapy for kids

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ABSTRACT 21

Green extraction of polyphenols from agricultural by-products and food waste

Ioannis Mourtzinis

ABSTRACT

Polyphenols are extracted from agricultural by-products and food waste due to their pharmacological and nutritional interest and used for several applications due to their antioxidant and antimicrobial properties. Extracts rich in polyphenols could be used to fortify conventional food or nutritional supplements. Common by products include: coffee by-products, grape and wine by-products, citrus by-products, apple by-products, pomegranate by-products, olive by-products and onion by-products. Common solvents used for the extraction of polyphenols are methanol, ethanol, acetone, ethyl acetate and their mixtures. Methods used are: solid-liquid extraction, in which plant tissues are extracted in liquid solvents with maceration or Soxhlet extraction, and liquid-liquid extraction. Although organic solvents are effective, they are involved in environmental and toxicological concerns and are relatively expensive. Safety, environmental and economic aspects are forcing industry to shift to "greener" solvents. Major disadvantages of the traditional extraction techniques are high amounts of solvent consumption, high energy consumption, thermal degradation of heat-sensitive components, and long extraction time. Moreover, the aforementioned techniques are not selective and other polar ingredients could be extracted along with polyphenols when the extraction is performed by polar solvents. Recently, non-conventional extraction techniques have emerged including enzyme-assisted extraction, supercritical fluid extraction, microwave-assisted extraction, ultrasonication, pressurized liquid extraction, pulse electric field extraction, ionic liquid extraction. Recently, in the direction of novel extraction techniques, cyclodextrins (CDs) are being explored to extract polyphenols, flavonoids and stilbenes from various types of plant sources. The use of CDs in aqueous solution as extraction media can be considered as green extraction since water is the main solvent and the existence of CD hydrophobic cavity boosts the extraction of polyphenols due to inclusion complex formation.

Keywords:

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ABSTRACT 22

High Phenolic Extra Virgin Olive Oil: How it is made and its contribution to health, beauty and well-being

Anita Zachou

ABSTRACT

Olive Oil has been the most important culinary ingredient for Greeks since antiquity. Homer called it the “Liquid Gold” and Hippocrates the “Great Healer”. It is considered to be the healthiest fat on earth, containing loads of antioxidants, fatty acids and vitamins contributing to our health, beauty and well-being. It is the core element of the Mediterranean Diet. It accompanies our lives every day during our lifespan. It is Mother Nature’s greatest gift to humanity. But are all olive oils the same? Do they have the same taste, characteristics or composition? Do all offer health and beauty benefits? Researchers have discovered that only extra virgin olive oils with a high phenolic profile can contribute to the protection of blood lipids from oxidative stress. “High phenolic” olive oil is a new category of olive oil recognized by the EU Health Claim Labeling Regulation. In 2012, the EU made the health claim labelling regulation 432-2012 where it states that olive oils with polyphenols over 250 mg/kg can bring a health claim indication on the label as it can reduce LDL oxidation. The health claim applies to olive oils with a minimum of 250 mg/kg of polyphenols and is based on the daily consumption of 20 g or 4 tsp. This indicates that the benefits of high phenolic olive oil are dose-dependent. How is it possible for a producer to create a high phenolic olive oil and offer an amazing health remedy to humanity? Does it have to do with the variety, the climate, or the milling procedure? What can go wrong during these stages? How can we understand if the olive oil we taste is rich in phenols? Does the quality of olive oil depend on its unique antioxidants? This presentation demonstrates all the necessary conditions, steps, and practices that will contribute to the production and right storage of High Phenolic Extra Virgin Olive Oils in order to maintain all their beneficial characteristics and become our daily allies against premature aging and chronic diseases.

Keywords: extra virgin olive oil, high phenolic olive oil, anti-aging, beauty, antioxidants, beauty benefits of olive oil, olive oil, olive oil tasting

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ABSTRACT 23

How to reduce UV-induced damage: a lesson from nature

Dima Lan, zviBentwich, Shiri Eshar, Guy Cohen and Nativ Dudai

ABSTRACT

Background: The skin is a vital homeostatic organ that acts as a physical, chemical, and biological barrier against the deleterious effects of the environment. Chronic exposure to solar radiation, and in particular to its ultraviolet B (UVB) region, directly affecting the epidermal surfaces and inner layers, is tightly linked to several pathophysiological alterations, including sunburns, erythema, modulation of the immune response and increases exogenous skin aging. Importantly, UVB may also harm the skin by inducing cellular apoptosis and damage the DNA of the epidermal cells, which is considered one of the major risk factors for the development of skin cancer. Therefore, adequate photoprotection is of constant need. Over the last century, sunscreens have been used extensively as the first line of defense against solar radiation. However, studies have shown that the currently used commercial sunscreens can penetrate and permeate the skin, reaching the circulatory system and producing both local and systemic adverse effects. For instance, topical application of sunscreens has been shown to increase ROS production after UV exposure, decrease the skin barrier functions and to modulate the endocrine system by direct binding of the estrogen and thyroid receptors. Sunscreens have also been shown to harm and pollute the marine environment by swimmers and wastewater. Thus, the need for new novel sun-care products is of need. We have hypothesized that plants grown in extreme conditions like the Dead Sea basin may possess potent active compounds that can reduce or even block the deleterious action of UV.

Materials and Methods: ethnobotanical screening of endemic plants from the Dead sea region were collected and samples were deposit in herbarium. The human skin organ culture was used to evaluate the ability of the extracts to attenuate UVB-induced damage. Apoptosis and epidermal viability were monitored by Caspase-3 activity and MTT assay, respectively. DNA damage was assessed by monitoring the levels of CPD and 6-4PP and validated in the COMET assay. Skin inflammation was monitored by ELISA.

Results: From the various plants screened, the sap extract *Commiphora gileadensis* showed the highest protection that reduced epidermal apoptosis. The extract showed a dose- and time dependency. Maximal protection was achieved when pre-applied topically for 24 h prior to UVB. The reduction in epidermal viability was attenuated by the extract as well as skin inflammation. Importantly, DNA damage was reduced by more than 90%, even at extremely high and super physiological levels of exposure flux. Surprisingly, the photo-protection was not due to SPF or chemical absorbance. Preliminary chemical analysis showed several unique volatile compounds.

Discussion and Conclusions: our results show a new and effective natural compound that can reduce the deleterious action of the sun. Of high interest in the new concept of sun-care that can reduce UVB damage at prolong exposure duration and in an SFP-independent pathway. This may reduce the need for multiple dosages of sunscreen recommended by experts (every two hours) and the concomitant adverse effect of the commercial compounds.

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Keywords: UVB, Medicinal plants, dermatology, human skin organ culture

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ABSTRACT 24

***Kalanchoe* species, skin care ingredients with anti-elastase activity**

Anna Hering

ABSTRACT

Background: Elastin fibers are responsible for elasticity of skin and with collagen they are one of the most important factors of skin structure. Reduction of elastin by elastase enzymes is a natural ageing process, though external factors, like long term sun exposition can activate elastin degradation. Natural and safe inhibitors of elastase enzymes are still demanded. *Kalanchoe* species (Crassulaceae family) are commonly used in folk medicine in different skin disorders. Its antibacterial, antifungal and wound healing properties are described, nevertheless anti-ageing activity is still not well known.

Materials and Methods: Water and ethanol extracts from three *Kalanchoe* species: *Kalanchoe daigremontiana*, *Kalanchoe pinata* and *Kalanchoe blossfeldiana* were analyzed according to Thring1 method. Each extract was incubated with elastase and after 10 minutes substrate (SANA) was added. The change in absorbance at 410nm was monitored by 20 minutes.

Results: The analysis revealed the ability to inhibit elastase enzyme by ethanol extracts from *Kalanchoe pinata* and *Kalanchoe blossfeldiana*. IC 50 for both extracts was similar to oleanolic acid. Water extracts exhibited no activity against elastase enzyme. Both water and ethanol extract of *Kalanchoe daigremontiana*, were neutral for elastase activity.

Discussion and Conclusions: Ethanol extracts from *Kalanchoe pinata* and *Kalanchoe blossfeldiana* could be used as anti-ageing ingredients to skin care products. Nevertheless, its ability to inhibit elastase, should be analyzed also *in vivo*.

Keywords: *Kalanchoe*, elastase inhibition, anti-ageing

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ABSTRACT 25

Natural deep eutectic solvents - New generation green liquids extraction of multi-functional polyphenols

Dimitris P. Makris

ABSTRACT

Currently, there is a great interest for the development and implementation of cutting-edge sustainable extraction methods for polyphenols from plants sources. In this direction, numerous green and low-cost approaches have gained acceptance as being more efficient and precise than traditional ones. In compliance with green chemistry principles, a crucial concern towards establishing eco-friendly extraction processes is the replacement of conventional petroleum-based volatile solvents with bio-based alternatives. The ideal candidate should display high extraction efficiency, low or no toxicity, low price, availability, and it should be produced from recyclable resources. Deep eutectic solvents (DES), are a new generation of liquids and have been characterized as the “solvents of the 21st century”. They are composed of inexpensive, recyclable and non-toxic materials, which can be natural substances (e.g., sugars, organic acids and salts, etc.) and their synthesis is benign, facile and straightforward. In addition, features such as low vapor pressure, absence of flammability and water miscibility make DES ideal solvents for a range of sustainable and eco-friendly applications. In recent years, the use of such solvents for the extraction of natural products has been gaining a very wide acceptance, on the recognition of their unique properties that allow for extraction yields higher than those achieved with conventional solvents.

Keywords: deep eutectic solvents, extraction, antioxidants, polyphenols

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ABSTRACT 26

Phytochemical and antioxidant evaluation of the *Campanula cretica* (A. DC.) Dietr. (Campanulaceae), a rare and protected local endemic plant of Crete (Greece)

Sofia Karavergou

ABSTRACT

The genus *Campanula* (Campanulaceae) includes herbaceous plants, shrubs, and rarely small trees, often with milky sap distributed almost worldwide (except arctic). Many of these are endemic to the Mediterranean Region and some of them have been used in the traditional medicine for the treatment of epilepsy, neurological disease, coughing and headache. Several secondary metabolites have been isolated from *Campanula* plants which mainly belong to the phytochemical class of triterpenes, sterols and cardiac glycosides. To date, studies concerning the pharmacological effects of *Campanula* plants are quite limited. *Campanula cretica* (A. DC.) Dietr. is a rare Greek native perennial species that is protected by the Greek Presidential Decree 67/1981. It is confined to the island of Crete (local endemic) where it grows exclusively on shady cliffs and rock crevices mainly in the 'Lefka Ori mountain range', Western Crete. In this study, hexane, dichloromethane and methanol extracts of fresh and air-dried leaves of *ex situ* cultivated *C. cretica* have been evaluated for their antioxidant activity and their phenolic compounds' content. The antioxidant activity was assessed employing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging method involving the Folin-Ciocalteu method for the total amount of phenols.

Keywords: *Campanula cretica*, antioxidant activity, local endemic

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ABSTRACT 27

Phytochemical and antioxidant evaluation of the local endemic plant of Crete (Greece) *Petromarula pinnata* (Campanulaceae)

Kyriakos Michail Dimitriadis and Sofia Karavergou

ABSTRACT

The family Campanulaceae contains almost 2400 perennial, biennial and annual species occurring on all continents except Antarctica organized in 84 genera. The genus *Petromarula* includes only one known species, *Petromarula pinnata*, which is endemic to the Greek island of Crete in the Mediterranean Region. Its name in Greek means “rock lettuce” as it grows in rock crevices, cliffs and old walls. The leaves of this perennial plant have been widely used traditionally for local nutrition as ‘wild green’. To date, there are no studies reported about the phytochemistry and pharmacological properties of *Petromarula pinnata*. The aim of this study is to present the antioxidant activity and the total amount of phenolic compounds of the hexane, dichloromethane and methanolic extracts of fresh and air-dried leaves of *ex situ* cultivated plant material at two different growth stages in order to evaluate their nutritional value and possible effects on health. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging method has been applied for the assessment of the antioxidant properties. The method Folin-Ciocalteu has been used for the determination of the total amount of phenolic compounds.

Keywords:

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ABSTRACT 28

Phytochemical characterization of the mesocarp of *Gonolobus edulis*, an ancient plant of the Costa Rican flora

Fernanda Rojas, German Madrigal and Valeria Jiménez

ABSTRACT

Background: The species *Gonolobus edulis*, known as cuayote in Costa Rica, belongs to the family Apocynaceae and is distributed from Guatemala to Costa Rica. Currently, there are no studies that elucidate the content and benefits from this plant. For this reason, it is reasonable to say that the study of this species is of utmost importance at the phytopharmacological area.

Materials and Methods: *Gonolobus edulis* fruits were collected in the provinces of San José and Heredia, between the months of October and January. The green fruits of *Gonolobus edulis* were peeled and the mesocarp separated from the shell and seeds. The mesocarp was then finely cut, subdivided and extractions were made with water, ethanol, methanol, chloroform and hexane. A phytochemical screening was carried out following the guidelines for Quality Control of Natural Products of the World Health Organization. The physicochemical properties of the aqueous extract were determined. They included pH, specific gravity, conductivity and osmolality, and an absorption spectrum from 240 nm to 700 nm.

Results: Based on the phytochemical analysis of the *Gonolobus edulis* mesocarp, the presence of flavonoids, carbohydrates, amino acids, steroids, triterpenes, saponins and cardiotoxic glycosides was demonstrated qualitatively. In addition, the aqueous extract had a pH of 5.84 ± 0.09 at 25°C , exposing its acid character, a specific gravity of 1.0017 ± 0.0028 and a conductivity at 25°C of $66.77 \pm 5, 49 \mu\text{S/cm}$, respectively showing a low concentration of dissolved solutes and electrolytes, and an osmolality of $8.44 \pm 1.96 \text{ mOsm/kg}$ at 25°C indicating that the solution is hypo-osmolal.

Discussion and Conclusions: It was elucidated in the mesocarp of *Gonolobus edulis*, the presence of valuable metabolites and characteristics that position it as an interesting candidate for formulations of cosmetics, natural products and even diet supplements.

Keywords: *Gonolobus edulis*, Costa Rica, mesocarp, phytochemical screening, formulation

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ABSTRACT 29

Post COVID-19 beauty trends

Mariliz Gkika

ABSTRACT

While the post- COVID-19 era is yet to commence, the new reality imposes new rules on our everyday life. Consumers are trying new beauty routines but still looking for safety and efficacy, with safety becoming number one priority as it is inextricably linked to health. But now, more than ever, consumers are concerned not only for their skin's health but also for their planetary impact as the new pandemic seems to have shaken everything taken for granted until now. Clean beauty has been leading the way in the cosmetics industry for the last 4-5 years, but now it seems to be one-way street. Most shoppers seek out products perceived as clean, ethical and community focused as this is a way to feel safe, clean and healthy. They are looking for a positive interaction between goods and people both on environmental and on social basis. Thus, beauty innovation should be focused on sustainability, ethically sourced ingredients and transparency. This also seems to be the way to get back to "normal" which has now become a wishful thought. And this is exactly what has become cosmetics request from the efficacy point of view. The eternal quest for anti-ageing has been transformed to a quest for a balanced routine to age well. High tech ingredients aim at unlocking and enhancing what skin has been programmed to perform following intrinsic restoring mechanisms and not interfering with abnormal methods. At most new studies, ageing is being perceived as a result of chronic inflammation, as a result of metabolic paths deregulation and thus structural degradation. Genomics, metabolomics, proteomics all in the service of beauty industry support that anti-ageing mechanisms perform better as a result of further wellbeing. Protection of the barrier, microbiome, circadian rhythms, proteasome, telomers are just some of the predominant trends in cosmetics industry which in the end of the day converge to a common conclusion: Youth preservation is rather a chimera but well ageing is the art of being in harmony with your age.

Keywords: anti ageing, sustainability, clean beauty

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ABSTRACT 30

Potential effect of *Salvadora persica* on SARS-CoV-2; molecular docking study

Asmaa Ibrahim Owis, Marwa el Hawary, Dalia El Amer, Usama Ramadan, Mohamed Kamel and Omar Sokkar

ABSTRACT

Coronavirus disease 2019 (COVID-19) outbreak commenced in Wuhan, China on December 2019 and spread all over the world in a matter of weeks. One of the promising treatments for virus infection is the search for enzyme inhibitors from natural source using molecular docking in order to obtain products with the least side effects. COVID-19 virus main protease plays a vital role in mediating viral transcription and replication, introducing it as a promising target for antiviral agent. Metabolic profiling of the aqueous extract of *Salvadora persica* L. (Salvadoraceae) aerial parts dereplicated eleven known flavonol glycoside using LC-HRESIMS. All the annotated flavonoids exhibited significant binding stability at the N3 binding site in different degrees except isorhamnetin-3-O- β -D-glucopyranoside when compared with the currently used COVID-19 main protease inhibitor, darunavir. Structural similarity between the identified flavonoids enabled the study of the relationship between their structure and the interactions with receptor in the N3 binding site of the COVID-19 main protease. The results indicate that the basic flavonol nucleus possesses activity itself. Moreover, the presence of rutinoside moiety at 3 position of ring C and absence of O-methyl group in ring B of the flavonol structure could increase the binding stability. This study provides scientific basis for the health benefits of the regular use of *S. persica* as it leaks the bioactive flavonoids in the aqueous saliva.

Keywords: COVID-19, *Salvadora persica*, molecular docking, metabolomics, flavonoids

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ABSTRACT 31

Stability studies of cosmetic products containing plant originated bioactive ingredients

Spyros Papageorgiou , Athanasia Varvaresou and Vassiliki Gardiki

ABSTRACT

Background: The use of bioactive substances such as, antioxidants and substances that induce collagen promotion, has led to the creation of a new generation of cosmetic products – dermatocosmetics, which aim to the treatment of aging and photoaging, as well as to medical devices, such as healing products, sunscreen products with enhanced antioxidant properties to cope with the effect of ultraviolet radiation on the biological macromolecules identified in the skin. The increasing trend of using such bioactive substances along with the need for environmentally sustainable and eco-friendly products, have led the cosmetics industry to fund the research and development of cosmetics containing natural ingredients.

Materials and Methods: As a part of our research focused on cosmetics with herbal ingredients, we developed at our laboratory, cosmetic ?/w cosmetic creams containing plant originated ingredients such as a balanced combination of activated forms of gallic acid and stem cells of wild olive tree. The products were studied and tested for their physicochemical stability (pH, viscosity, the separation of phases at different temperatures for various periods of time), their content and stability in BHT and phenoxyethanol using high-performance liquid chromatography (HPLC) as defined by international guidelines. They were also tested for the possible microbiological load of the products according to the European Pharmaceuticals guidelines.

Results: According to the preliminary results of our studies the cosmetic creams proved to be stable regarding their physicochemical characteristics and the preservative system was adequate.

Discussion and Conclusions: In conclusion we developed a series of phytocosmetics stable and efficiently preserved. The efficacy tests are in progress.

Keywords: phytocosmetics, antioxidants, bioactive substances, stability

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ABSTRACT 32

Study of anti-aging potential of hibiscus (*Hibiscus sabdariffa* L.)

Juliana Almeida, Marinna de Assis Fernandes, Bruna Inêz Carvalho de Figueiredo, Fernanda BarçantePerasoli, Luan Bianchini Silvestro and Rosana g Rodrigues das dores

ABSTRACT

Background: Skin aging is a process of progressive deterioration which may highlight ultraviolet radiation and the production of free radicals as important factors for this phenomenon. Chemical and enzymatic oxidations involving the formation of free radicals accelerate the phenomenon of skin aging due to the loss of the biological functions of collagen and proteoglycans, leading to increased flaccidity. The skin naturally produces substances and has structures to fight free radical species, however the efficiency of this protection system tends to decrease with age. Therefore, it is important to search for active substances that can fight free radicals and act as anti-aging structures. In this context the present study aimed to assess the anti-aging potential of ethanol extract from hibiscus.

Materials and Methods: The ethanolic extract of the flowers of *Hibiscus sabdariffa* L. was produced by the process of maceration in ethanol 96 ° GL for 10 days. In order to evaluate the anti-aging activity by free radical scavenging, the DPPH methodology was used, the extract being tested in concentrations of 5; 7.5; 10; 15; 20; 25, 30 and 35 microgram / ml. Flavonoid rutin was used as a standard and ethanol as a negative control. All tests were performed in quadruplicate. The result was expressed at the EC50 level that is the amount of extract needed to produce 50% of antioxidant activity.

Results: The evaluation of the antioxidant activity of the crude ethanolic extract of *H.sabdariffa* L. by the DPPH method showed EC50 equal to 25.66 micrograms /ml.

Discussion and Conclusions: Free radicals are important compounds in the skin aging process leading to the degradation of structural proteins causing sagging and the appearance of wrinkles. The ethanolic extract of the flowers of the species *Hibiscus sabdariffa* L. has shown to have antioxidant activity, being a good option for the development of cosmetic products with anti-aging potential. Studies with formulations containing the ethanolic extract of hibiscus are promising.

Keywords: hibiscus, anti-aging, DPPH

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ABSTRACT 33

Supercritical CO₂ extraction and phytocosmetics : advantages and disadvantages

Athanasia Varvaresou and Eleni Kalogria

ABSTRACT

Supercritical fluid extraction (SFE) can be used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g.essential oils). Carbon dioxide (CO₂), a non-toxic substance, acting as solvent, co-solvent, or anti-solvent, is the most important fluid used in the supercritical fluid technology in extraction, separation, fractionation, micronization, and encapsulation processes applied to obtain extracts concentrated with bioactive compounds for food, pharmaceutical and cosmetic applications. Supercritical extraction mostly uses carbon dioxide at high pressure to extract the high value products from natural materials. Unlike other processes, the extraction process leaves no solvent residue behind. Moreover, the CO₂ is non-toxic, non flammable, odorless, tasteless, inert, and inexpensive. Due to its low critical temperature 31°C, CO₂ is known to be perfectly adapted in food, aromas, essential oils, phytocosmetics and nutraceutical industries. The most cited drawback of using supercritical carbon dioxide as solvent is the high investment cost for equipment acquisition and operation. However, the extraction with supercritical CO₂ presents a lower extraction time because of its diffusivity and low surface tension, greater selectivity in the compounds of interest and little or no consumption of organic solvents.

Keywords: Carbon dioxide, supercritical fluid extraction, phytocosmetics

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ABSTRACT 34

Chemical composition of active compounds in *Hypericum* species native to Israel

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ABSTRACT

Background: *Hypericum perforatum* L. is a plant of the Hypericaceae family that is known for its medicinal properties. It contains several groups of biologically active compounds, among which, the most studied are hypericins and hyperforins. Hypericins and hyperforins are stored in dark and translucent glands, respectively, in different plant organs, including the leaves and the flowers. Hypericins and hyperforins have antiviral and anti-depressant effects and *Hypericum* extract is a prescribed psychiatric drug. *H. perforatum* also contains many other active compounds of which flavonoids are the major group, and the compound quercetin is a common backbone for many of them. The exact anti-depressant active compound is still unknown, and a synergistic effect probably exists to some extent. In Israel, *H. perforatum* is very rare, but three other *Hypericum* species, *H. triquetrifolium* Turra., *H. thymifolium* Banks & Sol., and *H. languinosum* Lam., are commonly found across several regions. It is unknown if they contain hypericin and hyperforin or other active compounds. In this research, we studied the composition of some active compounds in these three *Hypericum* species in comparison to *H. perforatum* to evaluate their potential as medicinal plants.

Materials and Methods: We have examined *H. perforatum*, *H. triquetrifolium*, *H. thymifolium*, and *H. languinosum* originated from Israel for the morphology of translucent and dark glands under an RH-2000 Stereomicroscope equipped with an MXB-050Z lens, to assess their likeliness to contain hyperforin and hypericin. Next, we performed 100% methanolic extraction (2 ppm umbelliferone was used as an internal standard) from young buds of all four species. The methanolic extractions were then analyzed on a high-performance liquid chromatography (HPLC) instrument coupled to a photodiode array detector. The analyses were performed at 40°C on a reverse-phase C-18 A100 column. The mobile phases were composed of three solvents [A = acetonitrile; B = water: 85% phosphoric acid (99.7:0.3, v/v) and C = methanol] with a flow-rate of 1.2 ml × min⁻¹. The absorption of the eluents was recorded at 200 - 700 nm. The following wavelengths were used for identification and quantification: 270 nm for hyperforin and 590 nm for hypericin and 370 nm for quercetin. The peaks were identified by comparing retention times and UV absorptions of the zcomponents with those of authentic reference standards that were spiked into the samples.

Results and Discussion: We found out that all four species display dark and translucent glands, but different tissues showed different distributions. *H. thymifolium* and *H. languinosum* show a pattern of dark glands on the edges of the sepals that might enable easy extraction of pure hypericin, and we will further test this in a follow-up study. The HPLC data showed that although translucent glands were observed in all species, *H. triquetrifolium* and *H. thymifolium* contain hyperforin only in trace amounts. All the species contain hypericin, but its levels in *H. perforatum* were the highest accumulated.

Conclusions: To conclude, it seems that *H. languinosum* might have a good potential for cultivation. We will test the levels of the active compounds in agricultural conditions in this species compared to *H. perforatum*. In parallel, we will examine the anti- depression and anti-stress activity of extracts of these species in zebrafish model.

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