3rd INTERNATIONAL CONFERENCE ON NATURAL PRODUCTS UTILIZATION:

FROM PLANTS TO PHARMACY SHELF

18 - 21 October 2017

Bansko • Bulgaria

BANSKO 2017

3rd INTERNATIONAL CONFERENCE ON NATURAL PRODUCTS UTILIZATION:

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ICNPU-2017

BOOK OF ABSTRACTS

18 - 21 October, 2017

Bansko, Bulgaria

The 3rd International Conference on Natural Products Utilization:
from Plants to Pharmacy Shelf (18-21 October, 2017), Bansko (Bulgaria),
is organized with the kind support of the following organizations and entities

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3rd INTERNATIONAL CONFERENCE ON NATURAL PRODUCTS UTILIZATION:
FROM PLANTS TO PHARMACY SHELF
ICNPU-2017, 18 - 21 October, 2017, Bansko, Bulgaria

Dear ICNPU Participants,

On behalf of the Organizing Committee, it is an outstanding pleasure and honor to welcome you to the **3rd International Conference on Natural Products Utilization: from Plant to Pharmacy Shelf (ICNPU-2017). ICNPU** meetings traditionally attempt to cover the wide spectrum of application of plants with special emphasis on the sustainable use of natural products, emerging -omics platforms and recent trends in (ethno)pharmacology, toxicology, molecular biology and biotechnology.

350+ experts from 60 different countries around the globe will share and discuss the latest developments on the field. The Organizing Committee assembled an exciting and diverse program.

The conference takes place in Bansko, the most popular winter resort of Bulgaria. Located in the foot of Pirin Mountains, with its incredible spirit and breathtaking landscapes, Bansko is definitely a place to explore. Rich social activities will be offered, including welcome reception, conference dinner and tour to historical monuments, besides the podium for informal discussions, networking opportunities and new insights.

We sincerely hope you will enjoy the scientific program and social activities.

Welcome to the **ICNPU-2017**!

Milen I. Georgiev, PhD
Chair of the Organizing Committee

CONTENT

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PROGRAMME

Tuesday, October 17, 2017

16:00 – 19:00	Registration

Wednesday, October 18, 2017

08:00 – 18:00	Registration
08:00 – 18:00	Exhibition opening
09:00 – 09:30	**Official opening ceremony of ICNPU-2017**
Session H	**Chairs: Alipieva & Bankova**
09:30 – 10:15	**KL1: Bharat Aggarwal** (US)
10:15 – 11:00	**KL2: Alisdair Fernie** (DE)
11:00 – 11:30	*Coffee break*
Session He	**Chairs: Fernie & Georgiev**
11:30 – 12:15	**KL3: Robert Verpoorte** (NL)
12:15 – 12:40	**IL1: Vassya Bankova** (BG)
12:40 – 13:05	**IL2: Maria Halabalaki** (GR)
13:05 – 13:15	*ICNPU-2017 group photo*
13:15 – 14:15	*Lunch*
Session Li	**Chairs: Aggarwal & Konstantinov**
14:15 – 14:50	**PL1: Marc Diederich** (KR)
14:50 – 15:15	**IL3: Albena Dinkova-Kostova** (GB/US)
15:15 – 15:30	**SL1: Cheorl-Ho Kim** (KR)
15:30 – 15:45	**SL2: Spiro M. Konstantinov** (BG)
15:45 – 16:00	**SL3: Anwar Rayan** (IL)
16:00 – 16:15	**SL4: Tung-Hu Tsai** (TW)
16:15 – 16:30	**SL5: Slavko Komarnytsky** (US)
16:30 – 17:00	*Coffee break*
Session Be	**Chairs: Dinkova-Kostova & Diederich**
17:00 – 17:25	**IL4: Atanas Atanasov** (PL/AU)
17:25 – 17:50	**IL5: Francesca Borrelli** (IT)
17:50 – 18:05	**SL6: Sylvian Cretton** (CH)
18:05 – 18:20	**SL7: Giulia Suarato** (IT)
18:20 – 18:35	**SL8: Milena Ignatova** (BG)
19:00 – 20:30	**Poster session I (PP1 – PP126)**
20:30 – 22:30	***Get-together party***

Thursday, October 19, 2017

08:00 – 18:00	Registration
08:00 – 18:00	Exhibition opening
Session B	**Chairs: Sieniawska & Atanasov**
08:00 – 08:25	**IL6: Ilkay Erdogan Orhan** (TR)
08:25 – 08:50	**IL7: Ajaikumar Kunnummakara** (IN)
08:50 – 09:05	**SL9: Pavlina Andreeva-Gateva** (BG)
09:05 – 09:20	**SL10: Severina Pacifico** (IT)
09:20 – 09:35	**SL11: Yusuf Öztürk** (TR)
09:35 – 09:50	**SL12: Nagendra P. Shah** (HK)
09:50 – 10:05	**SL13: Stefka Valcheva-Kuzmanova** (BG)
10:05 – 10:20	**SL14: Rumyana Simeonova** (BG)
10:20 – 10:35	**SL15: Kashif Iqbal** (PK)
10:35 – 11:05	*Coffee break*
Session C	**Chairs: Verpoorte & Komarnytsky**
11:10 – 11:45	**PL2: Richard Robins** (FR)
11:45 – 12:10	**IL8: Francisco A. Macias** (ES)
12:10 – 12:25	**SL16: Gertrud Morlock** (DE)
12:25 – 12:40	**SL17: Fanie R. van Heerden** (ZA)
12:40 – 12:55	**SL18: Dina M. El-Kersh** (EG)
12:55 – 13:10	**SL19: Anca Miron** (RO)
13:15 – 14:15	*Lunch*
Session N	**Chairs: Robins & Simova**
14:20 – 14:45	**IL9: Jean-Luc Wolfender** (CH)
14:45 – 15:00	**SL20: Temel Ozek** (TR)
15:00 – 15:15	**SL21: Simona Piccolella** (IT)
15:15 – 15:30	**SL22: Laura Torras-Claveria** (ES)
15:30 – 15:45	**SL23: Reneta Gevrenova** (BG)
15:45 – 16:00	**SL24: Raphaël Grougnet** (FR)
16:00 – 16:15	**SL25: Dimitrina Zheleva-Dimitrova** (BG)
16:15 – 16:30	**SL26: Oskan Tasinov** (BG)
16:30 – 17:00	*Coffee break*
Session O	**Chairs: Halabalaki & Macias**
17:00 – 17:25	**IL10: Michael Heinrich** (GB)
17:25 – 17:50	**IL11: Ionnna Chinou** (GR)
17:50 – 18:05	**SL27: Elwira Sieniawska** (PL)
18:05 – 18:20	**SL28: Maria Paola Germanò** (IT)

18:20 – 18:35 **SL29: Chao Zhao** (CN/US)
18:35 – 18:50 **SL30: Jelena Knežević-Vukčević** (RS)
18:50 – 19:05 **SL31: Fotini N. Lamari** (GR)
19:05 – 19:20 **SL32: Teresa Mencherini** (IT)
19:20 – 19:35 **SL33: Mohammad Hossein Mirjalili** (IR)
20:00 – 21:30 **Poster session II (PP127 – PP251)**

Friday, October 20, 2017

08:00 – 18:00 Registration
08:00 – 18:00 Exhibition opening

Session F	**Chairs: Popova & Wolfender**
08:00 – 08:25	**IL12: Jianbo Xiao** (MO)
08:25 – 08:40	**SL34: Valtcho D. Zheljazkov** (US)
08:40 – 08:55	**SL35: Perihan Gürbüz** (TR)
08:55 – 09:10	**SL36: Vessela Vitcheva** (BG)
09:10 – 09:25	**SL37: Gjoshe Stefkov** (MK)
09:25 – 09:40	**SL38: Vesela Balabanova** (BG)
09:40 – 09:55	**SL39: Tugba Boyunegmez Tumer** (TR)
09:55 – 10:05	**SL40: Siau Hui Mah** (MY)
10:05 – 10:20	**SL41: Alev Önder** (TR)
10:20 – 10:35	**SL42: Mingquan Guo** (CN)
10:35 – 11:05	*Coffee break*

Session Ne	**Chairs: Matkowski & Dajic Stevanovic**
11:05 – 11:30	**IL13: Efraim Lewinsohn** (IL)
11:30 – 11:45	**SL43: Carmela Gerardi** (IT)
11:45 – 12:00	**SL44: Nathalie Jullian-Pawlicki** (FR)
12:00 – 12:15	**SL45: Hélène Greige-Gerges** (LB)
12:15 – 12:30	**SL46: Smita Srivastava** (IN)
12:30 – 12:45	**SL47: Anna Szakiel** (PL)
12:45 – 13:00	**SL48: Soek Sin Teh** (MY)
13:00 – 13:15	**SL49: Satyanshu Kumar** (IN)
13:15 – 14:15	*Lunch*

Session Na	**Chairs: Lewinsohn & Miron**
14:15 – 14:40	**IL14: Zora Dajic Stevanovic** (RS)
14:40 – 14:55	**SL50: Pedja Janaćković** (RS)
14:55 – 15:10	**SL51: Ekaterina Kozuharova** (BG)
15:10 – 15:25	**SL52: Xinwei Jiang** (CN)
15:25 – 15:40	**SL53: Zhao-Jun Wei** (CN)
15:40 – 15:55	**SL54: Magdalena Kondeva-Burdina** (BG)
16:00 – 16:30	*Coffee break*

Session Mg	**Chairs: Heinrich & Chinou**
16:30 – 16:45	**SL55: Benita Hryć** (PL)
16:45 – 17:00	**SL56: Carolin Schulz** (DE)
17:00 – 17:15	**SL57: Radu Necula** (RO)
17:15 – 17:30	**SL58: Milena Vujanović** (RS)
17:30 – 17:45	**SL59: Jun-Young Park** (KR)
17:45 – 18:00	**SL60: Sun-Hyung Ha** (KR)
18:00 – 18:15	**SL61: Fukushi Abekura** (KR)
18:15 – 18:30	**SL62: Antonia Diukendjieva** (BG)
18:45 – 19:00	**SL63: Merve Badem** (TR)
19:00 – 19:15	**SL64: Blagica Jovanova** (MK)
19:15 – 19:30	**SL65: Chitta Ranjan Barik** (IN)
20:00 – 00:00	***Conference gala dinner and awards***

Saturday, October 21, 2017

09:00 – 16:00 ***Conference excursion***

KL – key-note lecture
PL – plenary lecture
IL – invited lecture
SL – short lecture
+ 5 min Q&A to all oral slots

INVITED LECTURES

KL 1

TARGETING INFLAMMATORY PATHWAYS LINKED TO CHRONIC DISEASES BY NUTRACEUTICALS DESIGNED BY MOTHER NATURE

Bharat B. Aggarwal

Inflammation Research Center, San Diego, California, USA
Professor (Retd.)of Experimental Therapeutics, Cancer Medicine and Immunology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Chronic infections, obesity, alcohol, tobacco, radiation, environmental pollutants, and high-calorie diet have been recognized as major risk factors for the most common types of chronic diseases including cancer. All these risk factors are linked to cancer through inflammation. While acute inflammation that persists for short-term mediates host defense against infections, chronic inflammation that lasts for long-term can predispose the host to various chronic illnesses, including cancer. Linkage between cancer and inflammation is indicated by numerous lines of evidence; first, transcription factors NF-κB and STAT3, two major cell signaling pathways linked to inflammation, are activated by most cancer risk factors; second, an inflammatory condition precedes most cancers; third, NF-κB and STAT3 are constitutively active in most cancers; fourth, hypoxia and acidic conditions found in solid tumors activate NF-κB; fifth, chemotherapeutic agents and gamma irradiation activate NF-κB and lead to chemoresistance and radioresistance; sixth, most gene products linked to inflammation, survival, proliferation, invasion, angiogenesis, and metastasis are regulated by NF-κB and STAT3; seventh, suppression of NF-κB and STAT3 inhibits the proliferation and invasion of tumors; and eighth, most chemopreventive agents mediate their effects through inhibition of NF-κB and STAT3 activation pathways. Thus suppression of these proinflammatory pathways may provide opportunities for both prevention and treatment of chronic diseases such as cancer. We will discuss the potential of various nutraceuticals derived from mother nature such as spices and from Ayurvedic medicine in suppression of inflammatory pathways and their role in prevention and therapy of cancer.

KL 2

TOWARDS FUNCTIONAL METABOLOMICS

Alisdair Fernie

Max-Planck-Institute of Molecular Plant Physiology, Germany

Two grand challenges face metabolomics that which is most frequently addressed is the poor coverage currently a couple of percent of the plant kingdoms metabolome. Less studied is what all these metabolites are good for. I will provide examples from our work on tomato and *Arabidopsis*. The former is based on fully elucidating the pathways of glycoalkaloids and phenylpropanoids the latter which will form the major focus went beyond this and characterized in vivo bioactivity of a new class of flavonols. Incidence of natural light stress renders it important to enhance our understanding of the mechanisms by which plants protect themselves from harmful effects of UV-B irradiation, as this is critical for fitness of land plant species.

Here I will describe natural variation of a class of phenylacylated-flavonols (saiginols), which accumulate to high levels in floral tissues of *Arabidopsis*. They were identified in a subset of accessions, especially those deriving from latitudes between 16° and 43° North. Investigation of introgression line populations using metabolic and transcript profiling, combined with genomic sequence analysis, allowed the identification of flavonol-phenylacyltransferase 2 (FPT2) that is responsible for the production of saiginols and conferring greater UV light tolerance in planta. This example of assessing the *in vivo* role of novel metabolites will be used as a case study to highlight the importance of assessing biological roles of metabolites both within plants and the species which depend upon them to meet their nutritional requirements.

KL 3

SYNERGY: EASIER TO SAY THAN TO PROVE

Robert Verpoorte, H.K. Kim, Y.H. Choi

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In the discussion on medicinal plants often synergy is used as an argument to explain activity, and even to argue that medicinal plants are superior to single pure compounds. Also in the resistance of plants against pest and diseases synergy might play a role. But how much real evidence is there for synergy? Synergy simply means that 1+1>2. The basis of nature! To proof synergy between two compounds for a certain biological activity isobolograms are used in which one can see that the activity of the mixtures of two compounds is higher than the sum of the activities of these two compounds separately. Many studies on synergy apply this method to proof synergy of two compounds, however, this requires that one knows the active compound(s) of a medicinal plant. If these are not known it becomes difficult, particularly when in bioassay guided fractionation the activity is lost, e.g. when activity is fully dependent on the presence of two or more compounds. The only solution is a systems biology approach. By measuring the metabolic profile of different extracts of a medicinal plant or fractions thereof and combining that information with the results of the bioassays of these samples, one may identify the signals that correlate with activity. These signals may be due to one or more compounds. After identification of these compounds, e.g. after isolation via metabolomics guided fractionation, one can test these compounds for synergy.

If synergy would play an important role in medicinal plants, the synergistic effect is on the system as a whole, and thus synergy may have many forms, also depending on the parameter that is the measure for activity, e.g. is it the cure of a disease or the effect on a single target to name the extremes. That means for studies on synergism one should use preferably in-vivo bioassays, and if possible even apply this approach in clinical trials, as besides synergy also prodrugs may be present in medicinal plants.

Obviously such an approach is totally different from the currently accepted approaches to drug development, which are based on the single target single compound paradigm. The fact that HIV now can be kept under control by using a combination of several drugs, are a first sign that a paradigm shift is on its way, bringing natural products and medicinal plants again to the forefront of drug discovery.

PL 1

ABOUT CANONICAL AND LESS CANONICAL CELL DEATH INDUCTION BY NATURAL COMPOUNDS WITH PHARMACOLOGICAL POTENTIAL

Marc Diederich

Department of Pharmacy, College of Pharmacy, Seoul National University, Seoul 151-742, South Korea

Natural compounds are the fundament of pharmacological treatments and more than 50% of all anti- cancer drugs are of natural origins or at least derived from scaffolds present in Nature. Over the last 25 years, molecular mechanisms triggered by natural anticancer compounds were investigated. Emerging research showed that molecules of natural origins are useful for both preventive and therapeutic purposes by targeting essential hallmarks and enabling characteristics described by Hanahan and Weinberg. Moreover, natural compounds could change the differentiation status of selected cell types. One of the earliest response of cells treated by pharmacologically active compounds is the change of its morphology leading to ultra-structural perturbations: changes in membrane composition, cytoskeleton integrity, alterations of the endoplasmic reticulum, mitochondria and of the nucleus lead to formation of morphological alterations that are a characteristic of both compound and cancer type preceding cell death. Apoptosis and autophagy were traditionally considered as the most prominent cell death or cell death-related mechanisms. By now multiple other cell death modalities were described and most likely involved in response to chemotherapeutic treatment. It can be hypothesized that especially necrosis-related phenotypes triggered by various treatments or evolving from apoptotic or autophagic mechanisms, provide a more efficient therapeutic outcome depending on cancer type and genetic phenotype of the patient.

In fact, the recent discovery of multiple regulated forms of necrosis and the initial elucidation of the corresponding cell signaling pathways appear nowadays as important tools to clarify the immunogenic potential of non-canonical forms of cell death induction.

PL 2

ISOTOPIC FRACTIONATION OF ^{13}C DURING BIOSYNTHESIS AND ENZYMATIC REACTIONS: CAUSES AND APPLICATIONS TO TRACEABILITY

Richard J. Robins[1], Gérald S. Remaud[1], Katarzyna M. Romek[1,2], Anna Grzybkowska[1,2]

[1] Elucidation of Biosynthesis by Isotopic Spectrometry Group, CEISAM, University of Nantes-CNRS UMR6230, F-44322 Nantes, France
[2] Institute of Applied Radiation Chemistry, Faculty of Chemistry, Lodz University of Technology, ul. Stefana Żeromskiego 116, 90-924 Łódź, Poland

Isotopic methods are important both in traceability and in understanding metabolic pathways. Studies at natural abundance are especially valuable in these contexts [1]. It has been recognised for some time that in the majority of natural products the $^{13}C/^{12}C$ ratio of *O*-methyl groups, and more recently of *N*-methyl groups, is depleted relative to the rest of the carbons in the molecule. These compounds all have in common that L-methionine (Met) is the donor of the methyl group via the activated intermediate *S*-adenosylmethionine (AdoMet). By using a combined experimental and theoretical approach, we have shown that fractionation during Met biosynthesis from homocysteine and 5-methyltetrahydrofolate contributes significantly to this fractionation: there is an impoverishment in the $^{13}C/^{12}C$ ratio of the *S*-methyl of Met which can be tied to the mechanism of the Met synthase reaction. Hence, it is apparent that the impoverishment in the $^{13}C/^{12}C$ ratios in *O*- and *N*-methyl groups can be due, at least partially, to the isotopomer selectivity of this enzyme. This is strengthened by an analysis of the ^{13}C distribution in xanthine alkaloids [2].

This phenomenon has been further probed by studying a wider range of natural products, measuring all *C*-positions by irm-^{13}C NMR. Not all products show impoverishment, a phenomenon for which we are seeking an explanation. A notable example is natural vanillin, which is thus readily distinguished from a nature-equivalent source.

An important application of this approach is in traceability, using irm-^{13}C NMR to distinguish geographical and biological origin of natural products, including caffeine. How and why different batches of product vary (or not) in their isotope values will also be discussed.

Acknowledgements: We are grateful to many colleagues and collaborators whose contribution has enriched this study.

References:

[1] Robins RJ, Romek KM, Remaud GS, Paneth P (2017) Phytochemistry Letters 20: 499-506.
[2] Diomande DG, et al. (2015) Analytical Chemistry 87: 6600-6606.

IL 1

FROM PLANTS TO PHARMACY SHELF VIA THE BEEHIVE

Vassya Bankova, Milena Popova, Boryana Trusheva, Svetlana Simova

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

The most popular bee products, most often used in prophylactics and treatments for numerous diseases, are honey and propolis, both derived from plants. Honey is consumed in large amounts and is a real nutrient; however, its healing and protective properties have been known for centuries. Propolis is another bee product with high potential in promoting human health. Honey and propolis have been the subjects of special attention by scientists, dedicated to their chemistry and pharmacological properties. Our studies on propolis from different geographic zones have revealed new molecules with valuable biological activities: cinnamic acid derivatives, prenylated benzophenones, stilbenes, di- and triterpenes, etc. Further, based on chemical knowledge about active principles, we suggested quality criteria for standardization of European type propolis. Standardization is crucial for the wide application of propolis in medical practice.

The potential of honey as a wound-healing agent has been known since ancient times. Of special interest is honeydew honey, considered to have higher antioxidant and antimicrobial activities than floral honeys. There is a growing demand and the prices of this honey type are growing. We developed an approach for rapid differentiation of oak honeydew honey from all other honey types. It is based on the identification of the signals of the cyclitol quercitol in the 1H and 13C NMR spectra of honey. Quercitol is a useful marker for oak honeydew honey; it also contributes to its health-beneficial properties: cyclitols are known to possess antiradical activity, quercitol itself has glucosidase inhibitor activity which blocks the absorption and metabolism of carbohydrates.

IL 2

PISTACIA: EXPLORATION OF A UNIQUE GENUS WITH EXCEPTIONAL PHARMACOLOGICAL PROPERTIES

Maria Halabalaki

Department of Pharmacognosy & Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis Zografou, 15771, Greece

Pistacia genus belonging to Anacardiaceae family encompasses plants with food, medicinal and ornamental value. The genus is comprised from about 20 species and the most common in Mediterranean basin are *P. lentiscus* var *Chia* and *P. vera*. Chios mastic, is the resinous secretion obtained from the wounds of the trunk and branches of *P. lentiscus* L. var. *Chia*, which is endemic to the Greek island of Chios. Since antiquity (500 BC), Chios Mastic has been well recorded for its medicinal and pharmaceutical properties such as gastrointestinal disorders. From 1997, Chios mastic has been identified as a product of Protected Designation of Origin (PDO) while cultivating mastic has been inscribed by UNESCO in 2014 in its Representative List of the Intangible Cultural Heritage of Humanity. In July 2015, mastic was recognized as a traditional medicinal product by the European Medicines Agency (EMA) with two therapeutic indications (mild dyspeptic disorders & skin inflammation/ healing of minor wounds). *Pistacia vera* L. is considered the only species in the genus that produces edible tree nuts, which are widely known as pistachios. The sensory, nutritional, and health attributes of this popular snack food make it increasingly valued and of significant economic importance for several countries including Greece, the sixth largest producer worldwide. Thus, valorization of the main side product derived from pistachio processing, the pericarp or outer hull, is of great interest and can be determined by the content in valuable phytochemicals that merit utilization.

In the frame of a continuation study on *Pistacia* sp. an integrated, complementary bottom up approach has been designed for the exploration of both species. This approach includes isolation of active, marker compounds from starting material with fast and state-of-the-art techniques (CPC-UV, SFC-UV-MS); profiling and disclose of composition via multiple analytical methods (HPTLC, HPLC-DAD, UPLC-HRMS & HRMS/MS & NMR); and validation of methods for quality control purposes. Additionally, pharmacokinetic characteristics of major mastic constituents have been determined after a human cohort and metabolomics approaches (LC-MS and NMR) have been implemented for revealing of biomarkers. The current work could be considered as an example of a complete workflow implemented in medicinal plants, from the plant to human organism.

IL 3

SULFORAPHANE: A MULTIFACETED PROTECTOR AGAINST UV RADIATION-MEDIATED SKIN DAMAGE AND PHOTOCARCINOGENESIS

Elena V. Knatko[1], Andrea L. Benedict[2], Maureen Higgins[1], Sally H. Ibbotson[3], Albena T. Dinkova-Kostova[1,2]

[1] Division of Cancer Research, School of Medicine, University of Dundee, Dundee DD1 9SY, Scotland, United Kingdom
[2] Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[3] Photobiology Unit, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, Scotland, UK

The plant isothiocyanate sulforaphane was isolated from broccoli extracts as the principal and potent inducer of mammalian cytoprotective responses, which protect against damage by electrophiles, oxidants, and pro-inflammatory agents, including ultraviolet radiation (UVR), the most abundant carcinogen in our environment and the major causative factor for skin damage and photocarcinogenesis. UVR causes direct DNA damage as well as generation of reactive oxygen species (ROS) and oxidative stress, which further damage DNA and proteins, and promote inflammation and immunosuppression. We have shown that sulforaphane-containing broccoli sprout extracts, administered either topically or in the diet, protect SKH-1 hairless mice against UVR-mediated skin damage and photocarcinogenesis. In a randomized placebo-controlled clinical trial, application of these extracts to the skin of healthy human subjects reduced susceptibility to erythema arising from acute exposure to solar-simulated UVR. Many of the protective effects of sulforaphane are due to its ability to activate transcription factor Nrf2. In addition, sulforaphane provides Nrf2-independent protection, such as suppression of NFκB signaling and direct inhibition of the activity of macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine implicated in UVR-mediated skin damage and photocarcinogenesis.

Thus, sulforaphane provides a paradigm for a natural product, which plays a multifaceted role in protection against the damaging effects of oxidative stress and inflammation.

Acknowledgments: We thank Cancer Research UK (C20953/A18644) and the British Skin Foundation (7015) for financial support.

IL 4

NATURAL PRODUCTS TO MODULATE MACROPHAGE PHENOTYPE IN THE CONTEXT OF ATHEROSCLEROSIS

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Cardiovascular disease represents number one cause of death in the world, and therefore the identification of compounds that can combat it is highly relevant. The phenotype of macrophages residing in the atherosclerotic plaque is a critical factor regulating cardiovascular disease pathogenesis. The ability of macrophages to efflux cholesterol represents an anti-atherosclerotic process counteracting the transformation of macrophages into cholesterol-enriched proatherogenic foam cells. Presented will be the characterization of small molecule natural products able to enhance macrophage cholesterol efflux in THP-1 macrophage-like cells used as an experimental model, as well as the identified underlying molecular mechanisms. Natural products have proven historically to be a rich source of compounds with promising therapeutic properties in general [1], and our new data confirm that they also represent a very promising pool for the identification of compounds with pharmacologic potential in the context of the modulation of the proatherogenic macrophage phenotype in particular.

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IL 5

EVIDENCE-BASED PHYTOTHERAPY IN ERECTILE DYSFUNCTION

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Clinical decisions, which include therapeutic treatments, are based on the evidence-based medicine (EBM) that integrates knowledge from the best available clinical evidence with individual clinical expertise. By systematically collecting and critically assessing clinical trials, systematic reviews and meta-analyses are at top of the pyramid of the clinical evidence.

Herbal medicines are a helpful alternative to synthetic drugs, although the rational basis for their clinical use as well as their safety are still far from being scientifically proven. Our goal is to assess the clinical evidence for or against the use of herbal medicines in the treatment of erectile dysfunction (ED). Through a systematic search of the literature, we retrieved randomized controlled trials (RCTs) on ginseng (*Panax ginseng*, five RCTs), saffron (*Crocus sativus*, three RCTs), *Tribulus terrestris* (two RCTs), L*epidium meyenii* (one RCT), *Pinus pinaster* (one RCT) and some herbal formulations (twelve RCTs). A critical evaluation of the identified RCTs seems to suggest efficacy of ginseng in ED patients. *Pinus pinaster* and *Lepidium meyenii* showed very preliminary positive results, saffron and *Tribulus terrestris* treatments produced mixed results. Several herbal formulations were associated with an improvement, although the results are preliminary. In conclusion, there is encouraging evidence suggesting that ginseng may be an effective herbal treatment for ED.

However, further, larger and high-quality studies are required before a firm conclusion can be drawn. There are also promising (although very preliminary) results generated from some herbal formulations.

IL 6

ELUCIDATION OF NEW ENZYME INHIBITORS FROM PLANTS: A COMBINATION OF *IN VITRO* AND *IN SILICO* DATA

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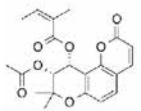
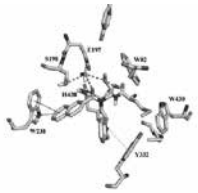
Natural compounds have always been attractive candidates for finding novel drug molecules since many years. In order to have an idea about their pharmacological activities, enzyme inhibition is a common mechanism of biological action. Enzyme inhibitors are the substances which revise the catalytic action of the enzyme and accordingly slow down or even stop catalysis sometimes. In this regard, many natural compounds have been shown to be potent and selective inhibitors of numerous enzymes as treatment strategy for human diseases.

In our extensive screening of natural products using *in vitro* methods using ELISA microplate reader, we have so far identified many promising molecules with notable inhibition rates against different enzymes including tyrosinase, elastase, collagenase, cholinesterases (acetyl- and butyryl- derivatives), xanthine oxidase, phosphodiesterase, carbonic anhydrase, urease, etc. The active compounds were proceeded to molecular docking (*in silico*) experiments to figure out the interactions between ligand and the enzyme at molecular level. During these endeavors, we have recently revealed a number of promising molecules such as tanshinones (diterpene derivatives) from *Perovskia atriplicifolia* Benth. and *Salvia glutinosa* L. [1], pteryxin (coumarin derivative) from *Mutellina purpurea* L. [2] as selective butyrylcholinesterase inhibitors, luteolin 5-*O*-*β*-glucoside as potent carbonic anhydrase type-II inhibitor, etc. In this talk, recent results of our enzyme inhibition studies with natural compounds will be presented.



Pteryxin



Pteryxin and enzyme (hBChE) interaction

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IL 7

MULTITARGETED AGENTS IN CANCER CELL CHEMOSENSITIZATION: WHAT WE LEARNED THUS FAR?

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Cancer, the second deadliest disease in the world kills over 8 million people worldwide annually. The research over the past several decades has developed numerous drugs for the treatment of this disease that targets a single gene or protein or a pathway, but most of them failed in clinic due to the emergence of chemoresistance. Therefore, the drugs that can sensitize cancer cells to the approved chemotherapeutic agents have high potential in the treatment of this disease. Several genes, proteins and pathways are involved in the development of chemoresistance in cancer cells such as MDR, NF-κB, Akt/mTOR, EGFR, STAT3, miRNAs etc. Therefore, targeting a single gene or protein or pathway is not effective in overcoming chemoresistance in cancer cells. Hence, the agents that target multiple genes, proteins or pathways that are responsible for chemoresistance have high potential in chemosensitizing cancer cells to chemotherapeutic agents. Recently, multitargeted agents made by "Mother Nature" have received much attention as chemosensitizing agents due to their safety, efficacy and affordability.

The present talk will summarizes the potential of these agents in chemosensitizing cancer cells to chemotherapeutic agents that are evident from many preclinical and clinical studies. The chemosensitizing potential of natural products such as curcumin, resveratrol, quercetin, soy isoflavones, fiscetin, sulphoraphane, ellagic acid, apigenin, ursolic acid, cucurbitacin etc. will be discussed. The present talk will also discuss the recently developed technologies for chemosensitizing cancer cells to chemotherapeutic agents.

IL 8

ALLELOPATHY, AN EFFICIENT APPROACH FOR BIOACTIVE COMPOUNDS

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The term "Allelopathy" involves the study of processes involving secondary metabolites preferably produced by plants, algae, bacteria and fungi, which may affect the growth or development of biological systems. In plants, these secondary metabolites can be produced by their own, or after exogenous stimulation, and are usually involved in the chemical defense mechanisms of the plants [1]. For this reason, this field has great importance in agriculture and pharmaciology, both for its ability to propose new alternatives to traditional synthetic herbicides, and for the production of new molecules leading drugs.

In the process of isolating, identifying, characterizing the structures of these metabolites, it is important to choose the appropriate extraction techniques. Although in ecological studies the most appropriate methodology involves imitating the natural conditions. This methodology has some important drawbacks. Advanced extraction techniques are currently being used, such as ultrasonic assisted, supercritical fluid and high pressure fluids extractions [2]. These methods allow the recovery of the compounds in shorter times and at lower temperatures, avoiding the degradation.

After the extraction process it is important to carry out a biodirected isolation of the extracts. Different types of bioassays can be carried out: cytotoxicity, phytotoxicity, etc. Once the active compounds are known, the total or partial synthesis of these metabolites can be proposed [3], as well as structure-activity relationship (SAR) studies that allow to improve their activity [4].

Knowledge of allelochemicals, as well as their mode and mechanism of action, allows the design of new applications in agriculture and pharmacology of natural products.

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IL 9

HOW FAR DEEP CAN WE ANNOTATE NATURAL PRODUCTS WITH MASS SPECTROMETRY? PERSPECTIVES AND ISSUES FOR LARGE SCALE METABOLOME PROFILING

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With the recent progresses made in metabolite profiling methods and miniaturization of bioassays, a question that arises is: do we still need to perform conventional large scale bioactive guided-isolation of natural products? All these rapid innovations may lead to a change of paradigm in natural products research towards more efficient and rational approaches in the field.

High resolution mass spectrometry (HRMS) and data dependent MS/MS analyses provide very valuable information on secondary metabolites for in-depth metabolome annotation studies [1]. The recent development of molecular network (MN) approaches for the mining of such data in combination with spectral database generated *in silico* [2] gives the possibility to establish relationships between metabolites thus significantly improving the efficiency of dereplication when combined with high quality chemotaxonomic data [3]. Such types of information can be generated with a few mg of extract only and are readily applicable to herbarium scale samples. In addition, such data can be statistically correlated with bioactivity data on extracts and potentially enable the localisation of interesting hits without the need for a bioactivity-guided approach. For complete *de novo* identification of new compounds MS-targeted micro-isolation can be performed and sensitive 1D and 2D microNMR with microgram amounts of purified metabolites can be acquired. For bioactivity determination, many bioassays fit also to this scale. Using an ideal combination of methods it is this virtually possible to fully identify any bioactive principles in this way. Integration of other filters to this approach such as permeation studies on extracts additionally provide key information on the possible bioavailability of NPs prior to their isolation. Furthermore the link of a given bioactivity results to those previously reported for compounds similar to those identified can be rationalised through *in silico* chemical space approaches.

Ideally a combination all these state-of-the-art methods should enable to identify and localise valuable NP efficiently at the analytical scale. In such a way large scale MS-targeted isolation of valuable NPs only can become a very rational way to conduct investigations. Different recent applications of our metabolomics and phytochemical investigations will illustrate these aspects.

A summary of what could be an ideal workflow will be presented and discussion on what is readily implemented and what is still required will be made.

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IL 10

FROM PLANT TO SHELVES: THE IMPORTANCE OF VALUE CHAINS RESEARCH IN THE PHARMACEUTICAL SCIENCES

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Medicinal (and food) plants are not only a source for new drug leads and high value medical products, but they also highlight a wide range of socio-cultural and pharmaceutical research challenges. Using series examples from our recent research this presentation focuses on ascertaining best practice in phytopharmacy.

In the last years and in an increasingly interconnected world value chains of medicinal plantshave also been highlighted as a core focus of research on medicinal plants. Research into these chains [1, 2] highlights problems like exploitation through middlemen, over-harvesting of wildmedicinal plants, adulteration and contamination of products at different stages along the chain and a general lack of traceability through the different stages of production. There are benefits to farmers that belonged to an integrated chain and the resulting products were subject to a higher standard of processing and storage. By using analytical and metabolomics methods, including HPTLC and 1H-NMR, we were able to correlate some variations in product composition for selected producers and identify strengths and weaknesses of some types of value chains. Examples from our research include *Rhodiola* spp., *Hypericum perforatum* L., *Ginkgo biloba* L. and *Curcuma longa* L.

Ethnopharmacology offers both a new framework for research and new strategies for resolving some of the key problems. We need to better understand what core values such products have to the primary producers and through the value chains leading to the final products.

The examples discussed highlight that aside from integrating modern analytical approaches we also have to critically engage with sociocultural and economic aspects of the field.

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IL 11

BORAGINACEAE - THE GOOD, THE BAD AND THE UGLY, NEW PHYTOCHEMICAL DATA

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Boraginaceae plant family consists of around 2000 species, occurring mainly in Europe (Mediterranean region) and Asia. Most of them are grown as ornamental plants, or as source of dye (alkanet) or have medicinal uses, like borage (*Borago)*, comfrey *Symphytum* and hound's-tongue *Cynoglossum*. Among the most characteristic compounds of the family, are: alkannins/shikonins and related isohexenylnaphthazarins, which are well-known natural red pigments and pyrrolizidine alkaloids (PAs) and N-oxides (PANOs) [1, 2].

Good: In the framework of our investigations on Boraginaceae plants, we have reported the results of the phytochemical investigations of *n*-hexane extract from natural plants, cell suspension cultures and transgenic roots of *Arnebia euchroma*, *Lithospermum canescens*, *Cynoglossum columnae* and *Rindera graeca*, resulted in the isolation of approx 20 isohexenylnaphthazarins and shikonofurans (several new natural products) and their biological properties (antimicrobial, antileishmanian, antimalarial and cytotoxic activities) [1, 2].

Bad: The phytochemical analyses led to the isolation of many structures of PAs and PANOs from Greek endemic plants *Onosma erecta*, *O. leptantha*, *O. kaiherei* and *Cynoglossum columnae* which will be presented.

Ugly: The Regulatory status of PAs/ PANOs themselves and containing products will be discussed as within the EU new lower limits have been proposed and already accepted by several EU National Competent Authorities. PAs have been detected through adulterations in many final products, as well as in foods such as honeys, salads, teas, spices etc [3].

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IL 12

THE DEGRADED PRODUCTS OF UNSTABLE POLYPHENOLS IN CELL CULTURE

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We have comprehensively investigated the structure–stability relationship of natural polyphenols in DMEM medium under the cell culture conditions [1-3]. Some polyphenols, especially flavonoid aglycones with catechol or pyrogallol structure and the pyrogallol-type glycosides were evidently instable. Herein, we further investigate the degraded products of instable polyphenols and its mechanism of degradation in cell culture.

Myricetin, myricitrin, kaempferol, quercetin, baicalin, and baicalein were incubated with DMEM at 37 °C in 5% CO_2 for 180 min. Then the degraded products were identified by LC-MS-MS.

Acknowledgements: This research was supported the Start-up Research Grant from University of Macau (SRG2015-00061-ICMS-QRCM), and the opening fund of the State Key Laboratory of Quality Research in Chinese Medicine of University of Macau (No. SKL-QRCM-2014-2016).

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IL 13

THE DISCOVERY AND CHARACTERIZATION OF GENES DIRECTING PLANT NATURAL PRODUCT BIOSYNTHESIS

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The study of the biosynthesis of secondary (specialized) metabolites and the genes involved in these processes has been greatly facilitated by novel genomic approaches developed during the last years. Many of the biosynthetic pathways dedicated to secondary metabolism, and the enzymes involved in these pathways have common evolutionary origins. Therefore by exploiting similarities between functionally-related genes, it has been possible to characterize many genes involved in the formation of unique natural products. Appropriate tissues where the compounds of interest are produced in significant levels are normally identified and the metabolome present is characterized. Deep sequence methodologies has facilitated the acquisition of corresponding transcriptomes and full genomes that in turn has resulted in the availability of huge amounts of sequence information on different structural and regulatory genes dictating the formation of specific specialized metabolites. The information obtained is examined using bioinformatic computer algorithms and, based on gene similarities and the patterns of expression of individual transcripts, predictions on their physiological and biochemical roles are made. Identity and biochemical function of the particular candidate genes are then confirmed by functional expression and complemented with genetic studies or silencing experiments.

A few examples of the isolation and characterization of genes involved in the formation of key terpenoid, alkaloid and other specialized metabolites in aromatic and medicinal plants will be reviewed. The potential of utilizing these genes for the improvement of the quality properties of agricultural produce and for the production of specialty natural products *in vitro* will be discussed.

IL 14

HERBAL EXTRACTS AND BIOACTIVE PLANT METABOLITES IN FUNCTIONAL DRINKS AND BEVERAGES

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Functional beverages are recognized among the fastest growing sector in the food industry today, referring to an unmodified natural food product, as well as product to which a bioactive component was added, removed, replaced or modified by technological, chemical or enzymatic means to provide an added value and/or health benefit. Medicinal and aromatic herbs are widely used in both homemade, traditional beverages and in industrial products of high commercial scale. Of great variety of secondary metabolites present in the plant extracts of functional beverages, enriched drinks and herbal spirits, the phenolic compounds, such as flavonoids and stilbenes, together with plant volatiles, are the mostly assessed, due to their biological activity and its role as natural additives, food stabilizers, flavors, and preservatives [1]. Obtaining of the herbal extracts and bioactive plant compounds for functional foods and beverages is dependent on optimization of extraction technique, solvent, temperature, extraction time, and plant material, as a mixture of many different phytochemicals. Therefore, metabolomics is proposed as a promising approach in chemical fingerprinting for evaluation of the quality, traceability, and safety of functional beverages.

Paper aims to review the current situation and prospects on characterization, use and significance of bioactive plant components and herbal extracts in functional beverages, enriched non-alcoholic and alcoholic drinks (e.g. beer, wine, herbal spirits) in relation to their antioxidant and sensory characteristics, especially in products developed upon richness and special features of indigenous Balkan flora [2]. Application of innovative technological tools in production of functional beverages, such as microencapsulation, was also addressed [3].

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SL 1

ESCULENTOSIDE B INHIBITS LPS-STIMULATED INFLAMMATORY RESPONSE THROUGH NF-KB, ERK1/2, P38, IL-6, IL-1B IN RAW264.7 MACROPHAGE CELLS

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The natural compound esculentoside B (ESB) is isolated from the roots of *Phytolacca americana* L. (Phytolaccaceae). Anti-inflammatory effects of the esculantoside B have been investigated in LPS-induced RAW264.7 macrophage cells. ESB inhibited the production of nitric oxide (NO) and prostaglandin E2 (PGE2) in a dose dependent manner in RAW264.7 macrophage cells. ESB inhibited the gene expression of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2). In addition, ESB suppressed the mRNA expression of inflammatory cytokines IL-1β, IL-6 and TNF-α in LPS-induced RAW264.7 macrophage cells. Moreover, ESB inhibited nuclear translocation of nuclear factor kB (NF-κB) and decreased the expression level of phospho-extracellular signal regulated kinase 1/2 (p-ERK), p-p38.

These results suggest that ESB exhibits anti-inflammatory effects in LPS-induced RAW 264.7 macrophage cells.

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SL 2

NATURAL PRODUCTS: THE ONCOPHARMACOLOGICAL POINT OF VIEW

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Natural products are broadly used food supplements for many reasons, e.g. malignant diseases, too. It is believed that they are not toxic and may have beneficial effects without considerable toxic reactions. More or less this is true for most part of the commercially available products. Standards for food supplements allow significant variations in the content of biologically active compounds. This is specifically true for curcumin preparations. Curcumin is one of the plant compounds which focused our attention as it has apoptosis inducing effects and NF-κB inhibition in many different types of human malignantly transformed cells. Despite of its relatively low bioavailability curcumin has some irrefutable effects in vivo. The talk will give a summary of the investigations performed and particularly published. Another topic of interest represents cannabidiol, which is in the focus of many research groups and preparations containing different quantities of cannabidiol are on the market.

A summary about most important pharmacological activities of cannabidiol will be given together with own experimental data concerning antineoplastic activity in vitro. Other plant products of interest for our research group are justicidin B and total chloroform extract from the stem bark *Kigelia pinnata* (*africana*). Noteworthy, the stem bark extract showed antineoplastic activity in vivo as well. Our experimental experience over the last decade indicates that natural products represent an attractive source of compound/cocktails of compounds with selective antitumor activity.

SL 3

HH_4R ANTAGONISTS FROM NATURAL SOURCES: UTILITY OF BIO-GUIDED FRACTIONATIONS AND IN SILICO TECHNIQUES FOR FACILITATING THE PROCESS

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Human Histamine H_4 Receptor (hH_4R) has attracted considerable interest as a potential target for the treatment of several inflammatory diseases. We have evaluated the activity of seventeen medicinal plant extracts at the hH_4R. The plant extracts displayed poor activity (Ki < 50% at 5 µg/mL), with the exception for the extract of *Curcuma longa* that showed strong activity at the hH_4R (complete inhibition at 10 µg/mL) [1]. Following a bio-guided fractionation, we have isolated and chemically identified three bioactive ingredients [curcumin (**1**), demethoxycurcumin (**2**), and bis-(4-hydroxycinnamoyl)methane (**3**), see Figure 1 below]. The compounds (**1**) to (**3**) showed activity at the hH_4R with estimated 95 % confidence intervals for the K_i value ranging between 4.26 - 6.26 µM (**1**), 6.66 - 8.97 µM (**2**) and 10.24 - 14.57 µM (**3**). The bioactive compounds, which were isolated from Curcuma longa, display different structural features than known ligands and they may provide new directions for selective and specific lead structures for drug development at the hH_4R. *In silico* approach capable to index chemicals for their hH_4R Antagonism, increasing drug candidates' pool, will be described.

Acknowledgements: Part of the work presented here was supported by COST Actions CM1207 and BM0806, as well as by the EU under the project ENPI/2011/259097 that was implemented by Leaders Organization and Galilee Society.

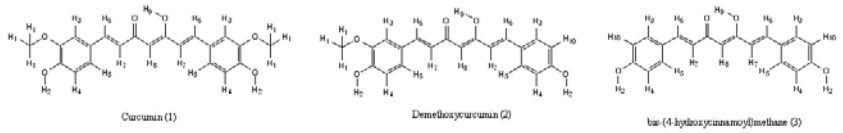

Figure 1. Chemical structures of new hH_4R antagonists.

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SL 4

PHARMACOKINETICS AND HEPATOBILIARY EXCRETION OF POLYHYDROXYLATED AND POLYMETHOXYLATED FLAVONOIDS IN RAT

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Based on the mechanism of metabolic pathway, the polyhydroxylated flavone would be easily conjugated to the phase II glucuronidation mediated by various UDP-glucuronosyltransferases (UGTs) in the liver and intestine. Our hypothesis is that the metabolic stability and oral bioavailability of polymethoxylated flavonoids will be increased. The aim of study is to investigate the pharmacokinetic mechanisms and oral bioavailability of 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-chromenone (luteolin), 2-(3,4-dihydroxyphenyl)-3,7-dihydroxychromen-4-one (fisetin) and 2-(3,4-dimethoxyphenyl)-5-hydroxy-3,7-dimethoxychromen-4-one (retusin), a parallel set of experiments was designed to study the structure–pharmacokinetics relationship of these flavonoids in rats. To determine the level of these flavonoids in the biological samples, a sensitive and rapid UHPLC-MS/MS method was developed according to the FDA's biological method validation guidelines. The experimental protocol was approved by the Institutional Animal Care and Use Committee of National Yang-Ming University, Taipei.

The pharmacokinetic results demonstrated that the elimination half-life of methoxylated flavones was prolonged when compare to the hydroxylated flavones. The oral bioavailability of luteolin was 26 ± 6%. The fisetin glucuronides and sulfates conjugated metabolites were mainly eliminated into bile for hepatobiliary excretion.

Acknowledgements: Funding for this study was provided in part by research grants from the Ministry of Science and Technology of Taiwan.

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SL 5

IMMUNOMETABOLIC EFFECTS OF BERRY ANTHOCYANINS AND THEIR COLONIC MICROBIAL METABOLITES

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Chronic disorders associated with low-grade inflammation are characterized by presence of pro-inflammatory mediators that induce the recruitment and activation of macrophages in the metabolically active tissues. Results from both cohort studies and randomized trials suggested that anthocyanins from berries may lower these risks [1], but the functional group in their structure responsible for this effect has not been characterized. The present study was designed to examine effects of six berries with structurally diverse anthocyanin profiles (normalized to 400 µg/g total anthocyanin content) on development of metabolic risk factors in the C57BL/6 mouse model of polygenic obesity [2]. Diets supplemented with blackberry (mono-glycosylated cyanidins), black raspberry (acylated mono-glycosylated cyanidins), blackcurrant (mono- and di-glycosylated cyanidins and delphinidins), maqui berry (di-glycosylated delphinidins), Concord grape (acylated mono-glycosylated delphinidins and petunidins), and blueberry (mono-glycosylated delphinidins, malvidins, and petunidins) showed a prominent discrepancy between biological activities of delphinidin/malvidin-versus cyanidin-type anthocyanins that could be explained by differences in their structure and metabolism in the gut. Different effects exerted by anthocyanins suggested that the presence of the methylated hydroxyls on the B ring might be critical for their greater biological activity. Numerous anthocyanin metabolites significantly reduced various cytokine and chemokine biomarkers of inflammation, with syringic and ferulic acids producing the greatest effects.

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SL 6

TRYPANOCIDAL ACTIVITY OF WALTHERIONES

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American trypanosomiasis, also known as Chagas disease, is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi*. About 7 to 8 million people are estimated to be infected worldwide, mostly in Latin America where Chagas disease is endemic. To date, benznidazole and nifurtimox are the only two available chemotherapeutic agents. These compounds are effective for the acute phase of the disease. In contrast, during the chronic phase their utility is limited due to low efficacy. In addition, both drugs produce severe side effects occurring in up to 40% of treated patients according to WHO.

Waltheria indica L. (Malvaceae) is a short-lived shrub widespread in subtropical and tropical regions which is used in traditional medicine. Thirty alkaloids were isolated from the roots and aerial parts and among them 20 are new compounds. Four of the most abundant alkaloids (waltheriones C, E, F and G) showed potent *in vitro* activity against the epimastigote (IC_{50} < 10 µM) and intracellular amastigote (IC_{50} < 4 µM) forms of *T. cruzi* with low cytotoxicity (selectivity index > 10) [1, 2]. Preliminary results indicated that waltheriones C, E, F and G were irreversibly cidal to *T. cruzi* amastigotes. Indeed, these waltheriones were able to completely clear out a pre-established infection. The targets of these compounds are not known so far. Several of the waltheriones exhibited a good permeation in the parallel artificial membrane permeability assay (PAMPA) [3]. Further investigations regarding the mechanism of action are ongoing.

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SL 7

ESSENTIAL OIL AND PLANT EXTRACT LOADED BIOPOLYMERS: NATURAL ANTIMICROBIAL AND ANTI-INFLAMMATORY STRATEGIES TO MANAGE SKIN TRAUMAS

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Recently, naturally occurring polymers have been explored in the biomedical and pharmaceutical fields for the development of new drug delivery devices to support tissue regeneration after skin injuries. Among them, sodium alginate and fibroin appeared the most promising, thanks to their biocompatibility, biodegradability and moist absorption behavior. In the skin wound management, absorption of fluids, prevention and control of infections and modulation of the inflammatory response are critical aspects in restoring the normal tissue physiology. Sodium alginate, an anionic polysaccharide derived from *Brown Algae*, and fibroin, extracted from silkworm *Bombyx mori* cocoons, constitute bioactive materials that can be easily processed to obtain various architectures, such as films [1, 2], fibers [3], nanocapsules and hydrogels. By tuning their mechanical properties and their porosity, and by modifying their surfaces with plant extracts and essential oils, engineered systems presenting multiple functionalities have been designed. Lemongrass, lavender, tea tree and cinnamon loaded alginate films [1, 2] and fibers [3] have shown antimicrobial and antifungal proprieties against *E. coli*, *S. aureus* and *C. albicans*. Moreover, pH-responsiveness functionality, crucial in monitoring the proper wound evolution, can be introduced by means of anthocyanins extracted from red-leaf vegetables and fruits. In addition, *in vitro* and *in vivo* anti-inflammatory activity of the above-mentioned systems, conducted by analyzing levels of cytokines (IL-6, IL-8, IL-1β, TNF-α), further confirmed the great potentiality of these naturally-derived engineered systems in assisting the wound healing process.

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SL 8

NOVEL CAFFEIC ACID-CONTAINING ELECTROSPUN FIBROUS MATERIALS COATED WITH POLYELECTROLYTE COMPLEX WITH ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY

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Phenolic compounds of plant origin are attractive for application in medicine and pharmacy due to the set of their valuable biological properties (antioxidant, anticancer, anti-inflammatory and antimicrobial activities). In recent years, the incorporation of natural phenolic compounds in electrospun fibrous materials intended for biomedical applications is increasing [1-3]. In the present study novel fibrous materials from poly(3-hydroxybutyrate) (PHB), quaternized chitosan (QCh), alginate (Alg) or k-carrageenan (Car) and natural phenolic compound caffeic acid (CA) of diverse design were successfully prepared. These materials were obtained by applying electrospinning or electrospinning combined with dip-coating and polyelectrolyte complex (PEC) formation. The release of CA was affected by the fiber composition. The bulk incorporated CA was homogeneously distributed in the fibers and was in the amorphous state, whereas CA was in the crystalline state when loaded in the PEC coating as evidenced by the performed differential scanning calorimetry (DSC) and X-ray diffraction (XRD) analyses. The non-coated and QCh/Alg(Car)-coated PHB fibrous mats loaded with CA were found to kill the Gram-positive bacteria *S. aureus* and the Gram-negative bacteria *E. coli*. Moreover, these materials had capability of suppressing the adhesion of pathogenic *S. aureus* bacteria. It was found that the antioxidant activity of fibrous mats containing a combination of CA and QCh/Car complex was higher than that of CA-containing mats or QCh/Car-coated mats. These properties make the obtained fibrous materials promising as antibacterial dressing materials in the treatment of wound infections.

Acknowledgements: Financial support from Bulgarian National Science Fund (Grant DFNI T02/1 12.12.2014) is gratefully acknowledged.

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SL 9

COMPARATIVE NEUROPHARMACOLOGICAL EVALUATION OF 2*H*-CHROMENE AND COUMARIN SUBSTITUDED HYDRAZIDE-HYDRAZONES

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Backround: Recently, we have synthesized and evaluated hybrid molecules, i.e. 4-chloro-coumarin-hydrazide/hydrazones and 2H-chromene-hydrazide/hydrazones with promising anti-seizure activity. Because of to the structural similarity between the coumarin and the 2H-chromene scaffolds, we wanted to compare the anti-seizure activity spectrum of the two series of hybrid molecules.

Methods: We comparatively tested two series of coumarin and 2H-chromene hydrazide/hydrazones substituted with p-chlorphenyl, 2-furyl, or p-methoxyphenyl groups with maximal electroshock (MES) test, subcutaneous pentylenetetrazole (scPTZ) test, 6Hz test and rotarod test in ICR mice.

Results: 4-chloro coumarin based hydrazide/hydrazone derivatives, which were further substituted either with p-chlorphenyl, 2-furyl, or p-methoxyphenyl, were protective against MES induced seizures (ED50 99.71 mg/kg, 68.66 mg/kg, and 81.29 mg/kg, respectively), and nonprotective against scPTZ. Two of the coumarin/hydrazone derivatives i.e. 2-furyl, and p-methoxyphenyl derivatives showed protective effect in 6Hz test (ED50 137.3 mg/kg and 94.37 mg/kg, respectively).
In contrast, 2H-chromene based hydrazide/hydrazones with either p-chlorphenyl or 2-furyl substitution showed protection both in MES (ED50 87.63 mg/kg and 12.51 mg/kg, respectively), and scPTZ tests (ED50 218.50 mg/kg and 127.10 mg/kg, respectively). The p-methoxyphenyl derivative of 4-chloro-coumarin-hydrazide/hydrazones was protective against 6Hz test only (ED50 1.08 mg/kg). Neither of the tested substances demonstrated positive rotarod test suggesting lack of neurotoxicity.

Conclusion: A different spectrum of neuropharmacological activities of 2H-chromene and coumarin substituted hydrazide-hydrazones was revealed. Further evaluations are needed for elucidating their corresponding mechanism of action.

Acknowledgements: Our work was partly supported with Grant D-74/2017 of the Council of Medical Sciences, MU-Sofia.

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SL 10

NEURO-NUTRACEUTICAL POTENTIAL OF THYME AND SAGE PHENOL-ENRICHED EXTRACTS

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As plant (poly)phenols could be a double-edged sword for human brain, the main goal of the present research activity was to evaluate the neuro-bioactivity of newly prepared phenol complexes from *Thymus vulgaris* and *Salvia officinalis*, two medicinal and aromatic plants with ancient use as culinary spices and herbal remedies. Dried leaves of the plants underwent UAM extraction using solvents with different polarity ($CHCl_3$ and MeOH). The alcoholic extracts were further fractionated to achieve a thyme (TPE) [1] and a sage phenol-enriched extract (SoA541) [2], which were metabolically profiled through LC-ESI-MS/MS techniques. Cytotoxic and oxidant properties of both the extracts were evaluated towards neuronal cell lines. TPE, consisting in sixteen polyphenols, exerted cytotoxic effect at doses of 62.5 and 125 µg/mL being able to increase intracellular ROS levels and to activate caspases. SoA541, which accounted for ~50% of abietane diterpenes and ~40% of phenylpropanoid constituents, exhibited antioxidant effectiveness at dose levels <125 µg/mL and inhibited AChE enzyme to a far greater extent than donepezil. The promising results suggest the need for further research to improve our data and investigations into the potential use of these natural products as neuro-nutraceutical remedies to counteract brain diseases using effective preclinical and clinical investigation tools.

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SL 11

NEUROPSYCHOPHARMACOLOGICAL ASPECTS OF SOME *CRATAEGUS* SPECIES OF TURKEY

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Crataegus species (fam= Rosaceae) have been known as Hawthorn having approximately 200 members worldwide. In Turkey, there are about 20 Crataegus species. *Crataegus* species have been used in traditional medicine for various purposes. Among these purposes, tranquilizing effect on the central nervous system has been traditionally known well.

In our institution's laboratories, we have also made experiments for anxiolytic activities with fruit pulp and seed extracts of *C. monogyna* using hole-board, activity cage and rota-rod tests. Based on these experiments, the extracts of *C. monogyna* have exhibited an anxiolytic-like action on mice [1]. Similarly vitexin, a constituent of *Crataegus* species, has exerted anxiolytic-like action in same experimental sets. We have also demonstrated an antidepressant-like activity using forced-swimming test, tail-suspension and plus-maze experiments [2], in accordance with the anxiolytic-like activity. As antidepressant drugs have analgesic activities, confirming the antidepressant activity, extracts prepared fruit pulps and seeds of *C. monogyna* have been found to possess analgesic activity in hot-plate, tail-clip and acetic acid writhing tests [1]. These effects seem to be related to antioxidant and antiradical activities [3] may be due to their flavonoid contents inhibiting the relevant phosphodiesterase activity.

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SL 12

SULPHONATED MODIFICATION OF POLYSACCHARIDES FROM *PLEUROTUS ERYNGII* AND *STREPTOCOCCUS THERMOPHILUS* ASCC 1275 IMPROVES HEALTH FUNCTIONALITIES AND UPTAKE OF ANTIOXIDANT ACTIVITIES USING CCD AND CACO-2 CELL LINE MODELS

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Polysaccharides extracted from *Pleurotus eryngii* (PEPS) and *Streptococcus thermophilus* ASCC 1275 (EPS) were sulphonated, and compositions were determined and the antioxidant activities of crude and sulphonated polysaccharides (S.PEPS and S.EPS) were investigated using ABTS radical scavenging activity and CCD and Caco-2 cell line models. Anti-inflammatory activities of PEPS and sulphonated PEPS (S.PEPS) were tested on RAW264.7 macrophages in four experimental models. Cytokines of IL-1β, IL-6, IL-10 and TNF-α were quantified by ELISA. Cytokine secretion ratios (IL-1β/IL-10, IL-6/IL-10 and TNF-α/IL-10) were significantly decreased by PEPS and S.PEPS treatments in a dose dependent manner in all four experimental models. The degrees of sulphonation for S.PEPS and S.EPS were 0.73 and 0.37, respectively. Cytokine secretion ratios (IL-1β/IL-10, IL-6/IL-10 and TNF-α/IL-10) were significantly decreased by PEPS and S.PEPS treatments in a dose dependent manner in all four experimental models. Anti-inflammatory activity of S.PEPS was significantly stronger than that of PEPS and the S.PEPS exhibited stronger reactive oxygen species (ROS) and nitric oxide (NO) inhibition activities compared with those of PEPS. Additionally, S.PEPS also had significantly stronger antiproliferative activities on both Caco-2 and HepG2 cells compared with those of PEPS. The results showed that S.PEPS and S.EPS had significantly higher scavenging capacities than those for PEPS and EPS using ABTS radical scavenging activity. Reactive oxygen species (ROS) and malondialdehyde (MDA) in H_2O_2 treated CCD 841 CoN (CCD) and Caco-2 cells were significantly inhibited by PEPS, EPS, S.PEPS and S.EPS as compared with that of H_2O_2 group as a control. Additionally, S.PEPS also had significantly stronger antiproliferative activities on both Caco-2 and HepG2 cells compared with those of PEPS. Results indicated that sulphonation improved the anti-inflammatory and antiproliferative activities of PEPS significantly. Additionally, S.PEPS and S.EPS significantly improved superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in H_2O_2 treated CCD and Caco-2 cells compared with those of PEPS, EPS and H_2O_2 groups. Results indicated that sulphonation was effective in improving the antioxidant activities of both PEPS and EPS.

SL 13

AMELIORATIVE EFFECTS OF *ARONIA MELANOCARPA* FRUIT JUICE IN A RAT MODEL OF INFLAMMATORY BOWEL DISEASE

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Trinitrobenzensulfonic acid (TNBS) is commonly used to induce an experimental inflammatory bowel disease model [1]. *Aronia melanocarpa* fruit juice (AMFJ) possesses potent antioxidant and anti-inflammatory properties due to its polyphenolic ingredients [2].

The aim of this study was to evaluate the effect of AMFJ in a rat TNBS-induced colitis model using criteria for microscopic scoring of colonic damage and to compare AMFJ effect with that of sulfasalazine.

Male Wistar rats (n=72) were divided into 6 experimental groups. Colitis was induced by TNBS applied in the colon. Control rats received the vehicle. Oral treatment was performed till the 14th experimental day with distilled water (groups Control and TNBS), AMFJ at doses of 2.5, 5 and 10 ml/kg (groups TNBS+$AMFJ_{2.5}$, TNBS+$AMFJ_5$, TNBS+$AMFJ_{10}$) or sulfasalazine 400 mg/kg (group TNBS+S). During the histopathological examination, colonic damage score was assessed applying the method of Gaudio [3].

The results showed that TNBS caused a diffuse or zonal destruction of intestinal wall and inflammatory cell infiltration reaching the muscularis mucosae or the submucosa or muscularis propria. AMFJ dose-dependently reduced the colonic damage. Thus, the inflammatory infiltration score of TNBS+$AMFJ_{10}$ group was significantly lower ($p<0.05$) and the mucosal destruction scores of TNBS+$AMFJ_5$ and TNBS+$AMFJ_{10}$ groups were also significantly lower ($p<0.01$) than those of TNBS group. Sulfasalazine decreased slightly but not significantly the microscopic criteria of colonic damage.

In conclusion, AMFJ ameliorated the microscopic signs of TNBS-induced colitis, the effect being higher than that of sulfasalazine.

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SL 14

GYPSOPHILA TRICHOTOMA EXTRACT AMELIORATES ETHANOL INDUCED BRAIN INJURY IN RATS

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The current study investigates the effect of defatted extract, isolated from *Gypsophila trichotoma* Wend. on behavioral and some biochemical parameters measured in the brain of rats with multiple ethanol treatment.

Methods and results: Thirty six male Wistar rats were divided into 6 groups (n=6): control; animals treated with ethanol (45%, oral-gavage, 14 days); animals treated with *G. trichotoma* extract (100 mg/kg oral-gavage, 14 days); animals treated with silymarin (100 mg/kg p.o. 14 days); the animals in group 5 and 6 have been treated with ethanol in combination with *G. trichotoma* extract and silymarin, respectively. Twenty four hours after the last treatment the animals in the ethanol group showed anxiety, piloerection, unusual body posture, associated with withdrawal. Such symptoms have not been observed among the animals in the combination groups. In the ethanol group this behavior was accompanied with biochemical changes in the brain, discerned by increased activity of the enzymes nNOS and GS and by an increased amount of MDA and decreased levels of GSH. It is interesting to be noted that *G: trichotoma* extract protected the brain against ethanol injury, evidenced by reversed activity nNOS and GS as well as GSH and MDA quantity to the control levels. The effects were commensurable with the effect of silymarin, used as a positive control.

Conclusion: On the basis of the results obtained under the conditions of this study we could conclude that the defatted extract, isolated from *Gypsophila trichotoma* ameliorates ethanol induced brain injury.

SL 15

ANTILEISHMANIAL ACTIVITY OF *CASSIA FISTULA* L., *MORUS NIGRA* L. AND *ZIZIPHUS JUJUBA* PLANT EXTRACTS

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Aim: To investigate the *in vitro* and *in vivo* antileishmanial activities of extracts of *Cassia Fistula* L., *Morus Nigra* L. and *Ziziphus Jujuba*.

Methods: The methanolic extracts of all three plant materials at concentrations of 500 – 2000 µg/mL were checked for their *in vitro* antileishmanial effects on *L. tropica* KWH23 promastigotes for 24-48 hours, having negative control and Amphotericin B as standard drug. For *in vivo* antileishmanial activity, the given leaves extracts were tested against *L. tropica* infected albino mice [1].

Results: For *Ziziphus jujuba* leaves, mean inhibition of extracellular promastigotes at 500, 1000, 1500, 2000 µg/mL after 48 hours were 92.1 ± 0.02, 95.00 ± 0.05, 97.09 ± 0.07 and 98.05 ± 0.05 % respectively. After 8 weeks, mean lesion size decreased from 0.8 ± 0.1 mm to 0.40 ± 0.2 mm ($p < 0.01$), and cure at 180 mg/Kg against intracellular amastigotes in albino mice was 90.00% (95% CI = 80.05-97.00)

Conclusion: The results obtained in this study show that *Ziziphus jujuba* leaves possesses significant antileishmanial activity.

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SL 16

ALL-ON-ONE-PLATE FOR THE NATURAL PRODUCT CHEMIST: FROM SAMPLE PREPARATION VIA CHROMATOGRAPHY VIA EFFECT-DIRECTED ASSAY TO HRMS

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Imagine a streamlined workflow to be more effective: From the extract to the sum formula of active compounds in half a day? Achievable. The chromatographic layer is used only once, sample preparation on the plate and chromatography can be combined in one step, nothing is lost or out-of-focus. This also means that samples can be analyzed as natural as possible. Matrix can remain at the start zone or be shifted to the front, even be applied as area to cope with a heavy matrix load. It is a robust technique that can cope with varying matrices and a wide range of analyte concentrations, as the application volume can be chosen from 0.1 µL to 1 mL. The whole extract is accessible for the non target effect-directed detection. The chromatogram is immersed into the cell suspension and this seeded plate is evaluated instantly or incubated for few hours, depending on the bioassay or the enzymatic assay selected. After a visualization step, only effective compound zones are detected in a complex sample. Thus, a complex sample consisting of up to 4000 single compounds can be reduced to the essential, the active compounds. Still from this (bio)autogram, HRMS spectra are directly recorded by scanning Direct Analysis in Real Time (DART)-MS. The evaluation is supported by statistical evaluation tools. The outcome is a hyphenated workflow highly streamlined for discovering novel natural products. If compared to other techniques, half a day is fast for parallel analysis ca. 18 more samples along this workflow. Effect-directed analyses or bioprofilings substantially support complex tasks.

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SL 17

STRUCTURE AND SYNTHESIS OF BIOACTIVE COMPOUNDS FROM SOUTH AFRICAN MEDICINAL PLANTS

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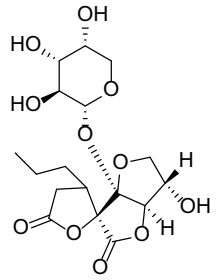
Many plant-derived natural products have anti-infective properties such as antibacterial, antifungal, antiviral and antimalarial activity. In our search for bioactive compounds from South African plants, we have investigated the phytochemistry of *Hypericum roeperianum* Schimp. ex A.Rich. (Hypericaceae), *Gnidia polyantha* Gilg and *Gnidia splendens* Meisn. (Thymelaceae). In this presentation the structure and activity of the isolated compounds will be discussed.

H. roeperianum yielded phloroglucinol and pyrone derivatives such as 3-geranyl-2,4,6-trihydroxybenzophenone and hyperenone B, known compounds with antimicrobial activity. The latter compound also has anti-HIV properties. In an effort to increase the activity of the compounds, a number of fluorinated derivatives have been synthesized.

The aerial parts of *G. polyantha* yielded a number of phenolic compounds and also novel ascorbic acid-derived polyols (e.g. **1**). From the roots of *G. splendens*, three daphnane-type diterpene ortho esters (e.g. **2)** with potent antiviral properties have been identified.



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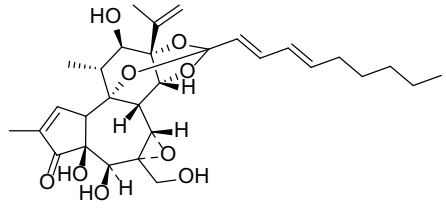

2

SL 18

ASSESSMENT OF ROASTING EFFECT ON FUNCTIONAL FOODS AROMA USING SPME AND ANALYZED USING CHEMOMETRIC TOOLS

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Aim of work: Assessment of the volatiles compositions of *Nigella sativa* seeds in the context of different origin and *Ceratonia siliqua* (carob) legumes using headspace-SPME coupled to GCMS to reveal the impact of oil extraction and roasting on their respective aroma as analyzed using chemometric tools.

Materials and methods: Specimens for both *Nigella* and Carob roasted and unroasted ones were subjected to aroma analyses using solid phase microextraction (SPME) coupled to GC/MS and analyzed using chemometrics.

Results: A total of 34 and 31 volatiles were identified in both *Nigella* seeds and Carob pods, respectively. In *Nigella*, aromatics was the major class for both raw and roasted seeds *ca.* 99.6 and 54%, respectively with thymoquinone amounting for the major volatile and found almost exclusively in *N. sativa* accessions suggesting it can serve as a potential marker for distinguishing *Nigella sativa* from its other allied drugs. In response to roasting, thymoquinone levels declined concurrent with increase in its precursor p-cymene and genetoxic volatile "estragole".

With regards to carob pods aroma profile, unroasted pods was found more enriched in volatiles compared to roasted ones. Short chain fatty acids were the major class (75-87%), whereas presence of *(E)*-cinnamldehyde in raw fruit is likely to mediate for spice cinnamon flavor of carob. In roasted seeds, increase in pyranone levels is likely to account for the characteristic malt sweet odor of heated carob fruits. Such analytical approach could be a key understanding for the organoleptic characteristics of raw versus heated pods used in beverages.

SL 19

PROANTHOCYANIDINS – A CHALLENGE IN NATURAL PRODUCT RESEARCH

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Proanthocyanidins (condensed tannins) have a huge chemical complexity and diversity derived from the hydroxylation pattern of their constitutive units, stereochemistry of the central ring, location and stereochemistry of interflavonoid bonds, esterification with gallic acid, glycosylation and different degrees of polymerization. Different extraction and purification methods were used to isolate proanthocyanidin-rich extracts from various plant sources. Important structural information for proanthocyanidin identification was obtained by LC-ESI-MS. RP-LC coupled with DAD-ESI-MS enabled the separation and identification of monomers to trimers as individuals in different plant extracts. B-type procyanidin dimers and trimers were separated and identified in extracts of *Pinus brutia*, *Pinus sylvestris* and *Cedrus brevifolia* bark, *Crataegus pentagyna*, *Viburnum opulus* and *Sorbus aucuparia* fruits [1, 2]. B-type prodelphinidin oligomers, made up of catechin/epicatechin and gallocatechin/epigallocatechin units, were detected in *Abies alba* bark [3]. By NP-LC coupled with DAD-ESI-MS, B-type procyanidins up to heptamers were separated according to their degree of polymerization in a *P. sylvestris* bark extract. Comprehensive two-dimensional LC significantly improved the characterization of proanthocyanidin complex mixtures. Despite a low bioavailability, proanthocyanidins proved to be effective in animal and human studies. In an animal model of L-NAME-induced hypertension, a procyanidin-rich extract isolated from *Pinus brutia* bark significantly attenuated the deleterious effects of L-NAME on antioxidant status, dyslipidemia and hypertension. Proanthocyanidin *in vivo* bioactivity seems to be mainly due to their gut microbial metabolites, many of them exhibiting local and systemic antioxidant and anti-inflammatory effects.

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SL 20

PHYTOCHEMICAL CHARACTERIZATION OF ENDEMIC *MARRUBIUM SIVASENSE* AYTAÇ, AKGÜL & EKICI AND ITS POTENT AGAINST OXIDATIVE DAMAGE, A-AMYLASE, LIPOXYGENASE, XANTHINE OXIDASE AND TYROSINASE ENZYMES

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The main goal of the present study was to get phytochemical characterization of volatile and non-volatile constituents of *Marrubium sivasense,* a local endemic species to Sivas province of Turkey, and investigate them *in vitro* for antioxidant, antidiabetic, antiinflammatory and antimelanogenesis activities. The essential oil (EO) and the methanol extract (ME) of *M. sivasense* were analyzed with GC-FID, GC/MS and LC-MS/MS techniques. The oil was characterized with high abundance of sesquiterpenes (66.5%) with bicyclogermacrene (19.0%), β-caryophyllene (19.0%), germacrene D (15.1%) and (*Z*)-β-farnesene (7.4%) and caryophyllene oxide (5.3%) as main constituents. From the fatty acids, hexadecanoic acid was detected in 6.6%.

The extract was found to be rich with phenylethanoid glucosides forsythoside B, verbascoside and leucoseptoside. The antioxidant activities of EO and ME were assessed by both chemical and enzymatic methods against $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals, linoleic acid hydroperoxides and superoxide anion radical ($O_2^{\cdot-}$) generated by xanthine - xanthine oxidase (XO) system. The ME showed free radical scavenging activity (IC_{50} 0.34 mg/mL), Trolox equivalent antioxidant capacity (2.3 mM±0.04) and inhibited lipid peroxidation (%Inh 43±1.26). The EO and ME demonstrated hypoglycemic activity (%Inh 81.25±1.69 and %Inh 49.67±1.09) *via* inhibition of α-amylase.

The antiinflammatory effect of the EO tested *via* inhibition of 5-LOX enzyme was found as %Inh 46.69±0.74. The inhibitory effect of *M. sivasense* on oxidation of L-DOPA was found as %Inh 23.3%.

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SL 21

LC-MS/MS PROFILING OF AN AQUEOUS CHERRY EXTRACT WITH PROMISING RADIOMODULATORY PROPERTIES

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Human exposure to ionizing radiation is ubiquitous because of its natural occurrence and widespread use in diagnostics and therapy. Therefore, developing radioprotectors and radiorecovery drugs is of great importance in view of their potential application during both planned radiation exposure (e.g. radiotherapy) and unplanned radiation exposure [1]. Indeed, the actual drug scenario lacks drugs acting as both tumour radiosensitizer and normal cells radioprotector [2]. In this context, an aqueous extract (PaDRw) from *Prunus avium* L. cv. Della Recca, a Southern Italy cherry cultivar, was investigated for its capacity to modify the response of SH-SY5Y cell line to ionizing radiation. PaDRw showed a double-sword behavior, as it seemed to act as radioprotector at lower doses (25 and 50 µg/mL) and radiosentisizer at higher tested doses (400 and 500 µg/mL). In particular, PaDRw 500 µg/mL dose level was shown to highly activate caspase-3, both in irradiated and not irradiated cells, showing promising apoptosis-inducing effects. In order to get insights into the chemical composition of PaDRw, the simplification of the complex sample in two fractions and their LC-UV-MS/MS analyses, proved to be efficient also in the disclosure of lower constituents. Quantitative analysis demonstrated that about 63% of the whole PaDRw extract was constituted by hexitol, followed by ~22.8% of fructose and ~10.7% of glucose. Phenol compounds, mainly chlorogenic acids and flavonoids, which accounted only for about 2.2%, were hypothesized to be the main actors in PaDRw-induced radiomodulation.

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SL 22

EXACT MASS AND ESI-QTOF-MS FRAGMENTATION OF BIOACTIVE AMARYLLIDACEAE ALKALOIDS

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Amaryllidaceae alkaloids, which belong exclusively to the Amaryllidaceae plant family, are isoquinoline alkaloids with a great variety of biological and pharmacological activities, including antiparasitic, antiviral, antitumoral and inhibition of acetylcholinesterase. Galanthamine, extracted from *Leucojum* or *Galanthus* species, is currently commercialised for the palliative treatment of mild to moderate states of Alzheimer's disease [1]. These compounds can be classified into 9 skeleton types, although recently dinitrogenous plicamine-type alkaloids have also been included.

As the number and quantity of these alkaloids can vary considerably among different species and populations, rapid and sensitive methods are required for their identification in plant extracts. GC-MS is frequently used for Amaryllidaceae alkaloid analysis without a derivatisation step [2], although some show low stability and thermal degradation under GC-MS conditions. ESI-Q-ToF-MS provides important structural information such as exact mass, molecular formula and ESI fragmentation [3]. There are few references in the literature to ESI fragmentation and exact mass of Amaryllidaceae alkaloids.

Amaryllidaceae alkaloids previously isolated from a variety of Amaryllidaceae plants and identified by NMR, CD and GC-MS in our laboratory were analysed using ESI-Q-ToF in the positive mode by direct injection in a methanolic solution.

High resolution exact mass and ESI-MS^n fragments of 56 alkaloids of lycorine-, homolycorine-, galanthamine-, haemanthamine-, and tazettine-types, together with some dinitrogenous alkaloids and epimacronine were determined with ESI-QToF-MS. With this structural information, it was possible to distinguish some stereoisomers of haemanthamine-type alkaloids, which is not possible by GC-MS.

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SL 23

A NEW LIQUID CHROMATOGRAPHY-HIGH RESOLUTION ORBITRAP MASS SPECTROMETRY-BASED STRATEGY TO CHARACTERIZE GLUCURONIDE OLEANANE-TYPE TRITERPENOID CARBOXYLIC ACID 3, 28-*O*-BIDESMOSIDES (GOTCAB) SAPONINS

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Glucuronide **O**leanane-type **T**riterpenoid **C**arboxylic **A**cid 3, 28-**B**idesmosides (GOTCAB) saponins are bioactive natural compounds spread in advanced groups such as Caryophyllideae, Rosideae and Asterideae. The high complexity of GOTCAB occurring as closely related isobaric and positional isomers is a challenge in their separation and identification. In this study, the fragmentation behaviors of 41 GOTCAB from methanol-aqueous root extract of *Gypsophila glomerata* Pall ex M. B. (Caryophyllaceae) were investigated using ultra-high performance liquid chromatography (UHPLC) coupled with hybrid quadrupole-Orbitrap high resolution mass spectrometry (HRMS). On the basis of the accurate mass measurements, fragmentation patterns in MS/MS analyses and comparison with previously isolated authentic references, a total of 41 GOTCAB saponins were identified or tentatively elucidated in *G. glomerata* roots, including 12 pairs of isobars and 17 new saponins. Possible fragmentation pathways for three groups of GOTCAB are suggested. The group I appeared to be GOTCAB of gypsogenin with two carbohydrate chains: branched trisaccharide at C-3 and tri- to hexa-saccharide attached to C-28 of the aglycone through a deoxyhexose residue. Saponins with monoacetylated (group II) or sulfated (group III) C-28 chain were evidenced, as well as quillaic and oleanolic acid GOTCAB. It seems that the most of *G.glomerata* saponins occur as isobaric pairs.

For the first time, triterpenoid saponins in *G. glomerata* roots were characterized. A new liquid chromatography – high resolution Orbitrap mass spectrometry acquisition strategy would be important for the structural elucidation of GOTCAB in plant extracts.

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SL 24

CHEMICAL DIVERSITY, NMR DATA AND BIOLOGICAL ACTIVITIES OF POLYMETHOXYFLAVONES FROM *GARDENIA* BUD EXUDATES

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The genus *Gardenia* (Rubiaceae) comprises about 500 species spread over tropical regions of Asia, Africa and Australia. Some species produce a yellowish exudate that covers buds and young leaves. This exudate, sometimes called oleoresin or gum, is used in traditional medicine especially in India, where it is called Dikamali, for several indications including gastric and CNS disorders [1]. Considered as a renewable plant part, its harvest in moderate amount does not damage the tree.

Chemical studies performed on this raw material have led to the identification of polymethoxyflavones (PMF), responsible for the yellow colour, along with several types of triterpenes.

This communication will focus on several aspects of PMF isolated from *Gardenia* bud exudates. First, chemical diversity will be described. Depending on the species, various substitution patterns were reported. Nevertheless, a methoxyle group in positions C-3 and C-6 of the flavone core often occurs, leading to santin-type derivatives.

NMR data of some PMF will then be presented, to depict especially the importance of some experiments, mainly COSY-LR and HMBC, in the structure determination of isolated compounds.

Biological activities on several targets have been evaluated, including antiproliferative, antioxidant, antiangiogenic or anti-parasite. In some cases, structure-activity relationships have been established and will be displayed as a conclusion [2, 3].

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SL 25

LC-MS PROFILING WITH *IN VITRO* BIOLOGICAL ACTIVITIES OF *GYPSOPHILA* SPECIES: NOVEL FUNCTIONAL PRODUCTS FROM NATURE

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Methanol-aqueous extracts of three *Gypsophila* species, *G. glomerata* (GGE), *G. trichotoma* (GTE), and *G. perfoliata* (GPE) were investigated for antioxidant potential using different *in vitro* models, as well as for phenolic and flavonoid contents. The possible anti-cholinesterase, anti-amylase, anti-glucosidase and anti-tyrosinase activities of extracts were also tested. The flavonoid variability was analyzed using ultra-high performance liquid chromatography (UHPLC) coupled with hybrid quadrupole-Orbitrap high resolution mass spectrometry (HRMS). On the basis of the accurate mass measurements, fragmentation patterns in MS/MS spectra and comparison with authentic standards, a variety of flavonoids was identified or tentatively elucidated in *Gypsophila* extracts. GGE was composed of 11 *C*-glycosylflavones and 4 *O*-glycosylflavonoids, including 2″-*O*-pentosyl-6-*C*-hexosyl-apigenin/methylluteolin, as well as their mono(di)-acetyl derivatives. Assayed extracts shared 2″-*O*-pentosyl-homoorientin together with the common homoorientin, orientin, isotitexin and vitexin. 6-*C*-hexosyl-8-*C*-pentosyl derivatives of luteolin, methylluteolin and apigenin were found in GPE. GPE exhibited remarkable DPPH (28.53 mgTEs/g) and ABTS (72.59 mgTEs/g) scavenging activity, as well as cupric (87.88 mgTEs/g) and ferric (48.99 mgTEs/g) reduction ability. Moreover, GGE demonstrated the highest antioxidant capacity in phosphomolybdenum test (1.13 mmol TEs/g) and acetylcholinesterase (1.59±0.09 mg GALAE/g) and α-glucosidase (18.01±0.02 mmol ACAEs/g) inhibitory potential. GPE was more effective as tyrosinase inhibitor (8.23 mgKAEs/g). Total phenolics contents were ranged from 17.07±0.23 mgGAE/g (GTE) to 21.60 mgGAEs/g (GPE). *C*- and *C,O*- glycosylflavones had function for the observed antioxidant and enzyme inhibitory activity.

Therefore, *G. glomerata*, *G. trichotoma* and *G. perfoliata* could be considered as potential candidates for designing novel functional applications.

SL 26

SAMBUCUS EBULUS L. FRUIT AQUEOUS INFUSION MODULATES NFKB, GCLC AND GPX GENES EXPRESSION IN A MODEL OF LPS CYTOTOXICITY IN J774A.1 MACROPHAGES

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Sambucus ebulus L. fruits have been used in the traditional medicine for treatment of disorders accompanied by inflammation and cell death. In the searched for scientific evidencefor the effect of *S. ebulus* fruit aqueous infusion (SEFAI) a model of lipopolysaccharides(LPS)–inducedcytotoxicity in J774A.1 macrophages was used to explore changes in gene expression levels of proteins related to oxidative stress-mediated signal transduction pathways: transcription factor NFκB and antioxidant enzymes glutamate-cysteine ligase catalytic subunit (GCLc) and glutathione peroxidase (GPx).

Real time qPCR was used to measurethe gene expression levelsin macrophages treated with increasing concentrations of SEFAI +/- LPS (*E. coli*, 026:B6). Cell viability was assessed by MTT reduction assay.

SEFAI in concentrations from 0.625% to 12.5% in theculture media was found to be non-toxic for the macrophages. LPS (200 ng/ml) andSEFAI/-LPS when applied alone in increasing concentrations significantly up-regulated NFκB ($p<0.001$), GPx ($p<0.001$) and GCLc ($p<0.001$) mRNA transcriptionmore than 300%. Pretreatment with SEFAI/+LPS significantly reduced LPS (200 ng/ml) stimulated transcription of NFκB ($p<0.01$) and GPx ($p<0.01$) by 70%, and GCLc ($p<0.05$) by 37%. NFκBmRNA expression significantly correlatedwith that of GCLc ($r=0.66$, $p<0.05$), and GPx ($r=0.79$, $p<0.01$) in all groups.

This study analyzes for the first time the effects of SEFAI in a model of LPS-stimulated cells. *S. ebulus* may alter the expression levels of oxidative stressand inflammation related genes, possibly by modulating NFκB mRNA expression.

SL 27

HOW NATURAL TERPENES INFLUENCE MYCOBACTERIAL CELL INTEGRITY?

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The natural terpenes are known for their antimicrobial properties and the detrimental effect on structure and function of microbial membrane and cell wall is thought to explain their antimicrobial action [1]. Our previous study showed that terpenes caused the morphological changes in mycobacterial cells suggesting that they may affect the cell wall synthesis/maintsenance pathways with the consequent changes on cell permeability and microbial death [2]. To verify this hypothesis we examined: 1) the expression of decaprenylphosphoryl-d-ribose oxidase (DprE1) involved in the biosynthesis of decaprenylphosphoryl-d-arabinose (DPA), an essential and abundant component of the mycobacterial cell wall, as well as 2) expression of ClgR which in *M. tuberculosis* controls a larger network of protein homeostatic and regulatory systems responding to stress conditions, both under the influence of natural terpenes. Additionally we checked if terpenes are removed from the cells by efflux pumps and if synergistic action of terpenes and tuberculostatic antibiotics exists in case of clinical strains. The quantitative PCR (QPCR) was used for determination of genes expression under the influence of sub-inhibitory concentrations of terpenes, while the log2 dilution method was applied for examination of combinations of terpenes and tuberculostatic antibiotics against panel of clinical strains of *M. tuberculosis*. The fractional inhibitory concentration index (FICI) was used for characterization of observed interactions. Verapamil was utilised as an efflux pump blocker.

QPCR experiments showed that some of the tested terpenes may significantly affect amounts of transcripts for dprE1 and/or clgR genes in *Mycobacterium tuberculosis* stain H37RAa. The most active compound-R-limonene increased expression of clgR gene by the factor of 1.93 and

lowered number of transcripts for dprE1 by the factor of 0,6 respectively. What is more, our experiment revealed that terpenes are not the substrates of efflux pumps. The synergistic action between terpenes and tuberculostatis antibiotics was observed only in case of reference avirulent strain H37Ra ATTC 25177 with regard to ethambutol and rifampicin, whereas for clinical isolates the result was indifferent.

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SL 28

ANTI-ANGIOGENIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF FRACTIONS FROM *VITEX AGNUS CASTUS* FRUITS

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Therapeutic angiogenesis inhibition is a promising strategy to treat cancer and other diseases [1]. Nowadays, it has been spent a great effort in discovering novel anti-angiogenic drugs. Although the anticancer activity of *Vitex agnus castus* fruits (chasteberries) has been already reported [2], no research on their potential anti-angiogenic activity has been yet addressed. To this purpose, *n*-hexane (EX), chloroform (CH), ethyl acetate (AC), *n*-butanol (BUT) and water (W) fractions obtained from a chasteberry methanol extract were preliminarily tested (50 µg/mL) on zebrafish embryos by the endogenous alkaline phosphatase (EAP) assay [3]. Results showed that only CH and AC fractions inhibited vessel formation in the exposed embryos (60% and 27%, respectively) as compared to control. The potential anti-angiogenic activity of chasteberry active fractions was also confirmed by the chick chorioallantoic membrane (CAM) assay, since the best anti-angiogenic effects were observed for CH fraction (50 µg/egg), due to the marked reduction of microvasculature in the area of application of the CAM and the decrease of the total haemoglobin content. The anti-angiogenic effects of the active fractions, CH and AC, encouraged us to screen their phytochemical composition by reversed phase-high performance liquid chromatography coupled to photodiode array and electrospray ionisation-mass spectrometry detection (RP-HPLC-PDA and RP-HPLC-MS). Well-known bioactive constituents, such as penduletin, casticin, orientin, and vitexin, were detected in both fractions, while hydroxytyrosol and tyrosol were abundantly found only in AC fraction.

In conclusion, based on the preliminary results here presented, chasteberry extracts can be considered a valuable source of angiomodulators.

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SL 29

BIOACTIVE COMPOUNDS FROM MARINE MACROALGAE AND THEIR HYPOGLYCEMIC HEALTH BENEFITS

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Diabetes mellitus is a group of chronic metabolic disorders characterized by hyperglycemia due to defects in insulin action and/or secretion. It is a worldwide problem which has led to ill health and premature mortality for many people, and the number of diabetes cases has been rising sharply. Unluckily, many conventional antidiabetic agents either show limited efficacy or serious mechanism-based side effects. Marine macroalgae possess tremendous nutritional value and have a wide range of bioactivities, including hypoglycemic property. Because of this, an increased interest in various bioactive natural products from marine macroalgae, as a potential source of effective antidiabetic agents, has been observed in recent years. This review focuses on marine macroalgae and macroalgae-derived bioactive compounds, e.g., fucosterol, phlorotannins, polyphenol, bromophenols, fucoidan, sulfated polysaccharides, carotenoid pigments and fucoxanthin, as potential antidiabetic agents, and also discusses their possible mechanisms of action [1, 2].

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SL 30

ANTIMUTAGENIC VS MUTAGENIC PROPERTIES OF MONOTERPENES

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The aim of antimutagenesis studies is to identify substances with antimutagenic potential and to understand the mechanisms of their action. The use of plant antimutagens as dietary supplements or auxiliary medicines could prevent mutation related diseases, including cancer.

In our laboratory differently prepared and chemically analyzed extracts of medicinal and aromatic plants and their pure constituents were screened for antimutagenic activity using bacterial reversion tests. Significant antimutagenic properties against UV-irradiation and 4-nitroquinoline N-oxide (4NQO) were established for essential oil of sage (*Salvia officinalis* L.), camphor, eucalyptol, thujone and linalool. In addition, essential oil of basil (*Ocimum basilicum* L.), linalool, eucalyptol and myrcene exhibited protective effect against oxidative mutagens.

For the evaluation of molecular mechanisms of antimutagenesis a comparative study in bacteria and mammalian cell lines was conducted and different genetic end-points were monitored. Obtained results indicated that protective effect of myrcene, linalool and eucalyptol against *t*-BOOH is mainly based on ROS scavenging activity; however, in high concentrations they acted as pro-oxidants and induced DNA damage. On the other hand, protective effect of camphor, eucalyptol and thujone against UV and 4NQO involved increased efficiency of error-free DNA repair. However, high concentrations of camphor, eucalyptol and thujone were genotoxic, indicating hormesis phenomenon: by making a small amount of DNA lesions, the three monoterpenes stimulate error-free DNA repair and induce adaptive response, providing protection against more potent mutagens such UV and 4NQO.

Although studied monoterpenes could be interesting for further chemoprevention studies, their genotoxicity must be carefully analyzed.

SL 31

SIDERITIS CLANDESTINA CHEMOTYPES AND ANXIOLYTIC EFFECTS IN AGED MICE

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Sideritis L. (Lamiaceae) comprises of more than 150 species; most of them are known as mountain tea and have been used not only for its exquisite flavor and aroma, but also as a calmative.

We focused our studies on *Sideritis clandestina* ssp. *peloponnesiaca,* an endemic taxon in northern Peloponnesus, Greece. Isolation of its essential oil via hydrodistillation of its leaves and flowers gave a low yield (<0.12%); about 65 components, mostly monoterpenes, were identified via GC-MS. To increase the yield and miniaturize the procedure, ultrasound-assisted extraction (UAE) as well as the effect of pretreatment with cellulase, hemicellulase and pectinase or just acidic buffer for 75 min at 37˚C was investigated. Both enzymatic and acidic treatments prior to hydrodistillation or UAE were more efficient in comparison to the respective techniques without pretreatment; however, the results of the enzyme-assisted procedures were not significantly different from acidic pretreatment. Acidic pretreatment prior to UAE was applied to screening various populations (3 g each sample), revealing their chemotypes.

The ethylacetate extract was administered intraperitoneally (30 and 100 mg/Kg body weight) to aged Balb-c mice (15-16 months old) for three consecutive days. After a 24 h period the open field test was repeated and anxiolytic activity of the extract was noticed only in the 100 mg/kg dose. The administration of the plant extract significantly reduced the activity of both isoforms (salt- and detergent-soluble) of acetylcholinesterase in the cerebellum and the striatum. In conclusion, the results showed that the ethylacetate extract provokes anxiolytic behavior in aged mice and inhibits in a tissue-specific manner the cerebral acetylcholinesterase activity.

SL 32

HAZELNUT (*CORYLUS AVELLANA* L.) BY-PRODUCTS: FROM WASTES TO RESOURCE OF CHEMOPREVENTIVE AND ANTI-*CANDIDA ALBICANS* POLYPHENOLS

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Hazelnut (*Corylus avellana* L., Betulaceae) is one of the most popular edible nuts used as whole fruit (raw or roasted) and as an ingredient in processed foods. A huge amount of by-products, namely shells and skins, are obtained from the kernel industrial processing. The disposal regarding this biodegradable waste material represents an environmental and economic problem for the hazelnut-industry, and its recovery and recycling may be of a great interest. In recent years, attention has been paid to the hazelnut wastes as sources of bioactive compounds with beneficial effects on human health [1]. The present research aimed to study polar extracts from the shells (HSE) and skins (RHS), kindly supplied by a food industry. The analytical investigation led to the isolation and NMR characterization of neolignans, phenolic compounds, and a diarylheptanoid from HSE. Among them the major constituents were quantified (HPLC-DAD method). RHS appeared rich in oligomeric proanthocyanidins (PAs) mainly constituted by B-type oligomers of (epi)-catechin. Also A-type PAs were detected and (epi)-gallocatechin and its gallate derivatives was identified as monomer units by UHPLC-UV, FIA-HRMS and UHPLC-MS/MS techniques. HSE exhibited a concentration-dependent inhibitory effect on melanoma cancer and cervical carcinoma cell growth showing a pro-apoptotic effect. The activity relies on the presence of neolignans and gallic acid. RHS was effective against *Candida albicans* SC5314, due to its PAs-rich fraction, as determined by the micro-broth dilution method and Candida morphological analysis [2, 3]. Our results showed that hazelnut wastes can be considered as newsworthy sources of bioactive compounds with promising chemopreventive and antifungal properties.

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SL 33

SCREENING AND IDENTIFICATION OF GALANTAMINE-PRODUCING ENDOPHYTES FROM *GALANTHUS NIVALIS* AND *NARCISSUS TAZETTA* (AMARYLLIDACEAE) GROWING WILD IN IRAN

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Galantamine (GAL) is an important acetylcholinesterase (AChE) inhibitor that is obtained naturally from the bulbs and flowers of Amaryllidaceae members [1]. GAL is well-known therapeutic agent in Alzheimer's disease (AD) because of its central cholinergic effects [2]. GAL has been currently extracted from the plant sources or produced synthetically for pharmaceutical use [3]. Limited supply of the natural source and high cost of synthetic production has led to a search for alternative sources of this valuable compound. In the present study, a total of 15 endophytic fungi (EF) from the bulb of *G. nivalis* and 10 endophytic bacteria (EB) from the bulb of *N. tazetta* grown in Iran were successfully isolated by the aseptic technique. Subsequently, fungal and bacterial extracts were analyzed by HPLC-MS. Two of 15 isolated EF from *G. nivalis* as well as two of 10 isolated EB from *N. tazetta* produced GAL. For the first time, *Penicillium verruculosum* and *Penicillium pinophilum* were identified as GAL-producing EF from *G. nivalis*; and *Burkholderia graminis* and *Bacillus thuringiensis* were identified as GAL-producing EB from *N. tazetta* according to their morphological characteristics, ITS nuclear rDNA gene sequence as well as HPLC analysis. *P. verruculosum* and *P. pinophilum* produced 0.07 and 0.3 g/g DW GAL, respectively, while *Burkholderia graminis* and *Bacillus thuringiensis* produced 37.8 and 61.0 µg/L GAL, respectively. Our results provide an opportunity in scale-up of GAL production through in vitro culture systems and isolated endophytes as well as conservation of genetic resources.

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SL 34

ESSENTIAL OIL FRACTIONS OF JUNIPER GALBULI ELUTED AT DIFFERENT TIMES HAVE DIFFERENT PROFILE AND ANTIMICROBIAL ACTIVITY

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The objective of this study was to evaluate essential oil (EO) composition, antimicrobial and antioxidant activity of EO fractions captured at different timeframes during the hydrodistillation of *Juniperus communis* and *J. excelsa* galbuli. The EO fractions were captured at eight sequential timeframes after the beginning of the hydrodistillation: (0-3, 3-5, 5-10, 10-20, 20-40, 40-80, 80-160, and 160-240 min). Essential oil fractions from 80-160 and 160-240 min had 2-5 times greater antioxidant capacity than fractions captured at the beginning of the distillation or from the whole oil. The strongest antimicrobial activity of *J. communis* EO against *Salmonella enterica* subsp. *enterica* was observed with the EO obtained at the 0-3 min distillation timeframe (DT, in minutes). The EO of *J. communis* obtained at the 0-3, 3-5, and 80-160 min DTs showed greater antimicrobial activity against *Klebsiella pneumonia*, compared to the EO obtained from the 160-240 DT. The strongest activity of *J. communis* EO against *Staphylococcus aureus* subsp. *aureus* and *Candida glabrata* was observed with EO from the 160-240 DT. *J. excelsa EO* from the 0-3 and 5-10 min DTs had greater activity against *S. enterica* and *K. pneumonia* compared to the EO from the 160-240 DT. Conversely, the *J. excelsa* EO from the 160-240 min DT had greater activity against *Clostridium perfringens* and *Candida glabrata*. This research revealed that EO with different profiles could be obtained from the same batch of galbuli, suggesting the possibility of generating natural unadulterated oils with specific targeted profiles and bioactivity.

SL 35

CASEIN KINASE 1δ, β-SECRETASE, GLYCOGEN SYNTHASE KINASE 3β INHIBITORY ACTIVITY OF SOME SELECTED LAMIACEAE PLANTS

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Alzheimer's disease (AD) is a progressive neurodegenerative disease with increasing prevalence in aging populations. Amyloid beta (Aβ) aggregates and hyperphosphorylated neurofibrillary tangles of tau represent the two main pathological hallmarks of AD [1, 2]. The search for compounds that act as inhibitors of CK1δ, GSK-3β and/or BACE1 has gained importance as it is understood that those enzymes plays a crucial role in beginning and progression of AD. In this study inhibitory properties of some culinary and medicinal Lamiaceae plants (*Salvia officinalis*, *Lavandula angustifolia*, *Mentha piperita*, *Melissa officinalis*, *Thymus vulgaris*, *Dorystoechas hastata*, *Origanum* spec. *and Sideritis congesta*) on CK1δ, GSK-3β and/or BACE1 enzymes were investigated. It's understood that GSK-3β is the most sensitive enzyme to the extracts between the all tested enzymes. With 8.84 ug/mL *Mentha piperita* showed the lowest IC_{50} value for GSK-3β inhibition. The chemical composition of *M. piperita* is determined with LC-MS/MS studies.

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SL 36

PROTECTIVE EFFECT OF ALCESEFOLISIDE, ISOLATED FROM *A. MONSPESSULANUS* SSP. *MONSPESSULANUS* L. AGAINST CARBON TETRACHLORIDE-INDUCED OXIDATIVE BRAIN INJURY IN RATS

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Carbon tetrachloride (CCl_4) is a model compound in experimental toxicology to induce oxidative stress in a number of tissues, including brain.

Aim: This study investigated possible beneficial effects of quercetin-3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyra-nosyl-(1→6)]-β-D-galactopyranoside (alcesefoliside, AF), isolated from *A. monspessulanus ssp. monspessulanus* L., against CCl_4-induced brain injury in rats.

Methods and results: Thirty six male Wistar rats were divided into 6 groups (n=6): control; treated with AF (100 mg kg^{-1}/p.o/ 21 days); treated with silymarin (100 mg kg^{-1}/p.o/ 21 days); animals challenged with CCl_4 (10% solution in olive oil); the animals in groups 5 and 6 were pre-treated with AF and silymarin in respective doses for 7 days, challenged with CCl_4, followed by curative treatment (additional 14 days). CCl_4-induced brain damage was discerned by a statistically significant decrease of superoxide-dismutase, catalase, glutathione-peroxidase activities and reduced glutathione levels as well as significant elevation in malondialdehyde production. The AF pre-treatment and consecutive curative treatment normalizes the activity of the antioxidant enzymes as well as the levels of GSH and MDA. The observed effects on tissue level were consistent with the histopathological observations of the brain and were comparable to the effects of silymarin, used as a positive control.

Conclusions: The results of our study showed that AF has a neuroprotective effect against CCl_4-induced brain injury in rats, possibly attributed to its scavenging potential and improved the activity of the antioxidant enzymes.

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CHEMICAL CHARACTERISATION OF CANNABIS FROM R. MACEDONIA

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Cannabis is a well-known drug and a controlled substance, which possession and use are illegal in most countries of the world. Nowadays, the use of cannabis and its legalization for medical use has become a worldwide trend. In many countries there have been initiatives to amend the existing laws in order to make drugs based on natural ingredients of cannabis, as well as other related products, synthetically produced, available to patients [1].

It is a matter of divided opinion whether the genus *Cannabis* (fam. Cannabinaceae) consists of one (*C. sativa*) or more species (*C. sativa*, *C. indica*, and *C. ruderalis*). Cannabis plants contain at least 489 distinct compounds distributed among 18 different chemical classes including cannabinoids, nitrogenous compounds, amino acids, proteins, enzymes, glycoproteins, hydrocarbons, simple alcohols, aldehydes, ketones and acids, fatty acids, simple esters and lactones, steroids, terpenes, non-cannabinoid phenols, flavonoids, vitamins, and pigments [2].

Therefore, due to the increased global necessity of research on the cannabis and for the sake of reinforcing the ability of the R. Macedonia to respond to the challenges arising from this issue, we have started a study for chemical characterization of various cannabis extracts and products, mainly cannabis oil, as well as plant material using GC-FID-MS and LC-MS.

THC or CBD were predominant cannabinoids in all analyzed samples, followed by THCV, CBC, CBG, CBN and Δ6-THC. Also, five terpenoid compounds (α-pinene, camphene, limonene, trans-E-caryophyllene and germacrene B) were predominant among the identified terpenes. Two types of samples can be distinguished: high THC and high CBD.

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SL 38

A PHYTOCHEMICAL INSIGHT ON *PORTULACA OLERACEA* L. (PURSLANE): FROM WEED TO FUNCTIONAL FOOD

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Portulaca oleracea L. (purslane) is a plant with cosmopolitan distribution, traditionally consumed in the diet of green leafy vegetables. Aerial parts of seven accessions from Bulgaria (4) and Greece (3) were analyzed for total polyphenols, flavonoids and polysaccharides by pharmacopoeial spectrophotometric methods. The optimal extraction conditions for the highest yields, polyphenols and saponins contents were evaluated using different extraction methods. It was found that the extraction by stirring at 80°C gave the highest percentage yield of 16%, while the maximum polyphenols (3.45% ± 0.83) and saponins (9.15% ± 0.17) contents were achieved by Soxhlet extraction. In addition, the ultrasound-assisted extraction, optimized by orthogonal design, was performed at 50°C 2 × 15 min with methanol-water (50:50, v/v) as the extracting solvent, and the solvent to solid ratio was 40:1. The total polyphenol and saponin contents as well as antioxidant activity (DPPH) were used as the criteria for selecting the optimal extraction condition. The results revealed that the Greek accessions are rich in flavonoids (up to 0.3%) and polyphenols (up to 1%), while the highest content of polysaccharides (up to 1.5%) was found in Bulgarian samples.

Principal component analysis (PCA) showed well defined cluster of three Bulgarian samples. *P. oleracea* with Bulgarian and Greek provenance have a significant potential to be a functional food as it provides a health benefit on plant-based diet.

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SL 39

A SYNTHETIC ANALOG OF NOVEL PHYTOHORMONES STRIGOLACTONES ATTENUATES INFLAMMATION AND IMPROVES INSULIN RESPONSE *IN VITRO*

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Naturally occuring phytohormones have demonstrated distinguished potential in chemoprevention and treatment of chronic inflammatory diseases. Strigolactones (SLs) are a novel class of carotenoid-derived lactones regulating many facets of plant development. Recently, it is reported that SL analogs induce cellular stress and apoptosis in tumor cells with minimal effect on survival of normal cells [1]. Here, we investigated the anti-inflammatory action of GR24, a synthetic analog of SLs, in lipopolysaccharide (LPS) stimulated RAW264.7 macrophages and 3T3L1 adipocytes. In macrophages, GR24 inhibited LPS induced NO production dose dependently with IC_{50} of 5.56 µM (% 95 CI: 4.671 to 6.619). GR24 at 10 and 20 µM doses also suppressed the LPS-stimulated iNOS expression at both mRNA (48% and 79%, respectively) and protein level (30% and 49%, respectively). It also attenuates mRNA expressions of IL-1β and COX-2. One of the main common features of chronic inflammatory diseases like obesity and insulin resistance is elevated levels of TNF-α in adipocytes. In present study, GR24 considerably downregulated TNF-α mRNA expression 70 and 83% for 10 and 20 µM, respectively, in LPS-stimulated 3T3L1 adipocytes. Additionally, we assessed the effects of GR24 on activation of AKT, one of the central molecules of insulin signalling pathway, in insulin resistant C2C12 myotubes. Accordingly, it significantly activates AKT protein 32 and 60% at 10 and 20 µM, respectively, through Ser473 phosphorylation up on insulin stimulation. Taken together, these results suggest that SLs can be developed as natural, multi-potent agents against several chronic inflammatory diseases.

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SL 40

ANTI-INFLAMMATORY PROPERTIES OF MALAYSIAN *CALOPHYLLUM* PLANTS

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*Calophyllum (*Clusiaceae) plants are known as Bintangor in Malaysia and used as traditional medicines by local communities for rheumatism, vein problems, and gastric ulcers. Previous studies showed that these plants possessed various biological activities including anti-HIV [1], cytotoxic [2] and anti-inflammatory [3] activities. IN this study, we perform a comparative study for the anti-inflammatory properties of six Malaysian *Calophyllum* species, *C. inophyllum*, *C. soulattri*, *C. lowii*, *C. teysmannii*, *C. benjaminum* and *C. javanicum*. Dried stem bark of the six plants were extracted consecutively with *n*-hexane, ethyl acetate and methanol. The anti-inflammatory activities of the extracts were evaluated by inhibition effects of NO production by LPS-induced RAW 264.7 cells and protein denaturation. The *n*-hexane, ethyl acetate and methanol extracts of *C. lowii* exhibited the most significant activities against NO production with IC_{50} values of 24.45, 38.02 and 24.48 µg/mL, respectively. On the other hand, *C. teysmannii* extracts showed the strongest inhibition effect of protein denaturation with IC_{50} values of 31.10, 62.44 and 88.61 µg/mL, respectively. Overall, the non-polar *n*-hexane extracts of these *Calophyllum* plants possessed stronger anti-inflammatory properties, if compared to ethyl acetate and methanol extracts. These activities are deduced to be contributed by the secondary metabolites present in the crude extracts, specifically flavonoid, triterpenes and xanthones.

Our present study confirmed the potential of *Calophyllum* plants to serve as lead agents in anti-inflammation, suggesting a future study on the detail mechanisms of the effects by these plant extracts.

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SL 41

RED ONION: THE RELAXATION EFFECTS OF ITS ESSENTIAL OIL ON THE ISOLATED RAT *CORPUS CAVERNOSUM*

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Erectile dysfunction (ED) is a common problem which has become increasingly prevalent in societies. Approximately, 5-20% of the general population in men suffered from moderate or severe ED [1]. It has been considered that many medicinal plants and secondary metabolites alleviate ED. The medieval Persian practitioners recommend some foods to help cure ED like *Allium cepa*. Onion (*Allium cepa* L.) belongs to genus *Allium* from the Amaryllidaceae family and is economically important as a vegetable. It includes sulfur containing active principles decomposed by allinase which are volatile and present in the onion essential oil (OEO) involving various important biological activities [2-4]. It is also popular in the cuisines of the world and has potential medicinal properties such as preventing cancer, reducing symptoms of diabetes, reducing coronary heart diseases, antibacterial activity and anti-inflammatory activity. The present study evaluated the relaxant effect of OEO on rat *corpus cavernosum* (CC).

Twenty-six adult male Sprague-Dawley rats were used in the experiments. Isolated rat CC strips were placed in organ baths containing Krebs solution, and functional experiments were performed. After preconstruction with phenylephrine (PE, 10^{-5} M), the relaxant response to OEO (25-400µL) was investigated in rat CC. The relaxant responses to OEO were repeated in the presence of nitric oxide synthase (NOS) inhibitor L-nitro-arginine methyl ester (L-NAME, 100µM) or soluble guanylate cyclase (sGC), [1H-1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 30µM) inhibitor. As a result, OEO caused relaxation of isolated CC strips in a concentration-dependent manner (maximum response: 98.0±3.5%). The relaxant responses to OEO were not altered in the presence of L-NAME and ODQ in rat CC.

OEO induced relaxation independent from NO/cGMP system in rat CC. We suggest that OEO may have a beneficial effect in ED patients who are non-responders to phosphodiesterase-5 inhibitors. In the future, we would like to investigate major components of onion essential oil and other extracts of the onion because of the health benefits.

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SL 42

SCREENING FOR BIOACTIVE COMPOUNDS TARGETING TOPOISOMERASE I FROM *TODDALIA ASIATICA* LAM

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The roots of *Toddalia asiatica* Lam are reported to exhibit significant anticancer activity. However, the specific components responsible for their anti-cancer activity and their targets still remain elusive. In this context, we strived to combine affinity ultrafiltration with topoisomerase I (Topo I) as a target enzyme aiming to fish out specific bioactive compounds from *Toddalia asiatica Lam*. 15 compounds from *Toddalia asiatica Lam* were thus screened out, among which peak 2 with the highest enrichment factor (EF) against Topo I exhibited good dose-dependent inhibition with IC_{50} comparable to that of camptothecin, a well-known Topo I inhibitor used in clinic for a wide spectrum of cancers. The *in vitro* antiproliferation assays revealed that peak 2 strongly inhibited the proliferation of HT-29 and Hep G2 cells in an intuitive dose-dependent manner with the IC_{50} values at 5.95µM and 5.14 µM, respectively, and also induced significant cellular morphological changes, which further validated the potent antiproliferative activities.

Collectively, these results suggested that peak 2 could be a very promising anticancer candidate for the therapy of cancer in the near future.

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PRUNUS MAHALEB, L. FERMENTED FRUITS: A POTENTIAL SOURCE OF ANTIOXIDANT ACTIVITY AND PROBIOTIC MICRORGANISMS

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Fermentation is a process traditionally adopted to preserve food which results in novel fermented products, provided with specific organoleptic properties. *Prunus mahaleb* L. is an autochthonous tree of the Mediterranean area. Its highly pigmented small-stone fruits are characterized for astringent and sour taste and therefore not used for fresh consumption. Recently, *Prunus mahaleb* L. fruits were found endowed with high bioactive compound content, especially anthocyanins, and strong antioxidant capacity [1]. With the aim to reduce their sourness, *P. mahaleb* fruits were dipped in water and fermented for 20 days at 25°C with four starter cultures of *Lactobacillus plantarum* strains alone or in mixture with a single strain of *Saccharomyces cerevisiae*. During this period, viable cell count of *L. plantarum* strains was never found lower than 10^7 CFU/ml that is the lower concentration request to probiotic microorganisms to promote beneficial effects on human health. *S. cerevisiae* in all trials reached a maximum population three days after inoculation and then, cells counts slightly decreased in a range from 10^7 to 10^3 CFU/mL depending on the co-inoculated bacteria strain. Among *L. plantarum* strains, only the FG68 survived after an *in vitro* digestion protocol specifically set up for the selection of potentially probioitic lactic acid bacteria [2]. As concerns the antioxidant activity, the fermentation medium always showed higher values when *L. plantarum* strains were co-coltured with *S. cerevisiae*. Conversely, no differences were found in fermented fruits even though their antioxidant capacity values were higher than the large part of fruits included in the human diet.

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SL 44

CYTOTOXIC LIGNANS IN HAIRY ROOT CULTURES OF *LINUM PERENNE*

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Cytotoxic lignans are plant secondary metabolites recovered within some botanical families. A plethora of pharmaceutically interesting properties, such as anti-cancer, -viral, -microbial, -fungal, -protozoal, -platelet and -inflammatory activities have been attributed to these molecules. As for many structurally complex plant molecules, their production through synthetic chemical approaches is not economically interesting and therefore, extraction from plant tissue is preferred. In the early 2000s it has been shown that cell and hairy root cultures of *Linum* species of the section *Syllinum* (*L. album*, *L. flavum*) accumulate aryltetralin-type lignans (e.g. podophyllotoxin, methoxypodophyllotoxin) [1], and *Linum* species of the section *Linum* (*L. perenne, L. austriacum*) accumulate arylnaphthalene-type lignans (e.g. justicidin B, diphyllin) [2]. Different *Linum perenne* hairy root lines were established using *Rhizobium rhizogenes* strain 15834. The justicidin B and isojusticidin B, as well as different glycosylated diphyllins are produced. Two diastereoisomers of 6-methoxypodophyllotoxin, an aryltetralin-related compound, are also observed, probably derived by the conversion of matairesinol and 7-hydroxymatairesinol as a hypothetical biosynthetic pathway**.**

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SL 45

CYCLODEXTRIN/NEROLIDOL INCLUSION COMPLEX FOR ORANGE JUICE PRESERVATION

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Plant essential oils are rich in bioactive molecules with various biological effects. Their addition to natural food may lead to functional food products. As fresh orange juice is widely desired, its preservation would be a challenge. Natural orange juice may undergo chemical and microbial deterioration. The latter may due to *Lactobacillus fermentum*. The aim of our study was to search for a natural antibacterial agent active against orange juice spoilage caused by *Lactobacillus fermentum*. *L. fermentum* was screened for susceptibility against 28 natural essential oil constituents under the optimal conditions for *L. fermentum* growth in MRS medium (anaerobic, 37° C, 22 hours). Among the screened molecules, nerolidol, a sesquiterpene alcohol, exhibited the strongest antibacterial activity with low minimal inhibitory concentration and minimal bactericidal concentration values of 25 µM and 50 µM, respectively. In orange juice stored at 4°C and inoculated with *L. fermentum* (10^6 CFU/ml), the total bacterial death was only achieved in 72 hours when nerolidol dissolved in dimethylsulfoxide was added at 2000 µM. As nerolidol is poorly soluble in water and light-sensitive, cyclodextrin/nerolidol inclusion complex was prepared by freeze-drying method and added to the fresh juice. The inclusion complex was proved to increase the aqueous solubility and photostability of nerolidol [1]. The complex containing nerolidol at 2000 µM maintained the antimicrobial effect against *L. fermentum* in orange juice at 4°C, but the total bacterial death was obtained upon 144 hours.

Non-toxic delivery systems encapsulating active molecules may constitute a promising strategy for the development of functional food products.

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SL 46

PLANT CELL BIOFACTORIES AS *IN VITRO* PRODUCTION PLATFORMS OF THERAPEUTIC CYCLOTIDES

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Cyclotides are a class of cyclic plant proteins with unique topology that is responsible for their exceptional chemical, thermal, and enzymatic stability. This makes them a potential candidate for diverse commercial applications as agrochemicals, pharmaceutical scaffolds for drug delivery, and therapeutic agents [1, 2]. In this study the Indian medicinal plant *Viola odorata,* commonly called as 'Banafsha' in traditional medicine, represented a rich source of known and novel cyclotides [3]. Successful identification and characterization of cyclotides in the plant was done using Liquid Chromatography and Fourier Transform Mass Spectrometry, which included confirmation based on their mass (2.5-4 kDa), hydrophobic nature, disulfide bonds, circular structure and amino acid sequence. A total of 71 known and 98 novel cyclotides were identified in the whole plants. Further, callus, cell suspension, somatic embryos and *in vitro* shoot cultures of *V. odorata* were established as alternative and sustainable production platforms of known and novel cyclotides. Out of the 49 novel cyclotides identified, 9 were exclusively being produced in *in vitro* cultures but not in the whole plant. The LC-MS analysis also revealed that the relative abundance of cyclotides in the crude extract from *in vitro* cultures was higher (maximum up to ~60%) in comparison to that from the whole plant biomass (~4%), which would reduce the cost of downstream processing at large scale. Hence, apart from germplasm conservation of the plant, *in vitro* cultures established in the study can serve as an alternative source for production of known and unknown cyclotides for potential commercialization.

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SL 47

EVALUATION OF PLANT MATERIAL QUALITY BASED ON THE ANALYSIS OF TRITERPENOID CONTENT – EXAMPLE OF CHAMOMILE (*MATRICARIA CHAMOMILLA*)

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Matricaria chamomilla L., commonly known as chamomile, is an annual plant of the family *Asteraceae*. Often called the "star among medicinal species", it has been used in herbal remedies from ancient times, mainly for anti-inflammatory, antiseptic, antispasmodic and mildly sudorific properties. Nowadays it is still a highly favored and much used medicinal plant in folk and traditional medicine due to its multitherapeutic, cosmetic and nutritional values.

The aim of the present study was the comparison of triterpenoid content in samples of chamomile flowers (floral heads, *Chamomillae anthodium*) of different origin, purchased in pharmacy or harvested directly from plantation, extracted with diethyl ether or ethanol. Analysis was made by GC-MS. The total content of triterpenoids accounted for 20 mg/g d.w. (the results obtained for diethyl ether extract) and 15 mg/g (for ethanol extract) in flowers from the plantation, whereas only for 15.2 mg/g (diethyl ether extract) and 11.3 mg/g (ethanol) in plant material purchased in pharmacy. Moreover, significant differences in abundance of the main identified compounds, e.g., β-amyrin, lupeol, moretenol, betulin as well as sitosterol and stigmasterol were observed, with the most striking fluctuation (more than 300%) in the amount of the predominating triterpenoid, taraxasterol.

The obtained results point to the unstable quality of herbal material that might be caused by genetic diversity of seeds and cultivation in different soil and climatic conditions. The content of bioactive compounds in plants of various origin can be substantially diverse and requires standardization if the material is used for pharmacological purposes.

SL 48

UTILIZATION OF PALM OIL MILL EFFLUENT AS POTENTIAL FOOD SOURCE

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The environmental impacts of waste water discharged from palm oil mill are alarming us. Various effective technologies have been exploited to treat palm oil mill effluent (POME) [1-3]. This study was aimed to recover the value-added products from the palm oil mill before being discharged into watercourses in order to achieve zero discharge of pollutants into watercourses. The regiospecific analysis by quantitative ^{13}C-NMR revealed that the samples collected from clarification tank have low levels of saturated fatty acids (SFAs) at *sn*-2 position of triglycerides (TG), but high levels of that at *sn*-1,3 positions. The positional distribution of FAs at TG implys their excellent profiles in terms serum cholesterols and fat deposition, respectively. The fatty acid composition of samples was analysed by GCMS and the results showed that these extracts contain predominantly oleic acid, linoleic acid, palmitic acid and stearic acid. The total flavonoid contents (TFC) of samples were exceptionally high if compared to that of palm olein. Moreover, the samples were found to be rich in phytonutrients including vitamin E, carotenoids, phytosterols and squalene. Proximate analysis indicated that both samples have high contents of fats and carbohydrates, suggesting their potential use as food source to boost energy. The samples also showed moderate antioxidant activity in FIC assay, attributed to the presence of tocopherols, tocotrienols, flavonoids and phenolic compounds. As a summary, this study revealed that the samples collected from palm oil mill before discharging into wastewater course could be used as an alternative food sources besides leading to zero discharge.

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SL 49

BIO-PROSPECTION FOR LAXATIVE PRINCIPLES SENNOSIDES FROM *CASSIA* SPECIES

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Cassia Lin is widely distributed in tropical and subtropical regions. About 45 species of *Cassia* are found in India. *Cassia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. They are well known in folk medicine for their laxative and purgative uses [1]. Twelve *Cassia* species having distinguished morphological and anatomical features were analysed for laxative principles sennoside A (SA) and sennoside B (SB) using a reversed phase HPLC-PDA detection method. Sennosides were detected in eight *Cassia* species. Total sennoside (%, SA+SB) varied in the following data order: *C. angustifolia* > *C. tora* > *C. javanica* > *C. occidentalis* > *C. alata* > *C. auriculata* > *C. fistula* > *C. surattensis*. The result of the present investigation could partly fill the gaps of sennosides from other potential sources.

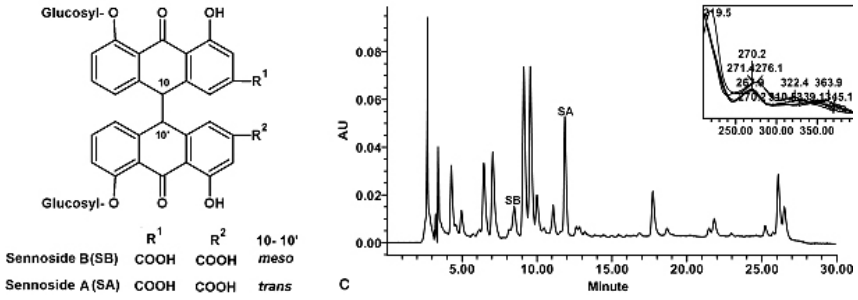

Figure 1. Chemical structures of SB and SA (left), and HPLC chromatograms with PDA spectra of *C. aunguistifolia* (right)

Acknowledgements: This work was funded through a research grant through ICAR-Network Project on High Value Compounds/Phytochemicals.

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SL 50

TRADITIONAL USE OF WILD PLANTS FROM THE AREA OF THE IRON GATES (SERBIA) - ETHNOBOTANICAL STUDY

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Plants have significant role in human life, even though traditional knowledge regarding wild plants is decreasing in many parts of the world [1]. However, interest for phytotherapy and investigations in this field has been increasing lately [2]. The aim of this study was collecting information on traditional use of medicinal and edible plants, as well as creating the list of medicinal and edible plants used by local community from the Iron Gates (Djerdap National Park, Eastern Serbia). The group of local inhabitants (60) was interviewed using semistructured questionnaires to identify the uses of the plants in folk medicine, veterinary, nutrition and in religious ceremonies. An inventory of 80 plant species traditionally used was created. At the family level, the most common are species of Rosaceae, Lamiaceae and Asteraceae. Most of the plants are used for medical purposes (67.5%), while some are used in human and animal nutrition (24.5%) and for other purposes (7.9%). Report states that *Hypericum perforatum* L., *Urtica dioica* L., *Achillea millefolium* L., *Matricaria chamomilla* L., *Tilia cordata* Mill., *Cotinus coggygria* Scop., *Plantago major* L. and *Sambucus nigra* L. are the most frequently used herbs. All parts of plants are equally used for different remedy treatments. The most reported medicinal uses of herbal drugs are in digestive (27.7%), skin (16.8%), genitourinary (16.5%) and respiratory (13.3%) diseases. Knowledge from this region needs to be documented and preserved as it represents promising source for further phytochemical and pharmacological studies which could provide new natural bioactive compounds.

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SL 51

NO BEES NO MEDICINAL PLANTS?

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Most people have heard that pollinators are in trouble, and with them agricultural products worth more than $200 billion annually (FAO 2017). Pollinators are fundamental to maintaining both biodiversity and agricultural productivity, but habitat destruction, loss of flower resources, and increased use of pesticides (particularly neonicotinoids) are causing declines in their abundance and diversity.

We constantly search new bioactive compounds and natural products. We put all our efforts to understand how they work in the human's body. We restlessly try to optimize the yield of plant bioactive compounds. However medicinal plants are living creatures and we relate on their reproduction to supply the necessity of plant substances. Many of the medicinal plants depend on bees for their seed set. What is the impact of pollinator decline on the resources of medicinal plants? The aim of this study is to evaluate the necessity of pollinator for the reproduction of medicinal plants. The plants recognized by Medicinal Plants Act (Bulgaria as a model) were classified as spore and gymnosperm plants, anemophylous (wind pollinated) plants and entomophylous (insect pollinated) plants. The plants that require insect vectors for their pollen transport are analyzed further. Entomophylous pollination syndromes are discussed according to the functional morphology and access to nectar and pollen of their "blossoms" (flower or compact inflorescence) in the following basic classes: "dish/bowl", "bell", "funnel" "flag" and "gullet". Adaptations like spontaneous self-pollination ability and apomixis are also discussed. This analysis only roughly predicts the pollinators. Detailed research results are presented about some medicinal plants (Fig. 1).

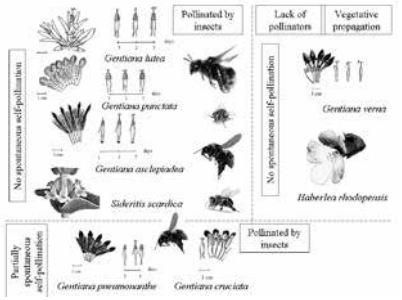

Figure 1. Ability for spontaneous self-pollination and reproductive strategies.

SL 52

CYANIDIN-3-*O*-GLUCOSIDE PROTECTED AGAINST 3-CHLORO-1,2-PROPANEDIOL INDUCED TESTIS INJURY AND IMPROVED SPERMATOGENESIS IN MALE RATS

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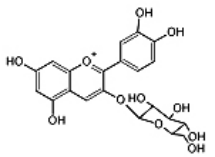
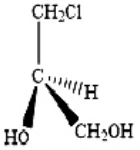
Recent years, the reproductive capacity of male is gradually reducing, which may be caused by the interaction between environmental factors and multiple genes. Studies showed that natural products in plant food may improve male fertility. In this study, we established a spermatogenetic failure rat model by the treatment of 3-chloro-1,2-propanediol (3-MCPD), which is one of the most widespread pollutants in food. Meanwhile, we investigated the protective effect of Cyanidin-3-O-glucoside (C3G) which is a typical monotype of anthocyanins, on the ability of spermatogenesis. Our results proved that intervention with C3G (250 mg/kg·diet) for four weeks significantly reduced the testis injury in 3-MCPD treated rats model. C3G effectively protected the spermatogenic epithelium structure, reserved the blood-testis barrier, which was proved by highly expressing of Claudin-11, Occludin and N-cadherin. C3G also improved the sperm motility, sperm count and decreased the deformity rate. In addition, C3G enhanced the antioxidant system by increasing the level of GSH, SOD, the ratio of LDH-X/LDH, and decreasing the expression of 8-OHdG in nucleus. C3G effectively promoted the spermatocyte meiosis via suppressing the activation of ERK, p38, JNK1 and the expression of GAPDH, whereas up-regulated the expression of BAX, CREM and p53. On the other side, the rate of cells apoptosis was reduced by C3G. Interestingly, the level of testosterone was not influenced by C3G treatment, but the expression of androgen receptor was significantly increased. In conclusion, C3G could effectively protect against 3-MCPD caused spermatogenic epithelial injury, spermatogenesis disorders, and consequently protected the male reproductive ability.

Chemical structures:



C3G



S-3-MCPD

SL 53

INSIGHTS INTO MORIN FROM MULBERRY DERIVED CELL-CYCLE ARREST AND APOPTOSIS IN HUMAN CERVICAL CARCINOMA HELA CELLS

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In the recent years, flavonoids are of great interest due to their antioxidant and anti-cancerous potential. The study aimed to investigate the anti-cancerous activity of Morin extracted from mulberry and its underlying mechanisms. Briefly, Morin inhibited the proliferation of HeLa cells at IC_{50} of 214.28 μM and led to morphological changes, followed by induction of cell cycle arrest in G2/M -phase and ultimately resulted into apoptosis. Morin-induced G2/M-phase arrest was accompanied by the increase of p53, p21 and Wee 1 genes mRNA expression and decreased levels of CDK1, Cdc25c, Survivin, cyclin B1 and CHK2. Moreover, Morin-induced apoptosis was regulated through multiple pathways, including intrinsic and extrinsic pathway.

The underlying mechanisms consisted of increased mRNA expression of Bax, Bad, cytochrome c, Apaf-1, caspases-9, DR3, DR5, FasL, FADD, caspases-10, PARP, PI3K, AKT, mTOR, P70S6K and Smac genes as well as decreased expression of Bcl-2, Bcl-xL, AMPK, cIAP-1, cIAP-2, PKC□ and NF-κβ.

In addition, Morin treatment resulted in the generation of intracellular ROS, which plays an essential role in the induction of apoptosis. Thus, the present study could provide the evidence of anti-cancerous effects of Morin against human cervical cancer.

SL 54

PHARMACOLOGICAL INVESTIGATION OF *ASTRAGALUS SPRUNERI* BOISS. (FABACEAE)

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Astragalus spruneri Boiss. is a perennial herbaceous endemic for the Balkan Peninsula. Volatile compounds from the aerial parts of the species and their pharmacological evaluation were reported up to date.

The aim of the present study was to investigate the *in vitro*/*in vivo* activity of extracts with different polarity as well as pure flavonoids from the plant.

Administered alone, the extracts of the aboveground parts of *A. spruneri* and the flavonoids isolated proved statistically non-toxic for the liver. Using *in vitro* models with different underlying mechanisms (metabolic bioactivation and oxidative stress), the defatted extract and the flavonoids proved statistically significant antioxidant and cytoprotective effects. The activity observed was stronger for the extract, compared to the pure compounds and comparable to those of silybin. In a model of non-enzyme induced lipid peroxidation *in vitro* all the tested extracts revealed statistically significant antioxidant effects. The ethyl acetate extract rich in flavonoids showed stronger antioxidant activity compared to the defatted and butanol extracts and silymarin. The *in vivo* investigation proved hepatoprotective and antioxidant effects, comparable to silymarin. The pathohistological study proved the effects observed.

This activity will serve as a perspective for further investigations as possible hepatoprotectors and antioxidants.

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SL 55

QUALITY CONTROL OF BAICAL SKULLCAP LYOPHILISATES USING ADVANCED LC/PDA/ESI-QTOF-MS/MS TECHNIQUES AND ANTIOXIDANT ASSAYS

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Baical skullcap (*Scutellaria baicalensis* Georgi., Lamiaceae) is the adaptogenic plant widely used in traditional systems of medicine (especially of China and Russia) in various types of biodegenerative diseases. In our studies, highly concentrated pharmaceutical formulations (lyophilisates) were obtained from the roots of this species using optimized ultrasound-aided extraction technique (UAE). As optimized extractants for UAE, ethanolic-aqueous (25% and 50%, v/v) solvents were used in order to withdraw both polar, medium-polar and non-polar bioactive flavone constituents from plant material.

Extracts were subjected to lyophilisation, giving vacuum-dried formulations, signed as SBR25 and SBR50. Detailed quality control of both lyophilisates was performed using standardized phytochemical protocol. For this purpose, two reversed-phase LC chromatographic systems were employed, hyphenated with PDA or PDA/ESI-QToF-MS/MS detectors, respectively. Methanolic (75%, v/v) solutions of SBR25 and SBR50 were analysed qualitatively using LC/PDA/ESI-QToF-MS/MS system both in negative and positive mode to identify precisely all bioactive flavone derivatives. As a mobile phase, gradient of acetonitrile with 10 mM ammonium formate and formic acid (0.1%) addition was used. Above 20 polyphenolic compounds were identified in both lyophilisates, mainly *O*-glycosidic derivatives of flavone aglycones (baicalein, wogonin and oroxylin A) as well as chrysin *O*- and *C*-glycosides. The concentration of predominant flavone: baicalin (baicalein 7-*O*-glucuronide) exceeded 10 and 15% and total flavone content was higher than 20 and 29% in SBR25 and SBR50, respectively.

Antioxidant (Folin-Ciocalteu) and antiradical ($ABTS^{\bullet+}$) assays additionally confirmed high quality of both lyophilisates and showed good correlation between the content of bioactive flavones and their free radical scavenging potential.

SL 56

HOP BETA ACIDS AND THEIR TRICYCLIC RANSFORMATION PRODUCTS SHOW HYPERFORIN-LIKE CELLULAR ACTIVITY

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β-Bitter acids (lupulones) of hops revealed sedative and antidepressant-like effects in animal studies [1]. Transformation of lupulones during beer brewing leads to the formation of various tricyclic products, which were recently identified and quantitated in commercial beer samples [2]. Lupulones and their transformation products have a close structural analogy to hyperforin, which is responsible for the antidepressant activity of St. John's wort. Thus, we hypothesized that the hop-derived substances could follow the same antidepressive principle of action as hyperforin. Previously, the latter was identified as a specific TRPC6 activator leading to a Ca^{2+} influx in cells expressing those cation channels. An increase in intracellular Ca^{2+} can finally lead to a stimulation of neuronal differentiation processes, contributing to an antidepressant effect [3].

The aim of the present study was to investigate the influence of β-acids and their tricyclic transformation products on the Ca^{2+} influx in neuronal-like PC12 rat phaeochromocytoma cells. Therefore, the major analogues co-lupulone and n-lupulone were isolated. By thermal treatment of lupulones nortricyclolupones, dehydrotricyclolupones and tricyclolupones were formed and subsequently isolated with good purity. Structures were elucidated by means of UHPLC-DAD, UHPLC-ESI^--MS/MS and 1D/2D-NMR spectroscopy.

We found out that lupulones and their degradation products induced a Ca^{2+} influx in TRPC6 expressing PC12 cells to the same extent as hyperforin. Application of a Ca^{2+}-free environment abolished the Ca^{2+} elevation, indicating that the increase is mediated by influx across the plasma membrane. Thus, it can be concluded that lupulones and their transformation products may have similar antidepressant effects as hyperforin.

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SL 57

ALKANE CONTENT IN THE CUTICLE OF TALL FESCUE (*FESTUCA ARUNDINACEA*) PLANTLETS EXPOSED TO METAL IONS

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Heavy metal pollution of terrestrial environments is of great concern, due to the persistence of metals in the ecosystem. In this regard, the mountainous pastures of Romanian Carpathians, especially those in the areas of abandoned mines, need particular attention. The aim of our study was to assess the effect of heavy metals and metalloids exposure on epicuticle wax composition of tall fescue leaves (*Festuca arundinacea*). Therefore, we particularly investigated the variations of long chain hydrocarbon fractions, which are acknowledged to act as a protection for plants especially in toxic environments [1]. The exposure experiments were conducted in triplicates for the following metals: Ba, Cu, Fe, Pb and also for the metalloid As. The 0.5 mM aqueous solutions of their corresponding salts were used as treatment solutions. Triplicate experiments were also performed for control samples using distilled water as exposure media. The plantlets were kept for growing in controlled environment for 15 days followed by *n*-hexane hydrocarbon fraction extraction from 0.5 g of sampled leaves [2]. The obtained extracts were semi-quantitatively analysed (identification followed by peak area measurement) through an optimised method based on gas-chromatography coupled with mass spectrometry detection. We focussed on the measurement of several long chain *n*-alkanes with the following number of carbon atoms: C21-C31. Significant differences were obtained between results performed for Ba, Pb and As when compared to Fe and Cu suggesting multiple mechanisms through which the tested plants could develop and adapt when exposed to various chemicals characterized by different degrees of toxicity.

Acknowledgements: The work was sustained from the BIODIVERS National Project (PN 16-190401, PN 16-190106).

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SL 58

THE APPLICATION OF MEDICAL PLANTS IN THE PRODUCTION OF FUNCTIONAL PRODUCTS

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Bread, as bakery product, is the basic staple food product around the world. With the development of science and technology, customer requirements for obtaining new, functional products are becoming even higher. Plants are traditionally used in folk medicine, due to the presence of various secondary metabolites (vitamins, phenolics, carotenoids, etc.), they are widely applied in the food industry. The aim of this paper is to show possibilities of usage of herbs in functional bakery products with additional value. Thanks to the proven biological activities and properties, as well as their chemical composition, stinging nettle shows great potential in creating new bakery products. Due to the high content of vitamins, minerals, polyphenols, carotenoids, and fatty acids [1]. Nettle may be applied in different forms for preparation of bread with enhanced nutritional and technological characteristics. Chemical profile of prepared bread is assessed using spectrophotometric and HPLC analysis. Beside chemical profile, biological activity of the products was evaluated using *in vitro* assays. Bread with addition of nettle and its extract showed high antioxidant activity (10.60-88.33%) and cytotoxic activity (IC_{50} < 30 µg/mL). Total phenolics (TPC) and flavonoids (TFC) contents were also determined and expressed as milligrams of chlorogenic acid (mg CAE/g) and catechin (mg CE/g) equivalents per gram of bread. Results were in following ranges: 2.90-11.88 md CAE/g and 0.35-1.49 mg CE/g for TPC and TFC, respectively.

The results showed interesting potential in the application of nettle in the formulation of new products with enhanced functional characteristics.

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SL 59

ASCOFURANONE INHIBITS LIPOPOLYSACCHARIDE-INDUCED INFLAMMATORY RESPONSE VIA NF-KAPPAB AND AP-1, P-ERK, TNF-A, IL-6 AND IL-1B IN RAW 264.7 MACROPHAGES

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The natural compound *ascofuranone* (AF, MW 420.93) isolated from *Ascochyta viciae* has been known to promote cell cycle arrest and inhibit invasion of tumor cells. We have previously studied a structurally similar compound *ascochlorin* (ASC; MW 404.93) with regard to its anti-inflammatory activity in LPS- stimulated RAW 264.7 macrophages. In order to examine the relationship between the anti-inflammatory activities and the molecular differences between AF and ASC, the activity of AF is herein studied, because ASC has a unique trimethyl oxocyclohexyl structure, while AF has a unique dimethyl-oxo-tetrahydrofuran structure. AF dose-dependently inhibited the production of NO and iNOS and the COX-2 mRNA and protein levels in RAW 264.7 cells. In addition, AF suppressed mRNA expression levels of inflammatory cytokines such as TNF-α, IL-6, and IL-1β, as assessed by RT-PCR. AF (30-50 μg/ml) treatment clearly inhibited the nuclear translocation of NF-κB, AP-1 (p-c-Jun) from the cytosolic space. Phosphorylation of IκB, which functions to maintain the activity of NF-κB, was decreased by AF treatment. Moreover, AF suppressed the binding of NF-κB (p65). Inhibition of IkBa phosphorylation and degradation inhibits nuclear translocation of p65. Immunofluorescence confocal microscopy analysis also revealed that translocation of NF-κB and AP-1 (p-c-Jun) was decreased upon AF treatment. AF specifically decreased the expression level of p-ERK, but not the expression level of p-p38 or p-JNK. Given these results, we suggest that AF suppresses the inflammatory response by targeting p-ERK. This indicates that AF is a negative regulator of LPS-stimulated nuclear translocation of NF-κB and AP-1 (p-c-Jun) in RAW 264.7 macrophages, and specifically it targets p-ERK. Therefore, AF and ASC exert their effects in different ways, most probably because their structural differences allow for specific recognition and inhibition of their target MAPKs. Our results further suggest that AF could be a natural bioactive compound useful for treating inflammation-mediated pathological diseases.

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SL 60

ESCULENTOSIDE H INHIBITS COLON CANCER MIGRATION AND INVASION BY SUPPRESSION OF MMP-9 GENE EXPRESSION VIA JNK1/2 AND NF-KB SIGNALING PATHWAY

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Eculentoside H (EsH), a water-soluble saponin, has been isolated from the *Phytolacca esculenta* roots. Although EsH known as an anti-cancer compound has a capacity for TNF release, the pharmacological capacity of EsH on tumor migration and invasion remains unclear. In this study, we investigated whether EsH suppresses phorbol-12-myristate 13-acetate (PMA)-induced migration and invasion in HCT116 human colon cancer and CT26 murine colon cancer cell lines. Interestingly, the wound healing and transwell assay showed that EsH suppresses PMA-induced migration and invasion capacities in HCT116 and CT26 colon cancer cells with unknown mechanism. EsH inhibited PMA-induced matrix metalloproteinases-9 (MMP-9) expression in mRNA and secreted protein levels in a dose dependent manner. In addition, the expression mode of MMP-9 gene was correlated with the activation of NF-κB signaling. EsH inhibits PMA-induced IκB phosphorylation, leading to suppression of NF-κB nucear translocation. Moreover, EsH repressed the PMA-induced phosphorylation of JNK, but not ERK and p38 signaling.

Taken together, these results demonstrated that EsH potentially blocks cancer migration and invasion through blocking of the JNK1/2 and NF-κB signaling-mediated MMP-9 expression.

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SL 61

REVISITING OLDENLANDIA DIFFUSA TO ANTI-METASTATIC AND APOPTOTIC POTENTIAL IN BREAST CANCER BY ANTI-MMP-9 AND ICAM-1VIA P38 AND ERK1/2 MAPK

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Oldenlandia diffusa (OD) has long been known as an apoptotic inducer in breast tumors in ethnomedicine. To scientifically confirm the anti-breast cancer effects of water, methanol (MeOH) and butanol (BuOH) extracts of O. diffusa on cell apoptosis, matrix metalloproteinases (MMPs), intercellular adhesion molecule (ICAM)−1 and intracellular signaling in MCF-7 breast cancer cells. MeOH extracts (MOD) and BuOH extracts (BOD) were prepared and examined for their ability to inhibit phorbol myristate acetate (PMA)-induced matrix metalloproteinase (MMP)−9 and intercellular adhesion molecule (ICAM)−1 expressions in MCF-7 human breast cancer cells. Additionally, transwell migration, invasion and transcriptional activity were assessed. Results of immunofluorescence confocal microscopy for translocation of NF-κB and p-ERK and p-p38 were also checked. Finally, apoptotic signals including processed caspase-8, caspase-7, poly ADP-ribose polymerase, Bax and Bcl-2 were examined. MOD and BOD specifically inhibited PMA-induced MMP-9 expression as well as invasive and migration potential via ICAM-1. The inhibitory activity was also based on the suppressed transcriptional activity in MCF-7 breast cancer cells. Results of immunofluorescence confocal microscopy showed that translocation of NF-κB decreased upon BOD and MOD treatments, with a decreased level of p-ERK and p-p38 phosphorylation.

In addition, treatment of MCF-7 cells with MOD and BOD activated apoptosis-linked proteins including enzymatically active forms of processed caspase-8, caspase-7 and poly ADP-ribose polymerase, together with increased expression of mitochondrial apoptotic protein, Bax and decreased expression of Bcl-2. The results indicate that OD as an anti-metastatic agent suppresses the metastatic response by targeting p-ERK, p-38 and NF-κB, thus reducing the invasion capacity of MCF-7 breast cancer cells through inhibition of MMP-9 and ICAM-1 expression and plays an important role in the regulation of breast cancer cell apoptosis.

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SL 62

MOLECULAR DETERMINANTS OF PPARΓ PARTIAL AGONISM AND RELATED *IN SILICO/IN VIVO* STUDIES OF NATURAL SAPONINS AS POTENTIAL TYPE 2 DIABETES MODULATORS

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Introduction: The metabolic syndrome, which includes hypertension, type 2 diabetes (T2D) and obesity, has reached an epidemic-like rise [1]. Saponins and sapogenins are considered as valuable natural products for ameliorating this pathology, possibly through PPARγ-mediated mode of action [2, 3].

Aim: The aim of this study is to investigate the molecular determinants of PPARγ partial agonism and a potential PPARγ participation in the anti-diabetic effects of a purified saponins' mixture (PSM) from *Astragalus corniculatus* Bieb.

Methods: The *in silico* studies include protein-ligand interaction fingerprint analysis of crystal structures of human PPARγ complexes with partial agonists from the Protein Data Bank (http://www.rcsb.org), pharmacophore modelling and molecular docking simulations. In the *in vivo* experiments spontaneously hypertensive rats with induced type 2 diabetes were treated with PSM and pioglitazone as a referent PPARγ full agonist, and pathology-relevant biochemical markers were analysed.

Results: In the *in silico* study we outlined key pharmacophoric features typical for the PPARγ partial agonists and performed pharmacophore-based docking of triterpene saponins/sapogenins from the tested PSM. Important protein-ligand interactions were identified. The *in vivo* experiments provide details on the PSM modulation of the glucose homeostasis and its potential mechanism.

Conclusions: The reported combined approach is intended to be used for development of drug-discovery protocol focused on natural T2D modulators by integrating mechanistically interpretable *in silico* analyses of a potential molecular initiating event and pathology relevant *in vivo* disease model.

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SL 63

ETHNOBOTANICAL AND ETHNOMEDICINAL STUDIES ON THE VILLAGES OF AKKUŞ DISTRICT (ORDU, TURKEY)

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The object of this study is to evaluate in terms of ethnobotanical and ethnomedicinal uses of the plants naturally growing in 35 villages of Akkuş District.

The study is the first report about the ethnobotany and ethnomedicinal of Akkuş District. Informations about the uses of the plants have been obtained from villagers using a questionnaire through face-to-face. The voucher specimens are saved in Ankara University Faculty of Pharmacy Herbarium (AEF). At the end of the identification of the used species from villagers, 58 taxa belong to 32 families have been determined [Asteraceae (7), Amaranthaceae (1), Boraginaceae (1), Caprifoliaceae (1), Caryophyllaceae (3), Chenopodiaceae (1), Convolvulaceae (1), Cruciferae (1), Cornaceae (1), Euphorbiaceae (1), Ericaceae (1), Fabaceae (5), Fagaceae (3), Hypericaceae (1), Hypolepidaceae (1), Juglandaceae (1), Lamiaceae (6), Loranthaceae (1), Malvaceae (1), Papaveraceae (1), Pinaceae (1), Plantaginaceae (1), Platanaceae (1), Polygonaceae (4), Primulaceae (1), Rhamnaceae (1), Rosaceae (5), Scrophulariaceae (1), Tiliceae (1), Umbelliferae (1), Urticaceae (1), Vitaceae (1)]. Depending on the data collected, the widespread species used by the villagers are *Plantago major* var. m*ajor*, *Pinus sylvestris*, *Malva neglecta*. It has been determined that the local people use mostly the plant in treatment of cough, rheumatic diseases and wounds.

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SL 64

CYTOTOXIC BIOACTIVITY AND ANTIOXIDANT POTENCY OF SELECTED PLANT EXTRACTS

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Plants have a long history of traditional use for treatment of numerous pathological conditions. Despite their widespread use in traditional medicine, there is insufficient data of their cytotoxic and antioxidant properties. The present work evaluated the cytotoxic potential and antioxidant activity of *Carum carvi* L. (CCF), *Citrus hystrix* DC. (CHF), *Lavandula angustifolia* Mill. (LAF), *Levisticum officinale* W.D.J.Koch (LOH), *Linum usitatissimum* L. (LUS), *Myristica fragrans* Houtt. (MFF) and *Origanum vulgare* L. (OVH).

Ethanolic extracts were examined for their antioxidant potential using 2,2'-diphenyl-1-pycrylhydrazyl and Ferric reducing antioxidant power assay. The cytotoxic bioactivity was determined by Brine Shrimp Lethality Assay and evaluated with Meyer's scale [1] and Clarkson's scale of toxicity [2] based on their LC_{50} values.

Obtained data indicate that all plant species possess cytotoxic properties except for LAF (LC_{50} = 1779 µg/mL). Highest cytotoxic potential was observed for OVH (LC_{50} = 97 µg/mL). Moreover, OVH manifested moderate antioxidant activity (IC_{50} = 0.058 mg/mL), whereas MFF showed strong activity (IC_{50} = 0.036 mg/mL) [3]. The ferric reducing power is decreasing as follows: OVH > MFF > LOH > CHF > LAF > CCF > LUS.

Results of the present study demonstrate that some extracts manifested high cytotoxic potential and a strong antioxidant potency. Due to their significant bioactivity, these species represent promising natural resources for prevention and as a complementary therapy for treatment of pathological conditions associated with excessive ROS.

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SL 65

HYPOLIPIDEMIC AND ANTIOXIDATIVE PROPERTIES OF NORTH-EAST INDIAN SEA BUCKTHORN (*HIPPOPHAE SALICIFOLIA* D. DON) LEAF EXTRACTS

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Sea Buckthorn (SBT), a dioecious plant has been explored traditionally for the treatment of various diseases, including diabetes. In the present study, methanolic (MT) extract of both male leaves (ML) and female leaves (FL) were examined for their in-vitro hypolipidemic activity due to their higher total phenolic content and antioxidant activities. Among all SBT extracts FL-MT and ML-MT extracts were the best antioxidants having least IC_{50} values. FL-MT extract exhibited better in-vitro hypolipidemic activity (13.92 ± 0.78% micellar solubility of cholesterol) than the ML-MT extract (37.13 ± 2.36% micellar solubility of cholesterol). FL-MT crude extract was further fractionated using column chromatography and among all fractions, aqueous-methanolic fraction showed better antioxidant activity (IC_{50} values of 2.83 µg/mL for DPPH and 12.23 µg/mL for ABTS) than the crude extract (IC_{50} values of 3.11 µg/mL for DPPH and 12.38 µg/mL for ABTS). However, none of the fractions imparted better in-vitro hypolipidemic activity than the crude extract. Hence, FL-MT crude extract was chosen for in-vivo hypolipidemic activity in ICR mice and Wistar rats. In single dose experiment, FL-MT extract and epigallocatechin-gallate (EGCG) standard depicted significant effect on decrease of plasma cholesterol and triglyceride level after 4h. In multiple dose experiment there was significant decrease in body weight gain and feed intake as compare to the control group after 7 days. Both EGCG groups and SBT groups showed decrease in serum triglyceride and plasma cholesterol level than the control group.

Therefore, Sea Buckthorn leaf extracts can be used as a potent antioxidative and hypolipidemic nutraceutical.

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PP 1

MANUKA HONEY TRIGGERS HUMAN COLON CANCER HCT-116 CELLS GROWTH INHIBITION THROUGH INDUCING OXIDATIVE AND ENDOPLASMIC RETICULUM STRESS, AND MODULATING MITOCHONDRIAL RESPIRATION AND GLYCOLYSIS

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Considerable researches have been made on plant bioactive compounds and cancer prevention in the recent decades. In the present study we investigated the growth inhibitory effects of Manuka honey (MH) on human colon cancer HCT-116 cells. MH represents a good source of phenolic compounds such as luteolin, kaempferol, quercetin, gallic acid and syringic acid. MH exhibits profound inhibitory effects on the growth of human colon cancer cells, without showing any toxic effect on normal non-cancer cells. At different concentrations, a strong induction of oxidative stress is observed after MH treatment since it increases the accumulation of reactive oxygen species (ROS) and elevates the damage of protein, lipid and DNA of the HCT-116 cells. Furthermore, MH suppresses the Nrf2 dependent antioxidant enzyme expression (Nfr2, SOD, CAT and HO-1) and the activity (catalase, SOD, GPX, GR and GST). Endoplasmic reticulum stress (ER) is observed, as suggested by the elevation of ATF6α and XBP1 protein expression. The HCT-116 cells metabolism is markedly disrupted after MH treatment. It decreases the maximal oxygen consumption and spare respiratory capacity, which may reduce mitochondrial function that is correlated with cell survival potential. Concurrently, MH decreases the extracellular acidification rate and glycolysis of HCT-116 cells. Furthermore, MH suppresses the p-AMPK/AMPK, PGC1α and SIRT1 activation which are involved in the HCT-116 cells survival under metabolic stress condition.

Above finding indicates that MH induces HCT-116 cell death partly by enhancing oxidative and ER stress, as well as by regulating the energy metabolism in both aerobic and anaerobic pathways.

PP 2

PHENOLIC EXTRACTS OF GALICIAN EXTRA VIRGIN OLIVE OILS INHIBIT CELL PROLIFERATION, PROMOTE APOPTOSIS AND ROS PRODUCTION IN MCF-7 BREAST CANCER CELLS

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The interest of Galician oil producers (NW Spain) in recovering the ancient autochthonous olive varieties *Brava* and *Mansa* has increased substantially in recent years. Previous studies have been developed to determine the chemical parameters and sensory analysis to classify olive oils according to EU Regulation 2568/91 and subsequent amendments. Recently their healthy properties are beginning to be studied.

In the present work, *Brava* and *Mansa* Galician Extra Virgin Olive Oils (EVOOs) were analyzed to determine the *o*-diphenolic and total phenolic content (TPC), the bitterness index (K_{225}) and the total antioxidant capacity (TAC). Phenolic extracts of *Brava* EVOO, which showed the highest values of all the studied parameters, were selected for treating MCF-7 breast cancer cells. Cell viability evaluated by MTT assay and intracellular ROS production and apoptosis rate by Tali® Image-Base Cytometer were determined. It was also determined the capacity of phenolic extracts from *Brava* EVOO to inhibit the ability of the MCF-7 migration.

The results showed that phenolic extracts from *Brava* EVOO significantly reduced MCF-7 cells proliferation in a concentration and time-dependent manner and induced apoptosis and ROS production. In addition they suppressed the ability of MCF-7 cells motility. As a conclusion, phenolic extracts from *Brava* EVOO could be a novel and promising chemotherapeutic agent in breast cancer treatment although more analysis is needed to confirm this hypothesis.

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PP 3

LEAF ANATOMY OF BRAZILIAN SPECIES OF *SOLANUM* (SOLANACEAE) USED AS MEDICINAL, AS SUPPORT TO THEIR QUALITY CONTROL

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Solanum L. is recognized for having species with ethnomedicinal importance, and biological activities, as well as for the presence of bioactive compounds, mainly steroidal alkaloids [1-2]. Popularly are known as "Jurubeba" and used in folk medicine in Brazil. The morphological similarities between *Solanum* species are remarkable, and a clear distinction between them is often very difficult. In this work a comparative study of the leaf anatomy of twenty Brazilian *Solanum* species was carried out in order to provide additional distinctive parameters for the quality control of their ethnodrugs, as well as to evaluate their taxonomic importance. The study was performed according to usual techniques of plant anatomy, with support of optical and scanning electron microscopy. As results, the species showed dorsiventral mesophyll, angular collenchyma and bicollateral vascular system, common characters to *Solanum*. The curved pattern of anticlinal cell walls was predominant on the adaxial surface, and the wave pattern was predominant on the abaxial surface. The leaves are amphystomatic in most of species, like *Solanum scuticum* M. Nee, and hypostomatic in some species, like *Solanum subumbellatum* Vell. Four different types of stomata were recognized: anisocytic, anomocytic, paracytic, and diacytic. The combination of some micro-morphological characters of leaf epidermis such as diversity, distribution and type of stellate trichomes, the anticlinal walls of epidermal cells, and the type and distribution of stomata proved to be the most useful and distinctive characters, which may contribute as an additional tool for the taxonomy of *Solanum* and quality control of its species with medicinal purposes.

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PP 4

ETHNOMEDICINE AND LEAF MORPHOANATOMY OF MEDICINAL AND POISONOUS APOCYNACEAE IN BRAZIL

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This work was performed with the objective to carry out an etnomedicinal and morphoanatomical study of ten species of Apocynaceae known as medicinal and poisonous: *Aspidosperma pyrifolium* Mart. & Zucc., *Allamanda blanchetii* A.DC, *Catharanthus roseus* (L.) Don, *Hancornia speciosa* Gomes, *Himatanthus bracteatus* A .DC.) Woodson, *Mandevilla moricandiana* (A.DC.) Woodson, *Mandevilla scabra* (Hoffmanns. ex Roem. & Schult.) K.Schum., *Nerium oleander* L., *Rauvolfia lingustrina* L. and *Thevetia peruviana* Schum. Paradermic and cross-sections were performed on samples of fresh and fixed leaves, according to usual techniques of plant anatomy, with support of optical and scanning electron microscopy. As results was observed epidermis with anticlinal cell walls sinuous on epiphyllo, and straight to curves on hypophyllo of *H. speciosa*, *T. peruviana* and *C. roseus*; straight to curves in *A. pyriifolium*, *A. blanchetii*, *N. oleander* and *R. ligustrina*; sinuous in *H. bracteatus* and *M. moricandiana*. All species showed hypostomatic leaves, except *C. roseus* with amphystomatic pattern. Paracytic stomata were observed in *T. peruviana*, *R. ligustrina* and *A. blanchetti*; paracytic and anomocytic in *M. moricandiana*; anisocytic in *H. speciosa* and *H. bracteatus*; and ciclocytic in *A. pyrifolium*. All species showed stomata at the level of epidermis, except *N. oleander* with encrypted stomata. The dorsiventral mesophyll was observed in all species, and six species showed the palisade parenchyma 2-stratified. Idioblasts type "druses" were observed in eight species, except in *M. moricandiana* and *A. blanchetii*.

The set of characters of the epidermis and its annexes together with those of the mesophyll were distinctive parameters for the species.

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PP 5

BARBATIMÃO (*STRYPHNODENDRON ADSTRINGENS* (MAR.) COVILLE) IMPROVES PROLIFERATIVE, ANTIOXIDANT, AND TELOMERASE LEVELS OF HUMAN DERMAL FIBROBLASTS

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Introduction: *Stryphnodendron adstringens* (barbatimão) is found in Brazil's savannah plains and has wound healing, anti-inflammatory, and antimicrobial effects associated with the rich chemical matrix of tannins. Although barbatimão presents no toxicity, its direct effect on human cells has not been evaluated. Therefore, in the present study, potential effects of barbatimão on health dermal fibroblast were determined.

Methods: Aqueous barbatimão stem bark extract was obtained and the main molecules were quantified by HPLC-DAD analysis, and its antioxidant capacity was estimated by DPPH assay. A human fetal fibroblast (HFF-1) cell line was used as an *in vitro* model, and bartimão's effects on its viability and cell cycle was determined by flow cytometry analysis. At different cell cultures moments (1, 3, and 5 days) the following parameters were also estimated in the two better barbatimão concentrations (0.49 and 0.99 mg/mL): modulation of ROS, DNA damage by 8-deoxyguanosine quantification, caspases (1, 3, 8), antioxidant enzymes levels. Levels of activity and gene modulation were also determined.

Results: Barbatimão extract was richest in polyphenols, and its effect on HFF-1 viability was observed at higher concentrations (3.99 mg/mL). The cell cycle indicated improvement of the HFF-1 proliferative state. In general, two barbatimão concentrations decreased levels of ROS, DNA damage, and apoptotic markers (caspases). Moreover, barbatimão improved levels of antioxidant enzymes and telomerase levels causing overexpression of this enzyme. The results suggested a non-toxic barbatimão effect on fibroblast cells and its potential use in the cosmetics industry.

PP 6

ANALYSIS OF AN AQUEOUS EXTRACT FROM *PICRALIMA NITIDA* FRUIT DEREPLICATION USING THE MOLECULAR NETWORKING

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We are interested in *Picralima nitida* (Stapf) Th. et H. Durand (Apocynaceae), a tree native to the forests of tropical Africa, which is widely used as an antimalarial, generally for its bark. Several investigations on leaves, bark, roots and seeds have shown very good *in vitro* antiplasmodial activities, with IC_{50} from 120 to 270 ng/mL [1], which were attributed to indole monoterpene alkaloids [2]. In Côte d'Ivoire, an original traditional preparation of the fruit is used and is the subject of this work.

Four different batches of the fruit were purchased in markets. According to traditional use, they were emptied of their seeds then filled with water for a maceration for 24 hours. These aqueous extracts were analyzed. After a gravimetric determination of the total alkaloids, the evaporated extract was subjected to LC-ESI(+)-Q-TOF MS/MS. The fragmentation data obtained were used, together with those of an "in house" database of indole monoterpene alkaloids [3], so as to build molecular networks, in a dereplicative approach. Several known compounds were thus identified, including picraline, serpentinine (confidence level: 2a), akuammine, burnamine, serpentine…, as well as analogues, while the presence of unknown alkaloids could be highlighted.

This study made it possible to highlight the presence, in this peculiar traditional preparation, of alkaloids described as being endowed with antiplasmodial activity [2], accrediting the allegation of "malaria remedy" proposed by tradition. Analyzes are under way to isolate molecules not yet described and determine their antiplasmodial activity, as well as that of the preparation itself.

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PP 7

IN VITRO ENZYME INHIBITORY POTENTIALS OF THREE *SALVIA* TAXA: *S. BLEPHAROCHLAENA*, S. *EUPHRATICA* VAR. *LEIOCALYCINA* AND *S. VERTICILLATA* SUBSP. *AMASICA*

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Salvia genus belongs to the family of Lamiaceae which contains about 99 species in the Turkey. The members of this genus are widely used to treat several ailments such as cold, and gastrointestinal disorders [1, 2]. At this point, the enzyme inhibitory potential of different extracts (dicholoromethane, methanol and water) from three *Salvia* species (*S. blepharochlaena*, *S. euphratica* var. *leiocalycina* and *S. verticillata* subsp. *amasica*) were investigated in the present study. Enzyme inhibitory effects were tested against cholinesterase, tyrosinase, amylase and glucosidase. For cholinesterase inhibitory effects, the dichlorometane and methanol extracts were exhibited stronger effects compared to water extracts. Also, the dicholoromethane extract of *S. verticillata* subsp. *amasica* had the strongest amylase and glucosidase inhibitory effect. However, the extracts exhibited moderate tyrosinase inhibitory effects.

On the basis of our results, these *Salvia* species could be considered a potential source of natural enzyme inhibitors for designing new pharmaceuticals or functional preparations.

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PP 8

A COMPARATIVE STUDY ON ANTIOXIDANT PROPERTIES OF *DIANTHUS CALOCEPHALUS*: DIFFERENT EXTRACTS AND TECHNIQUES

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Dianthus genus is represented by 67 species in Turkey [1]. *Dianthus* species are widely used for treating gastro-intestinal disorder, wound, and cough [2]. This work aimed to evaluated the effects of three various solvents (ethyl acetate, methanol, and water) and three different extraction methods (maceration, soxhlet, and ultrasonication-assisted) on antioxidant activity of *Dianthus caloceph-alus*. The antioxidant ability was evaluated with different methods including free radical scavenging (DPPH and ABTS), reducing power (CUPRAC and FRAP), phosphomolybdenum and metal chelating. Generally, the soxhlet technique was noted as the most effective technique and the methanol extracts from all techniques exhibited the strongest antioxidant effects in the test system. Also, the maceration technique had the lowest antioxidant ability. From these results, *D. calocephalus* can be utilized as a source of natural antioxidant in food and pharmacological areas.

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PP 9

ANTIOXIDANT POTENTIAL OF WATER EXTRACT OF TURKISH PROPOLIS

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Propolis is a natural, resinous hive product that has several pharmacological activities. The chemical composition of propolis depends on the vegetation, climate, season and environmental conditions of the area from where it was collected [1]. In this study, antioxidant activity of water extract of Turkish propolis was tested by different methods [free radical scavenging, reducing power, total antioxidant (phsophomolybdenum) and metal chelating]. Also, total phenolic and flavonoid contents of extract were detected [2]. Total phenolic and flavonoid content were found to be 113.69 mgGAE/g extract and 9.13 mgRE/g, respectively. The propolis sample was exhibited remarkable free radical scavenging activities on DPPH (182.25 mgTE/g extract) and ABTS (541.01 mgTE/g extract). Reducing power was determined as 546.07 mgTE/g extract (in CUPRAC) and 363.98 mgTE/g extract (in FRAP). Metal chelating and total antioxidant abilities were also 0.57 mgEDTAE/g extract and 1.17 mmolTE/g extract, respectively.

According to our results, the Turkish propolis may be noted as a valuable source of natural agents for discovering new functional products such as drugs and nutraceuticals.

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PP 10

ANTIOXIDANT ACTIVITY OF AERIAL PARTS OF *CAPPARIS OVATA*

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The aim of the present study is to determine antioxidant activity of *Capparıs ovata*. Antioxidant activity of the aqueous and methanolic extract was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and CUPRAC (cupric reducing antioxidant capacity) tests. Total phenol contents of extracts were calculated spectrophotometrically. The IC_{50} values for DPPH assay have been found as 0.3685 ± 0.0027 and 1.0126 ± 0.0082 (mg/mL); FRAP values are 192 ± 3.341 and 95 ± 1.470 (µM Trolox/g sample); CUPRAC values 384 ± 4.621 and 1638±13.789 (µM Trolox/g sample); total phenolic content values 5.3 ± 0.201 and 3 ± 0.0981 (mg gallic acid/g sample) for aqueous and methanolic extract of the plant, respectively.

Data from present results revealed that *Capparis ovata* act as an antioxidant agent due to its free radical scavenging and cytoprotective activity.

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PP 11

UV-BASED SPECTROSCOPIC DIFFERENTIATION OF PROPOLIS

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Buds of *Populus* species are the main source of propolis in Europe, Asia, New Zealand, North and South America [1]. The chemical composition of propolis is strongly related to its biological activity, because of this, it can be used for quality control of propolis-based preparations. The aim of this study was to develop a UV-based spectroscopic method for rapid characterization of propolis obtained from different *Populus* species. Samples were collected from February 2008 to October 2014, from hives located in Serbia, Macedonia, Bulgaria, and Bosnia and Herzegovina. Sampling locations varied in elevation from 100 to 1000 m. To correlate variability in *Populus* type propolis to altitude, multivariate data analysis using OPLS and O2PLS models were performed. Metabolites identification was done by LC-MS analysis.

According to OPLS and O2PLS analysis, the UV data showed strong correlation between *Populus* species and the corresponding propolis samples. Major components in propolis samples collected in temperate continental climate above 500 m, were phenolic glycerides, and this were originated from *P. tremula* buds. On the other hand, flavonoids were predominant in propolis samples collected below 400 m, originated from *P. nigra* and *P. x euramericana* buds. The samples collected from 400 to 500 m were produced by mixing different poplar species, showing presence of all metabolites detected in individual species.

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PP 12

APPLICATION OF LIQUID PHASE AND MICROWAVE EXTRACTION FOR THE DETERMINATION OF MULTI-COMPONENT PHENOLIC PATTERN IN DIFFERENT *GALIUM* SPECIES (*GALIUM VERUM, GALIUM PURPUREUM, GALIUM RIVALE, GALIUM PSEUDOARISTATUM*) AND COMMERCIAL PHYTOPHARMACEUTICALS

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Recently, using of herbal medicines for the prevention and preservation of health became a trend in modern medicine due to such benefits as safety, accessibility, efficacy with a wide range of therapeutic action and low costs [1]. *Galium* is a well-known genus gathering many medicinal representatives, which are rich sources of phenolic compounds [2, 3]. The aim of the current study is to develop liquid phase and microwave extractions (MAE) procedures in order to establish multi-component phenolic pattern of *Galium verum*, *Galim purpureum*, *Galium rivale*, *Galium pseudoaristatum* and analyze twelve commercially available phytopharmaceuticals of *Galium verum* using HPLC-PDA. Dispersive liquid–liquid microextraction (DLLME) in NaCl and natural deep eutectic solvent (NADES) medium and ultrasound-assisted DLLME in β-cyclodextrin medium were optimized. The optimal DLLME conditions were found to be: 10 mg of the sample, 10% NaCl, 15% NADES or 1% β-cyclodextrin, extraction solvent – 400 µL of ethyl acetate, dispersive solvent – 300 µL of ethanol, vortex time – 30 s, extraction time – 1 min, centrifugation at 12 000 rpm for 5 min. In the case of UA-DLLME, 5 min of ultrasonication is required. The developed microextraction procedures were applied for analysis of real samples and compared with results of MAE.

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PP 13

INHIBITION OF CELL ATTACHMENT OF HUMAN HERPES VIRUS TYPE 2 (CAUSATIVE AGENT OF GENITAL HERPES) BY WATER EXTRACT FROM *NEPETA NUDA* L.

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The genus *Nepeta* (Lamiaceae) comprises about 250 species distributed in the central and southern parts of Europe, Asia and the Middle East. Several *Nepeta* spp. are used in folk medicine as diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic, febrifuge, emmenagogue and sedative agents [1].

In our experiments we examined the antiviral activity of the water extract from *Nepeta nuda* L. against HHV-2 strains BA and ACV-resistant strain DD using modification of MTT assays at low MOI as well as the ability of the extract to inhibited viral yield production using yield reduction assay at high MOI as extract was added simultaneously with the virus. Our results from MTT assay show strongly inhibitory effect of the extract against the two examined viruses. The percentage of protection for HHV-2, strain BA is up to 90% (IC_{50} is ≈ 0.747 mg/ml), whereas the percentage of protection for HHV-2, strain DD is up to 80% (IC_{50} is ≈ 0.698 mg/ml). The results from yield-reduction assay also confirmed strong inhibitory activity. The water extract inhibited yield production of the two strain- BA and DD with 99.9 % at 4.5 mg/ml, and with 2 mg/ml with ~99%. We also examined the effect of the extract on virus attachment to the cell membrane. The results from this experiment show that the water extract from *Nepeta nuda* L. completely blocks the attachment of the virus to the cell membrane.

Acknowledgments: This work was financially supported by the grand № 80-10-211/2017 of Scientific Fund, University of Sofia "St. Kliment Ohridski", Bulgaria.

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PP 14

VARIATION OF MINERAL, POLYPHENOL AND FLAVONOID CONTENT BY DEVELOPMENT STAGE IN DIFFERENT PARTS OF *URTICA DIOICA* L. CULTIVATED ON THREE DIFFERENT SOILS

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Urtica dioica L. is a species with a long tradition of use as a herbal remedy and as a leafy vegetable. It has been claimed to be – *inter alia* – a "good dietary source" of calcium and iron [1], but iron absorption may be decreased by polyphenols. We here report on the variation of minerals, polyphenols and flavonoids in this species, in relationship to its development stage, herbal part and soil on which the plant has been cultivated. Atomic absorption spectrometry (AAS) was used to assess the mineral contents, whereas polyphenols and flavonoids were assessed spectrophotometrically. Calcium and iron contents were highest in leaves and lowest in stems. Iron contents increased along three development stages, whereas calcium decreased in the third stage. Polyphenol and flavonoid contents were considerably higher in leaf than in root and stem.

The development stage had a higher impact on contents in minerals, polyphenols and flavonoids than the type of soil.

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PP 15

CHEMICAL PROFILES OF AERIAL PARTS OF *THYMUS PANNONICUS* ALL. FROM DIFFERENT LOCATIONS IN SERBIA

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Aerial parts of *Thymus pannonicus* All. are traditionally used for the treatment of minor gastrointestinal and respiratory ailments, in which its previously demonstrated antimicrobial and antioxidant properties might have a role [1, 2]. In this work we investigated chemical profiles of essential oils and aqueous-methanolic extracts of aerial parts of *T. pannonicus* from six localities in Serbia, having in mind that thyme species often exhibit intraspecific variability regarding their chemical composition.

The aerial parts of *T. pannonicus* were collected from six localities (1-6) during flowering period, and air dried. Essential oils (EO1-EO6) were isolated by hydrodistillation and analyzed by GC-FID-MS. Extracts (ME1-ME6) were obtained using 80% aqueous methanol by sonication followed by maceration, and subsequently analyzed by HPLC.

Three types of EOs were recognized, based on the high content of: (1) oxygenated monoterpenes: EO1 (citral 74.6%); (2) oxygenated monoterpenes and sesquiterpene hydrocarbons: EO2 (thymol 38.0%) and EO4 (linalool 24.7%); and (3) sesquiterpenes: EO3 (spathulenol 16.8% and epi-*α*-cadinol 14.4%); EO5 (germacrene D 13.2%), (*E*)-caryophyllene 8.9% and caryophyllene oxide 7.5%), EO6 (germacrene D 29.4% and (*E*)-nerolidol 6.5%).

HPLC analysis of MEs revealed the presence of phenolic constituents. Rosmarinic acid was the most abundant compound in the all examined extracts (26.6–146.8 mg/g). The total luteolin hetrosides and total apigenin heterosides amounted for 4.1–17.3 mg/g and 1.4–8.5 mg/g, expressed as corresponding aglycon respectively. ME1 distinguished from the other extracts (ME2-ME6) by the presence of substantional amount of salvianolic acid H and markedly lower content of luteolin 7-*O*-glucuronide.

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PP 16

INTRA-POPULATION VARIATIONS OF THE FLOWER VOLATILE COMPOSITION OF HYSSOP (*HYSSOPUS OFFICINALIS*) PLANTS

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Hyssop (*Hyssopus officinalis* L.) has been traditionally and widely used in the food, cosmetic and phytopharma industries. The composition and biological activities of the hyssop essential oil have been a subject of several studies. However, the comparison of the essential oil is less efficient for characterization of larger natural or segregating hyssop populations at the single plant level. Here we describe a simple and efficient procedure for GC-MS characterization and comparative analysis of flower volatile composition of individual plants from a hyssop natural population located in the west part of the Balkan mountain in Bulgaria. The obtained results show low variation of the volatile composition and narrow range of variations in the relative abundances of individual volatile compounds.

The application of the developed procedure for comparative characterization of the volatile composition and metabolite biodiversity of naturally growing aromatic plants is discussed.

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PP 17

CHEMICAL CHARACTERIZATION OF SELECTED GREEK ROYAL JELLY SAMPLE AND COMPARATIVE STUDY OF 27 DIFFERENT ORIGIN SAMPLES. BIOLOGICAL ACTIVITIES

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Royal Jelly (RJ) is a thick, milky secretion of hypopharengeal and mandibular glands of worker bees *Apis mellifera* and it is a sole food of the queen bee during her larval and adult life. RJ has been demonstrated to possess several pharmacological activities as disinfectant action, antioxidant, antimicrobial, anti-hypercholesterolemic and anti-inflammatory activity, due to its unique fatty acids content [1]. Therefore it has considerable commercial appeal, ranging from the pharmaceutical and food industries to the cosmetic.

The objective of our research is the characterization of the quality of marketed RJ samples by GC-MS techniques. 27 samples of different origin have been studied, among which 2 Greek samples, which were further analyzed by chromatographic techniques. Seven secondary metabolites, including the main fatty acids (decanoic and decenoic acid derivatives), were isolated and their structures were determined by modern spectroscopic methods.

The Greek samples (Crete and Epirus, respectively), were also subjected i) in a clinical study at postmenopausal healthy women, where RJ exhibited a significant decrease in low density lipoprotein cholesterol (LDL-C) and an increase in high density lipoprotein cholesterol (HDL-C) without affecting the cardiovascular and the bone turn-over parameters [2] and ii) in an *in vivo* study on Wistar male rats, where the influence of RJ on the concentration of brain neurotransmitters by biochemical examination and the improvement of the spatial memory were observed, with very positive results [3].

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PP 18

EVALUATION OF PHENOLIC COMPOUNDS IN *CISTUS CRETICUS* BEE POLLEN FROM GREECE. BIOLOGICAL PROPERTIES

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Bee pollen is a raw material produced by flowering plants pollen, mixed with nectar and bee secretions by the honey-bees [1]. *Cistus creticus* is a Mediterranean evergreen shrub. Dried leaves have been traditionally used as infusion and/or decoction and have shown gastroprotective effect, while *per os* administration was used to treat cough and cold, as well as against mouth and throat irritations [2].

This study was carried out to evaluate the antioxidant properties of Greek *Cistus* (rock rose) bee pollen and define its phenolic compounds. The pollinic spectra of three Greek bee pollen samples from *Cistus* was obtained by Louveaux's quantitative microscopical analysis and it showed that one of them had *Cistus* sp. (Cistaceae) as abundant pollen (together with low percentage of *Brassica* sp., Cruciferae). Throughout the chemical analysis of the extracts, several secondary metabolites of flavonoid structure have been isolated and identified: quercetin-7-rhamnoside (**1**), quercetin-3-neohesperidoside (**2**), kaempferol-3-neohesperidoside (**3**), myricitetin-3-neohesperidoside (**4**), kaempferol-3-glucoside (**5**), quercetin-3-glucoside (**6**) and kaempferol-7-rhamnoside (**7**). Moreover, the total phenolic content was determined by the Folin-Ciocalteu method, total flavonoid content was estimated by the aluminium chloride colorimetric assay and the free radical scavenging activity was determined by DPPH and ABTS assays. The antimicrobial activity of the extracts was tested against six Gram-positive and -negative bacteria and three pathogenic fungi, showing an interesting antibacterial profile.

Acknowledgements: The authors would like to thank the Special Account for Research Grants and the National and Kapodistrian University of Athens for funding their participation in this meeting.

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PP 19

GENOME-WIDE PROFILING OF *LEUCOJUM AESTIVUM* L. (SUMMER SNOWFLAKE) AND *NARCISSUS CONFUSUS* PUGSLEY

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The two sets of naturally accessions were analyzed. A set of *Leucojum aestivum* L. individuals was collected from 31 habitats in Bulgaria and the other set of *Narcissus confusus* was collected from four different sites in Spain.

Genome-wide profiling of the accessions was carried out by several DNA marker systems: Random Amplified Polymorphism DNA, Amplified Fragment Length Polymorphism and Inter Simple Sequence Repeats (RAPD, AFLP and ISSR) which reveal the level of genetic diversity between and within populations, fingerprint of accessions as well as DNA markers linked to alkaloid pattern chemotypes.

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PP 20

ISOLATION OF GENES INVOLVED IN GALANTHAMINE BIOSYNTHESIS

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Future search for suitable markers should include both genome-wide analysis using AFLP as well as locus-specific analysis. The primary targets of such locus-specific screening could be the key enzymes of the proposed galanthamine pathway: phenylalanine ammonia lyase, tyrosine decarboxylase and hypothetical gene(s), homologous to S-norcoclaurine synthase. These three enzymes were identified as key factors in the biosynthesis pathway and were selected as most promising targets for gene cloning and analysis. Two of the enzymes are actually first ones in the aromatic acid pathway chain – Phenylalanine-ammonia lyase (PAL) and Tyrosine decarboxylase (TDC). Second enzyme was suggested to be an analog to S-norcoclaurine synthase (NCS) from *Papaver somniferum*. This enzyme catalyses similar condensation reaction thus providing a reasonable degree of confidence that a similar enzyme might function in *Leucojum* and/or *Narcissus*. Identification, cloning and characterization of these three genes will provide good starting point for further characterization of the galanthamine biosynthesis.

Of particular interest is the identification of allele variants as well as variations within promoter regions resulting in high levels of alkaloid production.

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ASSESSMENT OF REDOX-MODULATING EFFECT OF DOCOSAHEXAENOIC ACID AND CHOLESTYRAMINE IN HYPERCHOLESTERIEMIA-INDUCED KIDNEY DYSFUNCTION, USING A REDOX-SENSITIVE NITROXIDE PROBE

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Hypercholesterolemia could provoke a disruption of redox-homeostasis due to incorporation of abnormal amount of cholesterol in the membranes of endothelial cells of vessel wall, as well as in the tissues, and induction of oxidative stress. These events have a significant impact in the etiology of many diseases – cancer, neurodegeneration, diabetes, cardiovascular, renal dysfunction, etc.

The present study was designed to investigate the redox-modulating capacity and anti-lipidemic effect of cholestyramine and docosahexaenoic acid (DHA) in kidney of mice with hypercholesterolemia.

The mice (C57Bl/6) were subjected to a normal diet (ND) or a cholesterol diet (CD; 1.25% cholesterol, wt : wt) – in the absence or presence of the subsequent anti-lipidemic substance. Tissue redox-status was measured by nitroxide-enhanced MRI in vivo or EPR in vitro on isolated tissue specimens, using mito-TEMPO as a redox-sensitive probe. All experiments were conducted in accordance to the Guidelines of the Animal Investigation Committees of Chiba University (Chiba, Japan) and National Institute of Radiological Sciences, QST/NIRS (Chiba, Japan). The level of oxidative stress in kidney was also analyzed by conventional biochemical tests.

The results show that hypercholesterolemia induced oxidative stress in the kidney, which was clarified by the long-lived nitroxide-enhanced MRI/EPR signal. Cholestyramine and DHA shortened the lifetime of the MRI/EPR signal, which indicated a suppression of oxidative stress and restoration of the reducing capacity of renal tissue. These data were confirmed by the levels of superoxide, hydroperoxides, and reduced glutathione in the renal tissue, analyzed by conventional assays.

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EFFECT OF ETHYL ACETATE AND BUTANOL EXTRACTS OF *GEUM URBANUM* L. ON CELL CYTOTOXICITY AND CELL PROLIFERATION OF BURKITT'S LYMPHOMA CELLS

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The present study was directed to the investigation of concentration- and time-dependent cytotoxicity of ethanol and butanol extracts, obtained from roots and stems of *Geum urbanum* L. on Raji cell line, derived from patients with Burkitt's lymphoma.

The extracts were prepared by maceration of blade and radix of *Geum urbanum* L. in methanol-water 2 : 1 v/v, following by separatory extraction with ethyl acetate and butanol. The following incubation conditions were used: 1 x 10^6 cells/ml, plant extract – 50-1000 µg/ml, incubation time – 24-72 h, humidified atmosphere (37° C, 5% CO_2). The following parameters were analyzed: cell survival (trypan blue staining), intracellular superoxide (hidydroethidium test), hydroperoxides (OxySelect™ In Vitro ROS Assay), total antioxidant capacity (OxySelect™ TAC Assay), induction of apoptosis (FITC-Annexin V test).

It was found that butanol extracts from roots and stems of *Geum urbanum* L. (750 µg/ml and over) inhibited cell growth by 50% after 48 h incubation, increased the level of apoptosis ~3 times, and TAC more than 5 times, decreased the value of hydroperoxides by 50% versus controls, non-treated cells. Radix's ethyl acetate and butanol extracts increased intracellular superoxide more than two times.

Data obtained suggest that ethyl acetate and butanol extracts of *Geum urbanum* L. have a promising anti-cancer activity towards Burkitt's lymphoma B-cell line (Raji) *in vitro*.

PP 23

CHEMICAL FINGERPRINTING AND BIOACTIVITY OF ESSENTIAL OILS FROM ECUADORIAN'S AMAZON: *CHENOPODIUM AMBROSIODES* (AMARANTHACEAE), *SCHINUS MOLLE* (ANACARDIACEAE) AND *DACRYODES PERUVIANA* (BURSERACEAE)

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Three Amazonian essential oils (EOs) obtained through steam distillation of fresh leaves of *Chenopodium ambrosiodes* (Amaranthaceae), *Schinus molle* (Anacardiaceae) and *Dacryodes peruviana* (Burseraceae), named CA, SM, and DP, were chemically characterized by GC-MS [1]. Their chemical compositions were mainly characterized by the following compounds: CA, limonene 41.48%; SM α-phellandrene 13.62%; DP, -3-carene. All the essential oils were then checked for their antioxidant activity: These oils evidenced interesting results with both DPPH and ABTS assays, showing respectively DPPH-IC_{50} = 0.5 mg/ml and ABTS-IC_{50} = 0.15 mg/ml. Antibacterial properties of all EOs, verified through Broth dilution method [2], evidenced interesting bioactivity especially of SM against *Klebsiella oxytoca* (MIC = 500 µg/mL), *Pseudomonas aeruginosa* (MIC = 250 µg/mL), *Proteus vulgaris* (MIC = 500 µg/mL), *Enterococcus faecalis* (MIC = 500 µg/mL), *Micrococcus luteus* (MIC = 250 µg/mL) and *Staphylococcus aureus* δ (MIC = 500 µg/mL). Studies of synergistic effects are currently in progress. Valuable results against *Candida albicans* have been shown by DP with MIC = 1250 µg/mL. The research has been extended to dermatophytes and phytopathogens fungi through Agar vapour method [2]. CA showed 100% growth inhibition against the dermatophyte *Nannizzia gypsea*. This project gives an essential contribution to the valorization of biodiversity of Ecuadorian Amazonia and to the definition of possible industrial applications.

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PP 24

KAINARI - A GREEK TRADITIONAL HERBAL TEA, FROM LESVOS ISLAND. CHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES

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Kainari is a Greek spiced herbal tea traditionally prepared only in Lesvos and used as a warming beverage in winter. It was brought from Greek emigrants from Asia Minor to the island of Lesvos. In Turkey, a similar herbal tea called kaynar (means boiled) is used traditionally in childbirth [1]. It is produced mixing powder of several spices, but the exact recipe is kept secret in Lesvos.

This study was carried out to evaluate the antioxidant properties of three different samples of Kainari and to study the chemical composition of the mixture and the total phenolic content. Cinnamon, clove, nutmeg, pepper and ginger were identified as the main components. The rich aroma of the mixtures was studied and the volatiles were identified through GC-MS, as well as through Headspace Solid-Phase Microextraction (HSSPME)/GC-MS analyses. Cinnamic aldehyde, eugenol, β-caryophyllene, myristicin and curcumene were determined as the most abundant constituents and they were further isolated and identified through spectral means.

The total phenolic content was determined by the Folin-Ciocalteu method and the free radical scavenging activity by DPPH and ABTS assays. All extracts showed significant antioxidant activity probably due to the rich phenolic content of all contained spices [2, 3]. According to the antimicrobial assays against Gram-positive and -negative bacteria, oral pathogens and human pathogenic fungi, the Kainari extracts exhibited a broad spectrum of antimicrobial activity.

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SECONDARY METABOLITES OF *CREPIS INCANA* SM. (ASTERACEAE)

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The genus *Crepis* L. encompasses approximately 200 species and is well known for its abundance in sesquiterpene lactones. Due to the pharmacological and chemosystematic interest of these compounds [1], we investigated the chemical profile of *Crepis incana* Sm., native to southern Greece [2]. The fresh plant material was dried and extracted at room temperature with cyclohexane : Et_2O : MeOH (1 : 1 : 1) and MeOH : H_2O (5 : 1), successively. Two costus type guagianolides, grosheimmin and crepiside E and two germacranolides, taraxinic acid and its 1'-*O*-*β*-D-glucopyranoside and were isolated by RP_{18} HPLC from the non polar extract. The flavonoids, luteolin, luteolin-3-*O*-*β*-D-glucopyranoside [1, 3] and quercetin-7-*O*-*β*-D-glucopyranoside were isolated by column chromatography from the polar extract. The structures of the isolated compounds were elucidated by high-field NMR spectroscopy (^{1}H-NMR, ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC).

Acknowledgements: The authors wish to thank Associate Prof. Th. Constantinidis (Department of Ecology & Systematics, Faculty of Biology, NKUA) for the identification of the plant material and Dr. Michael Tsoukalas for the extraction of the plant material.

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CHARACTERIZATION OF SEA BUCKTHORN (*HIPPOPHAE SALICIFOLIA* D. DON) OILS FROM NORTH-EAST INDIA FOR VARIOUS APPLICATIONS

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Seed and pulp oils are considered as the most valuable components of Sea Buckthorn (SBT) berries comprising important bioactive phytochemicals. The pulp oil (PO) and seed oil (SO) extracted using petroleum ether (PE) and n-hexane (HX) showed very decent and identical physicochemical properties of odour, solubility, refractive index, optical rotation, acid value and peroxide value. The total unsaturated fatty acid contents of HX extracted PO and SO were found to be 76.15 wt% and 64.92 wt%, respectively. While for PE extracted PO and SO the values were 71.76 wt% and 59.97 wt%, respectively. PO exhibited high carotenoids content (513-579 mg/100 g) than the SO (24-29 mg/100 g) whereas total tocopherols content of SO was higher (186-196 mg/100 g) than PO (147-158 mg/100 g). In-vitro hypocholesterolemic actvity of SO depicted higher values for micellar solubility of cholesterol inhibition (61-63%) as well as for taurocholate binding ability (35-39%), compare to the standard soybean protein peptic hydrolysate (SPH) and PO. UV-VIS spectra of PO and SO exhibited strong absorbtion in the UV-B range (290-320 nm) and may therefore be used as natural sunscreen absorber. All the oil types imparted good cold flow properties, thermal and oxidative stabilty as studied by differential scanning calorimetry (DSC) and Thermo-gravimetric analysis (TGA). Therefore SBT oil endowed with numerous bioactive phytochemicals; exhibiting absorption maxima in UV-B range; having admirable hypocholesterolemic activites; imparting very decent and stable physico-chemical properties along with fantastic cold flow properties and thermal stability; demand a wide application in various nutraceuticals, pharmaceuticals and cosmeceuticals industries.

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PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF WILD *SCOLYMUS MACULATUS* L.

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One of the multi curable believed wild herbal mentioned in the list of native plants of Flora Palaestina is the spotted golden thistle, *Scolymus maculatus* (Asteraceate or Compositae). This wild plant is very common to the Mediterranean region [1]. *Scolymus maculatus*, collected from the farms of Kabul village in *north*-west of *Galilee*, was investigated for the first time for its antioxidant potential and phytochemical contents. Crude extract samples from methanol, ethyl acetate and hexane solvents were investigated using GC-MS in the EI mode. Some of the thirty-two compounds found in these extracts, Stigmasterol, β-Sitosterol, Lupeol, Lupeol acetate and α-Amyrin are known to exhibit various important pharmacological activities particularly against cancerous cells [2, 3]. Phytochemicals such as 2-linoleoylglycerol (5.87%), γ-Sitosterol (5.73%), β-Amyrin (15.98%), Lupeol (22.25%), (3α)-12-Oleanen-3-yl acetate (9.14%), Lupenyl acetate (18.11%) and Fagarsterol (11.26%) were found to dominate the methanol extract. Most of these compounds were also noticed in ethyl acetate and hexane extracts but at different levels besides some other minor compounds. The three extracts were tested for their antioxidant activity and we found that the methanolic extract possesses moderate antioxidant activity with an IC_{50} of 522.2 µg/ml, while both other extract are less active. The findings disclosed herein are important and maybe exhibit the need for development of natural products from *Scolymus maculatus* extracts that possess certain activities with primary applications for human health, and in a broader context, for the treatment of various diseases, mainly for cancer.

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PP 28

EFFECT OF ACETONE EXTRACT FROM STEM BARK OF *CANARIUM ODONTOPHYLLUM* MIQ (DABAI) ON REACTIVE OXYGEN SPECIES (ROS) PRODUCTION AND DNA INTEGRITY OF HUMAN COLORECTAL CANCER CELL LINE HCT 116

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Canarium odontophyllum is an indigenous plant in Borneo and acetone extract from its stem bark was proven to cause cytotoxicity on colorectal cancer cell line HCT116 by inducing cell death via apoptosis. This study was aimed to assess further cytotoxic mechanism of the extract against HCT 116. The IC_{50} value of acetone extract from C. *odontophyllum* stem bark against HCT 116 was determined using MTT assay at concentration ranging from 12.5 µg/ml to 200 µg/ml for 48 hours whereas ROS assay with dihydroethidium staining was conducted using flow cytometer at time points of 30 minutes, 1 hour and 2 hours. The DNA integrity of HCT 116 was evaluated using alkaline comet assay at IC_{10} and IC_{25} values of the acetone extract for 30 minutes of treatment exposure. The extract was cytotoxic towards HCT 116 with IC_{50} value of 82 µg/ml ± 9.3. At 2 hours of post-treatment, acetone extract from stem bark of *C. odontophyllum* has induced elevation of ROS level in the cell lines compared to control at 16 ± 7.2 % and 10 ± 0.9 %, respectively. DNA damage on HCT 116 was observed with elevated tail moment and percentage of DNA in tail when HCT 116 was exposed to low doses of extract at IC_{10} and IC_{25} values.

Acetone extract from stem bark of C. *odontophyllum* has induced cytotoxic activity against HCT 116 by increasing the ROS level and interfering the DNA integrity of the cell line therefore, has the potential to be developed as anticancer agent.

PP 29

ACTIVITY OF HONOKIOL FROM PLANT ORIGIN AGAINST ORAL *CANDIDA* ISOLATES

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Aim: Treatment of oral candidiasis has continued to be problematic because persistent resistance to the most common antifungals has increased greatly [1]. The aim of the study was to examine the antifungal potency of plant derived molecule Honokiol (HNK) [2].

Methods: The present work investigates the antifungal potential of HNK against four reference ATCC strains and 60 oral *Candida* isolates. The MIC for each isolate was determined as per NCCLS document M27-A3, 2008 [3]. Antibiofilm effect was estimated by MTT reduction assay. Cell damage was confirmed through Confocal Scanning Laser Microscopy (CLSM). Ultrastructural effects of HNK were examined through Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM), analysis.

Results: MIC of HNK against *Candida* species ranged from 32-128 µg/ml. MIC, MIC/2 and MIC/4 values of HNK showed the average biofilm inhibition (for four *Candida* species) by 781.2%, 63.21%, and 39.04%, respectively. CLSM results confirmed that the structure of the cell membrane and cell wall was disrupted. SEM results showed damage to the cell wall and plasma membrane which is sharply defined by a change in cell shape, deformity in cells, and formation of deep furrows and wrinkles on the cell surface. TEM analysis of the treated cells showed the complete disintegration of the cell wall, rupturing of the plasma membrane, and oozing out of intracellular content.

Conclusion: HNK is found to be an effective anticandidal agent, it eventually leads to damage of membrane and cell wall. These results taken together make HNK eligible for further development as an antifungal.

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PP 30

INVASIVE ALIEN SPECIES FROM FAMILY ASTERACEAE – POTENTIAL CHEAP AND ENDLESS RESOURCES OF PLANT SUBSTANCES FOR MEDICINAL USE

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Here we present our research data on the distribution and invasion level in Bulgaria of *Ambrosia artemisiifolia* L., *Xanthium strumarium* L., *Erigeron canadensis* L., and *Dittrichia graveolens* (L.) W. Greuter (Asteraceae). The high tolerance of various habitat conditions and potent propagation ability of these alien plants promote their aggressive invasive behaviour. Additionally, they not only over-compete the local vegetation but suppress the seed development. In the newly invaded habitats they might not have suitable herbivores to control their populations. The only effective enemy might be *Homo sapiens* which is known with its destructive power once an object has become significant for industrial utilization.

The aim of this study is to attract the attention towards these plants and their potential to be used as cheap sources of compounds with valuable pharmacological activities.

A growing body of scientific literature points to the therapeutic potential of chemical constituents of these alien invasive species (mainly sesquiterpene lactones). They posses different activities such as anticancer activity, as well as antitussive, antifungal, antiinflammatory, antinociceptive, hypoglycaemic, antimitotic, antioxidant, antitrypanosomal, CNS depressant activity, diuretic effects, contact dermatitis, insecticidal and herbicidal activities, hepatoprotective and hypolipemic activities etc.

Due to the fact that these are aggressive invasive species, they can provide abundant and cheap resources reach of plant chemical constituents which can be utilized for therapeutic purposes. Additionally, exploitation of the biomass for medicinal use might contribute to relieving the destructive impact of this species on natural habitats.

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COMPARATIVE QUANTITATIVE AND QUALITATIVE STUDIES OF EXTRA VIRGIN OLIVE OIL USING HPTLC, HPLC-DAD, NMR, LC-HRMS & MS/MS METHODS

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Olive oil has a unique, mazy chemical composition that makes it a very complex and intriguing matrix for in-depth investigation [1]. Alterations in the concentration of certain constituents, dependent upon various factors, make olive oil a medley of compounds that corresponds differently in every assay even without changes in the experiment conditions. Biophenols, molecules in considerably low, yet with high impact, concentration in extra virgin olive oil, have gained increasing interest leading to a major health claim by the European Food Safety Authority (EFSA) in 2011 [2]. According to this claim, biophenols in olive oil are proven to have protective effects over LDL particles from oxidative damage. However, despite the numerous studies on its chemical composition, health benefits or proposed analytical techniques, there is still a large vacancy of major significance to be filled. The present study, aiming to be the key piece missing from the puzzle, will develop a series of comparative qualitative and quantitative novel methods of extra virgin olive oil's biophenols using state-of-the-art equipment comprised by Nuclear Magnetic Resonance (NMR) spectroscopy, High Performance Liquid Chromatography (HPLC) with Diode-Array Detection (DAD), Liquid Chromatography combined with High Resolution Mass Spectrometry and Mass Spectrometry/Mass Spectrometry (LC-HRMS and MS/MS) and High Performance Thin Layer Chromatography (HPTLC). Additionally, each technique displays both positive and negative elements, while preliminary data already available shows very promising results with high compliance between the different techniques.

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EXPLOITING NATURAL ANTI-MYCOBACTERIAL PRODUCTS FROM THE CHINESE HERB *ARTEMISIA ANNUA*

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The Chinese have been using certain herbs as a part of medicinal practice for many centuries. At present, Traditional Chinese Medicine (TCM) is attracting considerable interest throughout the world as potential sources of novel drug leads. One example were a drug has been derived from a TCM to have global impact is Artemisinin.

Sweet wormwood (*Artemisia annua*) is native to temperate Asia, belonging to the family of Asteraceae. The plant is known to have antimalarial [1], anti-inflammatory, antitumor and allelopathic activities. Artemisinin, a sequiterpenes trioxane lactone, with an endoperoxide bridge, was isolated from *Artemisia annua* by Prof You-You Tu in the 1970's and she was awarded The Noble Prize in Physiology and Medicine in 2015. Although artemisinin itself is not active against tuberculosis, however, conjugation to a mycobacterial specific siderophore analog induces significant and selective anti-tuberculosis activity, including activity against MDR and XDR strains of *Mycobacterium tuberculosis* [2].

We here show new natural products with anti-mycobacterial activity from *Artemisia annua*. Several fractions, isolated using different chromatography techniques, showed very significant (<100 µg/mL) anti-tubercular activity against *M. smegmatis.* Purification, isolation and identification of compounds with the best activity have been performed along with toxicity assays. Mode of action studies are underway based on the innovative use of bacterial metabolomics.

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PHYTOCHEMICAL STUDIES ON SOME *RHAPONTICOIDES* SPECIES

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The genus *Rhaponticoides* Vaill. belongs to the family Asteraceae, which is one the genera previously segregated from *Centaurea* L. By the recent species descriptions and combinations, *Rhaponti-coides* currently comprises 40 species in the world and nine species in Turkey [1-3]. Of these, some species are endemic to Turkey including *R. mykalea*, whose capitulum is known to be consumed as food by the locals, generally named as "wild/bitter artichoke".

In the present study, it was aimed to investigate phytochemical contents of *R. mykalea* (Hub.-Mor.) M. V. Agab. & Greuter, *R. gokceoglui* Çinbilgel, Eren & Duman and *R. hierroi* Eren. Preliminary results of the 70% methanol extracts of mentioned species indicated that hydroxycinnamic acid derivatives are likely to be the prominent compounds. For the purpose of comparing the derivative profiles, these three endemic species were screened with LC-MS/MS system.

Acknowledgements: This study was supported by Adnan Menderes University Scientific Research Foundation.

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DETERMINATION OF TOTAL PHENOLIC CONTENTS, ANTIOXIDANT AND ANTI-LIPOXYGENASE ACTIVITIES OF *PRUNUS SPINOSA* L. SUBSP. *DASYPHYLLA* (SCHUR) DOMIN FRUIT EXTRACTS

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The genus *Prunus* belonging to the Rosaceae family is represented by 7 taxa in Turkey and is known with the local names "erik", "yabani erik", "çakal eriği", and "güvem". In traditional medicine, *Prunus spinosa* subsp. *dasyphylla* fruits are used for treatment of stomach discomfort, eczema, rheumatism, toothache, also as a laxative and tonic [1, 2].

In this study, antioxidant activities of ethanol extract (PSFE) of *Prunus spinosa* subsp. *dasyphylla* fruits and hexane (PSFH), chloroform (PSFC), ethyl acetate (PSFEA) and aqueous ethanol (PSFAE) fractions of its were investigated against DPPH and ABTS radicals. The total phenolic contents of them were determined by Folin-Ciocalteu method. Also, lipoxygenase inhibitory activity of extracts was evaluated was evaluated against 5-lipoxygenase enzyme.

PSFC had the highest DPPH radical scavenging activity with a IC_{50} value of 165.4 µg/mL, followed by PSFEA(195.0 µg/mL), PSFAE(497.6 µg/mL), PSFE(592.8 µg/mL), and PSFH(600.2 µg/mL). PSFC had the highest ABTS radical scavenging activity with a IC_{50} value of 57.84 µg/mL, followed by PSFEA (68.24 µg/mL), PSFH (139.0 µg/mL), PSFE(150.7 µg/mL), and PSFAE(312.1 µg/mL). When the total phenol contents of the extracts are compared, The highest amount of phenolic content was found in PSFC extract(19.56 mg/g), followed by PSFH(14.46 mg/g), PSFEA(10.86 mg/g), PSFE(3.36 mg/g), and PSFAE(2.76 mg/g). PSFH extract at a concentration of 156 µg/mL showed highest anti-lipoxygenase activity with inhibition rate of 52.34%, followed by PSFC(35.60%), PSFEA(34.57%), PSFAE(28.08%), and PSFE(25.54%) extracts.

These results indicate that the PSFC extract has significant radical scavenging and anti-lipoxygenase activities against free radicals and enzyme lipoxygenase, respectively.

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PP 35

EFFECT OF DRYING CONTIDITIONS ON BIOCHEMICAL PROPERTIES OF *CORYLUS AVELLANA* DIFFERENT PARTS

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Hazelnut is a very popular dry fruit in the world. In the present work, Turkish hazelnut (*Corylus avellana* L.) applied different drying condition, were characterized in respect to their chemical composition, antioxidant potential and antimicrobial activity. The samples were analysed for phenolic constituents HPLC and fatty acids profile by GC/FID. The highest total phenolic and flavonoid contents were obtained with shadow green kernel methanol extract and shadow green kernel acetone extracts, 56.26 ± 0.91 mg GAE/g extract and 20.12 ± 0.05 mg RE/g extract. The shadow green kernel methanol extract has the highest activity in DPPH, CUPRAC, FRAP and total antioxidant capacity test.

The present work demonstrates that Turkish nuts might be a natural source of bioactive compounds that can be incorporated into new health-related products or be substitutes of synthetic compounds of questionable safety, promoting human health and reducing disease risks.

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INVESTIGATION OF CHEMICAL COMPOSITIONS AND BIOLOGICAL ACTIVITIES OF *SALICORNIA EUROPEA* FERN. & BRACK. AND *HALOCNEMUM STROBILACEUM* (PALL.) BIEB. FROM LAKE TUZ (TURKEY)

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Bioactive compounds commonly found in fruits, vegetables have been shown to have possible health benefits such as antioxidative, anticarcinogenic and antimutagenic activities. The study was designed to evaluate chemical composition and some biological activities of *Salicornia europea* and *Halocnemum strobilaceum* species growing around Lake Tuz in Turkey. Antioxidant activities were evaluated using by different chemical methods including free radical scavenging (DPPH), ferric ion reducing power, cupric ion reducing power (CUPRAC), phosphomolybdate and β-caroten/linoleic acid bleaching tests. Also, total phenolic and flavonoid contents were determined. The fatty acid compositions and phenolic compounds were also evaluated by gas chromatography (GC) and high performance liquid chromatography (HPLC). Total phenolic and flavonoid contents of extracts changed between 95.61 - 228.39 mg GAE/g extract and 2.88 - 35.91 µg QE/g extract. Generally, dichloromethane extracts had higher antioxidant activity than methanol extracts. Twenty-two fatty acids were identified in the *Salicornia europea* and *Halocnemum strobilaceum* oils. Palmitic acid (C 16:0), linoleic acid (C 18:2 ω6), linolenic acid (C18:3 ω3) and oleic acid (C 18:1 ω9) and were major fatty acids for these plants. According to obtained data, *Salicornia europea* and *Halocnemum strobilaceum* different extracts had antimicrobial activity against fish and foodborne pathogens. Additionally, studied extracts had a high protective effect against DNA damage.

In conclusion *Salicornia europea* and *Halocnemum strobilaceum* extracts can be used in different areas such as pharmacology, food and medicine due to their beneficial natural phytochemicals ingredients.

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CHEMICAL COMPOSITION OF VOLATILE OILS OF TWO *CENTAUREA* (ASTERACEAE) SPECIES FROM CROATIA: CASE OF DIPLOID AND TETRAPLOID *C. SALONITANA* AND DIPLOID *C. RUPESTRIS*

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The Croatian Adriatic coast and islands form a unique and distinctive area as a habitat for 80 *Centaurea* (Asteracea) taxa out of which 27 are endemics; out of total 400 - 700 species, distributed in 27 sections in this genus [1]. Section *Acrocentron,* where both studied *Centaurea salonitana* L. and *Centaurea rupestris* L. belong, is one of the largest sections of *Centaurea* genus [2]. There are some studies connecting the cell nuclei ploidy and variation in volatile oil composition implicating possible connection [3]. The aim of the present study was to investigate the involvement of cell nuclei ploidy, determined using flow cytometry, in variation of essential oil composition in two species of *Acrocentron* section: *C. salonitana* and *C. rupestris*, from two different localities in Croatia, Adriatic region. The essential oil composition was determined using GC and GC-MS analysis of *C. salonitana* diploid and tetraploid and *C. rupestris* two diploid population's essential oils. The diploid and tetraploid *C. salonitana* showed very similar composition of essential oils, number of determined components and yield of essential oil. There were more differences among two different species than the same species with different ploidy level. However when comparing two populations of the same species from different ecological conditions, such as continental and Mediterranean climate we have noticed there are differences among the essential oils of the same species from two different localities.

The study presents the first results on *C. salonitana* essential oil composition, as well as first comparison of diploid and tetraploid population's volatile oils.

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THEORETICAL BASE FOR MULTIDIMENSIONAL CHARACTERISATION OF PLANT ESSENTIAL OILS - CASE OF *CENTAUREA* (ASTERACEA) ESSENTIAL OILS WITH GOOD ANTIMICROBIAL ACTIVITY

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Due to an antibiotic resistance around 700,000 people each year worldwide is life threatened and killed by drug-resistant microbes [1]. Therefore the new antimicrobial agents are needed and search for them presents a top priority in this area of science [1]. Recent interest in natural compounds has even risen due to a rapid development of resistant bacterial strains. This forced natural product re-evaluation as a source of novel chemical skeletons with antibacterial activity for elaboration in drug development [2]. *Centaurea* genus, Asteraceae family, is widespread in worldwide area comprising around 400-700 species [3]. They are well known, on medicinal purposes, in eastern Mediterranean ethno-pharmacology; due to a variety of bioactive compounds they comprise [4]. Good antimicrobial activity was previously reported for more than 20 *Centaurea* species essential oils, with more than 200 different compounds present. This presents a good model for development of mathematical tool in order to characterise the chemical compounds responsible for a good antimicrobial activity. Biological activity of essential oils is usually connected with the dominant components, but some compounds in minor percentage may also have a strong impact [3]. Therefore, we have used mathematical tool to detect the specific combination of compounds responsible for antimicrobial activity. For the theoretical base for multidimensional characterisation we have developed variance-covariance matrix and calculated its eigenvalues and eigenvectors. This tool may be used for a focused research of compounds that have good antimicrobial activity, but also for detection of any other essential oil characteristics that can be utilized in cosmetics, pharmaceutical, food or any other industry.

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BIOACTIVE ISOFLAVONES FROM *PUERARIA LOBATA* ROOT AND STARCH: DIFFERENT EXTRACTION TECHNIQUES AND CARBONIC ANHYDRASE INHIBITION

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Kudzu, the dried root of an important medicinal and edible plant (*Pueraria lobata* (Willd.) Ohwi), is used in Traditional Chinese Medicine for the important nutritional value strictly related to its isoflavone derivatives. These compounds characterize the quality of kudzu contained in different preparations, as pharmaceutical ingredient as well as dietary/food supplement (e.g. starch) [1]. The optimization of isoflavones recovery, monitored by HPLC-PDA, through different extraction techniques, e.g. microwave-assisted, ultrasound-assisted and conventional extraction represented a suitable challenge for natural derivatives in food compounds. We aimed to optimize the exhaustive recovery of isoflavones from kudzu through innovative and conventional extraction techniques. The impact on the isoflavone content by using ionic liquid-assisted extraction was also evaluated. The inhibitory activity of isoflavones, extracted from kudzu, was further tested *in vitro* using two tumor-related isoforms (IX and XII) of human carbonic anhydrase (hCA) in addition to their biological activity as estrogenic and anti-oxidant compounds. Results showed that the best extraction conditions for puerarin, daidzin and genistein were achieved by conventional extraction with water as the solvent for 30 minutes, and 1:5 (*w:v*) as solid:liquid ratio; while for daidzein the best conditions were achieved by MAE with ethanol as the solvent for 30 minutes, and 1:5 (*w:v*) as solid:liquid ratio. The resulting data demonstrated that the assessed isoflavones may represent new serious carbonic anhydrase inhibitors thus providing a potential therapeutic efficacy versus solid tumors both *in vitro* and *in vivo*.

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IN VITRO IMMUNOMODULATORY AND ANTIOXIDANT PROPERTIES OF *ANTIDESMA BUNIUS* (L) SPRENG AND *BAUHINIA PURPU*REA L. ETHANOLIC LEAF EXTRACTS

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Antidesma bunius (L.) Spreng (Euphorbiaceae), commonly known as "bignay" in the Philippines, has been known for its folkloric use against dysentery, diabetes, indigestion and constipation [1,2]. Meanwhile, the butterfly tree, *Bauhinia purpurea* L. (Fabaceae) is used in traditional medicine to cure fevers, headaches, diarrhea, dysentery, conjunctivitis, ulcerations, leprosy, lung and skin diseases [3]. However, very limited information is available regarding their potential use as immunomodulators; therefore, this study investigated the effects of leaf ethanolic extracts on *in vitro* phagocytic activity of murine peritoneal macrophage and splenic lymphocyte proliferation. In addition, some phytochemical tests were done to assess the presence of bioactive compounds and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was done to measure the antioxidant activity.

The extracts from *A. bunius* has terpenoids, flavonoids, steroids, and alkaloids while *B. purpurea* extract has alkaloids, terpenoids, flavonoids, steroids, phenols and condensed tannins. Both extracts showed strong antioxidant activity based on the DPPH scavenging activity at >80% at 250 µg/mL of the leaf extracts. Phagocytic activity significantly increased by 187% in macrophage treated with 100 µg/mL of *A. bunius* extract compared to lipopolysaccharide (LPS)-treated cells while 50 µg/mL of *B. purpurea* extract induced comparable phagocytic activity as LPS. Lymphocyte proliferation was stimulated by 100 µg/mL of *A. bunius* extract comparable to mitogen (ConA and LPS)-treated cells. The *B. purpurea* extract induced higher lymphoproliferation at 20 ug/mL than ConA and LPS-treated cells. These results highlighted the immunostimulatory and antioxidant activities of both plants possibly due to secondary metabolites found in their leaf ethanolic extracts.

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A STUDY ON ENZYME INHIBITORY PROPERTIES OF *HYPERICUM SALSUGINEUM*: ANTI-CHOLINESTERASE, ANTI-TYROSINASE, ANTI-AMYLASE AND ANTI-GLUCOSIDASE

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In millennia, some factors including modern lifestyle and oxidative stress are triggering to increase the prevalence of global health problems such as Alzheimer's disease and diabetes mellitus [1]. From this perspective, the effective therapeutic strategies are need to control these global problems. In these strategies, key enzyme inhibitory theory is considered as one of the most effective approaches. For this purpose, we investigated enzyme inhibitory properties of the methanol extract of *H. salsugineum* against cholinesterase (AChE and BChE), tyrosinase, amylase and glucosidase. The cholinesterase inhibitory abilities were detected 1.689 mg GALAE/g (on AChE) and 0.244 mg GALAE/g (on BChE). The tyrosinase inhibition ability was found to 65.29 mg KAE/g extract. Anti-amylase and glucosidase activity were 0.616 mmol ACAE/g extract and 19.466 mmol ACAE/g extract, respectively.

On the basis of our results, *H. salsugineum* might be considered as a potential source of natural enzyme inhibitors in food, medicinal and pharmacological areas.

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ANTIOXIDANT PROPERTIES OF DIFFERENT EXTRACTS FROM *BIDENS TRIPARTITA*: A POTENTIAL SOURCE OF NATURAL ANTIOXIDANTS

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In the past decade, an increasing interest in the use of natural bioactive compounds origin from plant for scientific research as well as different purposes such as pharmaceutical and food industries [1]. For example, plant antioxidants are very significant for aforementioned areas, because many synthetic antioxidants [butylated hydroxyanisole (BHA), butylated hydroxyltoluene (BHT) and propyl gallate (PG)] have possible activity as promoters of carcinogenesis [2]. From these perspectives, antioxidant inhibitory potentials of different extracts (ethyl acetate, methanol and water) from *Bidens tripartite* were tested by different methods (free radical scavenging, reducing power, phsophomolybdenum and metal chelating) in the study. Also, total phenolic and flavonoid content of each extracts were detected. Total phenolic and flavonoid content were found to be 50.95 - 84.24 mg GAE/g and 10.74 - 49.00 mg RE/g, respectively. Generally, the methanol extract exhibited remarkable antioxidant properties compared to ethyl acetate, and water extracts. However, the metal chelating ability can be ranked as ethyl acetate>water>methanol.

According to our results, *Bidens tripartita* may be considered as a valuable source of natural antioxidants for discovering novel functional products such as drugs and nutraceuticals.

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LONG TERM STABILITY OF A TRADITIONAL THAI ANTIHYPERTENSIVE HERBAL RECIPE

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There is a rising attention being paid to the wellness care benefits of herbs and their products in Southeast Asia. The World Health Organization (WHO) has been supporting countries to promote traditional medicine utilization so that this valuable resource is utilized safely and effectively [1]. In Thailand, many traditional herbal recipes have been established since ancient times and later have been carefully modified based on the wisdom of traditional Thai medicine [2]. In this study, a traditional Thai antihypertensive herbal recipe (TTAH) is selected and evaluated for its stability. The stability data is crucial since it recommends storage conditions of the product. Liquid chromatography mass spectrometry (LC-MS) was used as a tool to determine amount of piperine, a selective marker of the recipe. After long term storage, it was found that the TTAH exposed to light was deteriorated within 3 months. Under high humidity condition, the TTAH was deteriorated within 9 months. The dissolution profile of piperine in TTAH capsule was also changed after light exposure.

Thus, it was recommended to keep the TTAH in a well-closed container, protected from light which can be stored for at least 1 year.

Acknowledgements: The grant from the Research Institute of Rangsit University was acknowledged.

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RICE PROTEIN PROLAMIN INHIBITS LEUKEMIA GROWTH *IN VIVO* AND PROMOTES ANTI-TUMOR IMMUNE REACTION *IN VITRO*

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Background: Rice (*Oryza sativa*) is an important cereal for staple food worldwide. Prolamin is a heat-stable storage protein of rice with immunomodulatory activity. This study aimed to examine the effect of prolamin on anti-tumor immune response *in vitro* and leukemia growth *in vivo*.

Materials and methods: The prolamin-enriched rice fractions were prepared for *in vitro* test to stimulate the isolated peripheral blood mononuclear cells (MNC). The MNC-conditioned medium (MNC-CM) was collected to treat leukemia U937, hepatoma ML-1 and HepG2 cells followed by viability assessment. Purified prolamin was orally administered to syngeneic L1210-bearing DBA/2 mice with various dosages for consecutive 5 days. Weights of body, tumor, liver and spleen as well as peripheral blood neutrophil count were assessed. Cytokine levels in MNC-CM and mice serum and biochemistry profile in mice were measured.

Results: Prolamin-prepared MNC-CM, but not prolamin *per se*, inhibited the viability of leukemia and hepatoma cells, indicating an immune modulation effect. In leukemia U937 cells, the growth inhibition was accompanied by differentiation toward macrophages which expressed surface CD14 and CD68 and obtained phagocytosis activity. The growth inhibition activity of proplamin-prepared MNC-CM was partially blocked by neutralization of prolamin polyclonal antibody. In syngeneic L1210-bearing DBA/2 mice, oral administration of purified prolamin dose-dependently suppressed the tumor weight and attenuated the leukemia-induced reduction of liver and spleen weights. Prolamin inhibited the increase of peripheral blood leukocyte count in the L1210-bearing mice. The levels of tumor necrosis factor-α and interferon-γ in MNC-CM and mice serum were significantly increased by prolamin treatment. No significant change in body weight, serum alanine aminotransferase and creatinine levels was noted by prolamin treatment.

Conclusions: Rice protein prolamin could effectively promote anti-tumor immunity and inhibit leukemia growth without significant toxicity.

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STIMULATORY MECHANISMS OF ASTAXANTHIN IN PRIMARY CULTURED LYMPHOCYTES OF MICE

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Astaxanthin is a potent antioxidant carotenoid, may play a role in modulating immune response in animals. In this study, we investigated the immunomodulatory effects of astaxanthin in primary cultured lymphocyte in vitro and ex vivo. Direct administration of astaxanthin (70-300 nM) did not influence cytotoxicity in lipopolysaccharide (LPS, 100 μg/mL) or concanavalin A (Con A, 10 μg/mL) activated lymphocytes, whereas astaxanthin alone at 300 nM induces splenic lymphocytes proliferation ($p < 0.05$) *in vitro*. Despite astaxanthin alone or with Con A was not effective on INF-γ and IL-2 secretion in primary cultured lymphocytes, it enhances INF-γ production upon LPS stimulation. In an *ex vivo* experiment, oral administration of astaxanthin (0.28, 1.4 and 7 mg/kg/day) for 14 days, did not change in body and spleen weights of mice and also not induced toxicity of cells derived from mice. Moreover, treatment of astaxanthin significantly increased LPS induced lymphocyte proliferation *ex vivo* but it did not influence against Con A induction. Enzyme linked immunosorbent assay (ELISA) analysis revealed that administration of astaxanthin significantly enhanced INF-γ production in response to both LPS and Con A stimulation, whereas IL-2 production increased only in response to Con A stimulation. Also, astaxanthin treatment alone significantly increased IL-2 production in lymphocytes derived from mice, but did not significantly change INF-γ production.

These findings indicate that astaxanthin modulates lymphocyte-immune responses *in vitro*, and their *ex vivo* immunomodulatory effects are partly due to increasing INF-γ and IL-2 production without inducing cytotoxicity.

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PHENOLIC COMPOUNDS FROM NATURAL AND *IN VITRO* CULTURES (ROOTS AND SHOOTS) OF *RINDERA GRAECA* BOISS. & HELDR.

Katarzyna Sykłowska-Baranek[1], Konstantia Graikou[2], Christos Ganos[2], Pietrosiuk Agnieska[1], Jeziorek Malgorsata[1], Aligiannis Nektarios[2], Ioanna Chinou[2]

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Rindera graeca Boiss. & Heldr. (Boraginaceae, tribe Cynoglosseae) is an endemic plant of SE Europe listed in the WCMC Plants Database as "Rare". It grows on stony slopes within the main range 1700-2200 m, while to our best knowledge, has never been studied phytochemically before.

In the framework of our research on the Boraginaceae family [1-3], the presence of phenolic compounds in *R. graeca* has been established, from different sources: i) *in vitro* cultures of shoots (RG-Me/sh),, ii) natural and transgenic roots n-hexane extract (*in vitro* roots RG-Cy/r) and iii) plant harvested from natural environment (methanol extr RG-Me/nat).

All referred extracts were subjected in chromatographic separations to afford: two unusual phenolic naphthoquinone pigments (of *in vitro* RG-Cy/r), which have been isolated recently for the first time by our team from *Cynoglossum columnae* Ten. *in vitro* natural roots, while the presence of caffeic acid (CA), rosmarinic acid (RA), lithospermic acid (LA), lithospermic acid B (LAB) and of some flavonoids were confirmed. In particular, RA and LA were determined in all extracts (natural plant, natural and transgenic roots and in shoots), while LAB was found only in shoots. Moreover, a series of quercetin glucosides (mono and diglucoside derivatives) were also elucidated. The total phenolic content was determined by the Folin-Ciocalteu method, the total flavonoid content by the aluminium chloride colorimetric assay and the free radical scavenging activity by DPPH and ABTS assays showing an interesting profile.

Acknowledgements: The authors would like to thank the Special Account for Research Grants and the National and Kapodistrian University of Athens for funding their participation in this meeting

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PP 47

EXTRACTS OF ZINGIBERACEAE PLANTS INHIBIT HIV-1 PROTEASE ACTIVITY *IN SILICO* AND *IN VITRO*

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Various biological activities of Zingiberaceae plants have been discovered, particularly due to their medicinal properties such as immunomodulatory, anti-cancer, antioxidant and anti-viral activities. Herein, two Zingibercaceae plants; *Curcuma aeruginosa* Roxb. (Wan ma-ha mek, CA) and *Curcuma* sp. (Wan kong chak phra-in, KP) were extracted by sequential *extraction*. The phytochemical composition profiles of the extracts were analyzed by GC/MS (hexane extracts) and UPLC-HRMS (ethyl acetate and methanol extracts). Metabolic profiles were identified and classified into 9 groups based on their chemical structures. To elucidate the potential compounds from KP and CA profiles as HIV-1 protease inhibitors, *in silico* virtual screening approach operating by AutoDock 4.2 was then performed. The molecular docking results of all components suggested that 17 out of 225 (CA) and 20 out of 216 (KP) compounds could be potential inhibitors compared to pepstatin A, a positive control. The majority of the potential compounds were classified as terpenoid. Moreover, HIV-1 Protease Inhibitory activity of KP and CA extracts was investigated by screening kit using FRET assay. We found that KP methanol and ethyl acetate extracts as well as CA methanol extract at 1 mg/ml revealed high relative percentage inhibition against HIV-1 protease activity at 94.87 ± 4.14, 83.60 ± 2.24 and 82.44 ± 2.33 respectively, while pepstatin A at 1 mM displayed 82.23% inhibition. In conclusion, ethyl acetate and methanol extracts of KP effectively inhibited HIV-1 PR activity *in vitro*. Most of the potential compounds with highest affinity towards HIV-1 protease were correspondingly found in KP.

Additioanly, terpenoids have been reported to exhibit antiviral effect against herpes simplex virus type 2, hepatitis B virus, influenza virus or HIV. Thus, KP could be the best candidate for future drug development.

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PHYTOCHEMICAL INVESTIGATION OF *SALVIA FRUTICOSA* FROM THE ISLAND OF CRETE (GREECE)

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The genus *Salvia*, including about 900 species widespread all over the world, is the largest genus of the family Labiatae. Sage has been traditionally used for the treatment of excessive sweating, mouth and throat inflammations, bronchitis, cough, asthma, digestive and circulation disturbances, depression, skin diseases, etc.

In Flora Europaea, *Salvia* is represented by 36 species [1]. Among these, *Salvia fruticosa* is endemic to the Eastern Mediterranean basin, extending its native range from Sicily and Southern Italy, Cyrenaica, the Southern part of the Balkan Peninsula, Greece to West Syria. In all these regions, *S. fruticosa* has been used in traditional medicine practices [2, 3]. Even though there are a number of reports on the chemistry and biology of *S. fruticosa*, no thorough chemical investigation has been reported in the literature.

Aerial parts of *S. fruticosa*, collected in the area of Risoscaro, in Chania, Crete in March of 2015, were dried and exhaustively extracted with mixtures of dichloromethane and methanol. The organic extracts were subjected to a series of chromatographic separations to afford rosmarinic acid as one of the major constituents, as well as a number of terpenes and phenolic compounds. The structures of the isolated natural products were established on the basis of their spectroscopic characteristics, including NMR and MS data. Evaluation of the biological activity of the isolated compounds is currently in progress.

Acknowledgements: The authors thank the Special Account for Research Grants of the National and Kapodistrian University of Athens for funding to attend the conference.

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MOSQUITO REPELLENCY OF ESSENTIAL OILS FROM THREE *JUNIPERUS* SPECIES FROM GREECE

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The increased concern about human health has led to the use of natural products based repellents of blood-feeding mosquitoes (Diptera, Culicidae). The repellency of the essential oils from three *Juniperus* species (*J. oxycedrus* subsp. *macrocarpa*, *J. phoenicea*, *J. foetidissima*), collected from different areas in Greece, against the invasive Asian tiger mosquito *Aedes albopictus* was evaluated in laboratory bioassays. The essential oils obtained from different plant tissues were analyzed by means of gas chromatography-mass spectrometry (GC-MS). The terpene constitution showed a dominance of monoterpenes (α-pinene, myrcene, and limonene), followed by sesquiterpenes and their oxygenated forms. The mathematical analysis of the bioassays employed dose-response modeling with log logistic, log normal and all other functions in the *drc* library of R-system. It was found that all essential oils followed a dose - response model except for the foliage essential oil of *J. phoenicea*.

The highest repellency was found in *J. foetidissima* foliage essential oil, followed by *J. phoenicea* female cones essential oil and that of *J. oxycedrus* subsp. *macrocarpa* from female cones.

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PHYTOCHEMICAL EVALUATION OF SOME NATURAL POPULATIONS OF *ERODIUM CICUTARIUM* FROM ROMANIA

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Erodium cicutarium (L.) L'Hér. ex Aiton, syn. *Geranium cicutarium* L. (common stork's-bill), Geraniaceae, is a plant used in traditional medicine as astringent, haemostatic (uterine and other bleeding), antihemorrhageic, stomachic, antidiarrheic, diaphoretic, diuretic and abortifacient. Due to its phytochemical profile (tannins, catechins, flavonol glycosides, phenolic acids, vitamins K and C, essential oils), it possesses many pharmacological effects (antibacterial, antiviral, antioxidant, anti-inflammatory, analgesic, anti-proliferative etc.) [1, 2].

Starting from the ethno-pharmaceutical data provided by local inhabitants (a unique mixture of ethnicities in Romania) from Danube Delta surroundings, who use this plant as infusion and decoction [3], our research aimed the identification of some valuable populations of *E. cicutarium* from wild flora, in terms of bioproductivity (bioactive compounds with phytotherapeutic importance).

The vegetal material (aerial parts) was collected during the flowering stage, from different sampling points of Enisala (Tulcea County) (rocky soil, sandy soil), and naturally dried. Different types of extracts were analyzed by specific analytical methods (TLC, HPLC, UV-VIS spectrophotometry and iodometry), to evaluate the phytochemical profile: polyphenols (gallic acid), polyphenolcarboxylic acids (caffeic and chlorogenic), flavonoids (rutin, luteolin, hyperoside, isoquercitrin), vitamin C.

The results indicated the biosynthetic capacity for the analyzed active principles in natural populations of *Erodium cicutarium,* and also an interpopulational phytochemical variability depending on type of substrate. The content in antioxidant active principles confirms the ethno-pharmaceutical uses of *Erodium cicutarium* from Romania.

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HPTLC INVESTIGATION OF CONDENSED TANNINS IN BRANCHES OF *ROSA CANINA* L., *HIPPOPHAE RHAMNOIDES* L. AND *PRUNUS SPINOSA* L. SPECIES

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Rosa canina L. (Rosaceae), *Hippophae rhamnoides* L. (Elaeagnaceae) and *Prunus spinosa* L. (Rosaceae) species are used traditionally as medicinal plants due to phytotherapeutical and nutritional potential of their fruits [1]. The aim of this study is to identify and quantify the catechin, as a valuable biomarker in branches of these species and epicatechin in branches of *Prunus spinosa*, using qualitative (HPTLC) and quantitative (HPTLC densitometry) analises. This study was carried out as per the method of Dharmender et al. [2]. Methanol extracts of the branches were developed in the mobile phase of toluen: ethyl acetate: formic acid (12 : 12 : 2) and scanned under UV at 254 nm and under visible light. The presence of catechin and epicatechin was visualised as distinct black bands with 5% alcoholic $FeCl_3$ as visualising agent. HPTLC fingerprinting of crude methanol extracts showed specific peaks, with different *Rf* values, corresponding to phytocompounds catechin (*Rf* 0.46) evidenced in branches of the three species and epicatechin (*Rf* 0.43) highlighted in samples of *Prunus spinosa*. The screening at 254 nm has as result the quantitative determination of the biomarker catechin in the samples of the species, the HPTLC densitometry analysis revealing the amount of catechin in branches of *Rosa canina* (0.15% g/g), *Hippophae rhamnoides* (0.10% g/g) and *Prunus spinosa* (0.25% g/g) and the amount of epicatechin in branches of *Prunus spinosa* (0.22% g/g). The identification and quantification of catechin as a biomarker suggests an important potential of these species and can be considered a new source of catechins.

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ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS FROM COMMON SAGE AND ROSEMARY

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The presence of pathogenic and spoilage microorganisms in food and cosmetic products reduce their shelf-life and may cause several diseases among consumers. Nowadays, there is a trend to replace synthetic preservatives with others from natural sources. The objective of this research is to determine the antimicrobial capacities of essential oils (EO) obtained from four sage (*S. officinalis* L.) and six rosemary (*R. officinalis* L.) plants grown in Murcia (S.E. Spain).

The microorganisms used in this work were *P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739, *S. aureus* ATCC 6538 and *C. albicans* ATCC. The antimicrobial assays were carried out according to the M07-A10 [1] guideline for bacteria and M27-A3 [2] for *Candida*.

P. aeruginosa could not be inhibited with the EOs at the maximum concentration tested (20 µl/ml). This and previous studies reported that Gram-negative bacteria are usually more resistant to EOs than Gram-positive ones. The minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC) for *E. coli* were between 5 to 10 µl/ml with all EOs tested. As regard the inhibition of *S. aureus*, MIC and MBC values were lower with *S. officinalis* EOs (0.6 - 1.3 µl/ml) than with *R. officinalis* EOs (5 - 10 µl/ml). The MIC and minimum fungicidal concentrations (MFC) for *C. albicans* were similar or slightly lower than the obtained for *E. coli*, being *S. officinalis* EOs a little more active than *R. officinalis* EOs.

The knowledge of antimicrobial activities of EOs could be useful to identified new natural preservatives for several industries.

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ANTI-ENZYMATIC ACTIVITIES OF ESSENTIAL OILS FROM SPANISH SAGE AND SPANISH MARJORAM

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Lipoxygenase (LOX) and acetylcholinesterase (AChE) are enzymes related to inflammatory and Alzheimer's diseases, among others. Therefore, their inhibition could be considered as a potential therapy. In this work, we tested the inhibitory activities of four Spanish sage (*Salvia lavandulifolia* Vahl.) and four Spanish marjoram (*Thymus mastichina* L.) essential oils (SlEO and TmEO, respectively) obtained from plants grown in Murcia (S.E. Spain).

The LOX inhibitory activity [1] was described as degree of inhibition at 150 µg/ml EO. The AChE inhibitory activity [2] was assayed using 8 different concentrations of each EO to determine their IC_{50} values.

The degrees of LOX inhibition (%) were as follows: TmEO-1 (56.7 ± 1.6) > TmEO-4 (47.6 ± 1.5) ≈ TmEO-2 (46.25 ± 1.0) > SlEO-4 (42.4 ± 0.7%) ≈ TmEO-3 (40.8 ± 1.0) ≈ SlEO-2 (40.4 ± 0.8%) > SlEO-1 (36.6 ± 0.5%) ≈ SlEO-3 (34.2 ± 0.7%). Generally, TmEOs were more active against LOX activity than SlEOs. Some compounds such as bornyl acetate, limonene, linalool, 1,8-cineole, terpinen-4-ol and α-terpineol showed anti-LOX activity.

Regards to AChE inhibition, EOs showed the following IC_{50} (µg/ml): TmEO-1 (57.5 ± 2.8) < TmEO-2 (71.1 ± 3.1) ≈ TmEO-3 (72.3 ± 2.0) < SlEO-4 (108.0 ± 4.2) ≈ SlEO-1 (108.9 ± 3.5) ≈ TmEO-4 (117.2 ± 5.6) ≈ SlEO-2 (119.3 ± 10.4) < SlEO-3 (142.4 ± 6.1). The higher concentration of 1,8-cineole, the higher inhibitory activity, because this compound was tested to be the most active between all individual compounds.

The experimental results support further studies with these EOs.

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COMPARATIVE ANALYSIS OF CHEMICAL COMPOSITION AND CYTOTOXICITY OF WILD-GROWING AND *IN VITRO* CULTIVATED *RINDERA UMBELLATA* (WALDST. & KIT.) BUNGE

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Frequent tumor resistance towards conventional drugs, as well as their numerous side effects, encouraged continual search for new cytostatics. Plants, including rare and endangered species, are considered to be important source of new pharmaceuticals. For that reason, the *in vitro* culture of rare plants is necessary to provide the material for phytochemical analysis.

The subject of this study was *Rindera umbellata* (Waldst. & Kit.) Bunge scarcely distributed in sand habitats of Southeast Europe. We compared chemical composition and cytotoxicity of methanolic extracts of plants, wild-growing and *in vitro* cultivated in media containing 0.1 M and 0.3 M sucrose. Phytochemical analysis revealed that the highest amount of total phenolics was recorded at 0.3 M sucrose in cultivation medium, with rosmarinic and lithospermic B acids, being dominant in shoots and roots, respectively. On the other hand, pyrrolizidine alkaloids were abundant in extracts of wild-growing plants. Cytotoxic potential was determined in MTT assay applied on colon (HT 29) and lung (A549) cancer cells, as well as on fetal lung fibroblasts (MRC-5), used to evaluate the toxicity towards normal cells. While cytotoxicity towards A549 and MRC-5 cells was similar, the effect against HT-29 cell line was higher comparing to normal cell line. However, since obtained IC_{50} values ranged from 1.2-1.6 mg/mL, determined cytotoxic effect was denoted as weak.

Further research will be directed to identification of pyrrolizidine alkaloids and phenolics from *R. umbelata* and determination of their cytotoxicity, alone and in combinations with conventional cytostatics.

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SOLVENT EFFECTS BY THE EXTRACTION OF POLYPHENOLS

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The effect of solvent type by the extraction of polyphenols from plants was studied. The polyphenol composition was observed using HPLC-PDA and compared to this revealed by the chromatographic fingerprint profiles of 70% methanol extracts. The extracting media included hot water, 30% methanol, 60% methanol, 100% methanol, 66% ethanol, 80% acetone and ethyl acetate : methanol = 1 : 1. Ultrasound-assisted extraction (UAE) for 40min at 23^{0}C was applied for all extracts. The extracting power of the media was presented by the yields of main compounds in the extracts obtained in proportion 1 : 50. Two plants - *Clinopodium vulgare* L. and *Nicotiana tabacum* L. were subjected for this investigation. Chlorogenic acid (ChA) and rosmarinic acid (RA), rutin (Rut), luteolin (Lut) and apigenin (Apg) glycosides were the compounds of interest. 60% methanol extraction resulted in 97% yield for ChA. For RA and Lut/Apg glycosides, 66% ethanol as extracting medium showed the highest yield, while Rut was extracted from tobacco successful (100% yield) with ethyl acetate : methanol = 1 : 1 by minimum interference of solvent and plant matrix. The extraction with 70% methanol showed intermediate results for all components with acceptable yields.

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EFFECT OF *CLINOPODIUM VULGARE* EXTRACT ON THE PRODUCTION OF NITRIC OXIDE, REACTIVE OXYGEN SPECIES AND COX2 BY NEUTROPHILS

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Herein we evaluated how *Clinopodium vulgare* extract and its constituents (clorogenic acid, cafeic acid, cathechin) influence the functions of bone marrow neutrophils in respect to the production of nitric oxide (NO), reactive oxigen species (ROS) and COX2 production. We isolated neutrophils from bone marrow of healthy ICR mice or ICR mice with zymosan-induced shock. Cells were cultured in the presence of CV1 and of its constituents at concentrations ranging from 10 ng/ml to 10 µg/ml. After 18 hours of culture the production of NO and ROS was evaluated by colorimetric assays and cell apoptosis (Annexin V staining kit) and COX2 expression were assessed by flow cytometry. The *C. vulgare* extract (10 µg/ml) increased the apoptosis of healthy neutrophils (8 ± 2% in control) to 25 ± 5% and of neutrophils from mice with shock to 45 ± 9%. Despite that apoptosis was reduced twice at lower concentration (1 µg/ml) in both neutrophil populations *C. vulgare* extract decreased markedly COX2 production, NO and ROS secretion by healthy neutrophils but not by neutrophils from mice with shock. The two constituents – caffeic and clorogenic acid showed similar effects to *C. vulgare* extract while cathechin had specific mode of action. In comparison to controls cathechin increased 1.5 times COX2 expression, NO and ROS levels in both neutrophil populations.

Our data indicated that *C. vulgare* extract may have good potential to manipulate neutrophil functions, however, its action may depend on the cellular state, the inflammatory milieu and the relative content of caffeic and chlorogenic acid in it.

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ARONIA MELANOCARPA AFFECTS AGE-RELATED TISSUE REMODELING IN RAT THYMUS

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Progressive thymic involution, which is a sign of aging, leads to a loss of immune function, increased susceptibility to infections, risk of autoimmune diseases and neoplasms in adults. The use of antioxidants is a potential therapeutic approach for decelerating the aging of immune system. The aim of this study was to examine the effect of *Aronia melanocarpa* juice on age-related tissue remodelling of thymus in mature rats. A model of spontaneous aging rats supplemented with antioxidant beverage for 12 weeks was used. The thymuses were examined histologically using Azan and Methylene blue staining methods and morphometric analysis was applied. The number of mast cells and amount of connective tissue in the interlobular septa were evaluated. *Aronia* juice supplementation resulted in distinctive preservation of the thymic lobules structure. The morphometric analysis showed a significant decrease in the number of mast cells and reduction of the relative quantity of connective tissue in the interlobular septa.

Therefore, it could be concluded that *Aronia melanocarpa* affects beneficially age-related tissue remodelling in thymus.

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PROTECTIVE EFFECT OF DIFFERENT HERBS ON ORGAN DAMAGE CAUSED BY OXIDATIVE STRESS

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In today's world, diabetes mellitus (DM) and atherosclerosis are the leading examples of oxidative stress disorders. In oxidative stress disorders, free radicals are generated which are responsible for damage of various organs such as liver, kidney, heart, wall of blood vessels and skin. Over the last one decade, in our laboratory, we conducted experiments using natural products such as *Piper sarmentosum*, *Piper betle*, *Momordica charantia* and virgin coconut oil. We created animal model of atherosclerosis and DM and used various natural products to observe the effect on liver, kidney, wall of blood vessels and the skin wounds. Many organ damages could be seen to be reverted back to normal once the natural products were used. We also performed biochemical studies with various antioxidants and inflammatory markers.

Our histological findings under light microscope and electron microscope and biochemical analysis strongly suggest the protective role of various natural products on oxidative stress disorders such as DM and atherosclerosis.

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ANTIOXIDANT, ANTIMICROBIAL AND NEUTROPHIL-MODULATING ACTIVITIES OF BLACK CHOKEBERRY (*ARONIA MELANOCARPA*) POLYPHENOLS

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Aronia melanocarpa is extremely rich source of polyphenols rendering in one of the highest *in vitro* antioxidant activities among fruit. In our previous study, it was observed that chokeberry extract has strong antimicrobial effects against chosen foodborne pathogens and significantly inhibited the reactive oxygen species production from OZP-activated phagocytes [1]. In order to reveal which components are responsible for the observed effects, in the current study we investigated the antioxidant, antimicrobial and neutrophil-modulating activities of the major polyphenol components from *Aronia* berries: isolated proanthocyanidins, anthocyanins, hydroxycinnamic acids and purified polyphenol extract. Via the used methods (ORAC, TRAP and HORAC), it was observed that anthocyanins (cyanidin-3-glucoside and cyanidin-3-galactoside) have the highest antioxidant activity. Similarly, cyanidin-3-galactoside expressed the highest potential to inhibit the chemiluminescence response of OZP-activated human neutrophils while the effect on PMA-activated cells was less profound. On the other hand, none of the studied components affected the spontaneous production of reactive oxygen species in human neutrophils. The antimicrobial activity test against 12 pathogenes revealed that chokeberry proanthocyanidins are the most potent antimicrobial agents in the fruit.

The lowest minimum inhibition concentration for proanthocyanidins (0.156 mg/ml) was established against *Proteus vulgaris* G, whereas 0.312 mg/ml proanthocyanidins completely inhibited *Staphylococcus aureus* ATSS 25923.

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INVESTIGATION OF EXTRACTS DERIVED FROM THE PLANT *GEUM URBANUM* L. AS DRUG CANDIDATES WITH ANTIMICROBIAL AND CYTOTOXIC ACTIVITY

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Aim: To investigate the antimicrobial, cytotoxic and radical-scavenging activity of different extracts and fractions from *Geum urbanum* L., including their bactericidal effect, inhibition of bacterial motility and quorum sensing, as well as apoptosis induction in sensitive cancer cell lines.

Methods: Roots and aerial parts were used to obtain methanol extracts, petroleum ether, ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) fractions. Minimal inhibitory and bactericidal concentrations (MIC/MBC) were calculated by using serial dilution method. The dehydrogenase activity of sensitive bacterial strains was measured by MTT-test. Bacterial growth rate was determined by time-kill assay. Swarming motility and inhibitory potential for synthesis of pyocianin were investigated on *P. aeruginosa* (PA01). The cytotoxicity was tested on normal transformed and tumor cell lines by MTT assay. Apoptosis induction was measured by colorimetric enzymatic assay. IC_{50} values were calculated with GraphPadPrizm.

Results: All fractions exhibited antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus cereus*. The EtOAc fractions exhibited the strongest antimicrobial and radical-scavenging properties and were characterized by the highest amount of polyphenolic compounds. The IC_{50} values for bladder cancer cell lines corresponded to MIC/MBC. EtOAc and *n*-BuOH fractions inhibited the swarming motility and synthesis of pyocianin.

Conclusions: The antibacterial features of the investigated fractions from *G. urbanum* reveal the possible potential for treatment of certain skin infections. The established corresponding cytotoxicity supposes activity against bladder cancer with favorable antibacterial effect.

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IN VITRO ANTITUMOR ACTIVITY OF PROANTHOCYANIDIN-RICH FRACTIONS FROM *VACCINIUM* BERRIES ORIGINATING FROM BULGARIA AND NORWAY

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In recent decades, bioactive compounds of wild berries have attracted substantial attention and have been subjected to extensive research due to their antioxidant properties, potential in health promotion and disease prevention, thus improving safety and consumer acceptability.

The present study aims to evaluate *in vitro* antitumor and apoptotic potential of total extracts and proanthocyanidin-rich fractions of *Vaccinium myrtillus* L., *Vaccinium uliginosum* L., *Vaccinium vitis-idaea* L. and *Vaccinium corymbosum* L. harvested in Bulgaria and Norway on human breast (MCF7) and larynx (Hep2) cancer and non-malignant (Vero) cell lines. A total of 24 total extracts and respective number purified fractions of berries picked in Bulgaria and Norway were used. Antitumor effect was established by MTT cell viability assay and assessment of apoptotic potential was done through analysis of morphological cell characteristics (Fluorescence microscopy). The purified fractions of all berries have a strong dose-dependent inhibitory effect on survival of cells of the breast and larynx tumors. The strongest effect is observed in *Vaccinium vitis-idaea* L. samples. Cell viability of Vero was moderately decreased without visible dose-dependent effect. The mechanism of antitumour activity of Bulgarian and Norway berries involved apoptotic process. As a marker for early and late apoptosis in tumor cells after treatment there are morphological changes.

Evaluation of antitumor properties and pharmacological importance of Bulgarian and Norway wild berries could contribute to establish the natural substances useful for human health.

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IN VITRO CYTOTOXICITY AND ANTI-HERPES SIMPLEX VIRUS ACTIVITY OF *GRAPTOPETALUM PARAGUAYENSE* E. WALTHER

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Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) are common human pathogens associated with orofacial infections, genital lesions and encephalitis. The toxic side effects and the emergence of virus strains that are resistant to the drugs, enhance the need for new effective compounds against viral infectious diseases.

The objective of our examination is to evaluate the *in vitro* anti-herpetic and cytotoxicity of *G. paraguayense* extracts using colorimetric assay.

Three main fractions were obtained - A (lipids), B (amino and organic acids, carbohydrates) and C (phenolic acids). The composition of each was determined by GC-MS analysis. The capacity for inhibition the lytic activity of HSV-1 Victoria strain and HSV-2 Bja strain and the reduction of viability of infected or uninfected cell cultures were defined by MTT assay. Data were used to calculate CC_{50}. The cytopathic effect (CPE) was expressed as a percentage of the optical density of the sample compared with untreated virus-infected cells. Acyclovir was used as positive control.

Fraction **C** has not CPE on human cell lines RD and Lep and inhibited HSV replication in dose-dependent manner more efficiently against HSV-1, whereas its effect to HSV-2 was significantly lower. **A** and **B** fractions showed no antiviral effect.

The mechanism of the antiviral action of fraction **C** is not yet completely identified. Further studies are needed in order to verify which compounds could be responsible for this activity and how they exert their antiviral effects.

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DEVELOPMENT OF EXTRACTION AND MICROEXTRACTION TECHNIQUES TO CHARACTERIZE LEAVES OF DIFFERENT CULTIVARS OF *PRUNUS DOMESTICA* L.

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Despite the large number of synthetic pharmaceuticals, herbal products contain high amount of biological active compounds with a less harmful effect on human body than their synthetic counterparts. In the present work, leaves of different cultivars of *Prunus domestica* L were analyzed for the establishment of phenolic pattern in order to use them as potential sources of antioxidants in the pharmaceutical and food industry. Modern extraction procedures such as microwave extraction (MAE) [1], dispersive liquid–liquid microextraction [2] and sugaring-out liquid–liquid extraction technics were optimized in order to obtain full multi-component panel of phenolic compounds followed by HPLC-PDA analysis [3]. The best extraction was achieved using MAE in water : methanol (30 : 70) medium under microwave irradiation at 80°C for 13 min 8 s and it was applied for quantitative analysis of phenolic compounds in fourteen cultivars of *P. domestica*. The total amount of phenolic compounds varies from 101411.8–415772.3 µg/g extract. The antioxidant capacity of *Prunus* leaves was tested via several methods (DPPH, TEAC, etc.), as well as by assessing their total bioactive components (phenolics and flavonoids). The highest total phenolic contents were obtained for cultivars Ialomița (139.66 mg GAE/g extract), Alutus (139.15 mg GAE/g extract), and Tita (135.30 mg GAE/g extract). These findings suggest that leaves of *P. domestica* could be considered as potential sources of bioactive compounds for the design of novel phytopharmaceuticals.

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DETERMINATION OF PHENOLIC COMPOUNDS IN DIFFERENT SPECIES OF SALVIA (*SALVIA TRANSSYLVANICA*, *SALVIA OFFICINALIS*, *SALVIA GLUTINOSA*) USING HPLC-PDA ANALYSIS AND THEIR BIOLOGICAL ACTIVITIES

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Sage species are used in Romanian traditional medicine for coughs, rheumatism, inflammatory diseases, and antidiabetic remedies. In this study *Salvia glutinosa* and the endemic *Salvia* transsylvanica were compared with the well known *Salvia officinalis* in terms of chemical composition and biological activities. Previously validated HPLC method was applied for determination of 22 phenolic compounds in dry extracts of *S. glutinosa, S. transsylvanica,* and *S. officinalis.* In the samples the total amount of phenolics was 6675.9, 4937.7 and 8425.5 μg g^{-1}, respectively. The HPLC revealed that the main compounds of *Salvia* species are rutin (1357.9-4070.2 μg/g) and catechin (1112.6-1911.1 μg/g). The biological profile of the three sage extracts was evaluated towards several antioxidant assays by testing their inhibitory capacities against key enzymes involved in neurodegenerative diseases as well as diabetes mellitus. Concerning the antioxidant assays, generally *S. officinalis* exhibited high antioxidant capacity in all assays, the highest values being obtained for the CUPRAC assay: 400.01 mg TE/g extract for *S. officinalis*, 175.91 mg TE/g extract for *S. glutinosa*, and 118.11 mg TE/g extract for *S. transsylvanica*. Concerning the enzyme inhibitory assays, *S. officinalis* extract presented the highest inhibitory potential on butyrylcholinesterase (2.40 mg GALAE/g extract) followed by *S. transsylvanica* (1.43 mg GALAE/g extract). *S. officinalis* and *S. transsylvanica* extracts exhibited an important inhibitory potential against alpha-glucosidase (27.01 mmol ACAE/g extract, and 25.62 mmol ACAE/g extract, respectively).

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TOCOPHEROL CONTENT IN PARROTIA PERSICA WINTER LEAVES OBTAINED BY SUPERCRITICAL CO_2

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Parrotia persica, Hamamelidaceae, native to Caucasus, is planted for its autumnal colours. In autumn *Parrotia persica* leaves appear in red, purple, orange, yellow and gold colours. In winter the leaves are still on the branches and reach amber colour.

Amber coloured leaves of *Parrotia persica* were collected in February 2015. Collected *Parrotia persica* leaves were dried and grinded. Its extracts were obtained by supercritical fluid extraction at different temperatures. The obtained extracts were a subject to GC-MS analysis.

The results obtained indicated that the highest quantities of tocopherols are obtained when the extraction is done at the temperature of 40°C.

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COMPARATIVE STUDY OF ALKALOID PROFILE OF THREE *HYPECOUM* SPECIES

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The genus *Hypecoum* L. (Papaveraceae) is represented by 18 species growing in the Mediterranean region, Central Asia and China. The plants are used in Tibetan medicine as an antipyretic, analgesic and anti-inflammatory remedy [1]. The plants are known for their biologically and pharmacologically active isoquinoline alkaloids, such as protopines, protoberberines, aporphines, benzophenantridines, spirobenzylisoquinolines and secoberbines [2].

Alkaloid profiles of *Hypecoum pendulum* L. (Algeria), *Hypecoum procumbens* L. (Bulgaria) and *Hypecoum ponticum* Velen. (Bulgaria) - an endemic species for the Balkan region, were investigated and 10 isoquinoline alkaloids were determined. The main alkaloid of tertiary alkaloid mixtures in all analyzed samples were protopine. From quaternary alkaloid mixtures of *Hypecoum procumbens* and *Hypecoum ponticum* were identified the alkaloids N-methilcanadine and N-methylstylopine. These alkaloids were found for the first time from the species *Hypecoum ponticum*.

One new natural alkaloid with quaternary structure was isolated from *Hypecoum ponticum* and its structure was determined on the basis of detailed spectroscopic analysis, including1D and 2D NMR and EI MS.

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NON POLAR SECONDARY METABOLITES OF *CEDRUS BREVIFOLIA* NEEDLES

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Cedrus brevifolia A. Henry ex Elwes & A. Henry is an important narrow endemic tree of Cyprus flora and it is well-differentiated from other species of the genus based on morphological and eco-physiological traits, such as short needles and slow growth, resistance to aphids, and the highest tolerance to drought in all cedar species [1]. Theophrastus (371 - 287 BC) was the first to mention the existence of cedar in Cyprus [2]. The plant material was collected from Cedar valley near Paphos. *C. brevifolia* needles have been extracted with dichloromethane, methanol, methanol : water 5 : 1, successively. The dichloromethane extract has been subjected to further analyses by VLC and repeated CC and afforded four secondary metabolites, i.e. dehydroabietinal, dehydroabietic acid, manoyl oxide, phytol. The structures of the isolated compounds were elucidated by high-field NMR spectroscopy (^{1}H-NMR, ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC). The antioxidant and anti-inflammatory activities of the obtained extracts and isolated compounds are under investigation.

Acknowledgements: The author thanks Special Account for Research Grants and National and Kapodistrian University of Athens for funding to attend the meeting.

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RIGOR AND ADULTERATION OF ARGAN OIL IN NUTRITIONAL AND COSMETIC PRODUCTS

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Argan oil, the oil of *Argania spinosa* fruits is considered to be one of the most precious vegetal oil, especially the geographical rarity source [1]. From here, there is a great temptation to be adulterated [2].

The objectives of this study was: (1) to quantify by using GC the chemical composition of 7 argan oil samples, all originals from Maroccan manufactures, labeled as: *for cosmetic use*, (2) to compare those values with another sample of argan oil, labeled as an alimentary oil, (3) to measure and compare the chemical composition for a sample of cosmetic product, released on the cosmetic market by a multinational factory, labeled as: *hair protection with argan oil*.

The results show very similar values for the first 7 samples. Their average values are: palmitic acid: 16.8, stearic acid: 2.9, oleic acid: 22.5, linoleic acid: 40.9, linolenic acid 10.26, eicosanoidic acids: 1.5. Taking in consideration those averages values as standards, we remark the following deviations: (1) an inverted proportion *oleic acid/linoleic acid* for alimentary product: 47.6/23.6 (instead of 22.7/41), and (2) another inverted raport *oleic acid/linoleic acid* for the cosmetic product: 52.8/24.5 (instead of 22.7/41). These big differences raise the hypothesis of adding another oil, which could by organoleptically similar to argan oil.

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ANTIAGING PROPERTIES OF *SALVIA OFFICINALIS* OIL EVALUATED ON BOTH PHOTO- AND CHRONO- AGING

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The antiaging properties of essential oil extracted from *Salvia officinalis* have captured the researcher's attention because of a hypotetical anti-glycation effect [1].

In present study, the chemical extraction and analysis of *Salvia officinalis* oil was performed at Banat`s University of Agricultural Sciences. Then the data were compared and confirmed by those already existing in other studies [2].

Thus, 38 components were identified in volatile oil extracted from *Salvia officinalis*. The chemical composition shows that oxygenated monoterpenes (camphor, 1,8-cineol, α-thiona and borneol) are in the highest proportion. Camphor is a dominant component (20.64%), followed by eucalyptus (11.75%), borneol (8.80%) and α-thion (8.64%). Hydrocarbon monoterpenes are also in remarkable concentration (α-pinen 9.59%, β-pinen 5.04%). We have proposed and then realized a pharmaceutical form containing different concentrations of salvia oil. The cream was tested by using corneometry, after daily applications on 15 subjects. The entire study lasted 5 weeks, during which each patient was evaluated weekly. The obtained results were centralized and statistically processed using the StatPad application. So we can conclude that that the use of creams that contain Sage oil leads to an increase in the hydration degree both in the cases of cronoaging and in those of photoaging.

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POLYPHENOL-RICH EXTRACTS FROM CHESTNUT (*CASTANEA SATIVA*) BUR WITH ACTIVITY AGAINST PHYTOPATHOGENIC FUNGI

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Burs of *Castanea sativa* represent a significant byproduct of the edible chestnut productive chain, with a great potential to be valorized as a source of active phenolics with antioxidant properties useful in pharmaceutical, cosmetic or food packaging applications [2]. In the present research, the efficacy of organic (methanolic and hydrohalcoholic) and aqueous (decoction) extracts from chestnut burs against phytopathogenic fungi *Alternaria alternata*, *Fusarium solani*, and *Botrytis cinerea* was investigated [1]. Mycelial growth and spore germination rates of the mentioned above fungi were significantly reduced *in vitro* under exposure to all *C. sativa* burs extracts in a dose-dependent manner. In particular, *in vitro* growth assays with the extracts showed values of EC_{50} in the range 6-15 mg/mL for *A. alternata* and *F. solani* and from about 64 to >70 mg/mL for *B. cinerea*. The water-soluble fraction of the methanolic extract showed the highest inhibitory effects. Thus, its main components were isolated and their chemical structures characterized by NMR and MS. Phenolic acids, several flavonol glycosides (kaempferol and quercetin derivatives), phenol glucoside gallates (cretanin, chesnatin, chestanin) and C-glycosil ellagitannins (castacrenin A and B) were detected. The marker compounds were identified as quercetin-3-*O*- -D-glucopyranoside and chestanin, and their quantitative analysis was performed by HPLC-DAD. The major antifungal efficacy of the methanolic extract could be ascribed to its both higher total phenol (as determined by Folin Ciocalteu test) and chemical markers content, and radical scavenging activity (against DPPH and ABTS radicals) than hydrohalcolic and aqueous extracts of *C. sativa* burs.

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ANTIFUNGAL EFFECTS OF SOME MEDICINAL AND AROMATIC PLANTS WASTE

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To conform to the principles of sustainable development and bio-economy in order to close value chains in bio-economy even medicinal and aromatic plants wastes must be treated with a special attention. After industrial processing are obtained large quantities of wastes which can be transformed into greener everyday products such as food, feed, chemicals, materials and fuels - sourced and made in Europe [1]. Medicinal and aromatic plants play a valuable and important role in economic, social, cultural and ecological aspects of local communities all over the world, as more than 80% of the population of developing countries relies on traditional plant based medicines for their health requirements. Also, in the present *eco* and *bio* rush, materials such as essential oils, pharmaceuticals, colorants, dyes, cosmetics and biocides are obtained from plants. All over the world, especially in countries with traditions in the cultivation of such plants (Romania being a country with a very strong tradition regarding the medicinal plants), production of bio-products based on natural compounds leads to the generation of large quantities of plants waste, mostly unexploited.

In this respect, our research group conducted experiments for testing the antifungal effect of some extracts obtained from some medicinal and aromatic plants after industrial processing. Thus, was the antifungal effect of the main active principles of interest was tested from *Apium graveolens*, *Melissae herba*, *Leonurus cardiac*, *Satureja hortensis*, *Origanum vulgare*, *Salvia officinalis*, *Plantago media*, *Helianthus tuberosus.*

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MITODEPRESSIVE, ANTIOXIDANT, ANTIFUNGAL AND ANTI-INFLAMMATORY EFFECTS OF WILD-GROWING ROMANIAN HERBS

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The present study aims to evaluate, the potential uses of a hydroalcoholic extracts obtained from Romanian native wild-growing plants (*Arctium lappa* L. and *Veronica persica* Poiret). The hydroalcoholic extracts were obtained from the roots and respectively the aerial parts of burdock and birdeye speedwell by extracting 20 g of vegetal material in 1 : 1 mixture water-ethanol (100 : 100 mL). The extracts were characterized by HPLC, UV-Vis spectrometry and phytochemical assays (total terpenoids, total flavonoids and total phenolic content). Using the DPPH assay was evaluated the antioxidant potential of the extracts, while the antifungal potential was determined on two fungal lines (*Aspergillus niger* and *Penicillium hirsutum*); the cytogenetic effects were evaluated using the *Allium cepa* assay. The anti-inflammatory effect was evaluated in two inflammation experimental models (dextran and kaolin) by plethysmometry. The *in vitro* studies prove that both extracts possesses good antioxidant, antifungal and mitodepressive potential. The *in vivo* evaluation of the extracts (prepared as a microemulsion) shows a significant anti-inflammatory effect in the tested experimental models.

The *in vivo* and *in vitro* results recommend the *Arctium lappa* L. and *Veronica persica* Poiret hydroalcoholic extracts as promising biologically-active materials.

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THE USE OF THE DIFFERENT EXTRACTION METHODS FOR OBTAINING BIOLOGICAL ACTIVE COMPOUNDS FROM MEDICINAL AND AROMATIC PLANTS WASTE

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Obtaining extracts from plant material requires the use of intensive extraction technologies and techniques to obtain extracts rich in active principles. In order to obtain the active principles must be take into account a number of very important factors for the optimal development of the extraction process: the nature of the plant product (humidity, grinding, wetting), solvents used for extraction, the plant-solvent product ratio, the pH of the medium, the stirring, the extraction time and the temperature.

In this paper is presented the use of different extraction techniques, in order to obtain a plant extract with an optimum extraction yield of biologic active compounds. For different plants waste (industrial previously processed) were used solvent-free microwave method (Milestone Ethos X equipment - flavors mode), microwave hydro-diffusion method (Milestone Ethos X equipment - fragrances mode), microwave extraction (Milestone Ethos Easy equipment) and classical extraction methods. For the quantification of selected active compounds (galic, chlorogenic, ferrulic acid, apigenine, α pinene, bergaptene, quercitine, rutin) in the obtained extracts was used HPLC analysis. The management of these wastes is a major problem for industrial processors, so it is important to look for alternative solutions for the recovery of biologically active compounds. The results obtained showed that the chemical composition of the waste is similar with the fresh vegetal material, but in smaller quantities. This demonstrates that the extraction methods play an important role in the obtaining of biological active compounds.

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INNOVATIVE APPLICATIONS OF NATURAL EXTRACTS: FROM BIOTECHNOLOGY TO MATERIALS SCIENCE

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The plants constituted from ancient time a very rich source of food, fodder, fiber or medicinal remedies [1]. This traditional use, over time supported by scientific evidences, represents classical applications. Our group developed over time alternative applications for the natural extracts obtained following different recipes (classical extraction, hydro-distillation, solvent-free microwave extraction, microwave hydro-diffusion, accelerated solvent extraction) for different applications, ranging from biotechnology (control of fungal infestation of different supports) to materials science (phytosynthesis of metallic nanoparticles) [2, 3].

The present paper focuses on the potential use in those domains of several selected medicinal and aromatic plants (such as *Lavandula angustifolia* Mill., *Ocimum basilicum* L., *Rosmarinus officinalis* L., *Melissa officinalis* L. and others) extracted using different solvents and techniques.

The obtained results allowed us to propose, over time, new recipes for, for example, treating the microbial infestation of cultural heritage artefacts or for the phytosynthesis of biologically active noble metal nanoparticles.

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ACTION OF TYROSINASE ON ALPHA AND BETA-ARBUTIN: A KINETIC STUDY

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The derivatives from hydroquinone, α and β-arbutin, are used as depigmenting agents [1, 2]. In this work, we demonstrate that the *oxy* form of tyrosinase hydroxylates this compounds in *ortho* position of the phenolic hydroxyl group, giving rise to a complex formed by *met*-tyrosinase with the hydroxylated α and β-arbutin. This complex could evolve by oxidizing the originated *o*-diphenol to *o*-quinone and *deoxy*-tyrosinase, or by delivering the *o*-diphenol and *met*-tyrosinase to the medium, which would produce the self-activation of the system. Note that the quinones generated are unstable, so the catalysis cannot be studied quantitatively. However, if MBTH is used, the *o*-quinone is attacked, so that it becomes an adduct, which can be oxidized by another molecule of *o*-quinone, generating *o*-diphenol in the medium. In this way, the system reaches the steady state and originates a chromophore, which, in turn, has a high absorptivity in the visible spectrum. This reaction allowed us to characterize arbutins kinetically as substrates of tyrosinase for the first time, obtaining a K_m values of 6.5 ± 0.58 mM and 3 ± 0.19 mM, respectively. The data agree with those from docking studies that showed that the enzyme has a higher affinity for β-arbutin. Moreover, the k_{cat} obtained by the kinetic studies (4.43 ± 0.33 s^{-1} and 3.7 ± 0.29 s^{-1} for α and β-arbutin respectively) agree with our forecast based on ^{13}C NMR considerations. This kinetic characterization of arbutins as substrates of tyrosinase should be taken into account to explain possible adverse effects of these compounds.

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SPECTROPHOTOMETRIC CHARACTERIZATION OF THE ACTION OF TYROSINASE ON *P*-COUMARIC AND CAFFEIC ACIDS. CHARACTERISTICS OF THE *O*-CAFFEOQUINONE

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p-Coumaric acid (*p*-CA) is a phenolic acids widely distributed in nature, which have a hypopigmenting effect [1], and and caffeic acid (CAFA) is a derived from it [2]. In this work, new methods are proposed to determine the activity of tyrosinase on these compounds. Since the *o*-quinone from CAFA is unstable in its presence, it has been characterized through spectrophotometric measurements of the disappearance of coupled reducing agents, such as NADH. It has also been characterized by a chronometric method, measuring the time a known concentration of ascorbic acid takes to be consumed. The activity on *p*-CA has been followed by measuring the formation of *o*-quinone of CAFA at the isosbestic point originated between the CAFA and the *o*-caffeoquinone, and by measuring the formation of o-quinone at 410 nm, which is stable in the presence of *p*-CA (both of them in the presence of catalytic amounts of CAFA, maintaining the ratio between *p*-CA and CAFA constant, R = 0.025). The k_{cat} value of tyrosinase obtained for CAFA was higher (403 ± 8.30 s^{-1} and 381 ± 15.24 s^{-1}) than that obtained for *p*-CA (25 ± 0.34 s^{-1} and 15 ± 0.48 s^{-1}), while the affinity was higher (lower K_m) for *p*-CA (0.37 ± 0.02 mM and 0.44 ± 0.06 mM) than for CAFA (0.71 ± 0.05 mM and 0.64 ± 0.07 mM). These values agree with those obtained in docking studies involving these substrates and the oxytyrosinase.

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EFFECTS OF *ARONIA MELANOCARPA* FRUIT JUICE AND ITS COMPONENT CHLOROGENIC ACID ON LOCOMOTOR ACTIVITY IN OVARIECTOMIZED RATS

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Aronia melanocarpa, a plant rich in polyphenols, and one of its main bioactive components chlorogenic acid (CGA) are known to decrease locomotor activity and to exert antidepressant and anxiolytic effects in young/healthy rats [1-3].

The aim of this study was to investigate the effects of *Aronia melanocarpa* fruit juice (AMFJ) and CGA on locomotor activity in ovariectomized Wistar rats.

Female Wistar rats were divided into 5 groups, each of 14 animals. The daily oral treatment started 15 days after the operation. Groups SO (sham-operated) and OV (ovariectomized) were treated with saline (10 ml/kg). Groups OV+$AMFJ_5$ and OV+$AMFJ_{10}$ were treated with AMFJ (5 and 10 ml/kg, respectively). OV+CGA group was treated with CGA (20 mg/kg as a 10 ml/kg solution). After 30 days of treatment, open field test was performed. The indices recorded were: horizontal movements (HMs) and vertical movements (VMs) as measures of locomotor activity and central time (CT) as a measure of anxiety.

The recorded indices of OV group were decreased but not significantly compared to those of SO group. In OV+$AMFJ_5$ and OV+$AMFJ_{10}$ rats, HMs were significantly decreased compared to OV group while VMs and CT were not significantly different from those of OV group. HMs, VMs and CT of OV+CGA group did not differ significantly from those of OV group.

In conclusion, AMFJ but not CGA suppressed the locomotor activity in ovariectomized rats, probably due to sedation. AMFJ and CGA did not affect the state of anxiety.

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DEVELOPING AN EFFICIENT HAIRY ROOT TRANSFORMATION PROTOCOL TO AID FENUGREEK BIOLOGICAL ENGINEERING

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Trigonella foenum-graecum (fenugreek) is an annual legume originating in the Eastern Mediterranean region and India. It is a multi-purpose plant whose seeds and leaves are used in food as spice, flavoring agents and texture modifiers, and whose seeds are also used extensively for their medicinal, pharmaceutical and nutraceutical properties, due to their high content in bioactive flavonoids and phytosterols. Thus fenugreek is a significant target of biological engineering, with the aim of enhancing production of such valuable specialized metabolites. The main objective of this study was to design an efficient *Agrobacterium* mediated fenugreek transformation protocol. Hairy root cultures of fenugreek seedlings were established by two *Agrobacterium rhizogenes* strains, ARQUA and LBA9402. This resulted in different percentages of callus and root formation in the inoculated fenugreek seedlings. *A. rhizogenes* ARQUA showed greater root regeneration percentage as well as greater efficiency of hairy roots transformation compared to the LBA9402 strain. Furthermore, as an additional proof of principle, fenugreek seedlings were inoculated with *A. rhizogenes* ARQUA containing *Medicago truncatula* TSAR1 and TSAR2 expression constructs. These are basic helix-loop-helix transcription factors that selectively induce the expression of enzymes involved in mevalonate and triterpene saponin biosynthesis, resulting in the accumulation of the latter compounds in *M. truncatula*.

Thus heterologous expression of *Mt*TSARs will test whether TSAR-mediated signaling is conserved in fenugreek and could stimulate the production of steroid saponins by increasing the supply of their precursor mevalonate.

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ANTIMICROBIAL ACTIVITY OF HYDROGELS EMBEDDED WITH GREEN TEA AND PROPOLIS EXTRACTS AGAINST FOOD PATHOGENS

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The aim of this work was to verify the antimicrobial activity of propolis and green tea extracts against foodborne *Staphylococcus aureus* and *Escherichia coli*. Viability of the strains, assayed in broth against eight concentrations of glycerol extracts ranging from 0 to 25%, was clearly affected only at concentration higher than 6%. Then, the 24h microbial growth kinetic for both pathogens was drawn for both extracts only at 25 and 12.5%.

The values of inhibition index turbidity ratio [1] were found always higher for green tea than for propolis; in addition, the inhibition index turbidity ratio produced by green tea extract was found always higher than 90% for *S. aureus* strains whereas it was close to 70% for the *E. coli* strains.

Then, both extracts were incorporated into inert absorbent polysaccharide hydrogels at 10 and 70% and assayed, following the ASTM 2180, only against the most resistant *E. coli* O157:H7 strain. After 24 hours of contact, the concentration of viable cells showed a slight reduction for both extracts without any statistical ($P<0.05$) significant difference.

In conclusion, antimicrobial compounds occurring in natural extracts showed a loss of antimicrobial activity when included into hydrogels. The causes of this phenomenon, still under examination, could be ascribed to a reduction in the specific antimicrobial activity, or in the mobility, of different natural compounds.

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A LIQUID CHROMATOGRAPHY–HIGH RESOLUTION MASS SPECTROMETRY – BASED APPROACH COMBINED WITH BIOINFORMATIC ANALYSIS TO INTEGRATE CHEMISTRY WITH GEOGRAPHICAL ORIGIN: APPLICATION IN THE STUDY OF *PORTULACA OLERACEA* L. (PURSLANE)

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Portulaca oleracea L. (purslane) (Portulacaceae) is well known edible and ethno-medicinal plant currently included in the list of "World Economic Plants". The nutritional and therapeutic properties are related to the high level of organic and fatty acids, phenolics, polysaccharides, catecholamines and cyclodopa amides.

This study presents a strategy based on liquid chromatography – high resolution accurate mass spectrometry method (LC – HRAMS) and bioinformatic methods to analyze 33 purslane accessions originating from 15 floral regions in Bulgaria together with 5 accessions with Greek provenance. The aim was to develop a purslane metabolic database that was dedicated to LC-MS metabolic "fingerprints" of assayed samples. The extraction efficiency using microwave with solvent water, temperature of 120°C, solvent to solid ratio of 10/1 (v/w) and extraction time of 5 min. was higher than those achieved by ultrasound assisted extraction.

LC-MS data were subjected to differential analysis by SIEVE 2.2. software (Thermo Sci., USA). The identified compounds were used to perform a study of the biosynthetic pathways of purslane secondary metabolites using KEGG (Kyoto Encyclopedia of Genes and Genomes) software platform. The statistical treatments identified marker compounds that can be used to distinguish origin of studied accessions. Combining LC-MS data with multivariate statistical analysis was proven effective in studying the purslane metabolites, allowing for integration of chemistry with geographic origin.

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PHYTOBIOLOGICAL TESTING OF SOME VEGETAL EXTRACTS WITH PHYTOTHERAPEUTIC POTENTIAL FOR THEIR SAFETY EVALUATION

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Medicinal plants provide numerous bioactive compounds with phytotherapeutic importance, being an important source of new alternative therapies more efficient and safer; also, they could contain potentially toxic substances beside active principles, and also in certain dosages can induce negative effects. Thus, it is important to perform cytotoxicity assays for safety evaluation of plants used in human therapy.

The species of Polygalaceae (e.g. *Polygala vulgaris* L., common milkwort) contain many chemical compounds (xanthones, flavonoids, biphenyl derivatives) with biological activities, especially the antioxidant ones [1]. *Dracocephalum moldavica* L. (Moldavian dragonhead), Lamiaceae, is used in the treatment of stomach and liver disorders, headache and congestion, and as cardiotonic agent; it contains flavonoids, triterpenoids, steroids, phenylpropanoids, iridoids and polysaccharides which provide different pharmacologic properties (sedative, carminative, anti-inflammatory, spasmolytic, anti-microbial and fungicidal) [2]. *Nepeta cataria* L. (catnip), Lamiaceae, presents various chemical components (flavonoids, coumarins, glycosides etc.), being used in treating kidney diseases, flatulence and dysmenorrhea, and also as stomach tonic [3].

The research aimed the evaluation of the physiological and cytogenetic effects induced by the extracts (tinctures, teas) from these species on vegetal cells, and thus their safety for human use.

It has been revealed that these extracts (tinctures and teas) have no cytotoxic and cytogenetic effects on vegetal cells, if they are used in optimal concentrations. However, for more safety, it is recommended to perform experiments with these extracts on more complex biological systems.

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BENEFIT / RISK ASSESSMENT OF ESTRAGOL, FENCHONE AND T-ANETHOLE CONTENT IN FENNEL MEDICINAL TEA FROM ROMANIA

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In Europe Romania is recognized as a cultivator and exporter of bitter fennel, but in pharmacies and specialized firms, *Foeniculum vulgare* Mill. is marketed under the name of sweet anise no matter the variety (bitter or sweet). In this context, we aimed to assess the pharmaceutical quality of the fennel fruit marketed for infusions as sweet anise in Romania and the compliance with the specifications mentioned in the EFSA directive, especially since Ph.Eur. formalizes under the name *Foeniculi fructus* only the fruits of *F. vulgare* ssp. *vulgare* var. sweet. The safety issues are raised due to their chemical differences, bitter fennel containing 50-75% t-anethole and 3-20% estragol (the latter proving to be carcinogenic and genotoxic) [1]. In our study we investigated a total of 45 samples of *Foeniculi fructus* bought from pharmacies and medicinal tea producers, for which we established their volatile composition (GC-MS/FID) extracted in infusions, their antioxidant potential (scavenger capacity against free radicals) and a theoretical evaluations of estragol intake (margin of exposure) by the consumption of infused fennel samples [2, 3]. The results showed that the chemical composition of the investigated samples is closely related to bitter fennel, none of the samples is within the specified norms in terms of t-anethole, fenchone and estragole quantity. Noteworthy is that 86% of the investigated samples have higher quantities of fenchone, whereas 37 % contain more estragole.

Such results pose serious issues regarding the safety of fennel tea use in therapy, especially for babies and young children.

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NMR-BASED METABOLOMICS TO IDENTIFY CYTOTOXIC METABOLITES FROM *MAHONIA AQUIFOLIUM*

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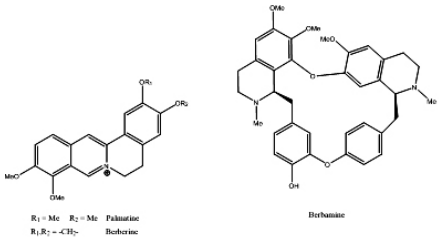
Mahonia aquifolium has been used in the American traditional medicine to treat different skin disorders [1]. Several studies indicate that *M. aquifolium* is effective in patients with mild to moderate psoriasis through activities on different components of inflammation process and on keratinocyte proliferation [2, 3]. Herein, we propose a ^{1}H NMR-based metabolomics method to reveal cytotoxic metabolites from *Mahonia aquifolium*. The powdered plant material was extracted in two different solvents: chloroform, and 62% aqueous ethanol. The part of the aqueous ethanol extract was further fractionated by dissolving in trifluoroacetic acid, and then neutralized and re-extracted with chloroform. All three type of extracts were used for NMR measurements and cytotoxic activity examination on Human cervical adenocarcinoma (HeLa) cell lines. Primary and secondary metabolites in the studied extracts were identified using 1D and 2D NMR spectra. An OPLS analysis was applied to correlate chemical composition of the *Mahonia* extracts with the results of the cytotoxic activity. Three compounds were identified as the most influential in the OPLS model, with the highest cytotoxic activity: protoberberine alkaloids berberin and palmatine, as well as bisbenzylisoquinoline alkaloid berbamine.



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EFFECTS OF LEAF EXTRACT FROM BULGARIAN *COTINUS COGGYGRIA* ON THERMODYNAMIC PROPERTIES AND EPIGENETIC EVENTS IN HUMAN BREAST CANCER CELLS *IN VITRO*

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As a medicinal plant with numerous valuable biological activities *Cotinus coggygria* is of great scientific interest for assessment of its pharmacological capacity. The antitumor properties of the herb extracts are poorly studied. Our previous investigations detected that leaf aqueous ethanolic extract from Bulgarian *C. coggygria* exhibits considerable *in vitro* antitumor activity on human breast cancer cells. The objective of the present research was to further elaborate the identification of the mechanisms of herb extract anticancer action on breast cancer cell line MCF7. The extract influence on the thermodynamic cell features was followed by differential scanning calorimetry and the extract-induced modulation of epigenetic DNA methylation process was studied through quantitative RT-PCR analysis of the transcriptional levels of genes coding DNA methyltransferases (DNMTs) and some methyl-CpG-binding domain proteins (MBDs). The obtained results indicated considerable alterations of the thermal transition in the low-temperature region in the calorimetric profiles of the extract treated cells, which is associated with denaturation of RNA, cytoplasmic and nuclear proteins, when compared with untreated controls. A trend of reduction in relative mRNA expression of *DNMT1*, *DNMT3a* and *MBD3* genes 72 hours after cancer cells treatment was observed. Further studies will be focused on the effect of the extract on other epigenetic processes as histone acetylation and deacetylation in MCF7 cells.

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SECONDARY METABOLITES FROM THE AERIAL PARTS OF *CENTAUREA PAPPOSA* (COSS.) GREUTER

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Continuing our research on the chemical constituents of *Centaurea* sp. (Asteraceae), we report here our results on the investigation of secondary metabolites from *C. papposa*, a species growing wild in Algeria [1], belonging to the Acrolophus (Cass.) DC section.

Their isolation was carried out according to the Bohlmann isolation method [2]. The residue was pre fractionated by VLC on silica gel, using cyclohexane-EtOAc-Me_2CO mixtures of increasing polarity as eluents to give several fractions. Further purification on RP_{18}-HPLC (MeOH-H_2O 2 : 1) and CC on silica gel yielded, so far, five sesquiterpene lactones, one germacranolide, cnicin, one eudesmanolide, malacitanolide and three elemanolides, 8a-*O*-(3□,4□ -dihydroxy-2-methylenebutanoyloxy)dehydromelitensin, its methylester derivative and 8α-*O*-(3□,4□ -dihydroxy-2-methylenebutanoyloxy)-15-oxo-5,7RH,6αH-eleman-1,3,11(13)-trien-6,12-olide. In addition to the isolated sesquiterpene lactones, one methylated flavonoid, eupatorin, was found. The study revealed that the chemical profile of *C. papposa* is similar to that of previous investigated *Centaurea* taxa belonging to the same section [3]. Moreover, the investigation of the potential biological activities of the plant and its secondary metabolites consists future goal.

Acknowledgements: The authors wish to thank Dr. Hamel Tarek (Département de Biologie, Université Badji Mokhtar Annaba, Algeria) for the collection and the identification of the plant material.

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PP 86

PHENOLIC METABOLITES OF FOUR GREEK ENDEMIC BORAGINACEAE SPECIES. ANTIOXIDANT ACTIVITY

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Boraginaceae is a family of herbs, shrubs and trees with a worldwide distribution. The family comprises ca. 130 genera and 2300 species, occurring mainly in Europe (especially in the Mediterranean region) and Asia [1]. A lot of plants from this genus have been widely studied with regard to their extensive use in traditional medicine but they are not only investigated for the presence of pharmacologically active compounds such as naphthoquinone pigments but also for toxic compounds, among which the most significant are pyrrolizidine alkaloids [1-3]. In the framework of our research on the Boraginaceae family [1-3], we report herein the comparative chemical analysis of the phenolic content of the aerial parts of four endemic Greek species *Onosma graeca*, *Onosma rigida*, *Onosma erecta* and *Alkanna corcyrensis*.

Qualitative phytochemical analysis of methanol extracts of the aerial parts of the above plants resulted in the isolation of several flavonoids such as luteolin and apigenin derivatives (luteolin-7-glucoside, luteolin-7-, apigenin-7- and quercetine-7-rhamnoglucosides, in forms rutinosides and/or neohesperidoside) as well as rosmarinic, caffeic and lithospermic acids, among which especially rosmarinic acid could be accepted as a chemotaxonomic marker in the Boraginaceae family.

Moreover, the total phenolic content was determined by the Folin-Ciocalteu method, total flavonoid content was estimated by the aluminium chloride colorimetric assay and the free radical scavenging activity was determined by DPPH and ABTS assays showing an interesting profile.

Acknowledgements: The authors would like to thank the Special Account for Research Grants and the National and Kapodistrian University of Athens for funding their participation in this meeting.

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PP 87

CHEMICAL CONSTITUENTS FROM THREE SPECIES OF THE GENUS *CORDIA* (BORAGINACEAE) FROM PANAMA AND THEIR BIOLOGICAL ACTIVITIES

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The genus Cordia (Boraginaceae) comprises approximately 300 species, mostly evergreen trees and shrubs with worldwide distribution. A lot of plants from this genus have been widely studied with regard to their extensive use in traditional medicine [1].

The aim of this work was the phytochemical analyses of three tropical species: *Cordia bicolor*, *C. megalantha* and *C. dentata*. as well as their biological evaluation.

Cordia bicolor afforded 9 compounds, namely allantoin **(1)** , rosmarinic acid **(2)**, caffeic acid **(3)**, isoquercetin **(4)**, rutin **(5)**, quercetin-3-*O*-β-D-neohesperidoside **(6)**, kaempferol-3-*O*-β-D-neohesperidoside **(7)**, helichrysoside **(8)** and kaempferol 3-*O*-(2″-*O*-α-L-rhamnosyl-6″*trans*-p-coumaroyl)-β-D-glucoside **(9)**.

Cordia megalantha yielded 2 compounds: rosmarinic acid **(2)** and quercetin-3-*O*-(6″*trans*-p- coumaroyl)-β-galactoside **(10)**, while six compounds have been isolated from *Cordia dentata:* rosmarinic acid **(2**), caffeic acid **(3)**, quercetin 3-*O*-(6″-*O*-α-L-rhamnosyl)-β-D-glucoside **(5)**, 4-hydroxyphenyl lactic acid **(11)**, sitostenone **(12)** and β-sitosterol **(13)**.

Compounds **(6)** and **(7)** are reported for the first time in the genus Cordia, while this is also the first report of compounds **(8), (9)** and **(10)** in the Boraginaceae family.

All extracts were evaluated for their total phenolic content as well as for their antioxidant profile (DPPH, ABTS, CURPAC, FRAP) and for enzyme inhibition against cholinesterases, a-amylase and glucosidase. *C. megalantha* showed the most interesting antioxidant profile and the highest glucosidase inhibition.

Acknowledgements: The authors would like to thank the Special Account for Research Grants and the National and Kapodistrian University of Athens for funding their participation in this meeting.

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PP 88

GREEN PROCESSING OF *KALANCHOE PINNATA* EXTRACT ACTIVE AGAINST HEMORRHAGE AND EDEMA INDUCED BY *BOTHROPS JARARACA* SNAKE VENOM

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Kalanchoe pinnata (Crassulaceae), known popularly in Brazil as "Saião" is used in traditional medicine for antiophidic properties. To confirm this indication, hydroethanolic leaf extracts of *K. pinnata* has been recently evaluated against local *effects* induced by *Bothrops jararaca* snake venom. A significant reduction of the hemorrhagic and edematogenic activities of the venom was observed, as well as inhibition of phospholipase enzymatic activity. High Performance Liquid Chromatography coupled with Diode Array Detection and Electrospray Mass Spectrometry (HPLC-DAD-MS/MS) were performed to characterize chemical markers of the extract, resulting in the identification, among others, of quercetin and kaempferol glycosides [1].
Considering the potential use of these flavonoid derivatives as new bioactive compounds against bothropic venom, a strategy was formulated mainly using several isolation techniques in accordance with the criteria of green chemistry and sustainable development. In these conditions, use of adsorption resin technology, centrifugal partition chromatography and recrystallization process enabled to access to pure metabolites. These methods have low environmental impact, high efficiency and high reproducibility for the purification of glycosides from polar extracts [2]. As the scale-up is feasible and the plant material easy to cultivate, this work may serve as a model for pilot and industrial production of natural products with antiophidic activity.

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PP 89

EFFECTS OF PURPLE POTATO [*IPOMOEA BATATAS* (L.) LAM] OLIGOSACCHARIDES ON GROWTH ABILITY OF *BIFIDOBACTERIUM ADOLESCENTIS*

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In this paper, purple potato (*Ipomoea batatas* (L.) Lam) oligosaccharides (PPOS) was separated by medium-pressure chromatography, and its components were identified via high performance liquid chromatography. The configuration of each component was analyzed through fourier transform infrared spectroscopy (FTIR), mass spectrum (MS) and nuclear magnetic resonance (NMR). The results indicated that PPOS consisted of six compounds at least. According to the fragmentation pattern of mass spectrum results, the molecular weights of its two components, PPOS2-1 and PPOS3-1, could be detected. The relative molecular weight of PPOS2-1 was identified as 342.08, approximately the same with sucrose. The molecular weight of PPOS3-1, a glycoside, could be ascertained as over 800. And their degrees of polymerization were two and three respectively. Disaccharide is major component of PPOS. PPOS was neutral, and it has the structure of pyranose. It is mainly configurated by α-glycosidic bond. The effects of PPOS on growth ability of *bifidobacterium adolescentis* were also investigated.

The results revealed that the proliferation of PPOS in-vitro on *bifidobacterium* was more significantly than glucose (GLU) and isomalto-oligosaccharide (IMO). Optimum concentration of PPOS in the culture medium was 7g/L. And growth curve of the *bifidobacterium adolescentis* implied that PPOS was more effective than GLU on shortening growth retardation of *bifidobacterium*. And the final concentration of the bacteria processed by PPOS was also higher. The results showed that PPOS was a valid proliferating factor for *bifidobacterium*.

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BIFLAVONOIDS FROM *FUMANA PROCUMBENS* (DUNAL) GREN & GODR.

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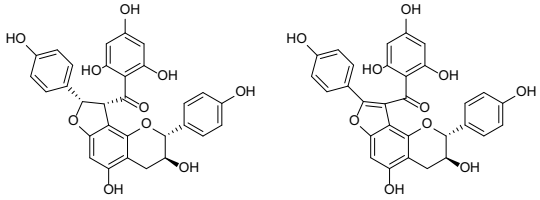
The genus *Fumana* (Cistaceae) comprising more than 20 species that are distributed in Mediterranean flora [1] and 10 species with 30% endemism in the Flora of Turkey. While, essential oil composition of *Fumana thymifolia* and methylated flavonol glucosides of *Fumana montana* were studied [2,3] nothing is known about chemistry of *Fumana procumbens*. In this study, phytochemical investigation on the aerial parts of *Fumana procumbens* (Dunal) Gren. & Godr. led to the isolation and identification of six compounds, including two biflavonoids dihydrodaphnodorin B (**1)** daphnodorin B (**2**) and, three flavonoids, quercitrin, myricitrin, quercetin and a flavan derivative epigallocatechin. The structures of the compounds were elucidated by extensive 1D- and 2D-NMR spectroscopic analysis in combination with MS experiments. This is the first report on the isolation of biflavonoids from genus *Fumana* and from family Cistaceae.



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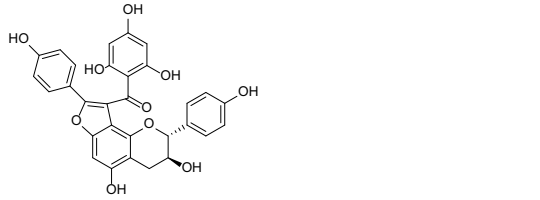

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DEVELOPMENT OF AN HPTLC AND AN HPLC-PDA METHOD FOR THE QUANTIFICATION OF SESAMIN AND SESAMOLIN IN FRESH SESAME SEEDS

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Sesame (*Sesamum indicum,* Pedaliaceae) has long been used as a highly nutritional traditional food in the countries where it is cultivated, with its applications varying from the production of oil to flour. Sesame seeds have been found to contain a group of antioxidant compounds named lignans, that have exhibited potent health-promoting properties. Among them, sesamin and sesamolin constitute the major secondary metabolites of sesame seeds and they have in recent years gained a lot of attention thanks to their numerous biological properties [1,2]. However, even though a great number of studies have focused on the quantification of sesamin and sesamolin in sesame oil, to our knowledge, no study has so far performed quantification of the two lignans by HPTLC in the fresh sesame seeds. In the present work, two methods were developed in compliance with the requirements of the European Pharmacopoeia for the quantification of the two lignans in the methanolic extract of fresh seeds, using HPTLC and HPLC-PDA. The results were subsequently subjected to statistical analysis in order to investigate a possible correlation between the two methods, indicating that they are adequately correlated in terms of performance.

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PP 92

NEW OLEANANE-TYPE BISDESMOSIDE SAPONIN FROM HAIRY ROOTS OF MARIGOLD (*CALENDULA OFFICINALIS*) ELICITATED BY JASMONIC ACID

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Marigold *(Calendula officinalis* L.*)* is a well-known medicinal plant containing significant amounts of oleanolic acid glycosides with sugar moieties bound to C-3 hydroxyl group in monodesmosides, or to both C-3 hydroxyl- and C-28 carboxyl- groups in bisdesmosides. These saponins can be classified as glucosides or glucuronides according to the first residue in sugar chain bound to C-3 hydroxyl group (glucose or glucuronic acid, respectively). Both groups exert anthelmintic, antibacterial and antifungal activities, however, glucosides are less potent than glucuronides. Glucosides are present in plant roots and they exert allelopatic activity. Glucuronides are present particularly in flowers, seeds and plantlets. They exhibit anti-inflammatory, hypoglycaemic, gastro protective and anti-tumour properties. Marigold hairy roots were obtained by transformation with *Agrobacterium rhizogenes* strain 15834 and were found to be capable of synthesis of oleanolic acid glycosides.

The aim of the present study was the isolation and identification of the most abundant saponin present in marigold hairy roots treated with jasmonic acid. Dried roots were extracted with ethanol, extracts were fractionated by CC and HPLC, and afterwards the isolated compound was analyzed with the use of a combination of 1D and 2D NMR techniques. The saponin was determined to be 3-*O*- -D-glucuronopyranosyl-28-*O*- -D-galactopyranosyl-oleanolic acid. The obtained result is very interesting because the identified compound does not occur in the native marigold plant. Thus, elicitation with jasmonic acid induced in hairy roots the enhanced biosynthesis of a compound not produced naturally by wild type plant, moreover, with more potent pharmacological activities than saponins present in native marigold roots.

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COMPARISON BETWEEN TWO LAVENDER OILS EXTRACTED FROM PLANTS BELONGING TO DIFFERENT ROMANIAN COUNTIES

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Lavender (*Lavandula angustifolia* L., Lamiaceae) have been largely used since antiquity in both cosmetics and therapy. While its use in cosmetics was well-known in the Western culture, in Eastern cultures it was also used for its use in aromatherapy. The use of lavender oil in muscular pain alleviation was largely documented, but also it has been claimed to have anxiolytic, antifungal and gastric syndromes alleviation effects [1].

Two lavender oils obtained by distillation from cultured plants with the same source, grown in different counties in Romania – Salaj and Dambovita – were assayed for their composition using GC-MS and HPTLC [2]. GC-MS analysis was performed on a TRACE TR-5MS capillary column, 5% phenyl polysilphenylene-siloxane, within the range 30–650 m/z. Identification was based on the mass spectrums from NIST library collection. HPTLC analysis was performed on silicagel, using ethyl acetate: toluene (5:95 V/V) as a mobile phase, and a migration distance of 8 cm.

Results showed linalyl acetate as the most important ester compound in both samples; other common identified compounds: cineole, linalool, caryophyllene oxide. However, the gas-chromatograms indicated slight differences between both qualitative and quantitative composition of the two oils, attributable to different cultivation conditions (soil, illumination, atmospheric). Results obtained were confirmed by the HPTLC analysis.

As a conclusion, the efficiency of the culture is definitely influenced in all terms related to the quality of the essential oils by the cultivation conditions and procedures, having as a result different economic proficiency.

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ALKALOIDS FROM *BERBERIS VULGARIS* AND THEIR BIOLOGICAL ACTIVITY CONNECTED TO ALZHEIMER'S DISEASE

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Alzheimer's disease is neurodegenerative disease with specific neuropathological changes, which are observed at molecular level. Nevertheless, ethiopathogenesis is still not clear and therapy is only symptomatic [1].

Berberine, an isoquinoline alkaloid from *Berberis* sp. is agent, which interferes with many processes present within the course of the disease [2]. The aim of this phytochemical study was to isolate tertiary alkaloids with potential neuroprotective activity, which could have less cytotoxic effect and better bioavailability.

The barberry root bark extract was was subjected to liquid/liquid extraction with different pH and treated by standard chromatographic methods. Structures of the alkaloids were determined by MS and NMR and alkaloids were subsequently tested *in vitro* for their inhibition activity in term of acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and prolyl oligopeptidase (POP); IC_{50} values were determined. The most active alkaloids were tested for a type of cholinesterase inhibition and ability to cross blood brain barrier (BBB).

Some of the alkaloids were weak AChE inhibitors: the berlambine, bersavine, obamegine and berbostrejdine (IC_{50} ranged from 55.3 to 97.4 μM). The last one inhibited also BuChE ($IC_{50} = 6.9 \pm 1.0$ μM). The most potent inhibitor of BuChE was aromoline with IC_{50} value of 0.82±0.1 μM. The type of inhibition of aromoline was determined at horse plasma BuChE model, it acted *via* a mixed mechanism in a dose-dependent manner. Based on parallel artificial permeation assay it is not able to cross BBB by passive permeation. The promising inhibition activity against POP was shown by new alkaloid bersavine (with $IC_{50} = 67.3 \pm 6.2$ μM).

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INHIBITORY MECHANISMS OF HONOKIOL, DERIVED FROM *MAGNOLIA OFFICINALIS*, IN PLATELET ACTIVATION

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Honokiol, derived from *Magnolia officinalis*, has various pharmacological properties. Platelet activation plays a critical role in cardiovascular diseases. Honokiol has been reported to inhibit collagen-stimulated rabbit platelet aggregation. However, detailed further studies on the characteristics and functional activity of honokiol in platelet activation are relatively lacking. In the present study, honokiol specifically inhibited platelet aggregation and Ca^{+2} ion mobilization stimulated with collagen or convulxin, an agonist of glycoprotein (GP) VI, but not with aggretin, an agonist of integrin $\alpha_2\beta_1$. Honokiol also attenuated the phosphorylation of Lyn, PLCγ2, PKC, MAPKs, and Akt after convulxin stimulation. Honokiol have no cytotoxicity in zebrafish embryos. Honokiol diminished the binding of anti-GP VI (FITC-JAQ1) mAb to human platelets, and it also reduced the coimmunoprecipitation of GP VI-bound Lyn after convulxin stimulation. The surface plasmon resonance results revealed that honokiol binds directly to GP VI, with a K_D of 289 μM. Platelet function analysis revealed that honokiol substantially prolonged the closure time in human whole blood and increased the occlusion time of thrombotic platelet plug formation in mice.

In conclusion, honokiol acts as a potent antagonist of collagen GP VI in human platelets, and it has therapeutic potential in the prevention of the pathological thrombosis.

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AMARYLLIDACEAE ALKALOIDS AS POTENT GSK 3B INHIBITORS

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Alzheimer´s disease (AD) is one of the most frequent causes of dementia in the world. During AD in the brain occurs to pathological changes of some enzyme systems that result in loss of neurotransmitter acetylcholine and formation of amyloids plaques and neurofibrillary tangles (NFTs). As a result are brain damage, development of dementia and loos of cognitive functions. NFTs consisting of paired helical filaments, with the main component being hyperphosphorylated τ-protein. Phosphorylation of τ-proteins is primarily dependent on glycogen synthase kinase-3β (GSK-3β) and cyclin-dependent kinase 5 [1]. Glycogen synthase kinase-3 is an ubiquitous serine/threonine kinase that plays a crucial role in numerous cellular functions, including cell cycle regulation, differentiation and proliferation, and gene expression by regulating a wide variety of substrates like glycogen synthase or tau-protein [2].

In our ongoing study focused on Amaryllidaceae alkaloids, we have investigated thirty previously isolated alkaloids from *Zephyanthes robusta, Nerine bowdenii, Chlidanthus fragrans* and various *Narcissus* cultivars for their GSK-3β inhibition potential. For all compounds was measured percentage inhibition at concentration 50 μM. Inhibitory activity IC_{50} was determined for three compounds (masonine: 28.25 μM ± 4.05, 9-O-demethylhomolycorenine: 27.20 μM ± 10.80 and caranine: 31.54 μM ± 1.26). Since galanthamine is used in therapy of AD, Amaryllidaceae alkaloids are still promising goal in searching for new bioactive compounds.

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ESSENTIAL OILS CHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITIES AND TOTAL PHENOLS OF THREE AROMATIC HERBS FROM APIACEAE FAMILY

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Chemical composition and antioxidant activity of three aromatic herbs from *Apiaceae* family were analyzed. The essential oils (EOs) were isolated by hydrodistillation from fruits of aniseed (*Pimpinella anisum)*, cumin (*Carum carvi)* and coriander (*Coriandrum sativum)* and their chemical compositions were determined by the GC-MS. Aromatic compound *trans*-anethole (90.46%) was predominant constituent of the aniseed EO. The main components of the EO of cumin were D-carvone (57.43%) and D-limonene (39.91%). In the coriander EO, linalool appeared as the main component (64.28%) followed by camphor (5.28%) and α-pinene (4.67%). Total polyphenolic content of the EOs was determined by Folin-Ciocalteu assay, and the antioxidant activity of EOs was evaluated using DPPH and ABTS tests. Based on their antioxidant activity, EOs can be sorted in descending order: coriander > cumin > aniseed. Values of antioxidant activity were correlated with the content of total phenols [1]. All of tested EOs are produced from herbal plants that are commonly used as spices or as food flavoring additive, therefore, they are safe to use, yet provide good defense against oxidative damage and associated health effects [2].

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PP 98

CYTOTOXIC ACTIVITY OF PODOPHYLLOTOXIN AND ITS ANALOGUES, PRODUCED BY PLANT TISSUE CULTURES OF *LINUM THRACICUM SSP. MULTIFLORUM* DEGEN, ELICITED WITH METHYL JASMONATE

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Cells of *Linum thracicum* ssp. *multiflorum* were aseptically cultivated in mediums with different auxin to cytokinin ratio. The growth *in vitro* introduced changes not only in the total amounts in the podophyllotoxin and 6-methoxypodophyllotoxin, but also in the ratio between them. The administration of methyl jasmonate to the cell lines boosted the lignan biosynthesis up to 4-fold. One of the cell lines, revealed as a promising source of 6-metoxy-podophyllotoxin as it produced high amount of the metabolite, and its accumulation was readily inducible upon methyl jasmonate treatment.

In this study the cytotoxic activity [1] of two naturally occurring cyclolignans, isolated from the in vitro cultures of *L. thracicum* ssp. *multiflorum* species was evaluated in a panel of human tumor cell lines, representative for some clinically important neoplastic diseases, namely HL-60 (acute promyelocyte leukemia), SKW-3 (a KE-37 derivative, T-cell leukemia), MDA-MB-231 (ER-negative breast carcinoma), MCF-7 (ER-positive breast adenocarcinoma), HT-29 (colon carcinoma). The results obtained indicate that despite the similar potency of tested lignans.

Acknowledgements: Financial support from SMN-Medical University of Sofia, Bulgaria (NoD-79/2017) is acknowledged.

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LAVANDULA STOECHAS: ANTI-INFLAMMATORY EFFECTS AND ACTIVE COMPONENTS

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Lavandula stoechas (LS) has distinct medicinal uses among Anatolian people. Rather than worldwide use of its essential oil, specifically aqueous portion has been traditionally used in Anatolia against type II diabetes and obesity, both characterized by a state of chronic inflammation. Ethyl acetate (EE) and n-butanol extract (BE) were prepared from LS and investigated for their potential anti-inflammatory activities on lipopolysaccharide (LPS) induced RAW264.7 macrophages. Accordingly, EE significantly suppressed LPS induced NO production 32 and 64% at 50 and 100 µg/mL, however, BE had no effect at same doses. Gene expression studies showed that EE was more effective for downregulating proinflammatory genes; iNOS, IL1β and COX-2 compared to BE. Therefore, EE was chosen for the bioactivity guided fractionation studies leading to 12 subfractions (E1-E12) by column chromatography. By NO screening test, E6 was identified as active subfraction subjected to column chromatography to get pure compounds which were then applied to NMR, IR, and UV analyses for identification. Apigenin, luteolin and p-coumaric acid were found in E6, and the first two compounds appeared to be primarily responsible for the anti-inflammatory activity. Apigenin and luteoline at 50 µM decreased NO production 66 and 80%- IC_{50}: 56 and 26 µM-by inhibiting iNOS gene expression 84 and 88% as well as protein expression 94 and 99%, respectively ($p<0.05$). Besides, apigenin and luteolin downregulated expressions of iNOS, IL1β and COX-2 considerably (31-85%) at 50 µM.

Therefore, the anti-inflammatory properties of EE can be attributed, at least in part, to apigenin and luteolin found in LS.

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STUDY ON THE EFFECT OF *CATHARANTHUS ROSEUS* EXTRACT ON VARIOUS BIOCHEMCIAL, ENDOCRINOLOGICAL AND OXIDATIVE PARAMETERS OF ALLAXON-INDUCED DIABETIC RATS

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Aim: The present study was designed to assess the effects of *Catharanthus roseus* extract on various biochemical, endocrinological and oxidative parameters in serum of allaxon-induced diabetic rats.

Methods: Diabetic rats were assigned to three groups *viz.* Group A: Negative Control fed basal diet; Group B: Positive Control non-supplemented; and Group C supplemented with *C. roseus* extract [1].

Results: The results indicated a statistically non-significant ($P>0.05$) elevation in blood glucose, TGs, ALT and urea concentration of group C (*C. roseus* supplemented) as compared to group B (non-supplemented) animals. Regarding cholesterol and AST, the results indicated a statistically non-significant ($P>0.05$) decrease in *C. roseus* supplemented group C. Blood cortisol level was increased in group C as compared to those animals in group B, though statistically non-significant ($P>0.05$). Regarding the levels of T^3 and T^4 hormones, it was noticed that their levels were non-significantly ($P>0.05$) decreased in herbal extract-supplemented group C as compared to Group B.

Conclusion: The results of present study revealed a gradually increasing though statistically non-significant ($P>0.05$) trend of catalase in group A, B and C animals, respectively. In a nutshell, we conclude that *C. roseus* had a restorative/corrective effect on levels of cholesterol and thyroid hormones; and a stress relieving effect through elevating catalase enzyme for potential scavenging of ROS in alloxan-induced diabetes rats.

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PP 101

PHYTOCHEMICAL INVESTIGATIONS AND BIOLOGICAL ACTIVITIES OF TANACETUM SPECIES FROM ROMANIA

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Tanacetum species are medicinal and aromatic plants used as flavoring for food and drink, insect repellent and herbal remedies with anti-migraine, anthelmintic, antimicrobial and anti-inflammatory activities. The present study aimed to evaluate the antimicrobial, antioxidant and cytotoxic activities of extracts from *T. vulgare*, *T. macrophyllum* and *T. corymbosum* and to assess their chemical composition in order to identify potentially bioactive compounds.
The impact of the extracts on cell viability was tested both on cancerous (HeLa) and non-cancerous (Vero) cells by MTT assay. The range of doses was between 25 and 200 µg/mL and the incubation time was established at 24 hours. The impact on cell viability was dose dependent, the normal cells being more sensitive to the action of the tested extracts; an important cytotoxic effect had arisen at lower doses than in HeLa cells.

All extracts were active against Gram-positive bacteria (*Staphylococcus aureus*) and fungi (*Candida albicans*, *Candida parapsilosis*) and lack activity on Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). The antioxidant activity assessed by DPPH method and reducing power assay was weak for all extracts.

HPLC-MS analysis revealed the presence of parthenolide only in *T. vulgare* flowers and leaves. Methoxylated flavones with anticancer properties, such as eupatorin, jaceosidin, hispidulin, eupatilin and acacetin, were identified in all tested extracts, in higher amounts in *T. vulgare*. Phytosterols were also determined and found to be in greater quantities in *T. vulgare*.

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ANTILYMPHOMA ACTIVITY OF CANNABIDIOL *IN VITRO*

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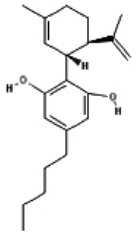
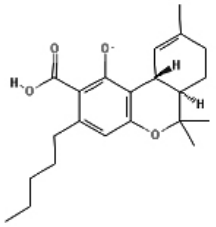
Cannabidiol (CBD) is a natural component in hemp. CBD is one of at least 113 active cannabinoids identified in cannabis. It is a major phytocannabinoid, accounting for up to 40% of the plant's extract. It has remarkable pharmacological activities such as tumor growth inhibition, pain perception modulation, and anticonvulsive, antipsychotic and antiemetic properties as well. It lacks any psychotropic activity and common toxicity and therefore could be present in food supplements. We studied the lymphoma growth inhibition in human NHL-derived cell lines such as U-266 multiple myeloma cells, MJ and HuT-78 cutaneous T-cell lymphoma (CTCL)-derived cells using the MTT-dye reduction assay. Concentration response curves showed IC_{50} values bellow 30 µM in all tumor cell lines investigated. Proteome analysis performed indicated up-regulation of pro-apoptotic signaling molecules such as Bad, Bax, caspase 3, cytochrome c etc., as well as down-regulation of anti-apoptotic proteins such as Bad, Bax, caspase 3, cytochrome c etc. Using specific ELISA cannabidiol was found to reduce the activity of the NF-κB transcription factor in a comparable with curcumin range. Western blot analyses did not show any changes in the CB2 receptor expression profile. In addition, using established HPLC method we determined the content of cannabidiol in hemp oil products on the Bulgarian market. Taken together our experimental data confirm the very promising antineoplastic activity of cannabidiol against multiple myeloma and CTCL NHL malignant cells.

Chemical structure of cannabidiol (CBD) and tetrahydro-cannabinol (THC):





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DETERMINATION OF TOTAL POLYPHENOL CONTENT AND *IN VITRO* ANTITUMOR ACTIVITY OF YELLOW DOCK (*RUMEX CRISPUS* L., POLYGONACEAE) FRUIT EXTRACT

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Apart from being considered a seriously invasive weed, young leaves of yellow dock (*Rumex crispus* L.) are edible and often used as vegetables. The use of its fruits has been described in Serbian and Turkish traditional medicine against stomach complaints.

The aims of this study were to selectively extract and identify several classes of compounds present in fruits of *Rumex crispus*, and to evaluate *in vitro* its antitumor activity.

Ripe yellow dock fruits were collected during summer from a meadow in Kumodraž. The plant material was reduced to a fine powder, extracted with water and evaporated. The phenolic compounds were analyzed by HPLC and identified by MS comparing to databases. The cytotoxicity of aqueous extract of yellow dock fruit was examined *in vitro* in human tumor cell lines HeLa, MCF-7, HT-29 and healthy MRC-5. The cytotoxicity was evaluated by measuring the total protein content by colorimetric sulforhodamine B assay.

Major constituents: gallic acid, catechin, hyperoside and miquelianin appear in examined extract in concentrations 0.35%, 0.048%, 0.79% and 2.45%, respectively. The most pronounced antitumor activity was observed towards cervix carcinoma (IC_{50} = 16.9 μg/mL) and breast adenocarcinoma (IC_{50} = 19.3 μg/mL) cells. The activity towards colon adenocarcinoma cells was lower, but IC_{30} value, obtained at low concentration (IC_{32} = 62.5 μg/mL), suggest high activity towards this cell type, also.

The results showed high and tissue-selective antitumor effects of yellow dock fruit extract *in vitro*, containing flavonoids, as substances which might have biological activity that should be investigated in further studies.

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[2] Četojević-Simin D, et al. (2015) Food Chemistry 166: 407-413.
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PP 104

IN VITRO ANTIOXIDANT POTENTIAL OF AEQUOUS EXTRACT OF *RUMEX CRISPUS* FRUITS

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The aim of this study was determination of *in vitro* antioxidant activity of aqueous extract of *Rumex crispus* fruits.

The ferric reducing antioxidant potential (FRAP) method, used to measure the total antioxidant capacity of examined extract, is based on the reduction: Fe^{3+}-TPTZ→Fe^{2+}-TPTZ by antioxidants. The free radical scavenging activity was measured using DPPH assay. Intensity of production OH• radicals was determined following reaction of degradation of deoxiribose, with main product malondialdehyde (MDA). The measurement of the end product of lipid peroxidation (LP), MDA, is used as indicator of LP. The protective effect of examined extract in LP and its ability to prevent forming MDA were performed by using TBA assay.

The free radical scavenging power of different concentrations of investigated extract increased with increasing amount of extract. There is a clear difference between control and sample containing 1%-extract ($t = 7.53$, $p<0.001$), suggesting that mentioned extract showed significant ability to reduce Fe^{3+}, and thus, evident ability to donate electrons. It has been determined a statistical significant difference of intensity of LP between control and group used 1% of investigated extract ($t = 3.127$, $p = 0.014$). The IC_{50} value for investigated extract was lower than 5%, while TROLOX (standard substance) was able to reduce DPPH free radical, reaching 50% reduction with IC_{50} more than 50%. It has not been determined statistical significant difference in intensity of OH• production between control and group of 1% extract ($p = 0.224$).

Taking into account that the aqueous yellow dock fruits extract possesses high antioxidant potential, further investigations have been recommended.

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PP 105

THE SESQUITERPENE LACTONE SELECTIVELY TRIGGERS SENESCENCE, APOPTOSIS AND CELL CYCLE ARREST IN TRIPLE NEGATIVE BREAST CANCER CELLS

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Sesquiterpene lactones (SLs) are plant-derived compounds that display anti-cancer effects. Some SLs derivatives have a marked killing effect on cancer cells and have therefore reached clinical trials. Little is known regarding the mechanism of action of SLs. We studied the responses of human triple negative breast cancer cells exposed to various concentrations of Guaianolide, a SL of the Guaianolide group isolated and purified from *Cyathocline purpurea*. Guaianolide was evaluated for their *in vitro* cytoxicity against three human breast cancer cell lines MDA-MB-231, MDA-MB-453, MCF-7 using MTT cell cytotoxicity assay. It showed IC_{50} values of 22 µM, 36 µM, and 38 µM against MDA-MB-231, MDA-MB-453 and MCF-7 cell lines respectively. However, the compound demonstrated low cytotoxicity on Human PBMCs, MSCs and mouse 3T3-L1 non-cancerous cells *in vitro*, which indicates selective cytoxicity towards tumour cells. Further studies also demonstrated that compound had significant effect on BrdU incorporation, senescence, apoptosis and cell cycle in breast cancer cells. Additionally, it showed anti-migration potential probably due to alteration of Wnt/β-Catenin signalling pathways were confirmed using RT-PCR. Statistical tests were performed by using Graphpad Prisom 5.0 and P values less than 0.05 were considered as significant for all data sets.

Our findings help elucidate the mechanism whereby Guaianolide triggers cell death and provide a basis for further exploration of the effects of Guaianolide in *in vivo* cancer treatment models.

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ANTIOXIDANT PROPERTIES OF SELECTED SPICES

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The pathology of many chronic diseases involves generation of excessive amounts of reactive oxygen species (ROS). Recent studies indicate the protective role of phytochemical compounds against oxidative damage of cells caused by ROS. Although plants are extensively used in traditional medicine, their medicinal properties remain largely unexplored. The purpose of our study is to investigate the antioxidant potential of some spices: *Apium graveolens* L. (AGR), *Cymbopogon schoenanthus* (L.) Spreng (CSH), *Thymus serpyllum* L. (TSH), *Urtica dioica* L. (UDS) and *Curcuma longa* L. (CLR).

The antioxidant activity was evaluated with 2,2'-diphenyl-1-pycrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay. Total phenolic content (TPC) and total flavonoid content (TFC) were determined by Folin-Ciocalteu method and $AlCl_3$ method.

In DPPH assay, most promising extracts were TSH and CLR, with moderate (IC_{50} = 0.073 mg/mL) and very strong activity (IC_{50} = 0.010 mg/mL), respectively [1]. The same extracts showed strong reducing power (31.46% and 74.27%) by FRAP assay. Results are in accordance with their TPC (22.81 - 642.97 mg GAE/g crude extract) and TFC (0.02 - 148.91 mg QE/g crude extract) values.

The obtained results revealed high antioxidant potential for *Thymus serpyllum* and *Curcuma longa,* suggesting these plant species to be considered for further investigation of their phytochemical composition in order to identify the bioactive compounds with antioxidant potency.

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PP 107

PENTACYCLIC TRITERPENES FROM OLIVE OIL MODULATE THE LIPID MEMBRANE PERMEABILITY OF LARGE UNILAMELLAR LIPOSOMES

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The beneficial effect of olive oil derived from bioactive constituents such as the series of pentacyclic triterpenoids. Oleanolic acid and its precursor erythrodiol are known for their anti-inflammatory, cardioprotective and anti-tumor effects. Being hydrophobic, they may enter the lipid bilayer modulating its fluidity and permeability. The aim of this study was to determine the effect of oleanolic acid and erythrodiol on the permeability of dipalmitoyl phosphatidylcholine liposomes prepared by reverse phase evaporation technique followed by extrusion and containing various cholesterol content (phospholipid:cholesterol molar ratios of 100 : 0, 100 : 2.5, 100 : 10, 100 : 20, 100 : 25, 100 : 30, 100 : 50 and 100 : 100). Liposome size determined by laser granulometry demonstrated the formation of vesicles with a mean diameter of 400 nm. The triterpenoid was added to the liposome suspensions at molar ratio phospholipid:triterpenoid of 100:2.5. The liposome permeability was then determined based on the release of a hydrophilic fluorescent agent, sulforhodamine B, incorporated in the aqueous core of the vesicles. The release kinetics of sulforhodamine B were studied in tris-HCl buffer containing NaCl (pH 7.4) during 48 hours at 37˚C. The results demonstrated that the effect of studied triterpenoids on the lipid membrane permeability depends greatly on its cholesterol content.

The findings are of great importance for the understanding of triterpenoid pharmacodynamics. Moreover they are useful in the development of liposome formulations loading both hydrophobic and hydrophilic bioactive agents.

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SYNTHESIS OF CAMPHANE-BASED COMPOUNDS AS POTENTIAL ANTICANCER AGENTS

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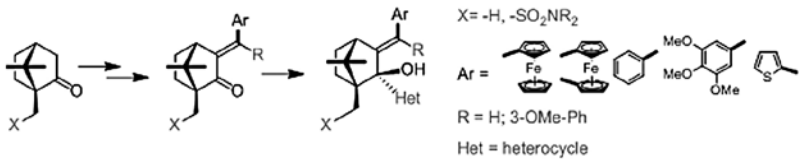
Camphor is a terpenoid found in the wood of the camphor laurel (*Cinnamomum camphora*) and other related trees of laurel family. It is a white crystalline substance and has been used for many centuries as a culinary spice, a component of incense, insect repellent and a flea-killing [1]. Camphor and its derivatives possessing bicyclic skeleton have shown promising biological activities as antioxidant, anti-inflammatory, anti-infective agents or anticancer [2].

The treatment of cancer diseases remains a significant problem due to the observed multidrug resistance. Therefore, the preparation of new and more effective agents with potential anticancer activity is object of considerable synthetic efforts.

Herein we present a small library of (+)-camphor-derived compounds realizing structural diversity through variation of substituents (i.e. sulfonamide groups, ferrocenylmethylidene and arylmethylidene moieties and heterocyclic substituents). The obtained derivatives were tested against seven human cancer cells lines and two normal human cell lines in order to determine their activity against malignant cells. Some of them exhibit IC_{50} values below 10 μM in at least one of the cancer cell lines [3].



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PP 109

DIFFERENT HPLC ASSAYS FOR DETERMINATION OF CANNABINOIDS' CONTENT IN CANNABIS FLOS

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The use of cannabis and its legalization for medical purposes has become a worldwide trend. In many countries there have been initiatives to amend the existing laws in order to make drugs based on natural ingredients of cannabis, as well as other related products, synthetically produced, available to patients [1]. Therefore, due to the increased global necessity of research on the cannabis, many different official methods for determination of cannabinoids content are published.

The aim of our work was to run and evaluate five different HPLC methods for quantification of eight cannabinoids (Cannabidiolic acid, Cannabidiol, Cannabigerol, Cannabinol, Δ9-tetrahydrocannabinol, Δ8-tetrahydrocannabinol, Δ9-tetra-hydrocannabinolic acid A and Cannabichromene). These five assays were according to: Ph. EUR. Liquid Chromatography (UPLC-UV) (2.2.29) - draft version; UNODC Recommended methods for the identification and analysis of cannabis and cannabis products 5.4.8 HPLC; Cannabisblüten Gehaltsbestimmung - german pharmacopoeia; Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material – published in Journal of Chromatography B and HPLC method for separation of eight cannabinoids in complex cannabis samples - unpublished data. As an outcome of this evaluation, we have developed a new HPLC method for determination of listed cannabinoids in cannabis plant material (flower) and preparations.

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PP 110

TECHNICAL MATURITY ASSESMENT OF WILD GROWING POPULATIONS OF *ARCTOSTAPHYLOS UVA-URSI* (L.) SPRENG FROM KORAB MOUNTAIN

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Arctostaphylos uva-ursi (L.) Spreng. from Ericaceae, also known as bearberry or bear's ear, is a small procumbent woody groundcover shrub, widely distributed on a global level. Nowadays, it is widely used as urinary tract antiseptic and diuretic, due to the presence of arbutin and hydroquinone [1]. Commercial forms that are used consist of whole or cut, dried leaf of *Arctostaphylos uva-ursi* that contains not less than 7.00% of anhydrous arbutin [2]. It is a common practic to collect the leaves during the flowering season of the plant (June-July). In Republic of Macedonia, there are no published data for the technical maturity of the bearberry natural populations, varying on the month of harvesting.

Therefore, the aim of the present study was to determine the content and to asses the arbutin variability regarding the month of collection of the bearberry leaves from Korab Mountain.

The asssay for determination of the content of arbutin was carried out according to European pharmacopoeia [2] on dried leaves, collected monthly (from April to November) from wild populations of *A. uva-ursi* during the two consequent years (2015-16).

Although, there were significant seasonal variations in the arbutin content (7.84 - 10.46% in 2015) and (3.83 - 6.44% in 2016), the highest percentage value of arbutin was determined in September, both in 2015 and 2016.

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ESSENTIAL OIL COMPOSITION AND AROMA COMPOUNDS OF CULTIVATED *HELICHRYSUM ITALICUM*, ASTERACEAE FROM REPABLIC OF MACEDONIA

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The genus *Helichrysum* (fam. Asteraceae) includes approximately 600 different species which are widespread all over the world. Among them, almost 25 species are native to the Mediterranean area [1]. Until now, the most common and investigated species is *Helichrysum italicum,* a small aromatic shrub with yellow flowers growing on dry cliffs and sandy oils, widespread along the coast and islands of the Adriatic Sea [2]. The plant has been widely used in folk medicine because of its approved anti-inflammatory, antioxidant, antiallergic, antibacterial, antifungal and anti-viral activity. This wide range of biological activity is probably due to the presence of various metabolite types [3]. Regarding literature data, chemical composition of *H. italicum* essential oil has been studied, but still there is insufficient data about its aroma components, thus this study was designed to examine the essential oil composition as well as aroma compounds of aerial parts of *H. italicum* cultivated in Republic of Macedonia.

GC/MS analyses of isolated essential oil revealed presence of 24 compounds representing 91.94% of the total oil. Two dominant fractions were recognized, mono- and sesquiterpenes. Among monoterpenes, limonene (3.58%), neryl acetate (6.63%) and α-pinene (26.93%) were predominant, while α-copaene (2.77%), *trans*-bergamotene (3.03%), italicene (4.20%), α-selinene (4.44%), *trans*-caryophyllene (5.32%), β-selinene (6.95%) and □-curcumene (20.45) were the major components among sesquiterpenes.

HS/GC/MS analyses of volatile aroma compounds, directly from dried *H. italicum* herb resulted in identification of 23 components belonging to the mono- and sesquterpene fraction, as well. Predominant of all components was α-pinene (87.80%).

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PP 112

PROXIMATE COMPOSITION ANALYSIS AND NUTRITIONAL VALUE OF MACEDONIAN PINE NUTS

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Pine nuts are known as a traditional food in many cultures and have an outstanding nutritional value as they are thought as a good source of lipids and proteins as well as carbohydrates [1]. Nowadays, nuts are part of healthy diet of many populations especially Mediterranean where mortality rates from coronary heart disease and cancers are low [2]. Regarding available literature, there is a lack of published data related to the composition of Macedonian pine nuts, thus the aim of this study was to investigate nutrient compounds of nuts collected from two Macedonian pine species, *Pinus peuce* and *Pinus nigra*.

Proximate ingredient analysis showed that the lipid content of pine nuts ranged from 32.61% for *Pinus nigra* to 38.56% for *Pinus peuce*. The percentage relative to the total fatty acids content varied from 61.18% to 68.88% respectively, where the most abundant were unsaturated w-6 and w-9 fatty acids like linoleic acid (54.81% in nuts of *Pinus nigra* and 61.95% in nuts of *Pinus peuce*) and oleic acid (22.80% for nuts of *Pinus nigra* and 24.98% for *Pinus peuce*). Carbohydrate content ranged from 8.28% in nuts of *Pinus nigra* to 11.21% in *Pinus peuce* while the content of protein fraction was from 19.77% to 27.91% for nuts collected from the two species, respectively.

Obtained results for the carbohydrate, protein as well as lipid content and composition revealed that pine nuts collected from Macedonian species can be considered as a good source of nutrients and be used in nutritional purposes.

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PP 113

AN ETHNOBOTANICAL STUDY OF MEDICINAL PLANTS IN ILAM PROVINCE, IRAN

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This paper reports an ethnobotanical study that focused on the traditional medicinal plants used by local communities to treat human and livestock ailments. A cross-sectional study was undertaken from March 2014 to December 2016 in Ilam Province, Iran. The aim of the study is to document medicinal plants used by local people of the study area and the threats currently affecting medicinal plants. A total of 117 medicinal plant species, distributed in 93 genera and 34 families, were collected together with their medicinal uses. Of the 117 species of medicinal plants collected from the study area, eighty six (68%) were obtained from the wild whereas thirty three (26%) were from homegardens. The most commonly medicinal plants represented families were Lamiaceae with 16 species, Asteraceae (Compositae) with 14 species, Rosaceae with 13 species and Fabaceae with 12 species. The number of reported medicinal plants and their uses by the local people of the Ilam province indicate the depth of the local indigenous knowledge on medicinal plants and their application. The documented medicinal plants can serve as a basis for future investigation of modern drug. The most observed cases of these plants were used for treatment of the digestive, respiratory, urinary tract and reduce pain, reduce sugar and fat. Due to the abundance and widespread use of medicinal plants, development of employment plans based on medicinal plants cultivation and development consistent with local ecological conditions, can be lead to maximum productivity and a proper strategy for conservation of these plant gene pool.

Acknowledgment: I would like to thank Vice-chancellor for Research and Technology of Lorstan University (Dr. Mohammad Feizian) for providing the equipments of this research.

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CHOLINESTERASE INHIBITORY AND LC-MS ANALYSIS OF THE BULBS OF EIGHT *FRITILLARIA* L. SPECIES FROM TURKEY

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Medicinal plants represent an important source of bioactive compounds that could be used for new drugs development. Most of the bulbous plants are known for their medicinal purposes in addition to their ornamental value. Turkey is one of the home country of many beautiful bulbous plants. In continuation of our extensive studies on finding new natural inhibitors from Turkish medicinal plants since the year of 2000, we have now aimed to screen the extracts of AChE and BChE inhibitory activity of eight *Fritillaria* species (*F. alburyana*, *F. armena*, *F. aurea*, *F. caucasica*, *F. crassifolia* ssp. *crassifolia*, *F. crassifolia* ssp. *kurdica*, *F. minima*, *F. persica*) (Liliaceae) of Turkish origin that were not well studied with respect to their neuroprotective potentiality. It should be also noted that oxidative stress plays a remarkable role in neurodegeneration, antioxidant function of anti-AD drug candidates is desired. In this regard, in order to determine preliminary antioxidant activity of the extracts, their DPPH radical scavenging effect was also measured using ELISA microtiter assay. Besides, LC-MS analysis was performed on the extracts to determine presence of steroidal alkaloids in *Fritillaria* species.

Our results indicated that the bulb extracts studied exerted high to moderate BChE inhibition between 71.33 ± 0.36 and 87.50 ± 2.08 (galanthamine 72.76 ± 0.82) at 200 mg/mL with no AChE inhibition. All of the extracts were found to possess low DPPH scavencing activity below 20%. These findings support the use of *Fritillaria* species in the treatment of neurodegenerative disorders such as Alzheimer Disease.

Acknowledgements: This study was financially supported under the project code KAMAG-110G007 by the Turkish Scientific and Technological Research Council (TUBITAK).

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COMPOSITION FLUCTUATIONS OF THE PHENOLICS IN THE *CISTUS INCANUS* L. HERBAL MATERIAL FROM THE POLISH PHARMACEUTICAL AND ALIMENTARY MARKET

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Introduction: The natural habitat of *Cistus incanus* L. from the Cistaceae family is the Mediterranean Basin and West Asia. Out of numerous herbal species and the interspecies hybrids, *Cistus incanus* L. has recently gained a lot of popularity in Poland. Due to an easy hybridization of *Cistus*, the species of this plant present in popularly available formulas are often not well-defined, so it is impossible to exactly determine their contents in commercial formulations.

Aim: Comparison of the phenolic fraction in the *Cistus incanus* L. samples originating from various manufacturers and available on the pharmaceutical and alimentary market.

Materials and Methods: The investigated material whose producers declared the presence of *Cistus incanus* L. originated from the pharmacies, groceries and the online herbal stores. Each sample was macerated and subjected to an exhaustive extraction in the Soxhlet apparatus. The herb extracts prepared in that way were subjected to a multistage extraction allowing isolation of individual fractions of the phenolic compounds including flavonoid aglycones, free phenolic acids and flavonoid glycosides. Each fraction was then analyzed by means of the thin-layer chromatography (TLC).

Results and conclusions: As shown in this study, the commercially available samples significantly differed in composition of the phenolic fraction. They demonstrated different polyphenolic profiles, which can cause different pharmacological effects [1, 2]. The conclusion is that the composition of the *Cistus incanus* L. containing herbal preparations is significantly diversified. Thus, proper standardization of chemical composition and the quality control of raw materials and herbal products should be implemented obligatorily.

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ESTIMATION OF THE EMBRYOTOXICITY OF ROOT EXTRACTS FROM SOME PLANTS OF LEGUMINOSAE

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The aim of this study was evaluation of the embryotoxicity of the extracts of the roots of some Leguminosae family plants (*Goebelia alopecuroides* Bge. and *Psoralea drupacea* Bge.)

The component composition of the extracts was examined by chromatography-mass spectrometry. The main component of the root extract from *G. alopecuroides* Bge. was a matrine, in addition, a significant amount of monomethylatinozitol and small amounts of sophocarpine, sophoramine, dehydromatrine, caulophyllin alkaloids were detected. 13 components have been identified in a root extract from *P.drupacea* Bge., mainly - inositol and isopsorbene.

To study embryotoxicity, preparations in a dose ranging from 10 to 0.04 mg dissolved in a phosphate buffered saline (PBS) solution with a volume of 0.2 ml were injected into chorionaleantoic embryo cavity. Control group was administered a PBS solution without the addition of drugs. The results were taken into account by the number of dead chick embryos (CE) during the entire period before the hatching of the chickens [1].

It is revealed that the root extract of *G. alopecuroides* Bge. in a dose of 0.04 to 2.5 mg/CE do not exert a pronounced toxic effect. Dose at 5 mg/CE causes death of embryos exceeding the control by 20%, administration of the maximum dose (10 mg/ml) leads to a 100% death of CE. Root extract of *P. drupacea* Bge. has insignificant toxic effect at a dose of 10 mg/CE, a dose at 5 mg/CE and lower concentrations does not show toxic properties relatively to CE.

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EXPLOITATION OF MASTIC GUM PROCESSING BY-PRODUCT FOR THE RECOVERY OF BIOACTIVE COMPOUNDS

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Mastic gum, the resin of *Pistacia lentiscus* L., has been used in traditional Greek medicine for various gastrointestinal disorders for over 2,500 years. The resin is a valuable market product itself but it is also used to produce another important product: mastic essential oil, which is widely used in the fields of pharmaceuticals, food and cosmetics. After distillation of the essential oil, a by-product remains, which contains a lot of bioactive substances, but till now it has only been used in some varnishes. In order to reveal the health promoting potential of the by-product, we performed chemical profiling using GC-MS and ^{1}H-NMR spectra and compared the profiles with the ones of the native mastic gum. The profiles were very similar, with only small quantitative differences. In both cases, the major constituents were moronic acid, masticadienonic and isomasticadienonic acid, oleanonic acid and oleanonic aldehyde. These compounds are known to possess valuable biological properties: moronic acid is active against HIV and Herpes virus [1], masticadienonic acid and oleanonic acid have demonstrated significant activity against *Helicobacter pylori* [2]. These results demonstrate that the by-product of mastica essential oil production has a great potential as a source of important biologically active compounds.

Acknowledgements: Financial and technical support from the EXANDAS project *(*MSCA-RISE-2015, Grant Agreement No 691247*).*

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PP 118

ELECTROPHORETICALLY MEDIATED MICROANALYSIS OF DIVICINE AND ISOURAMIL DEGRADATION AND QUANTIFICATION OF THEIR GLYCOSIDES USING CAPILLARY ELECTROPHORESIS – HIGH RESOLUTION MASS SPECTROMETRY

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Divicine and isouramil are pyrimidine derivatives present as glucosides in seeds of several plant species belonging to Fabaceae family. If ingested by predisposed individuals with low-activity variant of erythrocytic glucose 6-phosphate dehydrogenase, they can cause acute hemolytic condition known as favism. Due to fast autoxidation, both compounds are unstable upon hydrolysis of glycosides. Putative products of their degradation were recently investigated using in vitro enzymatic hydrolysis of parent glycosides: vicine and convicine, followed by HPLC-UV and HPLC-MS analyses [1]. However, no conclusive data on their structure were shown.

Capillary electrophoresis method presented here allows for nearly real-time monitoring of divicine and isouramil degradation process. Both chemical formulae and structural features of degradation products can be evaluated if the method is combined with high resolution mass spectrometry. With a few modifications, the method can also be used for rapid quantification of pyrimidine glycosides in plant material, with limits of detection and quantification comparable to these offered by HPLC-UV method.

Acknowledgements: This work was supported by grant from the National Science Centre (2014/15/B/NZ9/04302).

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ARUM: CHEMISTRY, BIOACTIVITIES, AND APPLICATION AS A CURE FOR HEMORRHOIDS

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Hemorrhoids become pathological when swollen or inflamed. They can be very painful and occasionally cause serious anemia as they often break and are hardly healed. Orthodox medicine usually treats hemorrhoids through surgery combined with medication containing the flavonoid diosmin. Our ethnobotanical and ethnopharmacological research in Bulgaria (Balkan Peninsula) revealed that *Arum maculatum* and possibly its relatives are effective remedies for the management of hemorrhoids. The aim of this study is to review the traditional use, the established pharmacological activities of *Arum* species and the biologically active compounds. We have traced the traditional uses and the medicinal applications considering geographical and cultural aspects. For convenience, we consider the Balkans as a milestone, as they have been recognized as a phytogeographical and cultural crossroad between Europe and Asia. *Arum* species possess pleiotropic pharmacological effects, such as analgesic, antimicrobial and antineoplastic properties.

The potentially active compounds identified in various parts of *Arum* species include: piperazine and pyrrolidine alkaloids, volatile and non-volatile terpenoids, phenolic compounds such as numerous flavonoids, as well as carbohydrate-binding proteins. The results of this analysis could provide a hypothesis which will allow designing further adequate experimental research with possible clinical translation.

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OREGONIN ALTERS THE EXPRESSION OF DNA METHYLTRASFERASES MRNA TRANSCRIPTS IN MOUSE EMBRYONIC FIBROBLASTS

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Oregonin is a polyphenol, obtained from the plants of genus *Alnus*, which possesses antioxidative, anti-inflammatory and anticancer activity [1, 2]. Experimental evidences of recent years support the idea that polyphenols target various epigenetic factors, such as histone acetyltransferasesases, DNA metyltrnasferases**,** and miRNAs [3].

Our goal was to evaluate whether the oregonin, obtained from *Alnus incana* (L.), can alter the expression of the DNA methyltransferases: Dnmt1; mtDnmt1; Dnmt3a and Dnmt3b mRNA transcripts in mouse embryonic fibroblast cells (MEF).

Initially the oregonin cytotoxicity was tested on MEF and measured by MTT assay. Subsequently, the MEF were incubated in 6-well plates at a density of 2 x 10^5 cells/well with different concentrations of oregonin - 50 μM and 100 μM for 24 hours. Samples were collected and subjected to quantitative PCR for determination of DNA methyltransferases mRNA transcripts.

The treatment of MEF with oregonin at concentrations of 50μM and 100 μM for 24 hours did not induce significant decrease in cell viability. Oregonin up-regulated the mRNA transcripts level of all Dnmt's at 24 hour after treatment. The effect was more pronounced at concentration of 100 μM.

The oregonin extracted from *Alnus incana* has a good potential for use as an epigenetic agent for the modulation of DNA methylation processes.

Acknowledgements: The research was supported by grant DKOST01/10, NSF-MES, Bulgaria, COST Action FA1403 and bilateral project between BAS and LAS.

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EFFECT OF PLANTS OLIGOMERIC PROANTHOCYANIDINS ON LIPIDS HYDROLYSIS IN MODEL OF DUODENAL PHASE

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In the Western Europe, cholesterol gallstones diagnosis was registered for 10 - 15% adult population. It is associated with different factors, including metabolic syndrome, diabetes, obesity, and high-carbohydrates. The plant originated substances, especially of plant polyphenolic pool, reveal prominent effects as the remedy for different metabolic diseases [1].

The present paper aims to evaluate the effects of the oligomeric proanthocyanidins (OPCs) extracts (OPCEs) on normalisation of the triacylglycerols digestions under pancreatic lipase in the bile absence - gallstones model.

The objects of study were OPCEs obtained from the bark of domestic deciduous trees using one step extraction with water or with 40% ethanol. The action of the OPCEs on lipid hydrolysis was estimated in a model of the duodenal phase.

The most promising results were obtained by standard set modelling of duodenal digestions: pancreatic lipase, 2.5 % milk, with bile or without bile, incubation for 1 hour followed by titration with 0.1 N NaOH. Activation of lipids breakdown with bile was more effective with water birch and goat willow OPCs extracts, than under the influence of 40% ethanol OPCs extracts. In gallstones model without bile in set, their reactivity was lower, but progressive hydrolysis of the triacylglycerols was detected in presence of birch ethanol extract and the black and grey alders water extracts.

The obtained data have shown that black alder and grey alder bark extracts could be used in perspective for the normalisation of lipids digestion in duodenal phase with bile malfunction.

Acknowledgements: COST Action FA1403, bilateral project BAS - LAS.

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GYPSOPHILA TRICHOTOMA EXTRACT AMELIORATES ETHANOL INDUCED BRAIN INJURY IN RATS

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The current study investigates the effect of defatted ethanol extract, isolated from *Gypsophila trichotoma* Wend. on behavioral and some biochemical parameters measured in the brain of rats with multiple ethanol treatment.

Methods and results: Thirty six male Wistar rats were divided into 6 groups (n = 6): control; animals treated with ethanol (45%, oral-gavage, 14 days); animals treated with *G. trichotoma* extract (100 mg/kg oral-gavage, 14 days); animals treated with silymarin (100 mg/kg p.o. 14 days); the animals in group 5 and 6 have been treated with ethanol in combination with *G. trichotoma* extract and silymarin, respectively. Twenty four hours after the last treatment the animals in the ethanol group showed anxiety, piloerection, unusual body posture, associated with withdrawal. Such symptoms have not been observed among the animals in the combination groups. In the ethanol group this behavior was accompanied with biochemical changes in the brain, discerned by increased activity of the enzymes nNOS and GS and by an increased amount of MDA and decreased levels of GSH. It is interesting to be noted that *G: trichotoma* extract protected the brain against ethanol injury, evidenced by reversed activity nNOS and GS as well as GSH and MDA quantity to the control levels. The effects were commensurable with the effect of silymarin, used as a positive control.

Conclusion: On the basis of the results obtained under the conditions of this study we could conclude that the defatted extract, isolated from *Gypsophila trichotoma* ameliorates ethanol induced brain injury.

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PYRROLIZIDINE ALKALOIDS *N*-OXIDES FROM *PULMONARIA OFFICINALIS*

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Pyrrolizidine alkaloids (PAs) are toxic to humans and livestock, their hepatotoxic, genotoxic, carcinogenic and teratogenic properties are well documented [1]. They are estimated to occur in approximately 3% of plant species, mainly belonging to Boraginaceae, Asteraceae and Fabaceae families. Poisoning outbreaks related to alkaloids typically involve ingestion of contaminated grains and other food products, such as honey. Recently, consumption of herbal tea and herbal dietary supplements was the leading cause of PAs poisoning. This is frequently because of insufficient quality control procedures employed by the manufactures, but also due to false sense of security coming from working with supposedly "safe", alkaloid-free plant.

We investigated contents of PAs in *Pulmonaria officinalis*, a member of Boraginaceae family allegedly containing only minute amounts of alkaloids in its aerial parts, which form commercially available *Pulmonariae herba*. Although sample originated from botanical garden indeed contained low concentration of intermedine and lycopsamine N-oxides, samples obtained from other sources had as much as 27 µg/g d.w. of alkaloids. Variability of alkaloid concentrations observed in samples from the nature is probably due to the fact that *P. officinalis* easily forms hybrids with other *Pulmonaria species* [2], which often contain high levels of alkaloids, like *P. saccharata*, *P. mollis*. Morphologically, these hybrids may well resemble *P. officinalis*, however their phytochemical composition seems to be much different. Therefore, batches of commercial *Pulmonariae herba* used for pharmaceutical and dietary purposes should be evaluated for their pyrrolizidine alkaloid contents.

Acknowledgements: This work was supported by grant from the National Science Centre (2013/11/D/NZ9/02771).

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PP 124

ETHYL ACETATE EXTRACT OF *LAVANDULA STOECHAS* INCREASED GLUCOSE UPTAKE, PHOSPHORYLATION OF AKT AND EXPRESSION OF LIPOPROTIEN LIPASE IN INSULIN RESISTANT C2C12 MUSCLE CELLS

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The aim of this work is to identify remedial effects of *Lavandula stoechas* (LS) against insulin resistance. Ethyl acetate extract (EE) of LS was investigated in free fatty acid (FFA) treated insulin resistant C2C12 myotubes for its effects on glucose uptake and activation of AKT (by pAKT/AKT ratio) molecule which plays a central role in insulin signaling through Serine (473) phosphorylation. In addition, the protein level of lipoprotein lipase (LPL) enzyme was also evaluated. C2C12 cells were made insulin resistant by FFA and effects of EE on p-AKT (Ser473)/AKT ratio and LPL level were determined by SDS-PAGE/Western Blot. The effect of EE on glucose uptake in insulin resistant cells was determined by the 2-deoxyglucose assay. EE at 25 and 50µg/mL significantly increased the glucose uptake 120 and 182% compared to insulin resistant control cells. Metformin at 2mM increased this parameter up to 132%. EE increased pAKTSer473/AKT level 43-37% and LPL expression 50-92% for 25 and 50µg/mL, in insulin resistant myotubes, respectively ($p<0.05$). EE of LS improved impaired insulin sensitivity through both enhancing glucose uptake and activation of AKT molecule through Ser473 phosphorylation. In addition, it also considerably increased LPL level which has very important function in lipid metabolism. Overall, results demonstrated that LS contain phytochemicals which can be effective for the prevention and also treatment of insulin resistance and associated conditions.

Our research group is on the way for the identification of these "bioactive" molecules with bioassay guided fractionation studies.

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VOLATILE COMPONENTS FROM *INULA ASCHERSONIANA* JANKA VAR. ASCHERSONIANA

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Inula aschersoniana Janka, family Asteraceae, is a subendemic species in the Balkan Peninsula and Anatolia with areas of distribution in Bulgaria, Greece, Macedonia, and Turkey [1]. There are three varieties of *I. aschersoniana* in Bulgaria: madarense, macedonica and aschersoniana [2, 3]. To the best of our knowledge, the chemical composition of volatile compounds from *I. aschersoniana* has not been investigated so far. Previous studies on this species reported the occurrence of sesquiterpene lactones and pseudoguaiane-type sesquiterpenoids [4].

Here we describe the volatile components in flowers of *I. ashersoniana* Janka var. *aschersoniana* The volatile fraction was obtained by micro steam distillation-extraction. Identification and quantification were performed by GC/MS. Fifty components were registered as chromatographic peaks in concentration more than 0.3%.

The studied sample was rich in fatty acids reaching 28.3%. The amount of dodecanoic (lauric) and hexadecanoic (palmitic) acids exceeded 8%. Characteristic for this fraction was the significant amount of aliphatic (C_{23} – C_{29}) hydrocarbons in concentration more than 15% in total. It should be noted that monoterpene hydrocarbons were not detected. Linalool was the main oxygenated monoterpenoid. Further, sesquiterpene hydrocarbons predominated oxygenated sesquiterpenoids. It was worth to mention the occurrence of β-damascenone, megastigmatrienone-2 and megastigmatrienone-4, compounds which have been detected rarely in Asteraceae family.

Acknowledgements: This work was supported by the National Science Fund, Ministry of Education and Science, Bulgaria, Project DN 09/11.

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PP 126

CHEMICAL PROFILE AND ANTI-LIPASE PROPERTIES OF *JURINEA TZAR-FERDINANDII* DAVIDOV

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Jurinea tzar-ferdinandii Davidov (genus *Jurinea*, Asteraceae family) is a Balkan endemic plant with limited distribution in Bulgaria and Romania. The literature survey did not show any reports concerning its chemical constituents and biological activity.

The aim of this study was to investigate chemical constituents of this species and to evaluate their anti-lipase properties. The aerial parts of *J. tzar-ferdinandii* were extracted with chloroform. CC and PTLC of the resulting chloroform extract led to isolation and identification of 26 compounds, belonging to 3 main groups of secondary metabolites: triterpenoids (α- and β- amyrin, lupeol, taraxasterol and ψ-taraxasterol, and the corresponding 3-O-acetates and 3-O-palmitates), sesquiterpene lactones (onopordopicrin and 5 biogenetically related germacranolides, eudesmanolides and an elemanolide) and flavonoids (pectolinarigenin, eupatorin and jaceosidin). Their structures were elucidated using spectral methods (NMR, MS, UV and IR).

Inhibitory activities of the fractions containing triterpene alcohols, triterpenyl acetates, triterpenyl palmitates, sesquiterpene alcohols and flavonoids against a bacterial lipase from *Candida rugosa* (CRL) and a lipase from porcine pancreas (PPL) were assessed in a spectrophotometric assay. The estimated half maximal inhibitory constants (IC_{50}) for the two enzymes were in the range of 70 - 150 μg/mL. The strongest inhibitory activity was found for the fraction of the triterpenyl palmitates, and the estimated IC_{50} values were 80.1 ± 2.4 μg/mL and 85.4 ± 3.5 μg/mL for CRL and PPL, respectively. The results are comparable with those reported in the literature for the tetrahydrolypstatin, an irreversible inhibitor of lipases from *Candida* sp. and human pancreatic lipase [1].

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PP 127

LC/MS AND GC/MS ANALYSIS OF MAIN COMPOUNDS OF FOUR *HYPERICUM* SPECIES INDIGENOUS IN THE PELOPONNESE (GREECE)

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Hypericum perforatum L. (Hypericaceae) is a species with numerous traditional uses especially treating mild depression [1]. In Greece other 40 taxa of *Hypericum* genus are indigenous [2]; however their phytochemical composition has not been investigated sufficiently.

In this study we focused on the chemodiversity of four species of *Hypericum* growing wild in Peloponesse (Greece), *Hypericum perforatum* subsp. *veronense* (Schrank) A. Fröhl**.**, *H. perfoliatum* L., *H. triquetrifolium* Turra, and *H. empetrifolium* Willd. subsp. *empetrifolium*. Aerial parts were collected during the flowering period and dried at room temperature. The phytochemical composition of the dry methanolic extracts was studied using Ultra Performance Liquid Chromatography (UPLC) coupled to Q-TOF Mass Spectrometer; compounds were quantified by HPLC - DAD on a C-18 column [3]. Essential oils were analyzed with GC/MS (with a HP-5MS capillary column) with a new method.

Several organic acids, flavonoids, naphthodianthrones and phloroglucinols were identified by LC-MS. The majority of them were common among the examined species; however some differences were detected especially as regards the phloroglucinol derivatives. *H. perforatum* had the highest % concentration of total flavonoids, naphthodianthrones and phloroglucinol derivatives. *H. empetrifollium* had the highest content of phenolic acids. In every essential oil, at least 45 volatiles were detected and quantified; *H. perfoliatum* essential oil had the highest complexity. More than 30 volatiles were common among species, with most abundant the caryophyllene E.

The results of this study shed a light on *Hypericum* chemodiversity and may even reveal other taxa which could be valuable as sources of the medicinal preparations.

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ISOMERIC RATIO OF *CIS-TRANS* CITRAL IN ESSENTIAL OIL OF *CYMBOPOGON CITRATUS* AS A FUNCTION OF SOIL COMPOSITION

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This study evaluated the influence of soil composition on the concentration of *cis-trans* citral isomers present in the essential oil of Lemongrass, *Cymbopogon citratus*.

The essential oil obtained by hydrodistillation of fresh leaves collected in 47 localities of Ecuador was analyzed by GC-MS chromatography determining different isomeric ratios of *trans*-citral (geranial) to *cis*-citral (neral). Through the soil analysis and the application of a generalized linear model it was established that for the *trans* isomeric form the coefficients of the factors: soil moisture, Nitrogen, Potassium, Phosphorus and pH are inverses to the corresponding of the *cis* isomeric form.

In conclusion, it is observed that the geranial isomer is found in a higher concentration in the essential oil of Lemongrass from little dry, acid soils rich in N, K and P, while the neral isomer is the preferred form in crops where plants are expose to greater stress due to lack of nutrients, lower humidity and greater soil alkalinity.

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PP 129

ANTI-OXIDATIVE, ANTI-INFLAMMATORY AND ANTI-SKIN AGING EFFECTS OF THE PHENOLIC COMPOUNDS FROM STEMS OF *CARPINUS TSCHONOSKII*

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The Carpinus species, commonly known as Hornbeams, belongs to the Betulaceae and consists of over 40 in the world [1]. *Carpinus tschonoskii* (CT) is distributed through in the southern regions of South Korea including Jeju Island.

A phytochemical study of CT identified six known compounds, including three ellagitannins, tellimagrandin I (**1**), pedunculagin (**2**), and gemin D (**3**), one gallotannin 2,3- digalloyl-O-glucopyranoside (**4**), and two flavonoids, afzelin (**5**) and quercetin (**6**). To determine the anti-oxidant activity, anti-inflammatory and anti-skin aging activities, the extract of CT and the isolated compounds (**1**-**6**) were evaluated by the measurement of DPPH, NBT superoxide scavenging activities, inhibition of NO production in LPS stimulated RAW264.7 and cytokines (IL-1β and TNF-α) in THP-1 cells about PCOLCE, Elastin, and MMP-2 in the CCD986sk human fibroblast cell.

These results showed that extract and the phenolic compounds (**1-6**) isolated from stems of CT have potent anti-oxidative, anti-inflammatory, and anti-skin aging activities. And it might be developed as active sources for the improvement of the sensitive skin and acne in the cosmetic industry.

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BIOINFORMATICS AND BIOCHEMICAL ANALYSIS OF WOUND-INDUCED SESQUITERPENE BIOSYNTHESIS IN KHAT (*CATHA EDULIS*)

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Khat (*Catha edulis*) leaves are commonly chewed for their mild psycho-stimulating properties, in parts of the Middle East and Eastern Africa. The psychoactive compounds in khat are thought to be the cathamine alkaloids such as cathine and cathinone but leaves also accumulate mono- and sesquiterpenoids and terpenoid-derived catheduline alkaloids.

Sesquiterpenes are accumulated in many plant species in response to mechanical injury or pathogen attacks. Metabolic profiling of volatile terpenoids present in khat leaves indicated that the main sesquiterpenes accumulated in intact leaves were (*E*)-caryophyllene, alpha-copaene, alpha-cubebene, alpha-humulene, alpha-muurolene and delta-cadinene. Wounding the leaves resulted in increased total sesquiterpene content, and increases in the levels of (*E*)-caryophyllene and alpha-copaene. Crude protein extracts incubated with farnesyl diphosphate as a substrate indicated an increased biosynthetic potential for (*E*)-caryophyllene, elemol, alpha-eudesmol beta-bisabolene and alpha-(*Z*)-bergamotene in extracts from wounded leaves as compared to non-wounded leaves. To get a broader view of genes that are up or down regulated in wounded leaves, we performed deep RNA sequencing of samples from wounded and non-wounded khat leaves. The analysis resulted in the identification of several novel putative terpene synthase genes. Functional expression of three of these genes indicated that they encoded proteins that catalyze the formation of various sesquiterpenes from farnesyl diphosphate *in vitro*.

Our results will help us to better understand the wound responses in khat leaves and the involvement of terpene metabolism in this process.

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IDENTIFICATION AND HYPOGLYCEMIC ACTIVITY OF FLAVONOIDS FROM *LOTUS* PLUMULE

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Lotus plumule is the young leaves and radicle in mature seeds of plant lotus (*Nelumbo nucifera* Gaertn), which belongs to the *Nymphaeaceae* family. As an important Chinese herbal medicine, lotus plumule has broad pharmacological applications and is of high research value. In this paper, we extracted and purified the lotus plumule, and determined its hypoglycemic activity. The microwave-ultrasound-assisted extraction of total flavonoids from lotus plumule and optimized the parameters were investigated. The optimal parameters were determined to be: ethanol concentration 70%, liquid/solid ratio 1 : 40, microwave power 355 W, ultrasonic power 423 W, extraction time 15min, ultrasonic/time interval 1/0. With STZ-induced diabetic mice as models, the hypoglycemic effect of lotus plumule flavonoids on blood glucose was determined. The results demonstrated that lotus plumule flavonoids could significantly reduce the level of glucose and lipid in blood, improve glucose tolerance, and increase insulin level. The optimal parameters were with sample concentration 2.085 mg/mL, sample quantily 10 mL, sample flow rate 1mL/min, water consumption 40mL, eluent was 50% (V/V) ethanol solution, eluent quantily 50mL, and eluent flow rate 1 mL/min. Flavonoids were extracted from lotus plumule with the solvents of petroleum ether, ethyl acetate and n-butyl alcohol. The results suggested the ethyl acetate phase demonstrated the highest inhibition on α-glycosidase enzymes, meaning the presence of highest hypoglycemic activity at the site. Chemical inspection and HPLC were employed to analyze and identify the structure of ethyl acetate phase in lotus plumule flavonoids. The results indicated that dihydrogen flavonoids, 5-hydroxy flavones, 2-hydroxy ketone of chalcone and aurones were present in the ethyl acetate phase. Rutin was also identified in the phase with a conceration of 77.74 μg/mg. Given the above, this study has been shown the theoretical basis and technological support for developing deep-processing products of total flavonoids from lotus plumule.

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BIOASSAY-GUIDED ISOLATION OF ANTHELMINTIC COMPOUNDS FROM *WARBURGIA UGANDENSIS*

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Parasitic helminths continue to pose problems in human and veterinary medicine. Due to increasing drug resistance, the need for new anthelmintic compounds is clear and urgent. Some traditional medicinal plants have been employed in the treatment of intestinal parasites for a long time, and they are simple and safe to use. However, the active compounds in some of these medicinal plants are still unknown. One such plant is *Warburgia ugandensis*. In this study, we aimed to identify the active compounds against helminths in *Warburgia ugandensis* using bioassay-guided purification. A *C. elegans in vitro* test using a WMicrotracker instrument (PhylumTech) was used to track the active compounds during purification. The acetone extract of the plant material was separated by a step-gradient silica gel chromatography, followed by high performance liquid chromatography (HPLC) purification using a C18 column. The structures of active compounds were elucidated by a combination of high-resolution mass spectrometry and NMR. Besides, the synergistic effect of active compounds in the plant was tested using a checkerboard assay. The bioassay-guided fractionation led to the isolation of three active compounds. The anthelmintic assay showed that their IC_{50} values were 28.2 ± 8.65 µM, 13.1 ± 5.32 µM and 58.2 ± 14.31 µM.

The checkerboard assay for two of these compounds showed a fractional inhibitory concentration index of 0.375, which suggests that they have synergistic effects against nematodes.

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PHYTOCHEMICAL PROFILE AND BIOLOGICAL ACTIVITY OF *VERBASCUM OVALIFOLIUM* DONN EX SIMS AERIAL PARTS

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Verbascum ovalifolium Donn ex Sims is a biennial plant of the *Scrophulariaceae* family, predominantly distributed in Bulgaria, Russia, Greece, Turkey and Romania [1, 2]. Beside the identification of 9 iridoids by TLC in this species [3], no other phytochemical and biological analyses were performed to our knowledge. The objective of this study was to investigate the phytochemical profile and biological activity of the crude methanolic extract from the aerial parts of *V. ovalifolium* and its hexane, ethyl acetate, butanol and aqueous fractions. The metabolite profile was determined by RP-HPLC-DAD-ESI-Q-TOF-MS/MS. The cytotoxicity of the extracts (25-200 µg/mL) was evaluated by MTT assay towards SK-MEL-2 human melanoma cell line. Caffeoyl quinic acids, flavonoids, catalpol-type iridoids and phenylethanoid glycosides were identified in the butanol and ethyl acetate fractions.

Application of high-performance counter-current chromatography (HPCCC) led to the isolation of pure luteolin-7-*O*-glucoside, verbascoside, saccatoside and 6-*O*-(3″-*O*-acetyl-2″-*O*-trans-*p*-coumaroyl)-*α*-*L*-rhamnopyranosylcatalpol (premnacorymboside A) from the ethyl acetate fraction. Premnacorymboside A was identified for the first time in *Verbascum* genus. The compounds were chemically characterized by 1D-NMR (^{1}H-NMR, ^{13}C-NMR) and 2D-NMR (COSY, HSQC, HMBC and NOESY) analyses. The viability of SK-MEL-2 melanoma cells treated with 200 µg/mL of each extract was significantly reduced between 82.52 ± 7.51% and 34.48 ± 1.15% compared to

non-treated cells ($p<0.001$). The IC_{50} values for the butanol and hexane fractions were 103.77 ± 28.39 µg/mL and 160.35 ± 6.91 µg/mL, respectively.

These preliminary results highlight the need for further biological investigations in order to identify the compounds responsible for the cytotoxic activity.

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PP 134

ANTIOXIDANT POTENTIAL OF OVAL-LEAVED MULLEIN (*VERBASCUM OVALIFOLIUM* DONN EX SIMS)

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Verbascum L. is the largest genus of *Scrophulariaceae* family. Many biological activities (antioxidant, anticholinesterasic, antimicrobial, anti-inflammatory, hepatoprotective, and immunomodulatory) have been investigated for different *Verbascum* species [1, 2]. The aim of this study was to evaluate the antioxidant potential of the crude methanolic extract of *Verbascum ovalifolium* Donn ex Sims aerial parts and its hexane, ethyl acetate, butanol and aqueous fractions. Total phenolic and flavonoid contents were also determined. The antioxidant potential was evaluated by DPPH, superoxide anion and hydroxyl radicals scavenging, reducing power and ferrous ion chelating assays [3]. Caffeic acid, quercetin and EDTA were used as positive controls. The highest total phenolic (185.82 ± 1.33 mg gallic acid equivalents/g extract) and flavonoid (150.40 ± 0.48 mg catechin equivalents/g extract) contents were found in butanol and ethyl acetate fractions, respectively. Butanol fraction was the most active as free radical scavenger and reducing agent, whereas hexane fraction showed the highest ferrous ion chelating capacity. The IC_{50} values of butanol fraction and caffeic acid were 29.40 ± 0.25 and 3.65 ± 0.02 µg/mL in DPPH assay, 490.49 ± 2.57 and 85.62 ± 0.90 µg/mL in superoxide anion radical scavenging assay, 785.10 ± 10.72 and 205.06 ± 1.34 µg/mL in hydroxyl radical scavenging assay and 15.56 ± 0.47 and 1.63 ± 0.13 µg/mL in reducing power assay. Hexane fraction had the best ability to chelate ferrous ion, with IC_{50} value of 94.39 ± 5.93 µg/mL *vs.* 6.65 ± 0.16 µg/mL for EDTA.

Strong negative correlations between total phenolic content and IC_{50} values of reducing power and free radical scavenging assays were observed. Pearson's correlation coefficients were significant at $p<0.05$ level and varied between –0.6862 and –0.9984.

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ANTIOXIDANT ACTIVITY OF POLYSACCHARIDES EXTRACTED FROM LEAVES OF *PLANTAGO* SPECIES

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Background: *Plantago* genus includes herbaceous plants used worldwide as a remedy for wound healing, inflammations, respiratory and digestive system disorders. The leaves of *Plantago* species have been known as a rich source of bioactive compounds like polysaccharides, flavonoids, phenolic acids and iridoids [1, 2]. **Objective:** The aim of the present study was to investigate the chemical composition and antioxidant activity of polysaccharides isolated from the leaves of *P. major*, *P. media* and *P. lanceolata* from Bulgaria. **Methods:** Polysaccharides were isolated from *Plantago* leaves by water and acid extraction. The monosaccharide composition was evaluated by HPLC analysis. Antioxidant activities were determined by DPPH and FRAP assays. **Results:** The phytochemical data revealed that the water-extractable polysaccharides (WEPs) were composed mainly of galacturonic acid (62.64% - 70.58%) and minor amounts of arabinose and rhamnose, while in total acid-extractable polysaccharides galacturonic acid (36.93% - 41.46%), galactose (22.80% - 46.11%) and rhamnose (16.96% - 35.74%) were detected. *Plantago* isolated polysaccharides showed significant antioxidant activity. Among them *P. media* WEPs exhibited the strongest radical scavenging ability (40.08%) and *P. lanceolata* WEPs showed the greatest ferric reducing power (137.83 μM TE/5 mg Ps). In comparison ethanol extracts of *Plantago* leaves showed DPPH radical scavenging ability between 55.21% - 75.48% and FRAP values between 51.85 - 159.48 μM TE/g dw.

Conclusion: The obtained results revealed that *P. major*, *P. media* and *P. lanceolata* leaves are promising natural sources of biologically active polysaccharides with significant antioxidant potential.

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ANTIOXIDANT PROPERTY OF WATER-SOLUBLE POLYSACCHARIDES FROM *DICTYOPHORA INDUSIATA* USING DIFFERENT EXTRACTION METHODS

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Dictyophora indusiata is a popular traditional medicinal plant that has invigorating activity. Water-soluble polysaccharides (DIPs) are its main active components. In this study, four different methods were used to extract DIPs, which include hot water extraction (DIP-H), ultrasonic-assisted extraction (DIP-U), ultrasonic-microwave-assisted extraction (DIP-UM) and microwave-assisted extraction (DIP-M). Their chemical com-positions and structure characterizations were compared. In vitro antioxidant activities were studied on the basis of DPPH radical, hydroxyl radical, reducing power and metal chelating ability. The results showed that DIP-UM exhibited reducing power and highest scavenging abilities on hydroxyl and DPPH radicals, while DIP-U exhibited the lowest antioxidant activities. Response surface methodology was used to optimize the extraction yield of DIP-UM by implementing the Box–Behnken design. Under the optimized conditions, the DIP-UM yield was 12.66%, which was well in close agreement with the value predicted by the model.

Overall, the ultrasonic-microwave-assisted extraction was an effective and mild method for obtaining antioxidant polysaccharides from *Dictyophora indusiata*.

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DETAILED LC/PDA/ESI-QTOF-MS/MS ANALYSIS AND BIOLOGICAL PROFILING OF ISOFLAVONES OCCURRING IN AERIAL PARTS OF *TRIFOLIUM MEDIUM* L. (FABACEAE)

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In contrary to popular belief, not only soy or red clover are considered as valuable sources of phytoestrogenic isoflavones. In our studies, aerial parts of *Trifolium medium* L. (zigzag clover), being a fodder plant from the family Fabaceae, were selected as a plant material. The efficient extraction technique, assisted by the operation of ultrasounds (UAE), was developed for obtaining 50% (v/v) ethanolic-aqueous extract, that was further subjected to the vacuum drying procedure. This process led finally to obtaining the lyophilisate (TML), that was purified from ballast compounds using solid-phase extraction on a phenyl J.T.Baker microcolumn. Then, TML was qualitatively analysed using LC-PDA/ESI-MS-QTOF technique in negative and positive ion mode to identify in detail isoflavone constituents.

A 6530B accurate-mass-Q-TOFMS apparatus (Agilent Technologies) with a Zorbax SB-C18 narrow-bore column (dp = 3.5µm, 2.1 x 150 mm) and gradient of acetonitrile (1%) + 10 mM ammonium formate (0.2%) (A) and acetonitrile (95%) + 10 mM ammonium formate (0.2%) (B) as a mobile phase were used. Isoflavone aglycones, namely formononetin, biochanin A, genistein, daidzein and their *O*-glycosides were identified and quantified together with small amounts of pseudobaptigenin, pratensein and irilone derivatives. Total content of all isoflavone compounds exceeded 12% of dry weight and it was above 2.5 higher than in red clover lyophilisate, used as the reference herbal preparation. The antioxidant potential of TML was evaluated using FCR method and $ABTS^{\bullet+}$ free radical assay. Mean IC_{50} values obtained in $ABTS^{\bullet+}$ test were 30.24, 2.75 and 0.66 ug/mL, for TML, Trolox and gallic acid solutions, respectively.

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ELECTROCHEMICAL INVESTIGATION OF THE BASIC REDOX BEHAVIOR OF RUTIN

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Rutin is one of the most common found flavonoid, containing quercetin as a flavonol aglycone. Its structure consists of a two aromatic (phenolic) rings connected through one heterocycle containing oxygen [1]. The high number of OH groups, as well as, its aromaticity justifies the use of an electrochemical method in its analyses [2]. Cyclic voltammetry has been applied in order to investigate the redox oxidation process of rutin on a glassy carbon electrode, as working electrode. Additionally, the platinum electrode has been used as a reference electrode and a graphite stick has been employed as counter electrode. Its electrochemical properties have been studied in different pH values by use of a few different buffer solutions in pH range from 2.8 to 8.8. The potential applied on the surface of the working electrode in the range of -0.4 to 0.6 V, was used in scanning the redox process of rutin. The reversible oxidation in physiological pH has occurred on E_{pa} = 0.198 mV and E_{pc} = 0.221 mV, but the results have shown that its oxidation is a pH dependent electron transfer process. Additionally, the adsorption of rutin was noted. The application of these data considering the redox behavior of rutin could be used in prospecting of its mechanism of oxidation and structure-antioxidant activity relationship, as well as developing a method for fast estimation of its antioxidant activity.

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THE IMPACT OF ANTIOXIDANTS ON THE CYTOTOXIC PROPERTIES OF CAPSAICIN

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Many studies revealed the cytotoxic properties of capsaicin on different types of cancer cells by discussing the mechanism of its toxicity. This study comprises the impact of antioxidants on the cytotoxic properties of capsaicin and therefore confirming or dismissing some of the thesis given previously.

The aim of this review is to examine the influence of a combination of a few common antioxidants, which possess particularly high antioxidative potential, as a reason to inhibit the cytotoxic activity of pure capsaicin. This is due to the synergistic antioxidative effect of capsaicin and other co-extracted bioactive compounds (vitamin C, vitamin E and some flavonoids). In a previous study, we have shown that *Capsicum* extracts did not showed cytotoxic activity on neuroblastoma cells, beside the cytotoxic properties of capsaicin, itself, at concentrations 0.5 mmol/L to 2.1 mmol/L. As shown in the literature, one of the mechanisms of capsaicin cytotoxicity that has been proposed support the production of reactive oxygen species on cellular level. This leads to disruption of mitochondrial membrane potential, activation of caspase-3 activity and successive apoptosis. We assumed that this phenomenon of synergism on the antioxidative effect between capsaicin and other bioactive compounds present in the extracts could be a possible reason for inhibition of the cytotoxic effect of capsaicin.

This data should stress out the importance of a balanced intake of antioxidants while using a cytotoxic agent, which acts as a prooxidnat in cancer cells.

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PHYTOCHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF THE SPECIES *LAGOCHILUS* (LAMIACEAE)

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Uzbekistan with unique climatic conditions has a large variety of plants, especially some unique endemic plants. The genus *Lagochilus* (Lamiaceae) consists of 44 species: 33 of them, which grow in Central Asia. About 18 species of *Lagochilus* found in Uzbekistan [1]. Many species of the *Lagochilus* genus have been used in folkloric medicine to treat hemorrhages and inflammation. *L. ilicifolius* and *L. leiacanthus* used for the treatment of hemostatic, inflammation and ulcer in Chinese folk medicine [2]. In Uzbek traditional medicine, people used *Lagochilus* species to make a tea from this herb for its unique sedative and intoxicating properties.

We performed phytochemical investigations on the aerial parts of 7 *Lagochilus* species (*L. acutilobus*, *L. gypsaceus*, *L. inebrians, L. olgae*, *L. proskorjakovii, L. setulosus* and *L. vvedenskii*) distributed in Uzbekistan. The chemical composition of the essential oil isolated from the aerial parts of *L. inebrians* was analyzed by GC-MS and GC-FID. Our investigation reveals that *Lagochilus* genus turned out to be a rich source of diterpens, flavonoids and iridoids. The structures of isolated compounds were elucidated on the basis of 1D and 2D NMR. Further, we performed the qualitative (HPTLC) and quantitative (UV/VIS spectrophotometry and HPTLC densitometry) analysis of the main constituents (lagochilin and 8-acetyl harpagide) of the methanol extracts from *Lagochilus* species. In addition, antimicrobial activity of 7 *Lagochilus* extracts on plant and human pathogens was also carried out.

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SEASONAL VARIATIONS OF THE ESSENTIAL OIL OF *SESELI RIGIDUM* WALDST & KIT.

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The biosynthesis of the essential oil is under influence of genetic, environmental factors and also depends on the phase of the plant development. The aim was to investigate the seasonal variations of essential oil of aboveground and underground parts of *Seseli rigidum* Waldst. & Kit. (Apiaceae), a herbaceous plant native to Southeast Europe [1]. The essential oils were isolated from plants in different phenophases, before flowering (rosette), the flowering and the fruiting phase. The composition of essential oils of two populations was determined by GC-FID and GC-MS [2]. In the essential oil of root of the population from serpentine soil, the content of falcarinol increased during vegetative phase (51.4-77.0%) and reached its maximum in flowering phase (88,8%). Meanwhile in the population from calcareous soil the concentration of falcarinol after slight decrease (47-40%) in vegetative stage, the content increased till the highest value in the fruiting phase (92.1%). Inversely to falcarinol, the contents of *n*-octanal and (*E*,*E*)-2,4-decadienal were the lowest in the roots of plants in generative stages in both populations. On the other hand the changes of main compounds of aerial parts essential oil (α-pinene, limonene and camphene) were quite similar in both populations. The concentration of α-pinene slowly increased during vegetative phase (54.0-58.6% serpentine soil and 57.5-61.1% calcareous soil) and remained relatively unchanged in the flowering period. The data about seasonal changes enable better insight into biosynthetic process in plants and determine the optimal period for plant collection, characterised by the highest content of desirable compounds.

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PLANTASYST: DEVELOPMENT OF A CENTER OF PLANT SYSTEMS BIOLOGY AND BIOTECHNOLOGY FOR THE TRANSLATION OF FUNDAMENTAL RESEARCH INTO SUSTAINABLE BIO-BASED TECHNOLOGIES IN PLOVDIV

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PlantaSYST aims at establishing a new Center of Plant Systems Biology and Biotechnology (CPSBB) in Plovdiv, Bulgaria. The CPSBB is registered as an autonomous legal research entity during TEAMING Phase I and is firmly supported by the Bulgarian and German PlantaSYST partners, the Governments of Bulgaria and Germany, and Plovdiv Municipality. The aim of the project is to position the Center as a leading research organization in Bulgaria and South-East Europe. CPSBB will implement cutting-edge genetics, functional genomics, metabolomics and bioinformatics technologies in order to unravel the plant biochemical machinery and translate the scientific knowledge into the food market and industrial applications for development of value-added products with potential markets in medicine, pharmacy and cosmetics.

The working plan of the 7-year conditions and specific agreement is designed in a way to fulfill all strategic objectives of CPSBB through: 1) Construction of a new modern building and campus in Plovdiv; 2) Acquiring state-of-the-art equipment for research; 3) Conducting cutting-edge basic and applied research; 4) Development of new products for the Bulgarian and the global market; 5) Performing services in the fields of metabolomics, bioinformatics, and soil fertility; 6) Attracting and keeping the best research, and technical personnel; 7) Educating next-generation scientists (PhD students, postdocs) in plant systems biology and biotechnology; 8) Connecting with academia and industry, and bringing different sectors together (policy, science and industry) for social and economic development.

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PHENOTYPE CHANGES OF CD4 T CELLS BY *RHODIOLA ROSEA* CALLI EXTRACTS

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Rhodiola rosea L. extract and its major bioactive constituents (salidroside, rosarin, rosavin and rosin) have been found to alter CD3 T cell growth and apoptosis *via* modulation of IFN-gamma production, expression of pro-apoptotic markers and phosphorylation of extracellular signal-regulated kinase [1]. In this study, the effect of various calli extracts from *R. rosea* on CD4 T cell population phenotype was investigated. CD4+ T cells from mouse spleens were stimulated in the presence of the calli extracts (originated from different calli lines and designated as L82, L84, L90, L91), as well as, p-tyrosol. The investigated extracts and p-tyrosol failed to inhibit markedly T cell proliferation at doses below 50 µg/ml. At a dose of 10 µg/ml they affected the production of IFN-gamma and changed the frequencies of IL-17+Foxp3+ cells *vs* single IL-17+ and Foxp3+ populations. The L82, L84 extracts and p-tyrosol significantly increased the frequency of IL-17+ cells while reducing the frequency of the double positive IL-17+Foxp3+ cells in comparison to control culture. L90 did not to elevate the number of Th17 cells but it decreased the frequency of cells at intermediate state - IL-17+Foxp3+ cells. L91 promoted the generation of IL-17+Foxp3+ cells without an effect on Th17 cells. These data suggest that *R. rosea* calli extracts and p-tyrosol at low dose may have potential to affect the phenotype profile of CD4 T cells and eventually the differentiation and functions of populations (IL-17+Foxp3+ T cells, T regulatory and Th17 cells) playing a role in the pathogenesis of immune-related diseases.

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PROTEINS FROM *FEIJOA SELLOWIANA* BERG: FRUIT: ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY BEFORE AND AFTER SIMULATED GASTROINTESTINAL DIGESTION

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Feijoa sellowiana Berg fruit is widely used for human consumption. In previous studies we showed that the extracts of *F. sellowiana* fruit exert a potent anti-bacterial and antioxidant activity. To deepen the study on activities of the fruit, in this work we analyzed the protein fraction for its antioxidant activity and its effectiveness against different bacteria both ATCC standard strains and clinically isolated. Our aim was also to study the effects of simulated gastrointestinal digestion on both activities. The results showed that *F. sellowiana* fruit proteins showed a surprising strong antibacterial activity against all bacteria strains tested and the corresponding clinically isolated bacterial strains with MIC and MBC values within the range from 3.9 to 62.6 µg/mL and 15.7 to 125 µg/mL respectively. Further, these proteins resulted active also on some bacteria strains tested, that are resistant to conventional antibiotics.

Simulated gastrointestinal digestion of *F. sellowiana* fruit proteins produced three proteins with molecular weights of about 15kDa, 33 kDa and 74 kDa that showed an antioxidant activity 10 fold and an antimicrobial activity from 2 fold to 4 fold higher respect to the undigested *F. sellowiana* fruit proteins.

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ANTIOXIDANT AND ELASTASE-INHIBITING ACTIVITIES OF EXTRACTS FROM *POLYGONI MULTIFLORI RADIX* AND *POLYGONI MULTIFLORI CAULIS* – THE TRADITIONAL CHINESE HERBS FROM *REYNOUTRIA MULTIFLORA*

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The Fleeceflower - *Reynoutria multiflora* (Thunb.) Moldenke (syn. *Fallopia multiflora*, *Polygonum multiflorum*) is a perennial vine from East Asia, used in Traditional Chinese Medicine. Tuberous roots (*Polygoni multiflori radix*) listed in European and Chinese Pharmacopoeias are known in Chinese as *He-Sho-Wu*. Additionally, the twining stems are used under different names *Ye-Jiao-Teng* or *Shou-Wu-Teng* and different traditional therapeutic characteristics.

Here, we compare these two organs using *in vitro* chemical antioxidant assays – antiradical by DPPH scavenging, reducing power using phosphomolybdenum test, and lipoperoxidation inhibition with TBARS detection. Inhibition of elastase activity was tested for potential in prevention of skin-aging and anti-inflammatory properties. Phytochemical composition was studied with total polyphenols and tannin assays. Polyphenol profile was analyzed using HPLC-MS^n.

The primary acetone extracts was successively fractionated with dichloromethane, diethyl ether, ethyl acetate, and butanol. The extracts differed in antioxidant activity and capacity to inhibiting activity of elastase. The materials contain a relatively large amount of polyphenols and tannins, but there was no linear relationship between the antioxidant or anti-elastase activity and content of total polyphenols and tannins. The highest antioxidant activity was observed in diethyl ether and ethyl acetate fractions of root extracts, and ethyl acetate and butanol fractions of stem extracts. In both tested materials, the greatest inhibitory activity of elastase was in water fractions, which were almost devoid of phenolics in the roots, but was quite rich in tannins in stems. The chromatographic profiling revealed several gallotannins and catechin mono- and oligomers as well as stilbenoids in the roots, whereas in stems, several flavonoids were also detected.

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INFLUENCE OF CULTIVATION CONDITIONS AND STRESS TREATMENT ON POLYPHENOL PROFILE AND ANTIMUTAGENIC ACTIVITY OD *STEVIA REBAUDIANA*

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Leaves of *Stevia rebaudiana* Bertoni have been used for the last 20 years in countries of South America and Southeast Asia as a low-calorie sugar substitute. The plant accumulates several tetracyclic diterpenoid metabolites that are natural sweeteners but also significant amounts of flavonols and caffeoyl-quinic acids recognized for their chemopreventive properties.

Here, we evaluated polyphenols content in stevia plants grown under different cultivation regimes: illumination with blue, red or white LEDs, osmotic treatment with polyethylene and sodium chloride as well as *in vitro* shoot cultures elicited with oxidative stress mediators. Polyphenol profiling was performed using UHPLC-ESI-MS with triple quadrupole mass analyzer. Eight caffeoylquinic acids and five main flavonol glycosides were determined, among which the individual flavonol glycosides showed the highest content variation (e.g. quercitrin from 0.25 to 756 mg/100 g dw), whereas the sum of flavonols was much less variable (ca. 5x between extreme values). Hence, the culture conditions influence the glycosylation pattern much more than production of flavonol aglycons or caffeic acid derivatives. Antioxidant activity was high but only polyphenol fractions were active, contrary to steviol glycoside fractions (EC_{50} 11.88 µg/mL *vs* 70.54 µg/mL).

Stevia can be a reasonable source of dietary polyphenols when used as whole/powdered leaves or crude extracts instead of purified steviol glycosides. The antioxidant and antimutagenic tests confirm that either polyphenol-enriched preparation or crude extracts have superior activity. A complex mixture of steviol glycosides also has antimutagenic properties. However, there is a great diversity of the polyphenols content and profile depending on the growth conditions and plant origin.

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PILOT SCALE EXTRACTION OF OLIVE OIL PHENOLIC FRACTION AND RECOVERY OF HIGH PURITY OLEOCANTHAL AND OLEACEIN

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The recognized health-benefiting properties of olive oil (OO) are mainly due to the total phenolic fraction (TPF) and specifically, to its ability to inhibit oxidative reactions that are involved in the beginning and progression of many human diseases. The most characteristic constituents of TPF are oleocanthal and oleacein, two secoiridoids with important biological properties. Those molecules have been investigated mainly on in vitro experiments due to the difficulty of recovery appropriate quantities in purity that required for in vivo and clinical studies. The aim of this study is to provide an efficient method for the recovery of TPF and isolated compounds in high purity with techniques that have the ability of pilot and industrial scale application. A pioneering liquid-liquid extraction, using the biphasic system n-Hex/EVOO/EtOH/H_2O 3 : 2 : 3 : 2 v/v [1] was employed on pilot scale Annular Centrifugal Extractor, BXP-190®, resulting on the production of large quantities of TPF in short time. A total of 240 L OO were extracted in approximately 3 h leading to the recovery of 255 g TPF. The second step of this procedure includes the treatment of TPF and purification of oleocanthal and oleacein. Initially 5 g of TPF were fractionated in a prep-CPC column using the biphasic system n-Hept/EtOAc/EtOH/H_2O 6 : 4 : 3 : 8 (v/v/v). Prep-HPLC was employed to isolate oleocanthal and oleacein from the CPC fractions and to reach purity up to 98%.

The proposed methodology for the recovery of bioactive compounds from OO is described for the first time and can be characterized as a process of high productivity.

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CHEMOPREVENTIVE POTENTIAL OF RED AND WHITE GRAPES FROM BANAT REGION

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Grapes products are correlated with a low incidence of cardiovascular and degenerative diseases. The skin and seeds of grapes, rich in phenolic compounds, with high antioxidant capacities, are known to be potentially active in various types of cancer by acting through different pathways, possible by modulating angiogenesis. In this study we evaluated ethanolic extracts of two varieties of grapes from Serbian Banat region (Srb) and two from Romanian Banat region (Ro): red grapes (*Othello-Srb, Muscat Hamburg-Ro)* and white grapes (*Grocanka-Srb, Chasselas Dore-Ro*). The content of polyphenols was expressed as GAE equivalents and antioxidant capacity was determined by the FRAP method. The chorioallantoic membrane (CAM) assay assessed by means of stereomicroscopy was applied for the evaluation of the antiangiogenic potential. Results showed that the red grapes are the richest in polyphenols while the white grapes indicated the highest antioxidant capacity. An antiangiogenic effect was correlated with the concentration of polyphenols and antioxidant capacity, being significant for both white and red grapes from Serbia.

As a result, the evaluated varieties of grapes can be considered as sources of active compounds useful in the prevention and treatment of cancer.

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LC-MS ANALYSIS OF PHYTOCOMPUNDS FROM *MELISSA OFFICINALIS* L.

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Melissa officinalis L. (MO) is known for its sedative, anxiolytic, antibacterial, antiviral and antiproliferative effects. Several types of extracts, in terms of the type of extraction solvent, extraction method and the origin of the vegetal part were thoroughly characterized by LC-MS method for the screening of polyphenolic and pentacyclic triterpenic compounds. The extracts were screened for 18 polyphenols by an LC-MS method using gradient elution and ESI ionization with SIM in the negative ion mode. All extracts were screened also for 3 pentacyclic triterpenic compounds by a different LC-MS method using isocratic elution and ESI ionization with SIM in the negative ion mode. Regardless of the type of extract, rosmarinic acid was the major polyphenolic compound, methanolic extracts exhibiting concentrations 10 times higher than ethanolic ones, accompanied by most of the polyphenolic compounds in much smaller concentrations (caffeic acid, rutin, etc). In terms of pentacyclic triterpenic compounds, ursolic acid was the major component found in equivalent concentrations both in the ethanolic and methanolic extracts, followed by oleanolic acid and traces of betulinic acid. We developed two complex LC-MS methods in order to conduct a complete screening of phytochemicals found in different vegetal parts from MO and also highlighted the best types of extraction methods in correlation with the concentration of phytocompounds which could be responsible for its therapeutical effects.

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ELICITATION OF CARROT CELL CULTURES TO INCREASE A-TOCOPHEROL AND PHENOLIC COMPOUNDS

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α-Tocopherol, is a bioactive compound associated with the prevention of several human diseases. Moreover, α-tocopherol is the most active form of vitamin E, which has strong antioxidant activity and therefore possesses a strong protective effect against lipidoxidation. On the other hand, vanillin is widely used for enhancing flavor in food and beverages, and as biopreservative because of its antimicrobial and antioxidant properties. Isoeugenol is an isomer of eugenol, which is sometimes used as precursor in the production of vanillin. Eugenol also has several pharmacological properties as an antioxidant, anti-inflammatory and anticarcinogenic agent, as well as other properties including antimicrobial and deterrent effect. This versatile compound is also a high value ingredient in perfumes and cosmetics.

A promising alternative to produce these compounds, which are difficult to be obtained by chemical synthesis or by extraction from plant raw materials, is the use of plant cell cultures [1]. However, the production of secondary metabolites by plant cell culture is still facing many biological and biotechnological limitations. One strategy to increase secondary metabolites in plant cell cultures is the use of elicitors such as methyl jasmonate [2] and β-glucan [3].

In this work, the content of α-tocopherol and phenolic compounds (vanillin, eugenol, isoeugenol) was analyzed in carrot cell suspension under elicitation condition. Elicitacion with ciclodextrins and methyl jasmonate was able to increase phenolic compounds while elicitation with β-glucan was the greater strategy to produce α-tocopherol.

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CARROT CELL CULTURES: A POTENTIAL BIOTECHNOLOGICAL SYSTEM TO PRODUCE BIOACTIVE COMPOUND

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Carrot (*Daucus carota*), is an important plant cultivated worldwide for its nutritive roots. Carrot has been used in traditional medicine due to a wide range of reported pharmacological effects. Carrot roots contain a wide variety of high-value compounds including phenolic compounds such as coumarins and p-hydroxybenzoic acid, volatile terpenoids and several isoprenoid compounds such as carotenoids, phytosterols chlorophylls and tocopherols [1].

Traditionally, secondary metabolites have been directly extracted from raw plant materials. However, these plant sources depend on geographical, climatic and storage conditions. A biotechnological alternative for their production is the use of plant cell cultures since some metabolites are accumulated in a higher extent comparing to their low levels found in intact plants. Moreover, elicitors such as ciclodextrins have been widely used to increase secondary metabolite production in plant cell cultures [2].

In this work, the content of isoprenoids (carotenoids, phytosterols, tocopherols and chlorophylls) was analized in two different cell lines obtained from carrot roots: one photosynthetic green cell line and other orange cell line. Significant differences in carotenoids, chlorophylls and tocopherol content were found. In addition, the effect of cyclodextrins on the production of these metabolites was analyzed. The results of these analysis showed that carotenoids were the mainly isoprenoid compounds found in carrot. Moreover, ciclodextrins were able to accumulated isoprenoids, mainly phytosterols, in the extracellular medium.

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CHEMISTRY AND BIOACTIVITY OF ESSENTIAL OILS ISOLATED FROM FIVE MOLDAVIAN *THYMUS* SPECIES AND CULTIVARS

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The purpose of this study was to examine the chemical composition and *in vitro* bioactivity of the essential oils isolated from five *Thymus* species and cultivars (*T. vulgaris*, *T. vulgaris* cv. *Faustini*, *T. citriodorus*, *T. citriodorus* cv. *variegata*, *T. calcareus*). Chemical composition was investigated by GC-MS and GC-FID. Antioxidant potential was evaluated by free radical scavenging and reducing power assays [1]. Comet assay was used to detect the genotoxic/antigenotoxic effects of thyme essential oils [2]. Anti-*Aspergillus flavus*, aflatoxin-producing fungus and anti-tyrosinase activities were also investigated [3]. The study revealed significant differences in chemical composition and bioactivity among *Thymus* species and cultivars. Thymol (55.4 and 55.45%) was identified as major constituent in *T. vulgaris* and *T. calcareus* essential oils, respectively. Lavandulol (54.27%) was the major constituent of *T. citriodorus* essential oil whereas geraniol (22.12 and 38.28%) was found to predominate in essential oils isolated from *T. vulgaris* cv. *Faustini* and *T. citriodorus* cv. *variegata*, respectively. *T. vulgaris* and *T. calcareus* essential oils showed significant anti-*Aspergillus flavus* (MIC = 0.25 µL/mL, MFC = 0.5 µL/mL) and antioxidant effects (EC_{50} = 0.15 and 0.37 µg/mL, respectively in DPPH assay, EC_{50} = 0.04 and 0.05 µg/mL, respectively in reducing power assay). At 25 µg/mL, *T. vulgaris* essential oil was more efficient than *T. calcareus* essential oil in protecting DNA against H_2O_2-induced oxidative damage (6.21 and 5.52% vs. 25.13 and 7.26% tail DNA in H_2O_2 pre- and post-treatment protocols, respectively). At 50 µg/mL, *T. citriodorus* essential oil inhibited tyrosinase activity by 35.75% whereas kojic acid, a well-known tyrosinase inhibitor, exhibited 42.85% inhibition.

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BIOLOGICAL ACTIVITIES OF SELECTED HERB EXTRACTS OF *POLYGONUM* AND *PERSICARIA* SPECIES

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Many plants from genera *Polygonum* and *Persicaria* are used as a food and spices worldwide. They are a significant source of plant polyphenols, especially flavonoids and anthraquinones, being proved to prevent free radical initiated processes caused by the imbalance in the production of free oxygen radicals (ROS) and antioxidant protection mechanisms. The use of food rich in polyphenols can reduce the risk of many serious chronic diseases. In addition, phytochemicals including polyphenols provided an abundant source of novel therapeutics for the treatment of human cancers. Taking this into account, searching for biological and pharmaceutical activities of *Polygonum* and *Persicaria* species could be of interest.

In this work we prepared ethanolic extracts of *Polygonum aviculare, Polygonum maritimum*, *Persicaria amphibia* and *Persicaria maculosa*. Phytochemical analysis included determination of phenolics, flavonoids and anthraquinones by LC-MS/MS analysis. Cytotoxic effect against lung adenocarcinoma (A549), hepatoma (HepG2) and fetal lung fibroblast (MRC-5) cells was monitored in MTT assay. Flow cytometric analysis of apoptosis and cell cycle phase distribution was also monitored. Furthermore, the effect of concomitant treatment with extracts and doxorubicin was determined by MTT assay. Obtained results indicate that all extracts possess pro-apoptotic effect, while IC_{50} values in MTT assay varied in the range 0.75 - 2 mg/mL. Synergistic effect of extracts and doxorubicin was obtained for certain concentrations in both cancer cell lines.

To examine the underlying mechanism of extracts activity, their influence on the enzymes of antioxidative defense, superoxide dismutase and catalase were determined by western-blot analysis.

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HR-ESI-QTOF-MS CHARACTERIZATION OF BIOACTIVE COMPONENTS FROM TWO GOJI (*LYCIUM BARBARUM* L.) BERRY CULTIVARS AND THEIR ANTIOXIDANT, ENZYME INHIBITORY AND CYTOTOXICOLOGICAL EVALUATION

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Goji (*Lycium barbarum* L.) berries have been widely used as a medicinal plant as well as in the preparation of different phytopharmaceuticals [1]. In this study, the antioxidant capacity, enzyme inhibitory, and cytotoxic effects of two newly developed Goji cultivars (Erma and Biglifeberry) were tested as well as their phytochemical composition by HR-ESI-QToF-MS characterized. Antioxidant capacity was assessed with different assays including free radical scavenging (ABTS and DPPH), reducing power (FRAP and CUPRAC), phosphomolybdenum and metal chelating assays. Enzyme inhibitory effects were tested against cholinesterase, tyrosinase, amylase, and glucosidase. iCELLigence real time cell analysis system was used to determine the cytotoxic activity of extracts from these cultivars on HEK-293 cells. HR-ESI-QToF-MS revealed a different chemical composition of the cultivars. However, Biglifeberry extract exhibited stronger antioxidant abilities with higher levels of total phenolic content (15.73 mg GAE/g extract) compared to the cultivar Erma (11.62 mg GAE/g extract). However, Erma extract had higher inhibitory effects on butyrylcholinesterase, glucosidase and tyrosinase. Moreover, these extracts showed no cytotoxic effects on HEK-293 cells in a time and dose dependent manner. LC-MS results showed the presence of several compounds including dietary important vitamin C and flavonoids such as rutin and phenolic acids such as coumaroyl- and caffeoylquinic acid derivatives and characteristic lycibarbarspermidines in both cultivars. These findings suggest that the two Goji cultivars could be considered as potential sources of bioactive compounds for the design of novel functional and health-promoting phytopharmaceuticals.

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HPLC-MS ANALYSIS OF INFUSIONS FROM *SENECIO GRAVEOLENS* FROM THE ATACAMA DESERT AND THEIR BIOLOGICAL PROPERTIES

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Senecio graveolens (Chachacoma) is a plant from the Atacama Desert used since ancient times by Aymara aboriginals in Northern Chile and Argentina for the treatment of several diseases, including gastrointestinal disorders and altitude sickness. Previous studies on this plant reported the presence of several acetophenones, which were the active components for altitude sickness and possessed cytotoxic activity. However, a complete metabolome analysis of the bioactive infusions including glycosylated compounds was not done yet. In this study we report the chemical fingerprint for an infusion of this plant, including glycosylated acetophenones, using UHPLC-PDA coupled to orbitrap high resolution mass spectrometry. LC-MS results showed the presence of vitamin C and malic acid, as well as several antioxidant phenolic acids such as: 3-methyl-4-methoxycinamic acid, dicaffeoylquinic acid, cinnamic acid, cafeic acid, caffeoyl-beta-D-glucopyranoside, p-cumaric acid, tetrahydroxydodecaenoic acid-*O*-glucoside, several myricetin derivatives such as 7,3'-dimethoxymyricetin, 3-*O*-glu-7-methoxymyricetin, and 7-acetyl-3-*O*-glu-3',4'-dimethoxymyricetin and 3-*O*-glu-7,3'-dimethoxyquercetin, plus the acetophenones: dihydroeuparin and 4-hydroxy-3-(3-methylbut-2-enyl) acetophenone and their glycoside derivatives. Beside the phytochemical analysis, the *in vitro* anti-oxidant and anti-inflammatory potential of *S. graveolens* extract and acetophenone were evaluated. The extract showed only moderate inhibitory effect on the pro-inflammatory transcription factor NF-κB, but strong anti-oxidant property on pyocyanine-induced reactive oxygen species generation *in vitro*. However, acetophenone did not show any of these activities. These findings support the ethno-medicinal use of *Senecio graveolens* that could be considered as a potential source of bioactive compounds for the design of novel health-promoting phyto-pharmaceuticals.

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LIPID COMPOSITION OF THE TWO MOST VALUABLE MOROCCAN SEED OILS – ARGAN (*ARGANIA SPINOSA* L.) AND CACTUS *OPUNTIA FICUS-INDICA* (L.)

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The argan tree (*Argania spinosa* L., Sapotaceae) and the cactus *Opuntia ficus-indica* L. (Cactaceae) are typical plants for the semiarid regions of Morocco, with vital ecological and socioeconomic significance. For centuries their fruits kernels have been processed to obtain valuable seed oils used as remedy, as food and recently mainly for cosmetics. Nowadays, the argan and cactus oils, especially the cold-pressed ones, are among the most expensive oils in the world. A part of their production still is obtained in a traditional way by Berber women through manual separation, cleaning and grinding of nuts in stone mills.

The objects of our investigation were two argan and two cactus oils produced by cold pressing and by solvent extraction. Their lipid composition was analyzed and compared in respect to the neutral lipid classes, fatty acids and tocopherols. Oxidative stability of the four oils was evaluated as well.

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OPEN-SOURCE DEVELOPMENTS FOR NATURAL PRODUCT SEARCH

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For open-source developments, the redistribution is free, the source code is available and modifications of the device are possible, if the open-source aspect is respected. Thinking about open-source developments, software comes first in mind, but there are several examples of open-source hardware (OSH), *e. g.* the Arduino microcontroller and 3D printers. Such tools allow the researcher to investigate new technologies in a rapid and cost-effective way. Only a limited knowledge on electronics, mechanics and programming is needed, letting still space to focus on the specific field of interest. Open-source solutions, both hard- and software, come along with a steep learning curve. During the self-assembly, the user will build valuable skills for trouble shooting and modification. Furthermore, the presence of a strong community of volunteers, answering questions on different forums, constitutes a valuable alternative to the customer support encountered in classic business models. The expansion of OSH is expected to trigger tremendous progress in the field of analytical chemistry, allowing small teams of experts to be as efficient as an entire department in a multinational company. The viral aspect of open-source developments is outlined, giving to the scientific community affordable and innovative tools to be included in their laboratories. Several already developed tools [1, 2] and latest achievements in open-source developments are illustrated. Similar to radical chain reactions, exponential in progress and highly dynamic in its outcome, open-source developments are integrative parts of our analytical toolbox.

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JUNIPERUS COMMUNIS ESSENTIAL OIL AND POST-DISTILLATION WASTE IMPROVE DOXORUBICIN CYTOTOXICITY AGAINST LUNG CANCER CELLS

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In this work we determined chemical composition, antioxidativity, genotoxicity and cytotoxicity of essential oil (EO) and post-distillation waste (PDW) of *Juniperus communis* var. *saxatilis*. GC-MS analysis identified 93.95% of total EO content and determined α-pinen as a dominant component (23.61%). LC-MS/MS analysis of PDW pointed at rutin (12.2 mg/g) and quinic acid (11.1 mg g^{-1}) as the most abundant among identified constituents. Antioxidant assays (DPPH and TBA, and additionally FRAP for PDW) indicated strong activity of PDW and negligible of EO. Comet assay, applied on human lung carcinoma (A549) and normal lung fibroblast (MRC-5) cells, indicated genotoxicity only for PDW, higher in A549 cells. Cytotoxicity of EO, PDW and doxorubicin, individually and in binary combinations, was determined in MTT assay applied on the same cell lines. Although cytotoxicity of EO and PDW was lower compared to doxorubicin, it was more pronounced in cancer than in normal cells. Obtained IC_{50} values were 69.4 and 120 µg/mL for EO, and 1.3 and 2.8 mg/mL for PDW, in A549 and MRC-5 cells, respectively. Flow cytometric analysis indicated that PDW increased apoptosis of A549 cells and arrested cell cycle in G_2/M phase. In binary mixtures with doxorubicin cytotoxicity of EO and PDW was remarkably higher. Combination index, calculated for IC_{50} values, revealed clear synergistic effect for all mixtures, stronger in cancer than in normal cell line.

Our results indicate that *J. communis* EO and PDW could improve doxorubicin activity against lung cancer cells.

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COMPARISON OF THE INHIBITORY EFFECT OF ANTHOCYANINS AND FLAVONOLS FROM FRUITS OF GENUS *VACCINIUM* ON KEY CARBOHYDRATE ENZYMES

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During the last decade, numerous investigations were made to identify α-glucosidase and α-amylase as potential inhibitors from natural sources in order to develop new alternatives for diabetes management. Fruits of *Vaccinium* genus were found to be effective in controlling postprandial hyperglycemia. These properties were also demonstrated for *Vaccinium* fruits extracts, which are a rich source of phenolic compounds, such as anthocyanins, flavonols (quercetin, kaempferol and myricetin), flavan-3-ols - catechins, stilbenoids and phenolic acid derivatives.

The studied aqueous extracts from *Vaccinium* fruits (*V. myrtillus*, *V. vitis-idaea* and *V. uliginosum*) were rich in chlorogenic acid, and rutin. Anthocyanin content in the aqueous extracts of the studied species varied from 130.2 mg C3GE/100 g (V. *myrtillus*) to 23.1 mg C3GE/100 g (*V. uliginosum*). *In vitro* assays showed that *Vaccinium* fruits aqueous extracts significantly inhibited the activity of the salivary α-amylase and α-glucosidase (*Saccharomyces cerevisiae*) enzymes in a dose-dependent manner. The extract of *V. vitis-idaea* fruits with both enzymes showed the strongest inhibitory effect, with IC_{50} values of 32.7 µg GAE/ml and 2.0 µg GAE /ml, respectively. The kinetic parameters Km and Vmax values of the studied enzymes were determined also in the presence of anthocyanins and flavonols.

This investigation of the inhibitory effect of anthocyanins and flavonols from *Vaccinium* fruits in different ratio revealed their potential for prevention of metabolic syndrome and diabetes type 2.

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INTEGRATED FT-ICR MS AND LC-HRMS METABOLOMICS STUDIED ON EXTRA VIRGIN OLIVE OIL (EVOO) BIOPHENOLS

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Over the last decade, extra virgin olive oil (EVOO) consumption has globally increased likely because of its nutritional and sensory properties. In combination with its importance for European Union's economy [1], it has been established as a product of high economic importance and the need for its quality and authenticity control has been emerged. Its chemical complexity enhances the hassle in investigating the most suitable methodology and consequently numerous analytical studies have been employed towards this direction [2]. In this study high resolution mass spectrometry (HRMS) techniques were transacted, in Greek EVOO biophenolic extracts. In particular, ultra-performance liquid chromatography (UPLC) coupled with a hybrid spectrometer ion trap and orbitrap analysers (LC-IT-Orbitrap-MS) and Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) using flow injection analysis (FIA) method were employed and provided considerable data for EVOO biophenolic extracts. The aim of this study was mapping Greek EVOO as well as evaluating the two HRMS workflows considering quality aspects. In brief, more than 300 EVOO samples were collected from the main Greek olive oil producing regions, for two harvesting years. After data pretreatment, multivariate data analysis (MDA) was subjected and revealed significant clusterings according to the main discriminants factor. The responsible for clustering metabolites-biomarkers were also investigated. According to our results, both techniques, under metabolic profiling concept, have been proven valuable tools for EVOO quality control purposes.

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PURIFICATION PROTOCOL OF HETEROLOGOUSLY PRODUCED BIOACTIVE PLANT PHENOLICS FROM METABOLICALLY ENGINEERED *E. COLI* BIOFACTORIES TO EXPLORE PHARMACOLOGICAL AND OTHER INDUSTRIAL USES-THE HYDROXYTYROSOL EXAMPLE

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One of the most abundant phenolic compounds traced in olive tissues is Hydroxytyrosol (HT), a well-documented molecule that has been attributed with a pile of beneficial effects [1, 2]. Due to its strong biological activities, HT is regarded as a potential supplement or preservative to be employed in the nutraceutical, agrochemical, cosmeceutical, medicinal or food industry. Although the HT biosynthetic pathway in olives was not fully decoded, *Escherichia coli* genetically engineered to overproduce the precursor molecule tyrosine and encompassing hydroxylation, decarboxylation, and deamination reactions, was able to *de-novo* produce HT. Such a redirection of the carbon flow towards the production of HT directly from glucose, achieved a reasonable HT concentration. One liter of various types of Minimal growth medium (M9) versions tested, resulted in an improved and optimal broth culture recipe for HT heterolgous production, containing appropriate combination of nutrients as well as antibiotics. Preliminary analysis using a Capillary Electrophoresis system coupled with a Diode Array Detector, showed an average concentration of 1.02 mM (157.2 mg/L). Extraction of the bacterial culture using ART technology and chromatographic techniques were employed for HT analysis and purification. LC-HRMS methodology was performed for the confirmation of the high HT production while significant molecules of the biosynthetic pathway were revealed. The final purification step of the initial crude extract of M9 optimal medium gave HT of high purity with no antibiotics and intermediate metabolites present that can be further used in medicinal, nutraceutical, agrochemical, cosmeceutical, or food industry applications to produce various commercial formulations.

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THE ESSENTIAL OIL OF *ALLIUM CEPA* INDUCED MARKEDLY RELAXATION ON THE RAT CORPUS CAVERNOSUM

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Erectile dysfunction (ED) is a common healthy problem, affecting more than half of men ages 40 to 70 [1]. It has been considered that many medicinal plants and secondary metabolites are used in the management of ED. Onion (*Allium cepa* L.) belongs to genus *Allium* from the Amaryllidaceae family and is economically important as a vegetable. The present study investigated the possible relaxant effect of OEO on rat corpus cavernosum (CC) [2, 3]. Therefore, to investigate the relaxant effect, a total of twenty-six male Sprague-Dawley rats were used in this study. Isolated rat CC strips were placed in organ baths containing Krebs solution, and functional experiments were performed. After preconstruction with phenylephrine (PE, 10^{-5} M), the relaxant response to OEO (25 – 400 µL) was investigated in rat CC. The relaxation responses to OEO have examined incubation with of nitric oxide synthase (NOS) inhibitor L-nitro-arginine methyl ester (L-NAME, 100µM) or soluble guanylate cyclase [1H-1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 30µM) inhibitor. OEO showed relaxant effect on rat CC strips in a dose-dependent manner (maximum response: 98.0 ± 3.5%). OEO caused relaxation of isolated CC strips independently of the NO/cGMP pathway. Further investigations are warranted to elucidate the mechanistic effects of OEO on ED fully.

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CAPITALIZATION OF FIBROUS PLANTS WASTE IN THE CONTEXT OF CIRCULAR BIO-ECONOMY

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The circular economy represents the economic model that focuses on reusing either the materials or the wastes from previous economic stages, aiming at creating added value through innovative solutions [1].

Bio-economy aims to create a more competitive resource-efficient economy, to create sustainable economic growth and, in the same time, to ensure the conservation of natural ecosystems [1]. The increase in urbanization encountered worldwide resulted in both material consumption and generation of huge amounts of waste [2].

Every company from the Medicinal and Aromatic Plants (MAP) industry produces tons of waste every year. These wastes usually end up as compost to be used in agricultural applications.

We propose an alternative approach, by evaluating the amount of fatty oils remaining in selected wastes (*Silybum marianum* L., *Helianthus annuus* L., *Linum*, *Carthamus tinctorius* L.) and their potential use for obtaining cellulose with potential further applications.

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INTEGRATION OF MEDICINAL AND AROMATIC PLANTS (MAP) INDUSTRY WASTES INTO NEW BIO-ECONOMICAL PROCESSES

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Waste from biostreams and different bio-based sources are being under-utilized as potential resource of valuable compounds. Raw materials from different industries can be capitalized in order to close value chains. Integrated biotechnological processes in order to convert co/by-products from bio-economy in products with multi target effect are the subject of research in the last decade. In the last years was observed an exponential increase of medicinal and aromatic plants waste (MAP), that most of the time remains unexploited. For example, just one of the MAP Industry company in Romania produces annually about 30 tons of medicinal and aromatic plants waste, only by aqueous extractions and about 23 tons of oleaginous plants waste (in the form of pomace), that are no longer processed and thus become useless waste. At international level, there are initiatives regarding the valorization of plant wastes: for example, a by-product deriving from apple juice pressing is considered a source of dietary fibers (about 50% of dry weight) and phenolics (from 1200 to 4000 mg/kg dry weight), including flavanols, hydroxycinnamates, and dihydrochalcone [1]. In this respect, the co/by-product of a phase becomes the raw material for the next process, according to the concept of industrial symbiosis. Plant waste constitutes strongly organic - enriched materials that can be further processed for environment reintegration in safe conditions. Thus, integrated and intensified biotechnological processes for the obtainment of the superior capitalization of co/by-product from agro-industries, offers to the direct beneficiaries from economic medium the possibility to implement the out-comes/ R&D&I services.

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EVALUATION OF INHIBITORY POTENTIAL OF *HERACLEUM VERTICILLATUM*, *H. SIBIRICUM* AND *H. ANGUSTISECTUM* EXTRACTS AGAINST ENZYMES INVOLVED IN ALZHEIMER AND TYPE II DIABETES

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In the present work the hexane extracts obtained from the leaf, fruit and roots of *Heracleum verticillatum* Pancic, *H. sibiricum* L. and *H. angustisectum* (Stoj. & Acht.) Peev were evaluated for phytochemical content, antioxidant potential, antiacetylcholinesterase and antidiabetic activities. The free radical scavenging potential of the extracts was evaluated against $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ radicals. Acetylcholinesterase (from *Electrophorus electricus*) inhibition of the samples was evaluated using Ellman's method. Antidiabetic potentials of the extracts were evaluated *via* measurement of inhibition of α-amylase enzyme involved in type II diabetes. GC and ^{1}HNMR analysis of the extracts allowed the identification of 10 furanocoumarins either by using authentic standards or by comparing spectral data with literatures. Pimpinellin was the main compound in the root and leaf extracts, while bergaptene was in the fruit extracts. The leaf extract of *H. angustisectum* (IC_{50} 0.58 ± 0.1 mg/mL) and the leaf (IC_{50} 0.68 ± 0.04 mg/mL) and the root (IC_{50} 0.92 ± 0.07 mg/mL) extracts of *H. sibiricum* demonstrated the highest free radical scavenging activity. The highest TEAC value was obtained for the root extract of *H. angustisecum* (1.83 ± 0.002 mM). The root extract of *H. verticillatum* was found to be effective against AChE with IC_{50} 0.30 ± 0.07 mg/mL. The extracts demonstrated weak inhibitory effect (%Inh. up to 29.7 ± 3.1) towards to α-amylase (from porcine pancreas). The present work is the first contribution about anti-AChE and antidiabetic potential of these three *Heracleum* species from Bulgaria.

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ISOLATION OF SECONDARY METABOLITES FROM *WIEDEMMANIA MULTIFIDA* (L.) BENTHAM

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Two species of genus *Wiedemannia* (Lamiaceae) grown in Turkey: *W. multifida* and *W. orientalis*. *W. multifida* is known colloquially in our country as "çok dallı ballıbaba" [1]. The object of the present study is to isolation of secondary metabolites from *W. multifida*. There is no any phytochemical study on *W. multifida*. In this study, aerial parts of *W. multifida* collected from the Horasan district (Erzurum) were dried and powdered and then extracted with methanol, the obtained crude methanol extract was suspended in water and partitioned with chloroform and ethyl acetate, respectively. 5 major compounds were purified using various chromatographic methods (thin layer chromatography, normal phase silica gel column chromatography, reverse phase silica gel column chromatography, Sephadex LH-20 column chromatography) in chloroform, ethyl acetate and remaining water subfractions. The structures of the pure compounds (lamiide, ipolamiide, ipolamiidoside, 5-hydroxy-8-epiloganin, luteolin 5-*O*-β-glucoside) were determined by Nuclear Magnetic Resonance (^{1}H NMR, ^{13}C NMR) method.

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PHYTOCHEMICAL STUDIES ON THE AERIAL PARTS OF *CIRSIUM DIRMILENSE*

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Cirsium (Asteraceae) genus is known as "Köygöçüren" and used for treatment of hemorrhoid, peptic ulcer, bronchitis traditionally in Turkey [1]. It has 66 species (78 taxon) in Turkey [2]. In this study, phytochemical studies were carried out on the methanolic extract of the aerial parts of the plant. Phytochemical studies have been performed on chloroform, etyl acetate and remaining aqueous subfractions which are obtained from methanolic extract using several chromatographic techniques. CDK-1, CDK-2, CDK-3, CDK-4 were isolated from chloroform subfraction as a mixture.

Using ^{1}H-NMR and ^{13}C-NMR spectroscopic methods, the structures of CDK-1, CDK-2, CDK-3 and CDK-4 were elucidated as *α*-amyrin, *β*-amyrin, lupeol and ψ-taraxasterol, respectively.

Acknowledgements: We would like to acknowledge the scholarship and funding by the Turkish Scientific and Technical Research Council (TUBITAK).

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PHENOLIC CONSTITUENTS OF CULTIVATED *GANODERMA LUCIDUM* IN TURKEY: A POTENTIAL SOURCE FOR PREPARING FUNCTIONAL INGREDIENTS

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Phenolic compounds are an important part of the human diet and have been largely studied due to their biological activities including antioxidant, antimutagenic, anticancer, antiallergenic, antiinflammatory, antiviral, antiulcer, antidiarrheal, anthelmintic, antihepatoxic, and antiproliferative [1, 2]. The *Ganoderma* species is considered one of the most important medicinal mushrooms and is traditionally used in the treatment of various ailments, including cancer, hypertension, gastric ulcer, tumor, kidney and cardiovascular problems [3]. From this perspectives, we investigated the phenolic constituents of water extract of cultivated *Ganoderma lucidum* in Turkey by HPLC-DAD technique. We used 24 phenolic standards and 11 of them were detected in the extract. Apigenin (1010 µg/g extract), (+)-catechin (334 µg/g extract), kaempferol (216 µg/g extract) were detected dominant compounds in the extract.

The results suggest that the cultivated *G. lucidum* species may be considered as a potential candidate for preparing new food supplements and drug formulations.

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SOME EFFECTS OF CULTIVATED *GANODERMA LUCIDUM* GIVEN *IN OVO* ON THE CHICK EMBRYOS

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Ganoderma lucidum, is a mushroom which used for alternative medicine worldwide particularly in China, also known as "Immortality Mushroom or Reishi". In recent years, cultivated *G. lucidum* is also produced in Turkey and consumed by people especially as tea, coffee and tablets [1, 2]. The aim of this study was to determine for the first time the some effects of the aqueous extract of cultivated *G. lucidum* in Turkey on the development of chicken embryos as an alternative experimental animal. *G. lucidum* aqueous extract at different three doses and distilled water as control group were injected into the air sac of fertilized chicken eggs at 8th day of incubation. Following parameters of each group were examined on 11th day of the incubation: rates of dead and abnormal embryo, malformation types, live embryo weights. In addition, some of the embryos were totally stained with Alizarin Red-S method for bone development. Data were analyzed with statistical methods. The extract of cultivated *G. lucidum* did not present significant embryotoxic and teratogenic effects on chick embryos. It also did not affect the bone development of chicken embryos at the macroscopic level. These results are consistent with another study used wild-grown *G. lucidum* [3].

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CHEMICAL CONSTITUENTS OF *ANAGYRIS FOETIDA* L. (FABACEAE)

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A. foetida L., a shrubby legume, is growing wild on rocky places and at sloping valleys. Its flowering season is during winter, from January to March. It is known since antiquity, due to its emetic properties [1]. It has been traditionally used as laxative, emetic and as abortive [2]. Aerial parts of the plant have been extracted with cyclohexane and methanol, successively. The cyclohexane extract has been fractionated by several CC; the obtained fractions have been analyzed by GC-MS and revealed the presence of several hydrocarbons and fatty acids, among which the main compounds were palmitic and linoleic acids. Furthermore, the methanol extract was subjected to CC and yielded diosmetin. It is noteworthy that this extract was poor on phenolic compounds of any type. The structures of the isolated compounds were elucidated by high-field NMR spectroscopy (^{1}H-NMR, ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC) and GC-MS analyses.

Acknowledgements: The authors wish to thank Associate Prof. Th. Constantinidis (Department of Ecology & Systematics, Faculty of Biology, NKUA) for the collection and the identification of the plant material.

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EVALUATION OF THE ANTIOXIDANT AND CYTOTOXIC EFFECTS OF *MELISSA OFFICINALIS* L. EXTRACTS IN BREAST CANCER CELL LINES

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Aim: The present study was aimed to evaluate different types of *Melissa officinalis* L. (MO) extracts (MOE) concerning their antioxidant capacity and cytotoxic effects on breast cancer cell lines.

Material and Methods: Multiple extraction techniques were applied in order to identify the most active type of extract from MO by means of LC-MS phytochemical profiling, antioxidant capacity and cytotoxic effects. Hydroalchoolic extractions were performed in ethanol (MOEE) and methanol (MOEM) from aerial parts of MO. The phytochemical profile was determined by HPLC-MS analysis considering as standards polyphenolic compounds and triterpenes. The most active extracts were compared to their main phytocompounds: rosmarinic acid (RA) and ursolic acid (UA). The antioxidant effect was assessed by DPPH free radical scavenging activity and the cytotoxic potential was evaluated by MTT assay, using two breast cancer cell lines: MCF-7 and MDA-MB-231.

Results: The highest concentration in both polyphenols and triterpenes were the ethanol 96% and methanol 80% extracts, with highest levels of RA in MOEM and of UA in MOEE. Antioxidant capacity was close to that of vitamin C and was higher for MOEM. MOEE showed a cytotoxic effect on both cell lines, being more potent in the case of MDA-MB-231 cells.

Conclusion: Ethanolic extracts of MO are more active as cytotoxic agents, containing high levels of UA, while methanolic extracts, rich in RA possess important antioxidant potential. Synergistic effects of the two extracts could enhance the chemopreventive effect on MDA-MB-231 breast cancer.

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ASSESSMENT OF THE CYTOTOXIC AND ANTIPROLIFERATIVE EFFECTS OF A BENZYLAMIDE DERIVATIVE OF MASLINIC ACID ON A375 HUMAN MELANOMA CELL LINE

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Background: Maslinic acid (MA), belongs to the class of pentacyclic triterpenes and can be forund mainly in olives, in *Olea europaea* L. In order to improve its activity, structural changes were made and led to the development of new derivatives with various effects. The present study is purposed to assess the effect of a benzylamide derivative of maslinic acid, "EM2" (Benzyl (2α, 3β) 2,3-diacetoxy-olean-12-en-28-amide) on A375 human melanoma cell line.

Materials and Methods: Increasing concentrations of EM2 (1, 5, 10, 25 and 50 μM) were tested on A375 human melanoma cells. Stock solution of the tested substance (10 mM) was prepared in dimethylsulfoxide (DMSO). For the viability assay, the cells were cultured in 96-well plates and stimulated for 72h with the compound. MTT assay was used for the assessment of cells viability. Furthermore, the antiproliferative effects of EM2 were assessed by scratch assay. Pictures were taken at different time points: 0h, 3h, and 24h.

Results: EM2 elicited a dose-dependent cytotoxic effect on A375 cells, the most potent being 50 μM. The antiproliferative capacity of the samples was evaluated, and the compound was able to inhibit cell migration.

Conclusion: Our preliminary results suggest that EM2 is a potential antitumoral agent against A375 human melanoma cells. Further studies will be carried out in order to elucidate the anticancer mechanisms and to evaluate the *in vivo* effects in animal models.

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BIOEVALUATION OF A POLYURETHANE CARRIER USED FOR EUGENOL DELIVERY

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Cloves are harvested from an exotic tree, originating from the Maluku Islands and the southern Philippines. It has been grown in special plantations since ancient times in Indonesia, Malaysia, Tanzania, Sri Lanka, China and India. In the old times, the products were used only for medicinal purposes. Due to the chemical content of the blooms and the oil, rich in eugenol, a set of therapeutic effects are exerted: antiseptic, antibacterial, antifungal, antiviral, anesthetic, analgesic, antiinflammatory, and others [1].

A polyurethane system, used as a transmembrane carrier of eugenol, with dimensions in the range of 135-170 nm was synthesized and characterized. It was obtained using the interphase polyaddition technique combined with a spontaneous emulsification in the presence of two main components: an organic phase (lysine-diisocyanate-based semi-prepolymer solubilized in acetone) and an aqueous phase (a aqueous mixture of polyethylene glycol and diols with short chains) [2, 3].

The obtained products have been characterized by: pH, solubility, aggregation size and surface charge, thermal behavior, and scanning electron microscopy. Products' harmfulness was assessed by non-invasive tests using CD1Nu / Nu mouse skin parameters such as: transepidermal water loss, skin-pH, sebum, erythema, melanin and *stratum corneum* hydration.

The results indicate the obtaining of weak acidic particles with low aqueous solubility and Zeta potential values between +25 and +30 mV, a good thermal stability over a wide temperature range (30-300°C), and no harm was observed (normal values of skin parameters, without any important change of these).

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BETULIN GOLD NANOPARTICLES: A PRELIMINARY NON-INVASIVE EVALUATION OF SPECIFIC CUTANEOUS PARAMETERS

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Topical-based betulin (Bet) formulations have proven to be effective in the treatment of actinic keratosis, Bet nanoemulsion in the antitumor therapy at the experimental level [1]. Gold nanoparticles (AuNPs) present a particular interest in dermato-cosmetic area especially due to beneficial anti-aging properties. Non-invasive skin tests, applied to both human and animals, have been developed to quantify and evaluate efficacy / toxicity of a finished product [2]. Bet, due to its hydrophobicity requires the nanotechnology for biological applications [1]. The present study was aimed to assess BetAuNPs toxicity by non-invasive techniques on human healthy volunteers correlated to *in vitro* toxicological studies on keratinocytes and fibroblasts.

Three types of formulations, namely: blank, with BetAuNPs and with BetPEG-AuNPs were prepared by homogenization method and applied on 20 human volunteers. Patch tests were conducted in the first 24h and specific skin physiological parameters (melanin, erythema, skin hydration and transepidermal water loss) were measured on 20 healthy female volunteers with ages between 20-25 years. All nanoparticles were properly solubilized and tested *in vitro* on keratinocytes and fibroblasts. The results indicated no relevant signs of toxicity both *in vitro* and *in vivo*. On cell lines the cytotoxicity rate was under 20% and *in vivo* skin parameters variation was in the interval acceptance as safe.

The main conclusion developed from the study is that nanostructures prepared by simple methods are safe and non toxic for skin applications. They could be applied and developed as skin treatment compunds.

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NEW TOCOTRIENOLS FROM COLOMBIAN PROPOLIS

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Propolis is a bee hive product providing social immunity to the bee family. It is well known to men for its preventive and healing properties and nowadays it is included in various products available on the market. As a mixture of plant resinous exudates and beeswax, its chemical composition depends strongly on the local flora and thus on the climatic characteristics at the site of resin collection. For this reason, many and structurally diverse compounds (flavonoids, phenolic acids, terpenes, stilbenes, xanthones, etc.) are isolated and many propolis types with well known chemical profiles are formulated, most of them for propolis originating from tropical regions due to the diverse tropical flora. Thus, the dealing with propolis from unexplored/scantily explored regions has the potential to uncover new biologically active compounds with important pharmacological effects.

We studied propolis from five regions of Colombia, a country with tropical climate from where propolis is scantily investigated. Following the propolis type dereplication strategy [1] GC/MS analysis of the total extracts was applied at the first step. The chemical profile of all samples studied was similar but did not match any known propolis type. Thus, through detailed chemical analysis two new natural compounds and one new for propolis delta-tocotrienols derivatives with oxidized chromanol ring and terminal side-chain were isolated. The structures were identified by means of UV, IR, MS and NMR methods.

The isolated compounds are the first tocopherol derivatives found in propolis. Their plant origin and biological activity are subjects of further investigations.

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ANTI-CANCER EFFECT OF *CRINUM ASIATICUM* EXTRACTS AGAINST U937 HUMAN MONOCYTIC LEUKEMIA CELLS

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Leukemia patients suffer from the abnormal production of white blood cells, particularly found in lymphoid and myeloid lineages. Other than modern treatments available, many natural products have been reported to show the anti-cancer effect against leukemic cells. Among these, crinum lily (*Crinum asiaticum* L.) was reported to show many properties such as analgesic, anti-inflammation, cytotoxicity, and anti-proliferation against tumor cells. We aimed to investigate the anti-cancer effect of a medicinal plant; *Crinum asiaticum* L against U937 human monocytic leukemia cells. 50 gram of dried crinum lily leaves were prepared and extracted by 70% ethanol using maceration method. U937 cells were cultured in RPMI1640 at 37^{O}C with 5% CO_2 and treated with 6 concentrations (100, 50, 25, 12.5, 6.25, 3.125 µg/ml) of crinum lily leaf extracts, with 1% DMSO in RPMI1640 medium and 10% 20 mg/ml cytarabine in RPMI1640 medium as negative and positive controls, respectively. The numbers of viable cells and cell metabolic activity were determined by MTT assay. Also, the cell morphologies were observed under the inverted microscope. The results showed that the crinum lily leaf ethanol extract exhibited the 70.57% inhibition against the U937 cells at 100 µg/ml. Also, the 50% inhibition concentration of crinum lily leaf ethanol extract was at 75.3 µg/ml. Moreover, the U937 cell death was demonstrated at 50 µg/ml and 25 µg/ml crinum leaf extracts. In conclusion, the *Crinum asiaticum* L. leaf extract showed the anti-proliferation and cytotoxicity against U937 cells in the concentration-dependent manner.

However, further studies should be done regarding the anti-cancer mechanism and to develop an alternative treatment for leukemia.

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EFFICACY OF C-10 MASSOIALACTONE AGAINST MULTISPECIES MICROBIAL BIOFILM

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Biofilms are communities of microorganisms that can be found in almost every habitat. They are attached to a surface and embedded in an extracellular matrix of biomolecules [1]. The matrix is substantially protect microorganisms and make them more resistant to anti-microbials, which makes them a progressive source of infections [2]. The discovery of anti-biofilm agents is required to deal with these biofilm-mediated infections. C-10 *masoaialactone,* a major component of *Massoia aromatica* Becc. essential oil has potential as antibacterial and antifungal [2]. This study aim to determine the efficacy of C-10 *Massoialactone* against multispecies microbial biofilm of *Candida albicans*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Massoia oil was obtained from the distillated of steam and water. C-10 *Massoialactone* was isolated using Preparative TLC method. Phytochemical analysis was performed using a Thin Layer of Chromatography (TLC) and gas chromatography - mass spectrometry (GC-MS). Microtiter broth dilution method was employed to assess antibiofilm activity of C-10 *Massoialactone* against microbial biofilm. Transmission electron microscope (TEM) and Scanning electron microscope (SEM) were used to analyze the biofilm structure. The GCMS result revealed the presence of C-10 *Massoialactone* (96.59%). C-10 *Massoialactone* showed a dose dependent activity in inhibiting biofilm formation as well as in breaking down established biofilms. Higher concentration of C-10 *Massoialactone* required to inhibit the mature phase biofilm of the test organism. TEM and SEM analysis showed cell lysis in the biofilm in the presence of C-10 Massoialactone.

Acknowledgement: The authors are gratefully acknowledging the funds support of this research by Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia.

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INFLUENCE OF DIFFERENT CONCENTRATIONS OF BILBERRY EXTRACT ON RAT CYTOCHROMES P450 AND ANTIOXIDATIVE STATUS

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Bilberries (*Vaccinium myrtillus* L.) are part of fruit juices, teas, foods and food supplements. Constituents of bilberry extract, anthocyanins, are natural antioxidants. This study evaluates the influence of bilberry extract on cytochrome P450 activity and antioxidant status of Wistar albino rats. Rats were fed by standard diet and drank *ad libitum* tap water (Control) or tap water with 0.15 g/L (Lower) or 1.5 g/L (Higher) of bilberry extract. After 29 days, rats were anesthetized and exsanguinated. Blood and inner organs were taken for further analyses. Microsomal fractions were prepared and used for study of xenobiotic metabolism. Activities of rat cytochromes P450 were measured using these substrates: ethoxyresorufin, testosterone, warfarin, bufuralol, diclofenac, phenacetin, diazepam and chlorzoxazone. The quantity of metabolites of these substrates was measured by HPLC with UV and fluorescence detection [1]. The results suggest increased activity of rat cytochromes P450 2E1 by 28% (Lower) and 2C11 by 34% (Higher). Slightly lowered activity was found in rat cytochromes P450 3A1/2. The blood was centrifuged to plasma and erythrocytes in which the selected parameters of oxidative stress were determined: reduced glutathione, glutathione *S*-transferase, glutathione reductase, glutathione peroxidase, malondialdehyde, superoxide dismutase. The most remarkable change was decrease in activity of glutathione *S*-transferase by more than 28% (Higher).

Taken together, the bilberries do not seem to influence significantly metabolism of xenobiotics and factors of oxidative stress (excluding aforementioned cases) but further research is needed.

Acknowledgement: This work was supported by GA ČR grant 303/12/G163 and IGA_LF_2017_011 project of Palacký University Olomouc.

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ANTIOXIDANT AND GENOTOXIC PROPERTIES OF EXTRACTS FROM FIVE *AGRIMONIA* AND *FILIPENDULA* SPECIES

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The species of Agrimonia and Filipendula have been traditionally used in folk medicine as anti-inflammatory herbs. Our recent studies revealed that the extracts of *F. vulgaris* and *F. ulmaria* leaves are rich in polyphenols (galloylglucosides, gallic and ellagic tannins and flavonol glycosides) possessing strong antioxidant capacity [1, 2]. This study is aimed at extending the knowledge on bioactivities of *F. palmata*, *A. eupatoria* and *A. procera*, *F. ulmaria* and *F. vulgaris*. Antioxidant properties of extracts were evaluated by $ABTS^{\cdot+}$, $DPPH^{\cdot}$ scavenging, and oxygen radical absorbance capacity (ORAC). Individual phytochemicals of examined extracts were identified by ultra-performance liquid chromatography - mass spectrometry (UPLC-Q-TOF-MS). Genotoxic activity of extracts was tested using alkaline single-cell gel electrophoresis (Comet) and cytokinesis-block micronucleus assays in human lymphocytes *in vitro* and the Ames *Salmonella*/microsome test. All investigated *Agrimonia* and *Filipendula* extracts showed negative results in the Ames test, indicating that they do not produce reverse mutation in bacterial cells. *Agrimonia* and *Filipendula* extracts were not genotoxic in the micronucleus test also. However, a slight though significant decrease of nuclear division index values was determined. In the Comet assay, extracts from *A. eupatoria* and *F. palmata* induced increase in primary DNA damage (expressed as mean percentage of DNA in the comet tail) in a clear dose-dependent manner, while extracts from *A. procera, F. ulmaria* and *F. vulgaris* in a dose-independent manner.

The results showed that studied extracts are a good source of bioactive compounds, thus *Agrimonia* and *Filipendula* extraxts may be classified as weakly genotoxic *in vitro* under conditions of the current study. These plants may be a promising material for the nutraceuticals and natural medicine.

Acknowledgments: The study was supported by the Research Council of Lithuania (SVE-06/11).

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CHEMICAL COMPOSITION OF VOLATILE AROMA COMPOUNDS IN FRESH *SATUREJA MONTANA* FROM ALBANIA AND KOSOVO

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Satureja montana (winter savory) is an important aromatic and medicinal plant [1]. In Balkans ethnobotany, it used for different purposes, such as a nutraceutical in Albania and a spasmolytic, anti-diabetic, respiratory tract infection treatment, antitussive and expectorant in Kosovo, whereas in the food industry, it is used as a flavouring agent [2]. The chemical composition of the volatile aroma compounds was defined in the fresh aerial part of *Satureja montana,* collected from 2 different localities, in Valbona (Albania) (S1) and Oshljak (Kosovo) (S2). The analysis were made by gas chromatography – mass spectrometry (GC-FID/MS) on HP5-ms column and equipped with automated headspace (HS) system with heated syringe sampler. 0.3 g of fresh plant material (homogenized samples from flower, leaf and stem) was put in sealed vials, heated (5 minutes, 80°C) and the gas phase was investigated. Total of 25 and 13 individual volatile aroma compounds were identified in the S1 and S2 samples of *S. montana*, representing 95.87% and 87.38% of the total content, respectively. Data analysis of the chemical composition revealed three different classes of components in S1 sample: monoterpene hydrocarbons (MH) 87.61%, oxygen containing monoterpenes (OM) 8.76% and sesquiterpene hydrocarbons (SH) 1.94%, as and two classes of components in S2 sample: MH (86.50%) and OM (0.44%).

The prevailing compounds in S1 and S2 samples were γ-terpinene (49.79 - 53.64%) and α-terpinene (9.05 - 16.33%), followed by α-thujene, myrcene and α-pinene. Carvacrol-methyl ether, cis-β-ocimene and carvacrol were found only in S1 sample and o-cymene in S2 sample.

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POLYPHENOLS IN DIFFERENT APPLE (*MALUS* SPP.) VARIETIES, CULTIVATED IN ESTONIA

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The aim of this study was to determine the composition and content of polyphenolic compounds in the peel, flesh and seeds of apple (*Malus* spp.) fruits and leaves. In total, 21 apple varieties were studied.

The polyphenols of ethanolic extracts of dried leaves, and peel (both 10% ethanol), flesh, and seeds (both 30% ethanol) were analysed by HPLC-ESI-MS/MS. For the identification and quantitation of individual polyphenols and calculation of total chromatographic polyphenol content (TP_{AUC}), the HPLC hyphenated with UV-Vis diode array and ion trap mass spectrometric (LC-ESI-MS/MS) detection (negative ionization mode) was applied.

The five apple varieties ´Antonovka´, 'Karksi renett´, ´Krista´, ´Cortland´ and ´Okero´ had the highest TP_{AUC} and their flesh, peel, seeds and leaves were further analysed.

Altogether 11 polyphenols were identified in the seeds, 33 in the peel, 23 in the flesh, and 25 in the leaves of these five apple varieties. The principal polyphenols were quercetin-galactoside in the peels (5.0 - 342.0 mg %), chlorogenic acid in the fleshes (77.0 - 398.0 mg %), phlorizin in the seeds (218.0 - 466.0 mg %) and chlorogenic acid in the leaves (147.0 - 446.0 mg %).

According to the differences in polyphenol contents (14 - 342 mg %), apples of ´Krista´ had the most valuable peel. Comparing fleshes, the best results were found in the case of ´Karksi renett´ (1 - 298 mg %), which also has the best leaves (37 - 333 mg %). The seeds of ´Okero´ are the richest (1 - 386 mg %) seeds in terms of polyphenol content. The principal polyphenols of different apple varieties were quercetin-galactoside in peel (5 - 342 mg %), chlorogenic acid in flesh (77 - 398 mg %), phlorizin in seeds (218 - 466 mg %) and chlorogenic acid in leaves (147 - 446 mg %).

The peel and flesh of local varietes ´Krista´ and ´Karksi renett´ were richest in polyphenols and thus a healthy supplement to everyday diet.

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PLANT *IN VITRO* CULTURES AS SOURCE OF COLLAGEN CROSS-LINKING SUBSTANCES

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Plants can be used as alternative source of potential protein cross-linking metabolites, so-called tanning agents [1]. These metabolites are relevant compounds for producers of collagen based biomaterials in the field of medicine technology and cosmetics as well as food and leather production. Cross-linking of collagen is performed almost exclusively using substances which bear a toxic potential or are produced on the basis of fossil fuels such as glutaraldehyde, isothiocyanates or chromium salts. Secondary plant metabolites show a less toxic behavior compared to glutaraldehyde for example. Furthermore, these substances can be sustainably obtained from plant resources. Plant biotechnological processes for the production of biological active ingredients provide a promising tool compared to traditional harvesting of field crops. However, in contrast to tanning processes based on chromium, procedures using plant material are more cost-intensive. Approaches to use plant-based cross-linking agents as alternative are mainly restricted by their availability, their stability as well as undesirable color reactions.

Within our work, in vitro cultures derived from *Gentianaceae* and *Oleaceae* have been established. Extracts of shoot and cell cultures have been tested for their cross-linking ability and the presence of secoiridoids. From these cultures, selected lines have been further investigated according to their growth and the production of the target metabolites. A process optimization for selected cultures will provide the basis for a scale-up of this bioprocess.

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INFLUENCE OF DIFFERENT MICROWAVE-ASSISTED EXTRACTION CONDITIONS ON TOTAL PHENOLICS CONTENT OF SOME MEDICINAL HERBS OF BALKAN AREA

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The Balkan is an area rich of natural resources, so we have to know how to explore antioxidant potential of medicinal herbs. Polyphenols are considered to be their dominant antioxidant ingredients which are the main reason to use them for the formulation of new cosmetic, food and pharmaceutical products [1]. The extraction of active components from natural sources depends on different factors. The knowledge of different extraction parameters effect is essential for the application of plant extracts and for prediction of extraction yield [2]. In this study, a microwave-assisted extraction (MAE) method was developed for the extraction of polyphenols from four herbs: *Morus nigra*, *Teucrium montanum*, *Teucrium chamaedrys* and *Symphytum officinale*. Various experimental conditions such as temperature (60, 100, 120 °C), solvent type and composition (ethanol, methanol, water and different proportions of ethanol : water and methanol : water), stirring speed (medium, high) and sample weight/solvent ratio (1 : 10, 1 : 20, 1 : 40) were tested. Total polyphenols content (TPC) of the prepared extracts was evaluated using the Folin-Ciocalteau procedure. All prepared extracts showed a significant difference in the TPC depending on applied conditions. Experimental TPC values were in the range 5.82 ± 0.69 to 68.37 ± 5.37 mg of gallic acid equivalent per gram of dry plant. The highest value of phenolic compounds was obtained in extracts of *Teucrium chamaedrys* prepared using following conditions: temperature 100 °C, ethanol : water (1 : 1; v/v), medium stirring speed and sample weight/solvent ratio of 1 : 40. Further research is ongoing based on the potential health benefits of Balkan area herbs.

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THE AMAZONIAN FRUIT *SOLANUM SESSILIFLORUM* (DUNAL) PRESENTS *IN VITRO* ANTITUMOR EFFECTS FROM APOPTOSE AND ANTIOXIDANT GENES MODULATION

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The aim of this study was to identify the potential antitumoral activity of *Solanum sessiliflurum* hydro-alcoholic extract. In this work chemical compounds were quantified in the extract, as well as their antioxidant capacity to confirm the presence of bioactivity. From this, it was performed an *in vitro* analysis of *S. sessiliflorum* anticarcinogenic effect against colorectal cancer (HT29) cell lines. Concentrations from 1 to 100 µg/mL decreased significantly the HT29 cell viability, as well as cell proliferation. The results show that *S. sessiliflorum* extract has important antioxidant bioactive molecules in its composition that probably contributes to protect the cells against oxidative stress. The results showed an acute effect (6 h of exposition) of low extract concentration (30 µg/mL) on antioxidant genes modulation. When compared to HT29 untreated cells, the cells exposed to *S. sessiliflorum* extract exposed cells presented a strong upregulation of MnSOD gene, which was not accompanied by a similar increase in the expression of GPX and CAT genes. The BAX/Bcl-2 gene expression ratio of HT29 cells treated with cubiu extract was 3.23 ± 0.39 indicating the initiation of apoptosis pathway. The CASP 3 and 8 genes also showed upregulation despite the short time of exposition to extract. These results corroborate the cytotoxic effect observed in MTT and LDH assay after 24 hours exposition.

The antitumoral effect against cancer cells it seems involves differential modulation of genes related to oxidative imbalance and apoptosis pathway.

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SPECIES OF THE *BAUHINIA* GENUS USED IN DIABETES THERAPY IN MATO GROSSO, BRAZIL

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Medicinal plants are alternatives to diabetes therapies. This disease reaches more and more people. From the beginnings, medicinal plants have been used. Scientific studies can validate or counter popular medical applications. Many botanical species, genera and families are used in folk medicine. It is revealed species of the genus Bahuinia (Fabaceae) used in diabetes therapy in Mato Grosso, Brazil. In a first stage [1], the study was based on bibliographic sources (FBi) existing in the Public Universities of Mato Grosso (UFMT, UNEMAT). In a second step [2], documents (DOC) have been retrieved from studies published online. We applied Google's Academic-GA search capabilities, instructing the search box (BOX) with [Allintitle:"*scientific name*" „diabetes"]. Of more than 500 FBi consulted on medicinal plants used in Mato Grosso (published until 2009), 92 FBi mention 133 species (52 families) used in diabetes therapy. The Fabaceae family is present in 70 FBi and with 22 species (16.54% of all). The genus *Bauhinia* contributes with 10 species (45.45% of Fabaceae) and on 37 occasions they are reported in FBi. In descending order the ten most mentioned species (FBi) are: *B. forficata* Link. (11); *B. rufa* (Bong.) Steud. (10); *B. nitida* Benth. (9); *B. cheilantha* (Bong.) Steud. (1); *B. glabra* Jacq. (1); *B. longifolia* (Bong.) Steud. (1); *B. longipetala* (Benth.) Walp. (1); *B. splendens* Kunth (1); *B. ungulata* L. (1); *B. rubiginosa* Bong. (1). The only species that recovered DOC online, in the years of 2012 and 2017, was the *Bauhinia forficata* Link (7 in 29dez20123; 21 in 30may2017). This species has mainly studies of ethnoboconduction, phytochemicals, pharmacological. Popular knowledge confuses the other species with *B. forficata.*

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THE MELÃO DE SÃO CAETANO (MOMORDICA CHARANTIA L.) IN THE CONTROL OF DIABETES

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The Melon of S. Caetano-MSC (*Momordica charantia* L.) is used for the control of Diabetes by popular medicine in Mato Grosso, Brazil [1]. However it is necessary to know if the use has effectiveness and safety. Scientific studies probably have these answers. We analyze documents (DOC) with scientific studies that confirm or not confirm safety and efficacy with the popular use of the plant (MSC) for diabetes. The analytical sources used are scientific DOC retrieved via the internet (through Google Academic-GA) using the toolbox [BOX] [allintitle: "scientific name" "diabetes"] [2]. Several DOCs with chemical and pharmacological studies were analyzed that could be related to the antidiabetic effects of MSC. Predominantly studies confirm hypoglycemic effect, but that depends on the part of the plant, phase, concentration, types of extract and of study. Those who do not confirm hypoglycemic effect recommend improving research. Excessive intake of MSC juice can cause abdominal pain and diarrhea. Excessive ingestion of seeds can trigger headache, fever and even coma in severe cases. The use of MSC is not recommended for pregnant women because the abortive effect. The beneficial bioactivity of MSC in diabetes therapy is confirmed by most studies, but use should be guided by practitioners specializing in herbal medicine. The most commonly used parts are ripe fruits and leaves of the plant. MSC depending on the dose and part ingested (mainly from the seed) may increase health risks rather than benefits.

Acknowledgments: FLOBIO Group (Bioactive Flora Study)/UNEMAT-CNPq-FAPEMAT.

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THE ANTICANCER EFFECTS OF LUPEOL AND BETULIN ON HUMAN BREAST CANCER CELL LINES

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An epidemiological study on the Mediterranean diet suggested that diets rich in terpenoids are related to low cancer cases [1]. Due to its promising antitumor properties, terpenoids as natural compounds extensively found in plants have attracted the attention of researcher the past few decades [2]. This study focuses on how betulin and lupeol, naturally occurring triterpenoids, affect the cell proliferation and induction of apoptosis in breast cancer cell lines. To examine the antiproliferative effect of these terpenoids, breast cancer cells were treated with lupeol and betulin, with the concentration ranging from 5 µg/mL to 50 µg/mL and 1 µg/mL to 40 µg/mL, respectively. From the MTTs results, the IC_{50} values obtained for lupeol on MCF-7 and MDA-MB-321 are 25 ± 1.2 µg/mL and 17 ± 1.1 µg/mL, respectively. Meanwhile, betulin possessed an effect on the proliferation of MCF-7 and MDA-MB-321 with the IC_{50} values of 14 ± 3.0 µg/mL and 12 ± 0.4 µg/mL, respectively. Besides that, the morphological changes such as cell shrinkage, and nuclear condensation were observed in both cells treated with betulin and lupeol, which acts as a proposition of apoptosis occurred during the treatment [3]. This preliminary data suggest that the selected terpenoids are capable of inhibiting cancer cell growth potentially through apoptosis pathway. However, further investigation is required to support this statement.

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THE IMPORTANCE OF MEDICINAL PLANTS IN THE DOMINICAN REPUBLIC: ORIGIN AND USES

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In the Dominican Republic, the Traditional Dominican Health System (TDHS) is one of the two medical systems that can be distinguished: the formal medical system and the informal medical system two. The formal medical system is based on the Western conventional medical system. The TDHS is based on the ancient concepts and practices in the Dominican culture, which has its basis in three separate cultures, the Taino (the original inhabitants), Spanish (the colonizer) and African culture (the enslaved African workforce). To formulate a national health policy based on the divers' Dominican culture, and hence integrate medicinal plants in the official health system, it will be necessary to know the uses of the medicinal plants in the country. The WHO stated, that a country, with an active traditional health system, wants to formulate a national health care policy, it should have a broad knowledge of the traditional health practices. In the Dominican Republic, until now, an extensive, systematic research on the use of medicinal plants has been lacking. To fill this gap we formulated a project: National Study of the Medicinal Plants of the Dominican Republic. In 2000, a questionnaire was developed in cooperation with the Pan-American Health Organization (PAHO). More than 8000 interviews with about 1600 persons in each of the provinces of the Dominican Republic were conducted. Preliminary results of this national Study will be presented and discussed. Also data on the origin of the medicinal plants and the formulation of a local pharmacopeia will be presented [1-4].

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COMPARATIVE ANALYSIS OF METHODS FOR EXTRACTION OF VOLATILE COMPOUNDS FROM FLOWER PETALS AND WHOLE FLOWERS OF ESSENTIAL OIL ROSE (*ROSA DAMASCENA* MILL)

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The progress in oil rose (*R. damascena* Mill) research in recent years related to the development of segregating populations, the application of DNA markers and GC/MS analysis of volatile compounds has created new opportunities for accelerated breeding of new lines and varieties. The latter requires the need to evaluate a large number of plants in segregating populations about their potential for production of high quality rose oil. In 2011 Rusanov et al. [1] published a method for extraction of volatile compounds from rose flowers. Despite the good results obtained, the method is relatively labor-intensive, related to the use of large volumes of liquid nitrogen and the grinding of a large number of samples using laboratory mill. Moreover, the observed broad segregation of flower traits like petal number in rose populations creates concerns about the suitability of working with extracts from whole flowers rather than from rose petals where the majority of rose oil is located which could potentially mimic the effect of QTLs. In the present study, we present results from the comparison of several methods for extraction of volatile rose oil compounds, including: 1) extraction of whole rose petals immersed in organic solvent; 2) extraction of rose petals frozen in liquid nitrogen and ground to powder and 3) extraction of whole flowers frozen in liquid nitrogen and ground to powder. The obtained results are the basis for successful implementation of accelerated breeding in the oil rose through early selection of plants with good potential for obtaining high quality rose oil.

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ENHANCEMENT OF TRIPERPENOID COMPOUNDS IN *SOLANUM LYCOPERSICUM* CV MICRO-TOM ELICITED CELL CULTURES

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The dwarf rapid-cycling of "Micro-Tom" (MT) tomato (*Solanum lycopersicum*) has been shown to be a representative plant model of the Solanaceae and other crops with fleshy, berry-type fruits. MT tomato is currently being used for plant genetic, genomic, and physiological and developmental studies. In tomato, terpenes are found in large quantities in the trichomes of leaves, stems, young fruits and partly in flowers. The triterpenoids have been studied extensively in the cuticle of tomato fruits and have found different terpenoids such as α, β, δ -amyrin, taraxasterol, lupeol, lanasterol, cicloartenol, cholesterol, stigmasterol and β-sistosterol [1].

The biotechnological production of valuable secondary metabolites in plant cell or organ cultures is an attractive alternative to the extraction of whole plant material. Multiple strategies have been used in order to raise of bioactive compounds produced from plant cell cultures. Among them, elicitation is the one most likely to result in an important increase on yield improvement. Previous studies have showed that the use of cyclic oligosaccharides like methyl-β-cyclodextrins and methyl-jasmonate as elicitors, are very effective in stimulating the production of phytosterols and taraxasterol in *S. lycopersicum* cv suspension-cultured cells (SCC) [2]. In this work, SCC of MT tomato were used to evaluate the effect of cyclodextrins, β-glucan and (Z)-3-hexenol separately or in combination under elicitation as a promising alternative for the sustainable production of triterpenoid compounds.

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THYMBRA CAPITATA CELL CULTURES AS SOURCE OF BIOACTIVE COMPOUNDS

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Thymbra capitata (L.) Cav. is a type of thyme species with circum-Mediterranean distribution. This species is characterized by a high chemical homogeneity in the composition of its essential oil, presenting a simple chemotype carvacrol in a relative proportion between 60-70%. Thus, because of the richness of its essential oil in carvacrol, it has been reported to exert antiseptic power for treatment of skin and oral diseases, besides having anti-inflammatory and antimicrobial properties [1].

Despite the importance of these compounds for the physiology of plants their production naturally by plants constitutes less than 1% of dry weight of the plant and depends mainly on the physiological and development state of the plant. Currently, most of these metabolites are directly obtained from plants by extractive techniques. Although obtaining essential oils from extracts of vegetable raw materials is the basis of a very important economic activity, there are increasingly more problems for the continuity in the use of these procedures due mainly to the overexploitation of wild materials. There are alternatives of biotechnological production that are based on the use of *in vitro* cultures. This system constitutes a source sustainable of high value compounds, due to the advantages of its use since these crops are independent of geographic, seasonal and environmental factors.

The aim of this work was to evaluate the capacity of *T. capitata* cell cultures to produce bioactive compounds such as phenolic and terpenoid compounds.

Acknowledgements: This work has been supported by CDTI (IDI-20150891).

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EXPLORATION OF DIETARY PLANTS FROM WORLDWIDE BIODIVERSITY AIMING THE DISCOVERY OF ACTIVE AGENTS WITH ANTI-AGEING EFFECTS

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Dietary interventions obtain a profound impact on human lifespan, while constitute edible plants a promising source of chemical entities that promotes healthy ageing [1]. Maqui (*Aristotelia chilensis*, Elaeocarpaceae [Molina] Stunz), is a small tree native to South Chile, deeply rooted in traditional dietary culture of indigenous. Moreover, carob tree (*Ceratonia siliqua*, Leguminosae L.) has been grown since antiquity in most countries of Mediterranean region, composing a staple in the diet of people in times of famine. Maqui berries and carob pods, have drawn attention due to their potential health benefits, which are largely attributed to their rich polyphenolic content.

A high-throughput phytochemical investigation of methanol and water extracts of the edible parts, berries and pods respectively, was performed. Fingerprinting procedures using HPTLC showed that both plants had rich chemical profile, while phenolic compounds prevailed. Profiling and dereplication procedures employing hyphenated techniques (HPLC/UHPLC-PDA, LC-(ESI)-HRMS/MS) were also applied and various putative bioactive compounds were identified. Hence, analytical and preparative techniques afforded compounds belonging to anthocyanins, flavonoids, flavonoid glucosides and phenolic acids, followed by structure elucidation (1D & 2D NMR). The *in vivo* evaluation of anti-oxidant and anti-ageing properties of maqui and carob pods water extracts in *Drosophila melanogaster* flies revealed, that oral administration of both activate the Nrf2/Keap1 antioxidant pathway and proteasome peptidase activities. Complementary to the above, the metabolism of both dietary fruit extracts was investigated *in vitro* using rat liver microsomes. Conclusively, maqui and carob pod water extracts could be a potent source of anti-oxidant/ anti-ageing nutraceuticals.

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FROM LEAVES, TO OLIVES AND OLIVE OIL. ARE DIFFERENT CULTIVARS POTENTIAL MARKERS FOR OLIVE PRODUCTS?

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Since olive products processing elevated over the past few decades and the world becomes more diet-conscious, the investigations of olive biophenols are attracting scientific attention so as to shed light to the diverse range of bioactivities such as antioxidant, antimicrobial and anticancer [1]. Olive leaves, olive processing residues constitute an enticing prospect for natural antioxidants and could also be used for olive-leaf extract production [2] as there is an observed increased demand regarding the use of olive leaf extract in foodstuffs, food additives and functional food materials. As the phenolic content of olive products is influenced by the variety, the main objective of this study was the determination of composition of the phenolic compounds in leaves, fruits and olive oil as well as on transfer between matrices. The phenolic composition was studied in twenty-two cultivars grown in the same grove under the same agronomical and environmental conditions in an effort to examine the effects of cultivars and determine possible varietal indicators. These indicators could be used as tools in order to confirm the provenance of unknown samples. The phenolic fractions were identified and quantified using liquid chromatography-mass spectrometry.

The results indicated qualitative and quantitative differences in phenolic composition among cultivars in both matrices, as well as regarding the transfer rate of phenolic compounds from fruits to oil.

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CHEMICAL SCREENIG OF ROMANIAN RED CLOVER EXTRACT

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Naturally occurring endogenous products from Romanian flora are vary diverse. And only a small percent was evaluated through modern techniques to highlight their chemical composition. It should be noted that a great majority from these plants are used for centuries in Romanian phytotherapy [1-3].

Red clover, a modest plant widely spread in Romania it is valued especially as animal feed. Its healing properties (dermatological diseases, osteoporosis, anticancer, menopause, etc) are not yet well known. However, recent studies had showed the presence of a series of bioactive compounds such as: isoflavons, isoflavonoids, volatile oils, sterols, phenolic acids, terpenes [4].

The aim of the present study was to investigate the bioactive molecules of red clover alcoholic extract profile using Ft-IR spectroscopy and LC-MS chromatography techniques.

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ANTIPSEUDOMONAL AND ANTIBIOFILM ACTIVITY OF QUERCETIN EXTRACTED FROM AJWAH DATE PALM (*PHOENIX DACTYLIFERA* L.)

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From the recent literature, there is not much information about the antipseudomonal and antibiofilm influence of Ajwah date palm. The current analysis was supported out to assess the antimicrobial of flavones extracted from the Ajwah date palm. Ajwah presented high total flavonoid contents were 159 mg QE/100 gram fw. The HPLC chromatogram exhibits the occurrence of peaks fascinating at 370 nm, which are probable conjugates arising from quercetin depend on considered masses. To get the dynamic constituent linked to the antibacterial activity, date was extracted with MeOH and fractionated sequentially with n-hexane, $CHCl_3$, and EtOAc. Antibacterial actions of these portions beside *P. aeruginosa* were experienced. The MeOH and $CHCl_3$ fraction exhibited important MIC and MBC values in contrast with the other fractions. Consequently, the $CHCl_3$ part was engaged for additional purification. Biofilms made by *P. aeruginosa* strains were totally destroyed subsequent coverage to 5% of quercetin for 1 h.

Quercetin extracted from Ajwah date palm (*Phoenix dactylifera* L.) presented noteworthy antipseudomonal and antibiofilm and denote unique principals studies may permit the progress of a pharmacologically satisfactory antimicrobial agent.

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CHEMOTYPE DIVERSITY OF NATIVE *VERBASCUM SONGARICUUM* POPULATIONS IN IRAN

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The genus *Verbascum* L. (Scrophulariaceae), commonly known Mullein, includes about 400 species in the Old World [1]. The plant species are rich sources of flavonoids and saponins which used in folk medicine [2]. The genus is widespread in Iran by 44 species of which 20 are endemic. The most of the species have a restricted distribution, except *V. songaricum* Schrenk which have the broadest distribution in the Irano-Turanian region [3]. To identify chemotypes of *V. songaricum* in Iran, the essential oils (EOs) composition of wild-growing populations was determined by GC-FID and GC-MS analyses. Aerial parts of *V. songaricum* populations (VSPs) were collected at full flowering stage from the three different geographical regions including Quchan (VSP1), Yasuj (VSP2) and Bastam (VSP3). The EO content (v/w %) was in ranging from 0.11 to 0.15%. The total number of compounds identified and quantified was 31 in VSP1, 33 in VSP2 and 29 in VSP3, representing 97.5%, 95.1% and 89.1% of the total oil, respectively. The major compounds of the EOs were hexacosane (0 - 35.4%), methyl chavicol (0 - 23.4%), hexahydrofarnesyl acetone (6.5 - 16.4%), heptacosane (0 - 15.3%) and α-bisabolol (0 - 13.6%). Aliphatic hydrocarbons (48.8 - 74.7%) were the main group of compound in the oil of studied VSPs. For the determination of the chemical variability as well as chemotypes, the EO components were subjected to cluster analysis (CA). The two different chemotypes characterized were *Chemotype I* (hexahydrofarnesyl acetone, heptacosane), and *Chemotype II* (hexacosane, methyl chavicol). The *in situ* and *ex situ* conservation strategies should concern all populations representing the different chemotypes.

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METABOLITE PROFILING OF TWO BULGARIAN COLCHICUM SPECIES

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The genus *Colchicum* is presented in Bulgaria by 12 species according to the recent taxonomical revisions.

In the present study, we have analyzed by GC-MS the metabolite profiles of methanolic extracts of seeds, leaves, and bulbs from 7 populations of *C. automnale*, collected from regions with different altitude and 2 populations of *C. diampolis* from the regions of Yambol and Karnobat, Bulgaria.

More than 80 metabolites, including alkaloids (colchicine and its derivatives), organic acids, amino acids, fatty acids, saccharides, sterols etc. have been identified in the samples. The results showed intra-species similarities in the metabolite patterns of *C. automnale* and *C. diampolis* and considerable differences between the species, indicating that the metabolite profiling can be used as a support for taxonomical decisions in the genus *Colchicum*.

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ANTIDIABETIC AND ANTIOXIDANT EFFECT OF THE AERIAL PARTS OF *LYSIMACHIA VERTICILLARIS* AND ITS ISOLATED PHENOLIC COMPOUNDS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Lysimachia genus (Primulaceae) has been represented by 8 species in Turkish flora. *Lysimachia* species have been used for wound healing and as antipyretic in Turkey [1, 2]. The goal of the present study is to evaluate antidiabetic and antioxidant effect of the aqueous extract and isolated compounds (gallic acid, myrcetin 3-*O*-*α*-L-rhamnopyranoside and quercetin 3-*O*-*β*-D-glucopyranoside) from *Lysimachia verticillaris* in normal and streptozotocin (STZ) induced diabetic rats. Male Spraque Dawley rats were divided into 6 groups. STZ (40 mg/kg) induced diabetic rats were treated with water extract (400 mg/kg) and isolated compounds (20 mg/kg) by orally, besides non-diabetic control group were treated with equal volume saline for 14 days. At the end of experimental duration, to interpret antidiabetic effect, blood glucose, serum insulin levels were detected. Serum samples from rats were also analysed in terms of some biochemial parameters, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) to investigate antioxidant effect. Oral administration of the extract and isolated compounds reduced high blood glucose levels ($p<0.005$). Morever, the treatments were resulted in increased serum insulin (($p\leq0.05$), cat ($p\leq0.05$), SOD ($p\leq0.05$) and GPX ($p\leq0.05$). According to results, the extract and isolated compounds of *L. verticillaris* displayed antidiabetic effect, while quercetin 3-*O*-*β*-glucopyranoside showed the highest antidiabetic effect. Thus, *L. verticillaris* can be potential a source of herbal medicine for diabaetes and its complications.

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IN VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY STUDIES ON AERIAL PART EXTRACTS OF ENDEMIC *TANACETUM ARGENTEUM* (LAM.) WILLD. SUBSP. *ARGENTEUM* (L.) ALL.

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Tanacetum L. is an important genus of Asteraceae family which is represented by about 160 species worldwide. The genus is also represented with forty six species in Turkey [1]. *Tanacetum* species are used in the treatment of arthritis, fever, migraine, menstrual disorders, stomach-ache, toothache, and insect bites in the folk medicine. Also, it is reported that different *Tanacetum* species have anticancer, antimicrobial, anti-inflammatory and antioxidant activity [2]. This species contain essential oils, flavonoids and sesquiterpene lactones as secondary metabolites [2,3].

In this study, total phenolic contents, antioxidant and anti-inflammatory activities of methanol extract (TAM) of *Tanacetum argenteum* (Lam.) Willd. subsp. *argenteum* (L.) All. and hexane (TAH), chloroform (TAC), and aqueous methanol (TAAM) fractions of its were evaluated by Folin-Ciocalteu, DPPH/ABTS, lipoxygenase inhibition assays, respectively.

TAM extract showed highest antioxidant activity in DPPH and ABTS assays with IC_{50} values of 154.7 and 115.3 µg/mL, followed by TAC (155.8 and 118.4 µg/mL), TAAM (160.9 and 196.0 µg/mL), and TAH (1140 and 464.5 µg/mL) extracts, respectively. The highest total phenol content was found in the chloroform fraction of methanol extract of *Tanacetum argenteum* subsp. *argenteum* (88.08 mg of GAE/g of dried extract). TAC extract at a concentration of 156 µg/mL showed highest anti-lipoxygenase activity with inhibition rate of 68.02%, followed by TAAM (43.54%), TAM (38.19%), and TAH (24.61%) extracts.

The results obtained in the present study indicate that all extracts except TAH, especially TAC extract, could be a potential source of anti-inflammatory and antioxidant agents.

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CHEMICAL COMPOSITION OF HEXANE EXTRACTS AND AROMA COMPOUNDS OF *HYPERICUM PERFORATUM* L., HYPERICACEAE

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In traditional medicine, *Hypericum perforatum* has been used for healing skin wounds and burns as well as for treatment of alimentary tract diseases and psychological disorders like depression [1]. Recently, *H. perforatum* has been shown to have antioxidant, anti-inflammatory and cytotoxic activity [2]. Chemical composition of *H. perforatum* extracts has been studied, but still very little is known about its aroma components, thus this study was designed to examine the chemical composition of the hexane extract as well as aroma compounds in the aerial parts of *H. perforatum* collected from different locations in Western R. Macedonia during the year 2015/16.

GC/MS analyses of the hexane extract prepared from leaves and flowers resulted in the identification of 64 compounds representing 80.55 - 93.34% of the total extract. Among hydrocarbon components, octacosane (up to 48.57%) and nonacosane (up to 44.06%) were predominant in all samples while tetratetracontane was identified only in flower extracts from Tetovo (9.13 - 15.61%). Monoterpenes were dominated by α-pinene (0.86%) while sesquiterpenes consisted mainly of caryophyllene oxide (0.40 - 3.20%).

HS/GC/MS analyses of volatile aroma compounds of dried aerial parts of *H. perforatum* resulted in the identification of 23 components representing 93.89 - 99.96% of total amount. In class of terpenoids, monoterpenoid fraction was dominated by α-pinene (8.06 - 35.08%), while sesquiterpenoids consisted mainly of caryophyllene E (7.94%). Compared to this, aliphatic hydrocarbons were present in higher amount (up to 88.66%) and were consisted mainly of alkanes and their derivatives. Among all identified components, octane-2-methyl was predominant (34.68 - 74.62%).

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FLAVONOIDS IN POPULATIONS OF *ASTRAGALUS MONSPESSULANUS* SUBSP. *ILLYRICUS* IN BULGARIA

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Astragalus monspessulanus L. subsp. *illyricus* (Bernh.) Chater is a rare plant in the Bulgarian flora known from few localities found at different altitude and floristic regions in the country. In continuation of an ongoing research we investigated the variations between two populations of the species both in morphological and phytochemical aspect. The populations showed differences in leaf morphology and fruit length. Flavonoids acylated with 3-hydroxy-3-methylglutaric acid were selected as chemotaxonomical markers. The flavonoid content in extracts obtained from aboveground parts of the species was analysed by UPLC coupled with HRESIMS.

The results of this study showed small differences in the genotype and significant variation in flavonoid composition of the subspecies collected from both populations. These data as well as the comparison with subsp. *monspessulanus* give us a reason to suggest a species rank for *Astragalus monspessulanus* L. subsp. *illyricus.*

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PHENOLIC COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF *HYPERICUM PERFORATUM* L. TRANSGENIC SHOOT EXTRACTS

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Hypericum perforatum transgenic shoots (TS) were spontaneously regenerated from the corresponding hairy roots induced by *Agrobacterium rhizogenes* A4-mediated transformation. The aim of this study was to evaluate the phenolic compounds composition and antimicrobial activity of *H. perforatum* transgenic shoot extracts. The HPLC/DAD/ESI-MS^n analysis of TS extracts revealed the presence of phenolic acids, flavan-3-ols, flavonol glycosides and aglycones, anthocyanins, naphthodianthrones, acyl-phloroglucinols and xanthones. The TS extracts exhibited strong inhibitory activity against *Staphylococcus aureus* ATCC 6538 due to the presence of xanthones such as padiaxanthone, 1,3,6,7-tetraxydroxyxanthone 2-prenyl xanthone and 1,3,6,7-tetraxydroxyxanthone 8-prenyl xanthone. The antibacterial activity of TS extracts against *Bacillus subtilis* ATCC 6633 was related to the content of γ-mangostin. Transgenic shoot extracts exhibited strong inhibitory activity against *Penicillium* sp. that was correlated with accumulation of 3-feruloylquinic acid. The antimicrobial activity of TS extracts towards *Pseudomonas aeruginosa* ATCC and *Saccharomyces cerevisiae* FNS YCC1 was attributed to the amounts of chlorogenic acid, hypericin, garcinone C and cadensin G. Anticandidal activity of TS extracts against *Candida albicans* ATCC 10231 was associated with the production of hyperforin, quercitrin, rutin, quercetin and dimethylmangiferin.

Present study demonstrates that *H. perforatum* transgenic shoots represent a promising biotechnological system for enhanced production of antimicrobial compounds that can be effectively used for treatments of bacterial and fungal diseases.

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ANTIOXIDANT ENZYMES AND OXIDATIVE STRESS MARKERS IN *HYPERICUM PERFORATUM* L. HAIRY ROOTS

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The activation of antioxidant enzyme system is one of the main defense responses of plant tissues against oxidative burst upon biotic stress. The objective of this work was to investigate the correlation between antioxidant enzymes and oxidative stress markers in *Hypericum perforatum* hairy roots (HR) induced by *Agrobacterium rhizogenes* A4-mediated transformation. When the antioxidant enzymatic system in transgenic roots was evaluated in response to genetic transformation, an up-regulation of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) resulted in suppression of oxidative stress markers hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet-}$). The SOD activity (2.48 - 19.77 U/mg proteins) in HR cultures correlated with decrease in $O_2^{\bullet-}$ levels [0.19 to 0.71 nM/(min·g FW)]. The activity of APX (14.42 to 223.15 pkat/mg proteins) and CAT (0.34 to 3.00 nkat/mg proteins) in HR extracts greatly contributed to the reduction of H_2O_2 amounts (0.21 - 0.74 μM/g FW). On the other side, the peroxidase (PX) activity (0.01 to 0.96 nkat/mg proteins) did not correlate neither with H_2O_2 nor $O_2^{\bullet-}$ levels. The PX activity in HR cultures was compensated by APX and CAT that acted concurrently to scavenge H_2O_2.

The process of genetic transformation was followed by strong perturbation of cell redox state in *H. perforatum* HR leading to the activation of antioxidant enzymes for protection of tissues from oxidative damage.

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BACTERIAL SUSCEPTIBILITY TO AGNPS SYNTHESIZED FROM EXTRACTS OF *TETRADENIA RIPARIA*

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Infectious diseases, especially those of bacterial origin, remain major public health threats globally, particularly in sub-Saharan Africa [1]. This is further exacerbated by the current global crisis of bacterial resistance to several drugs [2]. Breakthrough innovative strategies are therefore required to effectively manage bacterial-borne infections. Metal nanoparticles derived from plant extracts have been shown to have important applications in medicine. The leaves of *Tetradenia riparia* (Iboza), an important medicinal species in South Africa, are reported to contain various terpenoids and pyrones which have been used in the reduction of silver nitrate ($AgNO_3$) to nano-silver particles. Preliminary anti-bacterial testing using the micro broth dilution method confirmed that AgNPs synthesized using leaf methanol extracts [3] yielded positive results. The AgNPs were active and showed MIC against *Escherichia coli* ATCC-35218 (1.56 µl/ml), *Klebsella pneumonia* ATCC 700603 (1.56 µl/ml), *Pseudomonas aeruginosa* ATCC 27853, (12.5 µl/ml) and *Staphylococcus aureus* ATCC-43300 (Methicillin Resistant *S. aureus* - MRSA) (50 µl/ml). Methanol as a negative control showed no activity against MRSA. Subsequent testing using aqueous extracted and then freeze dried AgNPs yielded a MIC of 2 - 64 µg/ml against MRSA ATCC-43300. Vancomycin was used as the positive control. Further testing especially with *E. coli* and various strains of MRSA is ongoing and the results will be fully reported.

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POLAR CONSTITUENTS OF *STACHYS CANDIDA* BORY & CHAUB. (LAMIACEAE)

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Stachys candida Bory & Chaub. is a Greek endemic chasmophyte, growing on rocky places and calcareous habitats in Southern Peloponnisos [1, 2]; it is used in folk medicine against genital tumors, sclerosis of the spleen, inflammatory tumors and cancerous ulcers [2]. We report, herein, on the isolation and identification of the polar secondary metabolites of its aerial parts.

The isolated compounds have been identified as acteoside, chlorogenic acid, chrysoeriol-7-*O*-β-D-(3′′-E-p-couma-royl)-glucopyranoside, chrysoeriol-7-*O*-β-D-glucoside and iso-scutellarein-7-*O*-[6′′′-*O*-acetyl-β-D-allopyranosyl)-(1→2)-β-D-glucopyranoside.

The structures of the isolated compounds were elucidated by high field NMR spectroscopy (^{1}H-NMR, ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC).

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EARLY PREGNANCY TREATMENT WITH *LEPIDIUM MEYENII* L. RESULTS IN INCREASED POSTPARTUM MATERNAL CARE

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Lepidium meyenii, known as Maca, is a Peruvian hypocotyl growing exclusively between 4000 and 4500 m altitude in the central Peruvian Andes and is used traditionally to enhance reproduction. This study was aimed to evaluate effects of *Maca* on reproductive output and maternal care in NMRI pregnant female mice.

Pregnant female mice were treated with water extract of Maca on gestational days 8 to 10. Litter number, Litter weight and size, and male/female were measured in the birth day. On day 5, 10 and 15 after birth maternal behaviors were monitored in the home cage, two times a day from 9-10 am and 5-6 pm.

Our data shows that treatment with Maca extract during pregnancy results in increased male/female ratio, pups weight and increased maternal behavior such as nursing and licking of pops.

Maca is well known for its beneficial effects on sexual activity of adults. Our results suggest that treatments of pregnant mothers and prenatal exposure of fetus may also have beneficial effects on reproduction.

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EFFECTS *EPIMEDIUM GRANDIFLORUM* L. EXTRACT ON REPRODUCTIVE BEHAVIORS AND SPERMATOGENESIS IN ADULT MALE MICE

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Introduction: This study was designed to evaluate the aphrodisiac and spermatogenic potential of the aqueous extract of *Epimedium grandiflorum* L. in adult male mice.

Methods: Adult male NMRI mice were treated with mix of *Epimedium grandiflorum* L. leaves (0.2, 0.4 and 0.8 mg/kg) for two weeks. Sexual behaviors of treated and control mice evaluated, using receptive females and sperm samples were collected from cauda epididymis.

Results and Discussion: Our result showed that continues treatment with mixture of *Origanum vulgare* L. for two weeks resulted in increased sexual behaviors like, sniffing, following, jumping and ejaculation. Treated mice also showed increased spermatogenesis factors such as increased sperm count, motile sperms and increased progressive movement.

Conclusion: Results obtained in the current study reveals that *Epimedium grandiflorum* L. can improve aphrodisiac and reproductive factors in adult male subjects.

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SOLVENT-FREE MICROWAVE EXTRACTION OF ESSENTIAL OIL FROM CHERRY LAUREL (*PRUNUS LAUROCERASUS*) LEAVES

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Essential oil from cherry laurel (*Prunus laurocerasus*) fresh leaves was obtained by solvent-free microwave extraction. This "green" extraction method is a combination of microwave heating and distillation without adding any solvent or water. The process was carried out at different microwave powers (300, 450, 600 W), atmospheric pressure and with a Clevenger-type apparatus. The resulting oils were subjected to the GC-MS analysis.

The total content of essential oil in fresh leaves of cherry laurel was 0.38 ± 0.01 ml/100 g of fresh plant material, independently of the applied microwave power, but the time required to isolate the total amount of essential oil were 7.8 ± 0.2; 12.4 ± 0.1 and 18.9 ± 0.4 min under microwave power of 600, 450 and 300W, respectively. The most abundant compounds of obtained oils were benzaldehyde (over 80%), followed by mandelonitrile, benzoic acid and 2-hexenal. Composition of the analyzed oils was similar but depended on the applied microwave power. The content of benzaldehyde slightly increased, while the content of benzoic acid significantly increased by increasing the microwave power from 300 to 600 W. In contrast, mandelonitrile concentration was reduced under higher microwave powers.

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METABOLITE PROFILING AND THE INTRASPECIFIC VARIANCE OF *HYPERICUM PERFORATUM*

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The genus *Hypericum* comprises more than 450 species. *H. perforatum* L., commonly known as St. John´s Wort, is used for the treatment of mild to moderate depressions. Recently other bioactivities such as antiviral, antibacterial, and anti-Alzheimer have been described. The major active constituents are phloroglucinols (hyperforin), naphthodianthrones (hypericin) and flavonoids. The composition of these secondary metabolites is genetically determined and highly influenced by abiotic and biotic environmental conditions.

In this project, comparative metabolite profiles of *H. perforatum* accessions with different genetic background will be investigated to estimate the intraspecific variance caused by the genotype. Therefore, screening methods were developed, using 1D-NMR, 2D-NMR and LC-MS, to detect several hundred secondary metabolites simultaneously [1, 2]. The coherences between these complex chemical profiles and line characteristics such as genetic type, ploidy level and origin [3] were evaluated with multivariate data analysis. Furthermore, this approach can be applied to efficiently identify bioactive compounds in complex mixtures. To do so, the metabolite profiles will be compared with various bioassay results from the corresponding sample using computational correlation analyses to determine the bioactive constituents.

This untargeted metabolomics concept combined with multivariate data analysis enables the investigation of the natural product diversity within the species and the selection of genotypes with a suitable compound composition according to the proposed pharmacological application of the plant.

Acknowledgements: Leibniz Association (SAW-2015-IPB-2).

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EXPLOITATION OF BY-PRODUCTS DERIVED FROM HYDRODISTILLATION OF ROSE (*ROSA DAMASCENA* MILL) PETALS

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Rose oil is one of the most expensive essential oils, often referred as "liquid gold". During its production from fresh rose flowers by hydrodistillation, significant amounts of waste water are produced and discarded in the fields. The waste water from rose oil distillation is actually a water extract of rose petals, which is reported to extend the life span of Drosophila flies [1] and to exhibit moderate anti-HIV activity [2]. Moreover, this extract is rich in phenolic compounds, possesses significant antioxidant activity and thus could be as functional food ingredients [3-5]. So, it is rightly expected that the byproduct of rose hydrodistillation could be a readily available source of valuable compounds with potential to be applied in food and cosmetics industries. However, the data concerning the chemical composition of the waste water from rose distillation are very scarce.

Under the frame of the European project 'EXANDAS', several samples of the above mentioned byproduct were collected and their exploitation for the development of high-added value natural products was investigated. In more details, aqueous extract was treated with liquid-liquid extraction technique, using several ratios between the mixture EtOAc : EtOH 10 : 1 and the aqueous extract. The dry extracts obtained after the removal of organic solvents were evaluated for their Total Phenolic Content (TPC) and Total Flavonoids Content (TFC), while HPTLC analysis was performed. The most promising dry extract, obtained in ratio extract/organic solvent 2/1, was subjected to Centrifugal Partition Chromatography (CPC) resulting to the isolation nine pure compounds, which were identified as quercetin and kaempferol glycosides.

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FUNCTIONALITY OF THE AMY2 GENE CLUSTER IN *LOTUS JAPONICUS*

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Plants synthesize a variety of secondary metabolites (natural products), a major subgroup of which are triterpens. Triterpens are synthesized from mevalonate via cyclization of 2,3-oxidosqualene catalyzed by single enzymes, named oxidosqualene cyclases (OSCs). AMY2 is a multi-functional OSC, wich along with genes for 2 different classes of CYP450, belonging to CYP71D and CYP88D families, are clustered in the *Lotus japonicus* genome. *AMY2* transcript levels were highest in roots (including young nodules) 7 days post infection with *Mesorhizobium loti* [1]. Here, we investigated the role of AMY2 gene cluster in transgenic *Lotus japonicus* hairy roots lines overexpressing *AMY2* that will ideally accumulate AMY2 metabolic products in order to aid their identification. Hairy root *L. japonicus* lines, were obtained and tested for their symbiotic responses at different stages (20 and 30 days) after inoculation with its symbiont, *Mesorhizobium loti*. No obvious difference was observed in anatomy or morphology. We also investigated *AMY2* expression in plant mutants affected in the establishment of the symbiosis with both rhizobia and mycorrhiza in *L.japonicus*.

Our data thus far suggest that the potential involment of *AMY2* in nodulation merits further study.

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CYTOTOXICITY OF *MALUS SYLVESTRIS* MILL L. EXTRACTS ON ANIMAL AND PLANT CELLS

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Malus sylvestris (L.) Mill. (Rosaceae) is a spontaneous species, spread throughout Europe. Fresh fruits or as a decoction are traditionally used in Southwest Romania to treat inflammation and wounds. Leaves from other *Malus* species show antidiabetic effects in mice and rat models [1, 2].

Toxicity tests are performed on plant and animal organisms with hydroalcoholic (HAE) and an aqueous (AQE) extracts from leaves (5 %). The biological material (Buzau country) is firstly identified and characterized, both macroscopically and microscopically. The toxicity of the AQEs and HAEs is assessed on plant (*Hordeum vulgare* L.) and animal cells (lethality test on *Artemia franciscana* Kellog). In these tests are used diluted solutions of 0.03 ¸ 3.33% and 0.067 ¸ 1.0%, respectively. The influence of the extracts on *Hordeum vulgare* L. seed germination is also investigated. The polyphenolic and flavonoids content is spectrophotometrically evaluated. Excepting the highest concentration, the results of the toxicity assays on plant cells show that the AQE does not significantly affect the roots elongation. The HAE strongly inhibits the root elongation at concentrations higher than 1.66%. None of the two extracts affects the germination and none is toxic on animal cells at the concentration ranges evaluated.

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A FLAVONOID GLYCOSIDE FROM *GARCINIA GRACILIS* LEAVES INDUCES THE SYNTHESIS OF ANTIOXIDANT ENZYMES IN HEK-293 CELLS

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The enzymatic antioxidants play the important role of protecting the cells and organisms from the oxidative damage. However, only the cellular antioxidants may be inadequate to maintain reactive oxygen species at low levels. *Garcinia gracilis* Pierre (Clusiaceae) leaves are rich source of secondary metabolites and possess various pharmacological effects for health benefits [1]. The aim of this study is to investigate the effects of a major bioactive compound, apigenin-8-*C*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, from *G. gracilis* leaves on the mRNA expressions of antioxidant enzymes in HEK-293 cells. This compound was obtained from the chromatographic separation of *G. gracilis* leave extracts and its structure was determined through analysis of NMR and HR-ESI-MS data [2]. The cytotoxicity of the isolated compound on HEK-293 cells was evaluated by the MTT colorimetric assay. The mRNA expressions of antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and heme oxygenase were determined by RT-qPCR technique [3]. In this study, we found that treatment with apigenin-8-*C*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (20 µg/mL) significantly increased the mRNA expression levels of glutathione peroxidase, glutathione reductase, and heme oxygenase enzymes in HEK-293 cells. These findings first reveal the antioxidant effects of a flavonoid glycoside from *G. gracilis* through the induction of antioxidant enzyme synthesis in the cells.

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COMPARISON OF CAPSAICIN CONTENT IN *CAPSICUM* SPP. FRUITS, SPICES AND THERAPEUTIC PREPARATIONS

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Capsaicin, 8-methyl-*N*-vanillyl-6-nonenamide, is a well-known compound of plant origin studied very intesively due to its various pharmacological properties, including analgetic, antimicrobial and antitumor. Capsaicin is used for the treatment of diabetic neuropathy, osteoarthritis, post-herpetic neuralgia and psoriasis. It is also applied in numerous insecticides, pesticides, insect and animal repellents. Capsaicin is synthesized and accumulated in large quantities in fruits of plants of genus *Capsicum*, particularly in the placental tissue.

Optimized methods of thin-layer preparative adsorption chromatography (TLC) and gas chromatography combined with mass spectrometry (GC-MS) were applied in this work to qualitative and quantitative analysis of oleoresins obtained from *Capsicum* spp. fruits and derived products (culinary spices and macerates prepared with the use of linseed oil). In the materials and preparations studied, six capsaicinoids were determined: capsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin and nonivamide, along with steroids and pentacyclic triterpenoids. Among materials applied in our study, the highest content of capsaicin was demonstrated in dried fruits of *Capsicum chinense* Habanero extracted with ethanol (up to 2% d.w.). Spices available on the market contained considerably less capsaicinoids than dried fruits. Capsaicin usually consisted about 60% of capsaicinoid fraction, followed by dihydrocapsaicin (approx. 30%). Extraction of dried Habanero fruits made with the use of linseed oil was less efficient, yielding only 18% capsaicin and related capsaicinoids in comparison to extraction with ethanol. However, the final concentration of capsaicinoid fraction in linseed oil extract was significant and accounted to almost 6 mg/ml (i.e. 3.5 mg/ml capsaicin).

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HPLC AND HPTLC METABOLITE FINGERPRINTING FOR THE QUALITY CONTROL OF *SIDERITIS* SPECIES

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The genus *Sideritis* (Lamiaceae) is represented by more than 150 species, most of them mainly found in the Mediterranean area. It is characterized by a strong tendency to hybridize between species and thus its taxonomy is considered difficult [1, 2]. The current study aims to investigate the qualitative differentiation of *Sideritis* species using High Performance Liquid Chromatography (HPLC) and High Performance Thin Layer Chromatography (HPTLC) for chromatographic and densitometric fingerprinting. The samples evaluated herein were the hydroalcoholic (MeOH/H_2O 50 : 50, v/v) and ethyl acetate extracts derived from six of the most popular species (i.e. *S. scardica*, *S. raeseri*, *S. clandestina*, *S. syriaca*, *S. euboea*, and *S. sipylea*). The HPLC and HPTLC data obtained by phytochemical fingerprint analysis were treated with appropriate multivariate data analysis techniques, to extract the maximum relevant information occurring among *Sideritis* extracts of the same type [3]. By using specific chemotaxonomic markers, such as terpenoids, flavonoids and phenylpropanoids, the correlation or differentiation among different species was achieved. The results indicate the reliability of both techniques for the quality assessment of samples from these species, providing complementary information with respect to the phytochemical variability patterns. The potential of HPTLC combined with multivariate analysis is also highlighted for the rapid screening of *Sideritis* samples.

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ANTI-BACTERIAL PROPERTIES OF HYDRODISTILLED JAVA CITRONELLA (*CYMBOPOGON WINTERIANUS* JOWITT) ESSENTIAL OIL

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Citronella oil is an essential oil known for its natural insect repellent property and is of great importance in pharmaceutical and fragrance industries. This work has the objectives of citronella oil extraction and evaluation of its anti-bacterial activity. In the hydrodistillation process highest citronella oil of 2.38% was obtained at 180 min. GC/MS analysis of extracted oil revealed about 95% of commercially important compounds viz. citronellal (55.23%), geraniol (26.29%) and citronellol (13.41%). The anti-bacterial activity was performed using six bacterial strains (*Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*) by agar well diffusion method using different concentrations (10%, 20%, 30%, 40% and 50% v/v). The oil was found to be effective against all the bacterial strains. However, effectivess towards *Bacillus subtilis* and *Staphylococcus epidermidis* was relatively higher compared to other selected strains. The increase in anti-bacterial activity was observed with increased oil concentration in all the strains, irrespective of being gram positive or gram negative. Maximum zone of inhibition (23 mm) was observed for *Staphylococcus epidermidis* and *Klebsiella pneumonia* at highest concentration of citronella oil (50% v/v). The hydrosol obtained after hydrodistillation was analyzed using HPLC and was found to contain 0.07 mg/ml of erythritol and 0.03 mg/ml of mannitol. The above characteristics of the citronella oil therefore endorse its wide utilization in various nutraceutical and pharmaceutical industries.

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CHEMICAL COMPOSITION OF *BORNMUELLERA DIECKII* DEGEN (BRASSICACEAE) ESSENTIAL OIL AND AUTOLYZATE

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The genus *Bormuellera* Hausskn. is an endemic Balkan genus with only three species: *B. dieckii* Degen, *B. baldaccii* (Degen) Heywood and *B. tymphaea* (Hausskn.) Hausskn. *Bornmuellera dieckii* is a perennial herb or small shrub that grows on serpentine rocks on the Sharr Mountains in southern Serbia. It is an obligate serpentinophyte and therefore a steno-endemic species [1]. The chemistry of this species was poorly investigated. The only phytochemical study of this taxon was undertaken by Marin and co-workers twenty years ago [2]. The aim of the current study was set to perform a detailed chemical analysis of the essential oil and autolyzate of *B. dieckii*. Hydrodistillation of dry plant material yielded a small amount of a foul(cabbage-like)-smelling essential oil (0.01%, w/w). Autolysis experiments were conducted according to the procedure reported by Radulović et al. [3]. A GC-MS analysis of the essential oil enabled the identification of more than 100 constituents, among which nonacosane (18.3%), (*Z*)-3-hexene-1-ol (10.6%), 6-(methylthio)hexenenitrile (8.3%), hex-5-enenitrile (7.6%), and (*E*)-phytol (5.2%) were the major ones. A GC-MS analysis of the autolyzate, on the other hand, enabled the identification of 42 components, in total, among which the major ones were found to be nonacosane (50.5%), hentriacontane (22.7%) and pinoresinol (4.3%).
The isothiocyanates ((5-isothiocyanatopentyl)(methyl)sulfane and 1-isothiocyanato-5-(methylsulfinyl)pentane), corresponding to the nitriles detected in the essential oil, were also identified in the autolyzate.

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5-PHENYL-1,3-OXAZOLIDINE-2-THIONE, A VOLATILE FROM DYER'S ROCKET (*RESEDA LUTEOLA* L.), UNDERGOES THERMAL ISOMERIZATION UNDER GC CONDITIONS

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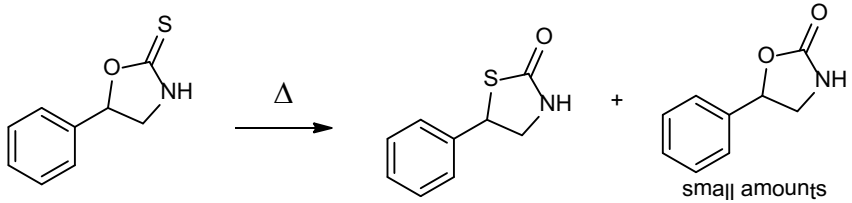
5-Phenyl-1,3-oxazolidine-2-thione, a known volatile goitrogen, was chromatographically isolated from an autolyzate of the flowers of *Reseda luteola* L. (Resedaceae) and its structure established by 1D and 2D NMR, and IR analyses. During a GC-MS analysis of a pure sample of this compound, an unexpected occurrence of two, interconnected by an elevated base line, dominant peaks in the TIC chromatograms was noted. Both peaks displayed the same M^+ values and differed only in the intensities of certain fragment ions. This led us to an assumption that elevated temperatures during the GC-MS analysis, in one or more of the three heated zones (injection port, column and transfer line), cause a thermal isomerization of this compound. To investigate this, we decided to undertake a preparative thermolysis (exposing to 200 °C for 10 min) of a sample of this compound, dissolved in DMSO-d_6, in a sealed glass vial, and analyze the contents directly (without prior separation) by NMR. ^1H, ^{13}C and 2D NMR spectra, unequivocally allowed us to identify the major isomerization product as 5-phenyl-1,3-thiazolidine-2-one. In addition to those from this compound, the spectra contained signals originating from the starting 5-phenyl-1,3-oxazolidine-2-thione, and another unexpected, minor product: 5-phenyl-1,3-oxazolidine-2-one (probably formed as a consequence of the presence of traces of moisture in DMSO). These results warn us of the possibility of artefact formation during the procedures of isolation and identification of natural products.



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PHENOLIC CONSTITUENTS FROM *ASPLENIUM CETERACH* L. - ASPLENIACEAE

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Based on ethnopharmacological data [1], we have undertaken the chemical investigation of *Asplenium ceterach* L (syn. *Ceterach officinarum* Willd.), commonly known as "rustyback". The fern is growing wild in Greece on rocks and on walls [1]; it is used in folk medicine against kidney stones, gallstones, and to treat benign prostatic hyperplasia [1, 2]. We report, herein, on the isolation and identification of secondary metabolites from the ethyl acetate and methanol extracts from its aerial parts. The ethyl acetate extract yielded caffeic and chlorogenic, while the methanol extract afforded quercetin-3-*O*-β-D-glucuronide [3], quercetin-3-*O*-β-D gentiobioside [3], kaempherol-3-*O*-β-D gentiobioside, p-coumaroyl-4-*O*-β-D-glucoside, 3-(1-*O*-β-D-glucopyranosyl-4-hydroxyphenyl) - propionic acid, 4-vinyl-phenol-1-*O*-β-D-gluco-pyranoside and 4-β-*O*-D-glucosyl-3,4-dihydroxybenzyl alcohol.

The structures of the isolated compounds were elucidated by high-field NMR spectroscopy (^1H-NMR, ^1H-^1H COSY, NOESY, HSQC and HMBC).

Acnowledgements: The authors thank Special Account for Research Grants and National and Kapodistrian University of Athens for funding to attend the meeting.

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TREATEMENT OF PSORIASIS WITH PHYTOTHERAPY – ABOUT 55 CASES

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Psoriasis is a common, chronic, skin disease, with no clear cause or cure. Psoriasis affects people of all ages, and in all countries. It is proposed in this work to define the place of phytotherapy in the treatment of psoriasis.

This is a retrospective study of 55 clinical cases with psoriasis. These patients were all treated by herbal medicine. The treatment is essentially based on medicinal plants according to the theory of integrative medicine [1].

The average age of our patients is 41.09 years. The majority of our patients (71%) developed psoriasis before the age of 40 years (psoriasis type I) with a male predominance (sex ratio M / F = 1.87). Clinically, psoriasis vulgaris is the most frequent form (61.29%). The risk factors studied are obesity (9 cases) and infections (17 cases). A total of 70 plants were prescribed in various preparations. *Lavandula officinalis* was prescribed in 89.09% of the cases, *Avena sativa* in 87.27% of the cases, *Silybum marianum* in 85.45% of the cases and *Eucalyptus globulus* in 80% of the cases. Of the 55 cases studied, the evolution of the disease was noted in 24 patients: A significant improvement or a complete disappearance of the symptoms of psoriasis was found in 79.41% of those patients. No adverse reactions have been reported.

Phytotherapy is an interesting approach in the management of psoriasis. The medicinal plant is then the therapeutic tool best suited for an integrative approach of the organism in its adaptive reactivity.

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NEW SESQUITERPENE LACTONES FROM *INULA OCULUS-CHRISTI* L.

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Genus *Inula* (Asteraceae) contains more than one hundred species widespread mainly in Africa, Asia and Europe. Sixteen *Inula* species are known as plants used in traditional medicine and some of them are constituents of medicinal herbal preparations. Plants belonging to this genus have shown to possess various biological activities, attributed to the presence of sesquiterpene lactones, phenolic acids, and flavonoids [1, 2].

Continuing our research on *Inula* species, growing in Bulgaria we have focused our attention on sesquiterpene lactones in *I. oculus-christi*. The aerial parts were extracted with chloroform. Further CC (Sephadex LH-20 and Silica gel) and PTLC purification of the extract afforded 9 sesquiterpene lactones – the new compounds 9β,10β-epoxy-gaillardin, 9a,10a-epoxy-2-*epi*-gaillardin, 2a-acetoxy-4a,9b-dihydroxy-1b-guaia-11(13),10(14)-dien-12,8a-olide, 9a-hydroxyinuchinenolide B, 9β-hydroxy-2-*epi*-inuchinenolide B and 4a,15a-epoxypulchellin E, in addition to known gaillardin, pulchellin C, pulchellin E. The structures and relative stereochemistry were determined by 1D and 2D NMR spectroscopy (^{1}H, ^{13}C, COSY, HSQC, HMBC and NOESY) and MS. The isolated compounds were with guaiane and eudesmane carbon skeleton. It was found that gaillardin was the principal one like in *I. oculus-christi* of other origins [3, 4]. Pulchellin C was detected in the plant from Iran [3], while pulchellin E – in *I. oculus-christi* from Montenegro [4].

Our results are consistent with the previous reports and enrich the knowledge on the lactone profile of *I. oculus-christi* with the newly identified components. The obtained results could be of chemotaxonomic interest.

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UPHLC-HRMS ANALYSIS OF *ROSA DAMASCENA* MILL. FLOWERS DRY EXTRACT – A NEW BY-PRODUCT FROM ROSE CONCRETE PRODUCTION

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Rosa damascena Mill. is considered as one of the most important economic products of Bulgaria. Rose concrete is one of the main products of rose, preparing from fresh rose flowers by extraction with n-hexane. It is a complex mixture of non-polar compounds, while polar compounds are retained in the waste material. Therefore, the recovery of valuable biologically active substances is an approach for reduction and valorization of the wastes generated in the rose oil industry.

In the present laboratory scale experiment, the processed rose flowers after concrete production were extracted with aqueous methanol followed by spray drying. The resulting rose flower dry extract was subjected to UHPLC-HRMS analysis. Twenty flavonoid glycosides were tentatively identified by comparison their full scan and MS/MS spectra with those published in the literature. It was found, that these compounds are derivatives of quercetin and kaempferol - diglycosides, their acyl derivatives, hexosides, methylpentosides, pentosides, and acylglycosides.
The results of this study are in accordance with those published in the literature for the flavonoid pattern of *R. damascena* extracts obtained from distilled petals [1] and distillation wastewater [2]. The new by-product obtained from waste after rose concrete production might be a good source of natural antioxidants in food industry and cosmetics.

Acknowledgments: The authors are thankful to Galen-N Ltd. for providing processed rose flowers and EXANDAS project (H2020-MSCA-RISE-2015) for partial financial support.

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QUALITATIVE ANALYSIS OF PHENOLIC COMPOUNDS IN THE ROOTS OF *SYMPHYTUM OFFICINALE* L. BY UPLC-DAD-QTOF-MS/MS

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Symphytum officinale L. (comfrey), Boraginaceae, has a long history as a traditional medicinal plant; comfrey root preparations are used for the external treatment of joint complaints, painful muscle, bone fractures, distortions and haematomas. The therapeutic properties of comfrey are based on its anti-inflammatory and analgesic effects, mainly due to allantoin and rosmarinic acid [1]. However, the key activity-determining constituents of comfrey root extracts and its molecular mechanisms of action have not been completely elucidated [2]. The aim of the present study was to evaluate the phenolic profile of a commercial sample of *S. officinale* roots. 65% ethanol was used for the extraction of the phenolic derivatives [3] and their profile was characterized using UPLC-DAD-QTOF-MS/MS. The individual compounds were identified by comparison of their UV, mass spectra, fragmentation pattern and retention times to those of authentical standards, online available databases and literature data. Rosma-rinic acid was identified as the main compound; the sample presented also other caffeic acid derivatives, such as trimers (isomers of lithospermic acid, salvianolic acid A and C) and tetramers (salvianolic acid B isomer). This is the first time that isomers of salvianolic acids A, B and C are described in *S. officinale*. In conclusion, comfrey root is an important source of phenolic compounds that could contribute to the overall bioactivity of *Symphytum* preparations.

Acknowledgements: We thank Alpinamed AG and Zurich University of Applied Sciences for financial support.

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EXPERIMENTAL ANTINEOPLASTIC ACTIVITY OF CURCUMIN LOADED POLYMERIC ELECTROSPUN MATS

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Malignancies are the second cause for death in humans worldwide. In many cases multidrug resistance, chemotherapy side effects and affected quality of life are seen. Curcumin is a practically non-toxic compound of plant origin with own antineoplastic and NF-κB inhibitory activities. We investigated the cytotoxic activity of curcumin loaded electrospun mats in human hepatic and colon carcinoma cell lines (HEP-G2 and HT-29). Disks with 5 mm diameter of the electrospun mats caused nearly 80% inhibition in HT-29 colorectal cells. The efficacy against the HEP-G2 hepatocellular carcinoma cells was weaker. The mats showed antibacterial features and were found to be biocompatible with human cells of different tissue origin. The tested polymeric mats increased the accumulation of curcumin inside of the malignant cells as estimated by fluorescent microscopy. Taken together our experimental findings indicate that the non-toxic yellow pigment curcumin after inclusion into electrospun polymeric mats can be used for topical treatment of liver lesions (e.g. colorectal cancer metastases) and these results are promising in terms of further clinical application.

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CHEMICAL CONSTITUENTS OF CYTOTOXIC EXTRACT OF PROPOLIS FROM PITCAIRN ISLAND

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Propolis (bee glue) is a resinous hive product collected by honeybees from certain plant sources. It has a long history of being used in traditional medicine and nowadays it is extensively used in food to improve health and prevent diseases such as inflammation, heart disease, diabetes, and even cancer. Because of its broad spectrum of biological activities there is a renewed interest in the composition of propolis, which depends on the vegetation of the area from which propolis was collected. There are numerous reports in the literature on the isolation and structural elucidation of biologically active phytochemicals from propolis collected in Europe, South America, Asia and the Pacific region. However there is no data about chemical composition and biological activity of propolis from Pitcairn Islands. In the last years it is of growing commercial interest, due to Pitcairn's bee population is disease free and their products are considered as clean of pollutants.

In our continued work on propolis of various origin we found that dichloromethane extract of propolis collected from Pitcairn Island possess *in vitro* cytotoxic activity towards triple-negative breast cancer cell line MDA-MB-231. The results showed that this extract inhibits the growth of the MDA-MB-231 line in a dose-dependent and time-dependent manner. Upon the cell growth inhibition propolis extract provokes apoptotic changes in cell nuclei. Since no previous research has been reported on it, we carried out a detailed chemical investigation. The work led to the isolation of 4 new cycloartane type triterpenes, together with 10 known individual compounds (di- and triterpenes) and five inseparable mixtures (triterpenes and phenolic lipids). Their structures were elucidated by extensive analysis of spectroscopic data (1D and 2D NMR, IR, HREIMS) and comparison with previously published reports. These findings certify the potential role of propolis in prevention and therapy of cancer and will strengthen the propolis implementation in different healthy products.

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CELLULOSE ACETATE ELECTROSPUN MATERIALS DECORATED WITH CURCUMIN/PVP PARTICLES WITH ANTIBACTERIAL ACTIVITY

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Curcumin (Curc) is a biologically active substance found in the roots of *Curcuma longa* plant. This naturally occurring polyphenolic compound manifests remarkable antibacterial, antifungal, antioxidant, anti-inflammatory, anticoagulant and antitumor activity. However, Curc is poorly soluble in water and body fluids, and this impedes attaining effective therapeutic concentrations. Incorporation of curcumin in suitable nanoformulations may provide means to obviate this obstacle. Electrospinning has been shown effective in preparing nanofibrous materials with modulated curcumin release [1, 2]. The aim of the present study was to obtain novel electrospun materials from cellulose acetate (CA) and polyvinylpyrrolidone (PVP) for curcumin delivery. The morphology of the obtained fibrous materials was observed by scanning electron microscopy (SEM). The SEM micrographs showed that separated and evenly distributed particles of Curc/PVP were deposited on the surface of the mats and on the inner layers of the mat. X-ray diffraction studies showed that Curc was in amorphous state. *In vitro* studies demonstrated that Curc release was facilitated from the Curc/PVP-*on*-CA mats. Moreover, the curcumin-containing materials exhibited antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*.

Acknowledgements: The authors thank the National Science Fund of Bulgaria for the financial support (Grant number DFNI–T02/1-2014).

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PP 227

ETHNOPHARMACOLOGICAL NOTES FOR THE TRADITIONAL USES OF THE BLACK ELDER (*SAMBUCUS NIGRA* L.*)* MEDICINAL PLANT IN THE FOLK MEDICINE OF THE AREA OF DRAMA, NOTHERN GREECE

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Black Elder is currently one of the most-used medicinal plants worldwide.

The aim of the present paper is to summarize the information occurred after the extended ethnopharmacological research in the area of Drama, Greece about the traditional uses of the Black Elder (*Sambucus nigra* L.) in the folk medicine practiced for centuries in this area in comparison to the known and accepted uses of this plant as a research study.

The summary of those studies is compared with the ethnopharmacological information collected with interviews to accept or decline the folk information, about the ethnomedicinal uses of the Black Elder in the region.

In conclusion to the research evidence and the information from the field research in Drama, Greece, the plant parts of Black Elder in various forms and conditions can be used safely as a natural remedy, anti-inflammatory, antivirus for internal and external use in human.

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CHEMICAL COMPOSITION AND TOXICITY ASSESSEMENT OF ESSENTIAL OILS FROM TWO GREEK *CONIUM* (APIACEAE) SPECIES

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The essential oils from various plant parts of two Greek species of the genus *Conium* L., namely *C. maculatum* L. and *C. divaricatum* Boiss. & Orph., were analyzed by GC-FID and GC-MS [1]. After chromatographic separations of a *C. divaricatum* alkaline extract, three new natural products, which constitute the main components in the majority of *C. divaricatum* essential oils, were isolated and characterized through NMR and MS data analyses. In the quality control that was conducted for the presence of alkaloids, the extracts from the aerial parts of both taxa gave positive reactions. Moreover, quantitative determination of the total alkaloid content was performed in hydroethanolic extracts of the dried aerial parts with *C. maculatum* displaying a higher percentage compared to that of *C. divaricatum*. The essential oils and extracts, along with the main component of *C. divaricatum* essential oils, a new natural product, were evaluated for their toxicity against nauplii of *Artemia salina* L. The completely different chemical profile of *C. divaricatum* essential oils, compared to that of *C. maculatum*, in combination with the differences that were observed in the total alkaloid content, the toxicity and the morphology of the two taxa, suggest that *C. divaricatum* should be classified as a separate species in the genus [2], as initially proposed by Boissier and Orphanides (1856), and not as a synonym of *C. maculatum*, as currently used in some floras.

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COMPOSITION OF THE ESSENTIAL OILS OF *DAUCUS CAROTA* L. AND *DAUCUS GUTTATUS* SM. FROM GREECE

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The genus *Daucus* (Umbelliferae) is a cosmopolitan genus of herbaceous plants and comprises 22 species, ten of which are found in Europe [1]. Most widely known is *Daucus carota*, an extremely polymorphic species, including many subspecies such as *D. carota* subsp. *carota* which is also known as wild carrot. *D. guttatus* is found in Italy and the Balkan Peninsula [2]. Extracts and essential oils of *D. carota* have been used in traditional medicine as diuretic, carminative and against kidney diseases [3]. The essential oil from different plant parts of *D. carota* is used in cosmetics, as a functional ingredient in food industry while it is also known to exert a plethora of bioactivities such as antioxidant, anti-inflammatory, hepatoprotective, and anti-parasitic properties [3]. Stems, leaves, flowers and roots from *D. carota* and *D. guttatus*, collected in Greece, were subjected to hydrodistillation. The composition of the obtained essential oils was analyzed by means of GC-FID and GC-MS. Comparison among the essential oils from different plant parts of the same species showed qualitative similarity, yet quantitative differences were distinctive. Between the two species, however, not only quantitative, but also significant qualitative differences were observed. The monoterpenes, sabinene (2.3-32.2%), α-pinene (tr-23.9%), terpinen-4-ol (tr-7.7%), and limonene (tr-6.6%), characterized the essential oil of *D. carota*, followed by sesquiterpenes in lesser amounts, while the sesquiterpene hydrocarbon acora-3,7(14)-diene (9.2-60.1%), and the phenylpropane derivatives, epoxy-pseudoisoeugenyl isobutyrate (18.6-64.9%), and epoxy-pseudoisoeugenyl 2-methylbutyrate (0.6-33.0%) were the main constituents of *D. guttatus* essential oil.

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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF FLOWER ESSENTIAL OILS OF THREE *HERACLEUM* L. TAXA

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Chemical composition and antimicrobial activity of flower essential oils of three *Heracleum* L. (Apiaceae) taxa, *i.e. H. pyrenaicum* subsp. *pollinianum* (Bertol.) F. Pedrotti & Pignatti (HPEO), *H. ternatum* Velen. (HTEO) and *H. orphanidis* Boiss. (HOEO) were investigated for the first time. Previously, the constituents and notable antimicrobial activity of their root, leaf and fruit essential oils were demonstrated, justifying further research of these plants [1, 2].

The essential oils were obtained by hydrodistillation using Clevenger-type apparatus. By GC and GC-MS, ninety-one components were identified in both HPEO and HTEO, and fifty-nine in HOEO (comprising 95.8, 95.6 and 96.9% of total oils, respectively). The major constituents of HPEO were sesquiterpenes (51.0%), with (*E*)-nerolidol (30.2%) being the most abundant. HTEO contained lower quantity of sesquiterpenes (27.5%), and was dominated by phenylpropanoids (48.4%), mostly (*Z*)-isoelemicin (22.4%) and methyl eugenol (19.3%). The composition of HOEO was significantly different, *i.e.* aliphatic esters (77.7%), predominantly octyl acetate (51.0%), were its main components.

In microdilution method, HPEO showed the best antimicrobial activity. It exhibited stronger effect against all eight tested fungi belonging to *Aspergillus*, *Penicillium* and *Trichoderma* genera (MIC = 0.03 - 0.08 mg/mL, MFC = 0.08 - 0.20 mg/mL), and against two of eight investigated bacteria, *Bacillus cereus* and *Micrococcus flavus* (MIC = 0.04 mg/mL, MBC = 0.08 mg/mL), than tested reference antibiotics. The activity of HTEO and HOEO was lower. Observed differences were in agreement with the composition of these oils [1, 2].

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VOLATILES AND FLAVONOIDS OF *GONIOLIMON TATARICUM* (L.) BOISS. (PLUMBAGINACEAE)

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Goniolimon tataricum (L.) Boiss. (Plumbaginaceae) is a perennial plant with glaucous, coriaceous, sparsely whitish-punctate leaves arranged in a basal rosette, corymbulose synflorescence with laxer, often racemiform terminal spikes and subulate calyx lobes. It is distributed in European Russia, Transcaucasia, Ukraine, Romania and Balkans [1].

In this work, volatile compounds and flavonoid glycosides of *G. tataricum* were analyzed for the first time. Plant material was collected in Central Serbia (Mt Vujan). Volatile fractions of the roots and flowering aerial parts were obtained by hydrodistillation. GC and GC-MS analysis revealed twelve volatiles in the roots and forty-five in the aerial parts (comprising 91.1 and 92.8% of total fractions). The dominant was hexadecanoic acid (50.4 and 38.5%). Terpenes were identified as well (7.0 and 17.7%), and among them, polygodial (4.8%) was the most abundant in the roots and hexahydrofarnesyl acetone (10.4%) in the aerial parts.

To analyze polar constituents, leaves, stems and flowers were pre-extracted with dichloromethane and then extracted with methanol. Obtained dry methanol extracts were subjected to LC-MS analysis. Using standards, isoorientin, hyperoside and quercitrin were identified in all three extracts, and additionally isoquercitrin in leaf and stem extracts. Identified phenolics were quantified using external standard method. The leaf extract was the richest in all detected flavonoids. The dominant in each extract was quercitrin (30.0, 27.4, and 20.4 mg/g in leaf, stem and flower extract, respectively), followed by isoorientin (13.4, 11.4, and 6.6 mg/g).

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INFLUENCE OF *CURCUMA LONGA* L. ON TWO CYTOKINES PRODUCTION IN CHRONIC MILD STRESS MODEL

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Introduction: Extracts of *Curcuma longa* L. are beneficial in chronic stress and depression [1]. These conditions are often accompanied with altered immunoreactivity [2]. **Aim:** To evaluate the effect of *Curcuma* extract on behavioral changes and lipopolysaccharide-induced cytokines` production in rats subjected to a chronic mild stress (CMS) model. **Methods:** The standardized commercial *Curcuma* extract was characterized by high performance liquid chromatography (HPLC). Male Wistar rats (n = 32) divided in 4 groups were treated orally with: distilled water 10 ml/kg (control and CMS groups); *Curcuma* 250 mg/kg or 500 mg/kg. All groups except the control were stressed daily according to a CMS protocol. Changes in body weight, locomotor activity and spatial memory were recorded. At the end the animals were challenged with LPS and rats` sera were obtained for ELISA evaluation of TNF-α and IL-6 levels. **Results:** The animals from the model group decreased their weight, locomotor activity and spatial memory compared to controls. The group exposed to stress and treated with *Curcuma* 500 mg/kg increased their locomotor activity compared to the model group. On water maze test *Curcuma* in both doses decreased the latency time compared to the model group. High expression of the pro-inflammatory cytokines TNF-α and IL-6 were found in all groups exposed to CMS and challenged by LPS.

Conclusions: Depression-like behaviour was found in all groups subjected to the CMS protocol. Increased cytokines` levels showed that CMS is affecting rats` immunity. Repeated administration of *Curcuma* reversed the stress-induced changes. Its anti-stress effect probably modulates the excessive cytokines` production.

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FLAVONOID GLYCOSIDES EXTRACTED FROM *INULA* REORGANIZE RAFT-LIKE MEMBRANE DOMAINS

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The research in modern pharmacological therapy is focused on drugs of plant origin. The herbs of the genus *Inula* are used in folk medicine because of its properties - expectorant, antitussive, antimicrobial, anti-cancer, antidiabetic and etc. We have previously showed that the extracts from *I. oculus-christi*, enriched with flavonoid glycosides (FGs), inflict cell morphology impairments which is an indicative for disrupted intercellular contacts [1]. The mechanism by which FGs exert their effects on cells is considered to include multiple pathways. We are particularly interested in cellular membrane domains, called rafts, enriched in sphingomyelin, cholesterol and proteins. Before to approach the complex cellular life, we developed a model membranes exhibiting ordered raft-like domains surrounded by lipids in liquid-disordered phase. Fluorescence spectroscopy was used to monitor the effects of FGs on membrane structure and organization by incorporating two fluorescent molecules reporting lipid order and fluidity at different depth of the membrane. Fluorescence quenching method allowed us to compare the fraction of raft-like domains with and without FGs. We demonstrated that FGs influence rather the deep hydrophobic core of the lipid bilayer than the bilayer interface. FGs demonstrate an ability to tune the membrane fluidity because, on one hand, they reduce the fluidity by increasing the sphingomyelin phase transition temperature, and on the other hand, they enhance the fluidity in the presence of cholesterol. FGs stimulate the formation of rafts and make them more fluid compared to control conditions. Thus, it can be concluded that the FGs affect the membrane organization and functions.

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INVASIVE ALIEN SPECIES FROM FAMILY ASTERACEAE – POTENTIAL CHEAP AND ENDLESS RESOURCES OF PLANT SUBSTANCES FOR MEDICINAL USE

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Here we present our research data on the distribution and invasion level in Bulgaria of *Ambrosia artemisiifolia* L., *Xanthium strumarium* L., *Erigeron canadensis* L., and *Dittrichia graveolens* (L.) W. Greuter (Asteraceae). The high tolerance of various habitat conditions and potent propagation ability of these alien plants promote their aggressive invasive behaviour. Additionally, they not only overcompete the local vegetation but suppress the seed development. In the newly invaded habitats they might not have suitable herbivores to control their populations. The only effective enemy might be *Homo sapiens* which is known with its destructive power once an object has become significant for industrial utilization.

The aim of this study is to attract the attention towards these plants and their potential to be used as cheap sources of compounds with valuable pharmacological activities.

A growing body of scientific literature points to the therapeutic potential of chemical constituents of these alien invasive species (mainly sesquiterpene lactones). They posses different activities such as anticancer activity, as well as antitussive, antifungal, antiinflammatory, antinociceptive, hypoglycaemic, antimitotic, antioxidant, antitrypanosomal, CNS depressant activity, diuretic effects, contact dermatitis, insecticidal and herbicidal activities, hepatoprotective and hypolipemic activities etc.

Due to the fact that these are aggressive invasive species, they can provide abundant and cheap resources reach of plant chemical constituents which can be utilized for therapeutic purposes. Additionally, exploitation of the biomass for medicinal use might contribute to relieving the destructive impact of this species on natural habitats.

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LC-HRMS PROFILING OF HYDROXYCINNAMIC ACID AMIDES AND STEROID ALKALOIDS FROM LEAVES EXTRACT OF *SOLANUM SCHIMPERIANUM*, HOCHST.

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Hydroxycinnamic acid amides (HCAAs) are a diverse family of phenylpropanoid amine conjugates, considered second metabolites, found widely in higher plants. A variety of HCAAs have been found throughout the genus *Solanum*. HCAAs have been reported to possess good activity against wide range of microbial pathogens [1]. Genus *Solanum* is famous for the numerous diverse steroid alkaloids isolated from different species. In the present study, twelve known hydroxycinnamic acid amides (HCAAs) were detected by a previously reported method [2] using LC-MS, as well as several 3-amino steroid alkaloids were tentatively assigned, in the leaves extract of *S. schimperianum*. Mass analyses of the extract were carried out on a Q Exactive Plus mass spectrometer (ThermoFisher Scientific) equipped with a heated electrospray ionization (HESI-II) probe (ThermoScientific). The targeted acquisition of the 12 HCAAs were carried out on parallel reaction monitoring (PRM) mode. Also, the antioxidant activity of the total leaf extract was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) scavenging radical methods. The methanol extract displayed antioxidant activity in both assays with IC_{50} value of 156.1 ± 3 and 521.3 ±3 µg/ml, respectively.

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COMPARISON OF POLYPHENOL PROFILE AND ANTIMUTAGENIC AND ANTIOXIDANT ACTIVITIES IN TWO SPECIES USED AS SOURCE OF *SOLIDAGINIS HERBA* – GOLDENROD HERB

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European Pharmacopoeia accepts two equivalent species *Solidago gigantea* Ait. and *S. canadensis* L. as Goldenrod herb (*Solidaginis herba*). Both species are highly invasive in Europe. We compared phytochemical profile of both species from typical invasive population in Poland. Further, we compared *in vitro* antimutagenic and antioxidant activities of various solvent extracts from *Solidaginis herba* as well as from underground parts of both species. In *S. gigantea*, flavonoid profile was dominated by several quercetin glycosides, with major quercitrin, whereas in *S. canadensis*, the kaempferol rutinoside - nicotiflorin was the main constituent, followed by rutin. Caffeoylquinic acids (CQAs) were less diverse than flavonoids with 5-CQA as a main compound in both species. *S. gigantea* contained exactly 2x more total flavonoids than *S. canadensis* but the latter species contained 1.7x more total hydroxycinnamoyl-quinic acids. In roots of both species, over 20 putative diterpenoids were detected, mostly unidentified. Also, several CQAs were present in higher amounts than in aerial parts. Antioxidant and antimutagenic activities of all extracts were different between species and organs, with the strongest inhibition of lipid peroxidation by ether and ethyl acetate fractions from herb (IC_{50} 13.33 - 16.88 µg/mL, quercetin 9.89 µg/mL). Chemical mutagenesis was inhibited by almost 100% by non-polar fractions, but oxidative mutagenesis was inhibited only by 20-40% only by *S. canadensis* extracts. No clear relationship could be found between class of phytochemicals and antimutagenic activity.

In conclusion, both species are likely to have varied activity and their phytochemical profiles should be taken into account in quality evaluation of thereof herbal drugs. Pharmaceutical unapplied underground organs of these weeds can also provide potential chemopreventive substances for further studies.

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ANTIOXIDANT AND ANTIMUTAGENIC ACTIVITY OF MAIN ISOFLAVONES, XANTHONES, AND STILBENES FROM *BELAMCANDAE CHINENSIS RHIZOMA*

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Iris domestica (L.) Goldblatt & Mabb (syn. *Belamcanda chinensis*) provides rhizomes which are extensively used in East Asian traditional phytotherapy. Recently, the monograph of *Belamcandae chinensis rhizoma* has been included in the European Pharmacopoeia. The main group of phytochemicals identified in the dried rhizoma is polyphenols such as isoflavones, xanthones and stilbenes. The available literature indicates that *Belamcandae rhizoma* can prevent oxidation of biomolecules based on various antioxidant mechanisms: transition metal ions reduction, inhibition of substrate peroxidation, free radicals scavenging, there is also a number of *in vitro* studies which confirm its antimutagenic properties.

The goal of this study was to compare the antioxidant and antigenotoxic efficiency of compounds representing three major polyphenol classes from this drug. We would like to verify, how the different structures can contribute to antioxidant and antigenotoxic properties.

The compounds were isolated using classic preparative column chromatography on silica gel, Sephadex LH-20, ODS adn preparative HPLC and identified by spectral (UV/VIS, IR, MS, NMR). Irigenin -isoflavone, *trans*-resveratrol - stilbene as well as mangiferin, isomangiferin and neomangiferin - xanthone glycosides were examined in antiradical, antioxidant and antimutagenic assays. Xanthone glycosides exhibited a high antioxidant potential comparable to the well known antioxidant resveratrol. Conversely, irigenin was weakly active in these assays. All tested polyphenols exhibit antimutagenic activty against environmental mutagens but in diverse manner.

Funding: Wroclaw Medical University grant ST-909.

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SUDANESE MEDICINAL PLANTS- A SOURCE OF POTENTIAL BIOLOGICAL ACTIVITY

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Aim of the study: This study was conducted to investigate the traditional plant knowledge on medicinal uses in Sudan and the ethnomedicinal value of selected species was demonstrated through phytochemical and biological activity studies.

Methods: The information was collected using semi-structured interviews with traditional healers from eastern, western and south-eastern Sudan.

Results: The most represented families are Fabaceae, Combretaceae and Euphorbiaceae. The most frequently mentioned indications were digestive system disorders, skin diseases, diabetes, and urinary system diseases. Study on the roots of *Hydnora johannis* (traditionally used for the treatment of dysentery, diarrhea, and cholera) suggested that, the mode of action of water extract which is rich in tannins was not connected to their inhibition to the diarrhea bacteria but to their action on the digestive tract [1]. The antidiabetic effect of *Geigeria alata* is mediated by enhanced insulin secretion, modulation of β-cell function, and improvement of antioxidant activity in streptozotocin-induced diabetic rats [2]. Bulb of *Pancratium tortuosum* showed significant antimalarial activity (IC_{50} 0.5 µg/mL). Bulb of *Urginea grandiflora* showed tumor-specific antiproliferative activity against MCF-7 cells. *Grewia* spp. contained remarkably high amounts of iron with values 20.8 mg/100 g (*G. tenax*), 26.9 mg/100 g (*G. flavescens*) and 29.6 mg/100 g (*G. villosa*) supporting their traditional use in the treatment of anaemia [3].

Conclusion: The medicinal plants reported in this paper reflect a well-preserved traditional popular knowledge of people living in these areas. Some of these plants could be a natural source for bioactive agents.

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THE NOVEL NEUROPROTECTIVE EFFECT AGAINST CEREBRAL ISCHEMIC STROKE IN MICE BY PLATONIN, A THERAPEUTIC IMMUNOMODULATING MEDICINE

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Objective: Thrombosis and stroke are major causes of disability and death worldwide. However, the regular antithrombotic drugs may have unsatisfactory results and side effects. Platonin, a cyanine photosensitizing dye, has been used to treat trauma, ulcers and some acute inflammation. Here, we explored the neuroprotective effects of platonin against middle cerebral artery occlusion (MCAO)-induced ischemic stroke in mice.

Methods: The middle cerebral artery occlusion was adapted to induce regional brain damage and evaluate excitotoxic events after hypoxic challenge. Indexes including morphological infarct area, neurological scores, and biochemical indicators were analyzed to reflect hypoxic susceptibility. Platonin (200 µg/kg) were applied intraperitoneal 30 minutes after the MCAO surgery.

Results: We found Platonin (200 µg/kg) substantially reduced cerebral infarct volume, brain edema, neuronal cell death and neurological deficit scores, and improved the MCAO-reduced locomotor activity and rotarod performance. Platonin (5 - 10 µM) potently inhibited platelet aggregation and c-Jun NH2-terminal kinase (JNK) phosphorylation in collagen-activated platelets. The antiaggregation effect did not affect bleeding time but increased occlusion time in platonin (100 and 200 µg/kg)-treated mice. Platonin (2 - 10 µM) was potent in diminishing collagen- and Fenton reaction-induced ·OH formation. Platonin (5 - 10 µM) also suppressed the expression of nitric oxide, inducible nitric oxide synthase, cyclooxygenase-2, interleukin-1β, and JNK phosphorylation in lipopolysaccharide-stimulated macrophages. MCAO-induced expression of 3-nitrotyrosine and Iba1 was apparently attenuated in platonin (200 µg/kg)-treated mice.

Conclusion: Platonin exhibited remarkable neuroprotective properties against MCAO-induced ischemia in a mouse model through its antiaggregation, antiinflammatory and antiradical properties. The observed therapeutic efficacy of platonin may consider being a novel medicine against ischemic stroke.

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SAPORIN (TYPE-1RIP) PRODUCTION IN *IN VITRO* CULTURES OF THE BALKAN ENDEMIC SPECIES *SAPONARIA STRANJENSIS* D. JORD.

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Saporins are type 1 ribosome-inactivating proteins (RIPs: EC 3.2.2.22) produced by soapwort, occurring in all plant organs, most abundantly in seeds. These toxins inhibit protein synthesis through a site-specific deadenylation of the large ribosomal RNA subunit at the level of the conserved α-sarcin/ricin loop. Type 1 RIPs are developmentally regulated in distinct organs and gene expression responds to biotic and abiotic stresses. Saporins have been of considerable interest and largely investigated in biomedical research because of their use in immunotoxin constructions, on the contrary, their biological function and physiological role *in planta* remain still controversial.

This study aimed to evaluate the production of saporin during the *in vitro* cultivation of *Saponaria stranjensis* plants and in the process of root culture growth. The Western blot analysis using saporin polyclonal rabbit antibody showed low saporin accumulation in the initial phase of root cultivation followed by a progressive increase during the linear growth phase (after the 6th day) and reaching a maximum at the end of the stationary phase (after the 21st day), which suggests that saporin might have a role in the arrest of culture growth. Comparison between saporin expression in differentiated leaves of *in vitro* propagated plants and root culture revealed difference in the protein pattern and intensity suggesting putative presence of additional protein isoforms or enhanced saporin accumulation in leaves. *S. stranjensis in vitro* cultures has a potential to be utilized as a promising model system for the production of pharmaceutically valuable toxins and also to study the regulation of RIP biosynthesis, processing and targeting within the plant.

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EXPOSURE OF HISTONE DEACETYLASE 2 INHIBITOR CURCUMIN AND ITS ANALOGUES PGV-0 AND PGV-1 CHANGE MEMORY AND COGNITIVE FUNCTION, ANXIETY, AND SOCIAL INTERACTION BEHAVIOR IN MOUSE

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The inhibitors of class 1 and 2 histone deacetylases (HDACs) have been reported as novel therapeutic approaches to treat neurodegenerative disorders, depression and anxiety and the cognitive deficits that accompany many neurodevelopmental disorders [1]. HDAC inhibitors ameliorated deficit in cognition and stress-related behaviors in a wide range of neurologic and psychiatric disorders. Preclinically, behavioral bioassay can be used to predict the influence of compounds for the treatment of pschyatric illness [2]. Curcumin, PGV-0 and PGV-1 have been reported to inhibit HDAC2 [3]. However, reports regarding the effect of curcumin and its analogues on the memory and cognitive function, anxiety and social interaction behavior are as yet to be examined.

Mice were divided into control and treated groups. They received sodium carboxy methyl cellulose (as control) and curcumin, PGV-0, PGV-1 orally once a day for 21 days. The behavior tests social interaction, open field, radial 8-arm maze and passive avoidance were performed after the induction of brain disorder by oral administration of 10% ethanol for 7 days (at day 29). To increase the dissolution and the bioavailability of the test compounds, they were formulated in Self-Nanoemulsifying Drug Delivery System (SNEDDS).

In different doses, curcumin, PGV-0, and PGV-1 increased social interaction capability of mice in their groups, reduced depression in open field test, and increased long term memory and cognitive function in passive avoidance test.

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NEW POSSIBILITIES FOR TARGETING PROTEIN KINASE B AND NUCLEAR FACTOR KAPA B IN CUTANEOUS T-CELL LYMPHOMA

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Aim: To investigate combinations between alkylphosphocholines and nanoencapsulated curcumin for treatment of cutaneous T-cell lymphoma (CTCL) by affecting different factors of proliferation, inflammation and skin related infections.

Methods: Photon correlation spectroscopy and electrophoretic laser Doppler velocimetry for determination of nanoparticle size and zeta-potential of nanosystems containing curcumin; HPLC for determination of curcumin concentration; MTT and CFU tests on CTCL cell lines for evaluation of cell viability, clonogenicity and combination effects; Cell death and NF-κB ELISAs; Light microscopy; Hoechst staining; Western blotting; antimicrobial activity by ISO 20776-1:2006(E); Chou&Thalalai software for calculations of drug-drug interactions; possible further response surface analysis of combination effects.

Results: Two copolymeric carriers were used to prepare nanosized curcumin delivery systems with diameter less than 200 nm and negative surface charge. Encapsulated curcumin penetrated through the cell membranes of CTCL cells faster than curcumin solubilized in ethanol. Combination effects were evaluated as additive to synergistic. Induction of apoptosis was observed at higher extend after incubation of cells with combinations than with curcumin. The expression of protein kinase B and NFkB was inhibited after treatment with the combinations. Erufosine exhibited antimicrobial activity against Gram-positive bacteria in concentrations ranging from 32 to 100 μM.

Conclusion: Our experimental findings indicated that the combinations of alkylphosphocholines and the non-toxic natural compound curcumin, when included into innovative nanosized drug delivery systems were promising and may enhance therapeutic potential for the treatment of CTCL as orphan disease.

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QUANTITATIVE VARIABILITY OF SESQUITERPENE LACTONE PARTHENOLIDE AMONG WILD POPULATIONS OF *TANACETUM PARTHENIUM* (ASTERACEAE) FROM IRAN

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Tanacetum parthenium (L.) Schulz-Bip. (Asteraceae-Anthemideae), also known as feverfew, is one of the most important aromatic and medicinal plants [1]. The principal compounds present in the aerial parts of the plant are sesquiterpene lactones (SLs) which are found in the glandular trichomes on leaves, flowers and achenes [2]. The predominant SL present in the plant is a germacranolide, parthenolide (PN), which has been indirectly linked to the anti-migraine action of feverfew preparations [3]. Feverfew herbal medicinal products for migraines have been available for more than three decades in the Europe. More and more pharmaceutical industries in Iran are interested in a PN and anti-migraine preparation from the plant; therefore, there is a need for its quantitative determination in existing natural populations. Feverfew is distributed in various regions from the North to the center of Iran [1]. Identification of elite germplasm having higher PN content can be multiplied through conventional or biotechnological means. In the present study, quantitative determination of PN among twenty-two *T. parthenium* populations collected from their natural habitat was assessed by HPLC for the first time. Results showed that the concentration of PN is ranging from 0.01 to 2.81 mg/g DW. Highest concentration of PN was found in the sample collected from Dizbad-e-bala, Neshaboor road, Khorasan Province. High PN producing populations can be play an important role in designing mass propagation as well as conservation and breeding strategies.

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BIOLOGICAL EFFECTS AND CHEMICAL HARACTERIZATION OF *IRIS SCHACHTII* MARKGR. EXTRACTS: A NEW SOURCE OF BIOACTIVE CONSTITUENTS

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Iris L. genus (*Iridaceae*) is widely distributed in Eurasia, North Africa, and North America gathering about 210 species. *Iris* rhizomes have been used as diuretics, carminatives, and laxatives as well as for the treatment of coughing and pharyngitis in different folk medicine systems [1]. In this study, the biological and chemical fingerprints of different extracts (methanol and water) from rhizomes and aerial parts of *Iris schachtii* were investigated. For the biological assays, antioxidant, enzyme inhibitory and mutagenic/anti-mutagenic effects were tested. Moreover, for the chemical investigations total and individual bioactive components were detected by spectrophotometric and HPLC-DAD techniques, respectively. Antioxidant capacity was assessed with different assays including free radical scavenging (DPPH and ABTS), reducing power (FRAP and CUPRAC), phosphomolybdenum and metal chelating assays. Enzyme inhibitory effects were tested against cholinesterase, tyrosinase, amylase and glucosidase. Furthermore, mutagenic/antimutagenic effects were evaluated by Ames test. Apigenin, luteolin and kaempferol were determined as predominant compounds in the studied extracts, the highest amount of apigenin being detected in the rhizome-water extract (4734 µg/g extract). Methanolic extracts from both aerial parts and rhizomes exhibited higher results in terms of antioxidant capacity and enzyme inhibitory effects. Particularly, methanolic extracts of rhizomes and aerial parts presented an important lipase inhibitory effect (26.55 and 23.14 mgOE/g extract). Furthermore, molecular docking was performed to better understand interactions between dominant phenolic compounds and selected enzymes. On the basis of our results, *I. schachtii* might be considered as a valuable source for designing of novel phytopharmaceuticals or additives in existing food products.

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PHYTOCHEMICAL, *IN VITRO* AND *IN SILICO* STUDIES ON *ONONIS NATRIX SUBSP. HISPANICA*: MULTIDIRECTIONAL PERSPECTIVES FOR NOVEL FUNCTIONAL PRODUCTS

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Ononis genus belongs to the family of Fabaceae which contains about 75 species in the world. The members of this genus have shown to possess remarkable biological activities such as antibiotic, antipyretic, anti-inflammatory [1]. At this point, the biological and chemical fingerprints of different extracts (ethyl acetate, methanol and water) from *Ononis natrix* subsp. *hispanica* were investigated in the present study. For the biological assays, antioxidant, enzyme inhibitory, DNA protection and antimicrobial activities were examined. Moreover, for the chemical investigations the total and individual bioactive components were detected by spectrophotometric and HPLC-DAD techniques, respectively. Antioxidant capacity was assessed with different assays including free radical scavenging (DPPH and ABTS), reducing power (FRAP and CUPRAC), phosphomolybdenum and metal chelating assays. Enzyme inhibitory effects were tested against cholinesterase, tyrosinase, amylase and glucosidase. The scavenging effect of extracts was determined in plasmid nicking assay for DNA protection assay. In addition, antibacterial effects of water, methanol and ethyl acetate extracts were detected by the way of disk diffusion method. Apigenin, luteolin and quercetin were found as predominant compounds in the studied extracts. Ethyl acetate and methanol extracts exhibited higher results in terms of antioxidant capacity and enzyme inhibitory effects. These two extracts also indicated strong antibacterial activity against to *S. aureus* and *S. epidermidis.* On the other hand, water and methanol extracts had DNA protection activity and protected supercoiled form of plasmid DNA from Fenton reaction. Furthermore, molecular docking was performed to better understand interactions between dominant phenolic compounds and selected enzymes. On the basis of our results, *O. natrix* subsp. *hispanica* could be providing a new perspective for designing of novel phyto-pharmaceuticals or functional ingredients.

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STUDY OF *PISTACIA LENTISCUS* VAR. *CHIA* FRUITS IN THE FRAMEWORK OF THE EUROPEAN PROJECT EXANDAS

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Exandas is a European project that unites 12 Academic and SME partners from European and North-African countries. Its aim is to study and exploit aromatic plant's by-products in order to develop new cosmeceuticals and food supplements.

Pistacia lentiscus var. *chia* (Anacardiaceae) is an evergreen tree, endemic of the Greek island of Chios. Its unique resin (mastic gum) is widely known and used for more than 2,500 years for its medicinal properties, especially against gastrointestinal disorders. A wide variety of products based on mastic gum are commercially available [1]. Renewable parts of this species, such as fruits, are not used in Greece, but can be found in the traditional medicines of other Mediterranean countries such as Algeria for the treatment of inflammation, burns and ulcer [2].

To the best of our knowledge, the fruits of the Chios variety have never been studied before. Extensive extractions using ASE with c-Hex, EtOAc, MeOH and H_2O were performed and the obtained extracts were evaluated for anti-oxidant, tyrosinase inhibitory, anti-fungal against the phytopathogenic fungus *Fusarium graminearum* and cytotoxic against the Bb2 microglia cell line activities.

Due to promising results combined with low toxicity, bio-guided fractionation was then conducted and phenolic compounds such as anacardic acid derivatives have been identified and isolated from non-polar and polar fractions.

Acknowledgements: We thank the European project EXANDAS (MSCA-RISE-2015) for financial support.

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ANTI-DIABETIC AND INTESTINAL MICROFLORA MODULATION ACTIVITIES OF LESSONIA NIGRESCENS ETHANOLIC EXTRACT IN STREPTOZOTOCIN-INDUCED TYPE 2 DIABETIC MICE

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To investigate the effects on anti-diabetic activity and intestinal microflora of *Lessonia nigrescens* ethanolic extract (LNE) in streptazotocin-induced type 2 diabetic mice fed a high-sucrose/high-fat diet. Fasting blood glucose level, body weight, oral glucose tolerance and histopathology of liver and kidney in mice in the lasted 28 days were determined to evaluate the anti-diabetic effect of LNE *in vivo*. The diversity of intestinal microflora was analyzed by high-throughput next-generation 16S rRNA gene sequencing. mRNA and protein expressions of phosphatidylinositol 3-hydroxy kinase (PI3K) and c-Jun N-terminal kinase 1 (JNK1) in liver were detected by real-time PCR and western blotting to explore the possible mechanism. Fasting blood glucose levels was significantly decreased after LNE administration in comparison with diabetic mice ($p< 0.01$). LNE controlled the normal body weight and ameliorated the impaired glucose tolerance of mice. The histology revealed that LNE could protect the cellular architecture of liver and kidney as shown in hematoxylin-eosin stain. Analysis of intestinal microflora showed that LNE significantly increased *Bacteroidetes* bacteria and decreased *Firmicutes* populations. Specifically, LNE treatment may enrich the amounts of beneficial bacteria, *Barnesiella*, as well as reduce the abundances of *Clostridium* and *Alistipes*. The increased mRNA expression of PI3K in liver was observed in LNE groups, while the mRNA expression of JNK1 was significantly down regulated ($p< 0.01$). LNE could be considered as a functional food for reducing blood glucose and regulating intestinal microflora.

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SEQUENTIAL ELUTION OF ESSENTIAL OIL CONSTITUENTS DURING DISTILLATION OF HOPS (HUMULUS LUPULUS L.); OIL YIELD AND ANTIMICROBIAL ACTIVITY

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The profile and bioactivity of hops (*Humulus lupulus* L.) essential oil, a complex natural product. We hypothesized that compound mixtures eluted sequentially and captured at different timeframes during the steam distillation process of whole hop cones would have differential chemical and bioactivity profiles. The essential oil was collected sequentially at 8 distillation time (DT) intervals: 0 - 2, 2 - 5, 5 - 10, 10 - 30, 30 - 60, 60 - 120, 120 - 180, and 180 - 240 min. The control was a 4-h non-interrupted distillation. Nonlinear regression models described the DT and essential oil compounds relationship. Fractions yielded 0.035 to 0.313% essential oil, while control yielded 1.47%. The oil eluted during the first hour was 83.2%, 9.6% during the second hour, and only 7.2% during the second half of the distillation. Essential oil (EO) fractions had different chemical profile. Monoterpenes were eluted early, while sequiterpenes were eluted late. The Gram-negative *Escherichia coli* was strongly inhibited by essential oil fractions from 2 - 5 min and 10 - 30 min, followed by oil fraction from 0 - 2 min. The strongest inhibition activity against Gram-negative *Yersinia enterocolitica*, and Gram-positive *Clostridium perfringens*, *Enterococcus faecalis*, and *Staphylococcus aureus* subs. *aureus* was observed with the control essential oil. To the best of our knowledge, this is the first study to report significant activity of hops essential oils against *Trypanosoma brucei*, a parasitic protozoan that causes African trypanosomiasis (sleeping sickness in humans and nagana in other animals). Hops essential oil fractions or whole oil may be used as antimicrobial agents or for the development of new drugs.

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PHYTOCHEMICAL ANALYSIS OF *PINUS ELDARICA* BARK EXTRACTS

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Introduction: Pycnogenol®, a standardized extract of *Pinus pinaster* contains numerous phenolic compounds such as catechins, taxifolin, and phenolic acids (caffeic and ferulic acids). These compounds have received considerable attentions because of their anti-inflammatory, antimutagenic, anticarcinogenic, antimetastatic and high antioxidant activities.

Objective: To analysis of chemical composition of Iranian *P. pinaster* and *P.eldarica* bark.

Methodology: Reversed-phase-HPLC method was applied in order to identification and quantification of catechin, caffeic acid, ferulic acid, and taxifolin in. A mixture of 0.1% formic acid in deionized water and 0.1% formic acid formic acid in acetonitrile was used as mobile phase and chromatographic separation was achieved on a Nova pack C18 (Waters Association, Milford, MA) at 280 nm.

Results: An easy and simple RP-HPLC method for determination and quantification of catechin, caffeic acid, ferulic acid and taxifolin in pine bark extract was developed. The two studied Pinus species contained high amounts of polyphenolic compounds. Among four marker compounds, the main substances identified in *P. pinaster* and *P. eldarica* were taxifolin and catechin, respectively.

Conclusion: Our results indicated that Iranian *P. pinaster* and *P. eldarica* bark extracts other than French *P. pinaster* could be used as sources of polyphenolic compounds in the food and pharmaceutical industries.

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IDENTIFYING THE PHYTOCHEMICAL AND PHARMACOLOGICAL PROFILE OF DIFFERENT *HYPERICUM PERFORATUM* ACCESSIONS

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Hypericum perforatum is a native species to Europe, Middle East and North Africa, which has been adapted itself to different climatic and ecological conditions, often reaching weed status. Due to the widespread distribution of the plant, local cultivars or varieties have been developed, which may differ regarding their agronomic traits and the profile of secondary metabolites. The great natural variability may serve as a sound basis for breeding development but, at the same time, is the main reason of the high chemodiversity of the species. Considering the pharmacological properties and the traditional uses of *H. perforatum,* a phytochemical investigation regarding phloroglucinols, naphtodianthrones and polyphenolic compounds was carried out on different accessions (cultivated and collected from the wild), by using advanced chromatographic tools. Liquid Chromatographic analysis (UPLC-DAD, LC-MS/MS MRM) revealed significant variability in the secondary metabolites content of the examined methanolic extracts. The most common derivatives detected, belong to 9 groups i.e. benzoic acids, phenylpropanoids, coumarins, flavones, flavonols, flavan-3-ols, anthocyanins, phloroglucinols and naphtodianthrones. Apart from hypericin and hyperforin, the main characteristic compounds of methanolic extracts were catechin, epicatechin, quercetin, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-glucoside, neochlorogenic acid and proanthocyanidins (A and B series). Following such phytochemical analysis, the cytotoxicity profile of all herbal methanolic extracts was assessed in vitro, using primarily human colorectal adenocarcinoma Caco-2.

The data obtained thus far could permit a detailed profile characterization of *H. perforatum* plant raw material.

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BIOPHYSICAL AND FUNCTIONAL ESTIMATION OF *CALENDULA OFFICINALIS* EXTRACTS AND PROBIOTIC *LACTOBACILLUS* CULTURES

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Calendula officinalis is a well-known from centuries medicinal plant. Under different forms, it has been used to fight different problems in gut – inflammation, mall – digestion/absorption and help to heal pelvic and bowel infections and detox. However, little is known about the beneficial or cumulative effects of *Calendula* extracts (CEx) and probiotic lactic acid bacteria (LAB). With this aim the effects of freshly prepared extracts of *Calendula officinalis* and 11 probiotic mix, commercial and containing original Bulgarian strains, have been studied. A strain-specific effect on the LABs' growth in MRS broth, supplemented with 10% (v/v) of CEx prepared from fresh or dry plants was observed. Moreover, a stimulation of the growth of some *Lactobacillus plantarum* strains has to be pointed. In parallel, functional evaluation of extracts and probiotics alone and/or in combination was carried out. The Electro-Acupuncture according to Voll, also known as EDS, Electro-Dermal Screening test was applied. This is a method for individual, non-invasive detection of healthy imbalances used for prophylaxis, drug testing or adjustment of therapeutic schemas. Despite the fact that EDS testing is not a "standard medical procedure" it has great value in giving practitioners a "hands on" assessment. Thus, putative beneficial effects of several *Lactobacillus* cultures, their lysates and two *Calendula*'s extracts were estimated in case of patients with serious gastrointestinal disorders and chronic/non-communicable diseases.

These results are initial, but promising for further characterization and establishment of new integrative medical approaches for healthy prophylaxis.

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ISOLATION OF OLIGOSTILBENES WITH DIFFERENT DEGREES OF CONDENSATION

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The leaves of *Neobalanocarpus heimii* were investigated for their oligostilbene contents. Prior to isolation process, the determinations of compounds were based on mass spectrometric fragmentation patterns. Three compounds, heimiol B, hopeaphenol and vaticaphenol A were identified directly from the crude extract. Preparative high performance liquid chromatography (HPLC) was used to isolate and purify the other compounds. The purified compounds were then analyzed using NMR spectroscopy to identify the compound structure and stereochemistry. The method employed for the research modified to comply with different HPLC techniques such as preparative and analytical techniques. The crude sample was injected into preparative HPLC to obtain several fractions which consist of oligostilbene mixture. The fractions were further isolated using analytical HPLC to obtain four pure compounds. The compounds then were characterized using nuclear magnetic resonance (NMR). The result shows that the leaves extract of *N. heimii* contain three oligostilbenes, namely vaticanol A, balanocarpol and vaticaphenol A, and a galactopyranose.

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NOBILETIN, A CITRUS FLAVONOID, ACTIVATES VASODILATOR-STIMULATED PHOSPHOPROTEIN IN HUMAN PLATELETS THROUGH NON-CYCLIC NUCLEOTIDE MECHANISMS

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Vasodilator-stimulated phosphoprotein (VASP) is a substrate for cyclic nucleotide-regulated protein kinases (i.e., PKA). In the present paper, we show that, VASP is phosphorylated by nobiletin, a bioactive polymethoxylated flavone, in human platelets via non-cyclic nucleotide mechanisms. This result was proved by the inhibitors of adenylate cyclase SQ22536 and guanylate cyclase ODQ, as they did not response on VASP phosphorylation induced by nobiltein. Besides, this event was also not changed by the specific inhibitors of PKA (H-89), PKG (KT5823) and PKC (Ro318220), representing cyclic nucleotides-independent pathways to nobiletin-induced VASP phosphorylation. Similarly, the inhibitors of p38 MAPK (SB22536), ERK2 (PD98059), JNK1 (SP600125), Akt (LY294002), and NF-κB (Bay11-7082) did not modify nobiletin-induced VASP phosphorylation. Moreover, electron spin resonance (ESR), dichlorofluorescein fluorescence (DCF) and Western blotting techniques revealed that nobiletin did not response on hydroxyl radical ($OH^{\bullet}$), intracellular ROS and protein carbonylation, respectively. Furthermore, nobiletin-induced VASP phosphorylation was surprisingly reversed by the intracellular antioxidant N-acetylcysteine (NAC), but not by the inhibitor of NADPH-oxidase diphenyleneiodonium chloride (DPI). Thus, it is astonished to observe the differential effects of nobiletin and NAC on VASP phosphorylation in human platelets, since both they have reported antioxidant properties. The likely explanation of this effect that NAC may bind to allosteric sites on the receptor different from that nobiletin binds in the human platelets. Together, these data suggest that nobiletin is able to induce VASP phosphorylation in human platelets through non-cyclic nucleotide mechanisms. Nevertheless, the exact mechanisms of this hypothesis need to be confirmed in future study.

PARTICIPATION BY CORRESPONDENCE

PbC 1

THE ESSENTIAL OILS COMPOSITION OF *THYMUS PRAECOX* SSP. *POLYTRICHUS* COLLECTED FROM FOUR DIFFERENT ALTITUDES

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In order to highlight the cumulative effect of altitude and plant development on the chemical composition of volatile oils, *Thymus praecox* ssp. *polytrichus* was chosen as a study species. This subspecies grows in the Parang Mountains at different altitudes. For this purpose, the plant material was collected from 4 different locations, at different altitudes (950 m, 1600 m, 2069 m and 2145 m), in 2 consecutive years. *Thymus praecox* ssp. *polytrichus* is a subspecies quite rare, and studies on the chemical composition of volatile oil are scarce.

The volatile oil has been extracted using a Clevenger hydro-distillation process, according to European Pharmacopoeia. The separation and the identification of the components have been carried out with GC-MS (gas chromatography coupled with mass spectrometry) Agilent 6890 N with a spectrometric mass detector 5973 and an auto sampler. The separated compounds were identified by means of the NIST Mass Spectral Library, and the peak position was confirmed by the Kovats retention index.

The main chemical constituent of the volatile oils of *Thymus praecox* ssp. *polytrichus* is δ-cadinol, a compound obtained in high percentages in both years. This compound possesses replicative activities [1, 2]. In quite large amounts, the following compounds were also obtained: germacren D-4-ol, β-caryophyllene, α terpineol, terpinen-4-ol, cis-sabinene hydrate. Noteworthy is that germacren D-4-ol was identified in large quantities in first year, and it was not identified in volatile oils obtained in second year. Linalool was also produced in large quantities in first year, compared to second year.

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PbC 2

THE ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF *THYMUS DACICUS* COLLECTED AT THREE DIFFERENT PHENOPHASES

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Among the species of *Thymus* genus, *Thymus dacicus* is less studied in terms of antimicrobial activities and chemical composition of secondary metabolites. In the present work the authors investigated the antimicrobial activities of *Thymus dacicus,* a species that grows spontaneously in Romanian flora. For this purpose, the vegetal material was collected in 3 different phenophases (vegetative, anthesis and fruiting), during 2 consecutive years, from Novaci, Gorj County, Romania. The volatile oil was extracted using a Clevenger hydro-distillation process, according to European Pharmacopoeia. The separation and the identification of the components were carried out with GC-MS (gas chromatography coupled with mass spectrometry) Agilent 6890 N with a spectrometric mass detector 5973 and an auto sampler. To test the antimicrobial activity of the volatile oils studied, two methods were used: the Kirby-Bauer diffusion method and the microplate method. Antimicrobial testing was done on test microorganisms *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans*. Regarding the studies on *Thymus dacicus* only few data on the chemical composition of the volatile oil are available [1].

Our studies have shown that the essential oils of *Thymus dacicus* present antimicrobial activity in all in 3 phenophases (vegetative, anthesis and fruiting). Minimal inhibitory concentration is 1% in the case of the vegetative and anthesis stage and 10% in the fructification stage for *Staphylococcus aureus*; 1% for *Escherichia coli* for all stages and 0.1% for *Candida albicans*, except vegetative stage where the minimal inhibitory concentration is 1%.

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PbC 3

CONSERVATION AND SUSTAINABLE USES OF NATURAL RESOURCES IN SOME PROTECTED AREAS FROM THE NORTH - EASTERN PART OF ROMANIA

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This paper is a part of a broader study which aims to identify and assess the value of the ecosystem services of some Natura 2000 sites in the north-eastern part of Romania, focusing on information regarding the current usage of plant medicinal resources (included in the provisioning ecosistem service), in accordance with the conservation demands of the studied areas.

The first step was taken considering the rapid assessment methodology of ecosystem services at the site-scale, including habitat's description, identification and description of ecosystems and their services in their actual and alternative state, based on evolutionary scenarios. In the second step, taxonomic analysis was conducted focusing on species with economic value, including the medicinal plants.

Based on the collected data, conclusions were made regarding the tendency of evolution of each ecosystem service, the possible threats and necessary recommendations in connection with the sustainable use of biologic resouces, in accordance with habitat's conservation objectives. Moreover, there was created a database with posibilities in the use of biological resources, including the medicinal ones, in connection with the service and the sustaining ecosystem. The current usage rate was analysed in balance with ecosystem's ability to regenerate, ensuring long-lasting sustainability.

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PbC 4

THE INFLUENCE OF *EUPATORIUM CANNABINUM* L. EXTRACTS ON CANCER CELLS PROLIFERATION

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Eupatorium cannabinum L. (Asteraceae), commonly known as hemp-agrimony, has long been used for medicinal purposes since ancient times, due to its antitumour, antiinflammatory, antibiotic, hepatoprotective and immunomodulatory properties. These diverse therapeutic indications are correlated to richness in active compounds – phenolics, sesquiterpenes, pyrrolizidine alkaloids, polysaccharides. The present study aimed to determine the effects of two *E. cannabinum* extracts – in chloroform (EC) and water (EW), respectively, on human cell functions, with particular regard to their anti-proliferative properties in three cancer cell lines (Jurkat, BT-20, Caco2). In all assays, 5-fluorouracil was used as positive control. HPLC analysis confirmed the presence of eupatorin, eupatilin and quercetin in the non-polar extract while in the aqueous extract high amount of caffeic acid and rutin were found. Antiproliferative potential was evaluated by MTS assay against selected cell lines. The EC extract displayed higher tumour cell proliferation inhibitory activity in all cell lines tested. Jurkat cells were more sensible to both extracts (IC_{50} ranging from 7.35 ± 0.35 for EC to 13.77 ± 2.16 µg/ml for EW) while BT-20 and Caco2 cells were susceptible only on EC extract (IC_{50} 88.27 ± 1.34 on BT-20 cells and 231.54 ± 1.38 µg/ml on Caco2 cells) after 24h exposure.

The obtained data indicate that certain flavones are at least partially responsible for the observed cytotoxicity.

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OPTIMIZATION OF *LAPIEDRA MARTINEZII* LAG. (AMARYLLIDACEAE) PROPAGATION USING *IN VITRO* CULTURE SYSTEMS

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Lapiedra martinezii Lag. is a species endemic to the South-West Mediterranean area with high potential in the pharmaceutical and chemical industry as well as in ornamental gardening. We have optimised the *in vitro* propagation protocol for this species, starting from adult wild bulbs and seedlings. Sterilization and establishment of *in vitro* cultures were shown to be extremely difficult due to the presence of fungal contamination, endogenous bacteria and the subsequent inability of explants to restore growth. Once established *in vitro*, explants showed rapid and vigorous growth with spontaneous rooting when cultured in solid media, whereas culture in liquid media under permanent and temporary immersion yielded abnormal, hyperhydrated and lower amount of bulblets per explants than solid cultures, contrasting to other geophytes [1, 2]. In the solid media, the presence of benzylaminopurine in combination with low concentrations of auxin (mainly naphtalenacetic acid) gave better results than other cytokinins, paclobutrazol, ancymidol and the controls. The acclimatization showed a very high success, with 86% and 89% of survival for microplants obtained from seedlings and bulblets, respectively, after transference to *ex vitro* conditions. Fully acclimatized micropropagated plants were phenotypically indistinguishable from their wild relatives and 100% plants survived after one year of growth in outdoor conditions. These results provide an optimized protocol for *in vitro* propagation of *L. martinezii*.

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PbC 6

CRYOPRESERVATION OF *LAPIEDRA MARTINEZII* LAG. (AMARYLLIDACEAE) SEEDS

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Seed banking is widely used for economically important crop plants, crop wild relatives and native flora in general. Cryopreservation is one of the most effective methods for the long-term conservation of seeds [1-3]. *L. martinezii* is a South-West Mediterranean endemism with chemical and pharmaceutical potential and efforts on its germplasm conservation and propagation are needed. In this work we determined the desiccation tolerance of various seed samples, as well as the germination following [2], after preservation under three different sub-zero temperatures. Seed moisture content of desiccated seeds was 4.44 (desiccation chamber) and 4.10 (silica gel). These values are within the recommended international standards for seed storage at sub-freezing temperatures[3]. Desiccation only affected significantly the germination onset, but this did not have any consequence in the germination speed and final germination rate. The final germination did not show any significant difference after the two levels of desiccation combined with cold storage at -20, -80ºC (90 days in freezer) and -196ºC (one-week immersion in liquid nitrogen). A significant reduction in onset was obtained for all samples at all subzero temperatures tested. However, this did not finally affect germination speed. Since orthodox seeds are those that can tolerate drying to very low moisture contents (≤3 - 7% fresh weight), the obtained results suggest the orthodox storage behaviour of *L. martinezii* seeds and thus, they can be successfully preserved by 'conventional' seed banking approaches.

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PbC 7

A NEGATIVE EFFECT OF AN ULTRASOUND-ASSISTED EXTRACTION ON MEROTERPENE BAKUCHIOL YIELD

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Bakuchiol is a meroterpene phenol, which has many biological activities and is contained in some species of genus *Psoralea* (Fabaceae) in high amounts, and in much smaller quantities - in some other genera of plants of different families.

Ultrasound is often used in the process of an extraction of plant biomass. It is widely assumed that an ultrasound-assisted extraction can increase the yield of target compounds. However, the negative effect of ultrasound has been revealed in our research, when studying the extraction of bakuchiol from *Psoralea drupacea* shoots. Discoloring of some of studied chloroformic extracts has been observed after ultrasound treatments. The content of bakuchiol has decreased significantly (by 5 - 20%) in 60% samples in comparison with untreated ones.

These results emphasize the importance of control of possible negative effects of ultrasound on target compound yield during the development of plant extraction protocols.

On the other hand, it has been revealed that the content of one of the components of the studied extracts has proportionally increased. This substance has UV-Vis spectral characteristics which are very similar to such ones of bakuchiol, whereas the retention time of the one is much shorter. So, it can be suggested that bakuchiol has transformed into its more hydrophilic derivative during the ultrasound-assisted extraction process. Whether this transformation has been caused by an ultrasound itself or by some ultrasound-derived processes, remains unknown.

PbC 8

COMPARATIVE STUDY OF DOPAMINE CONTENT IN CALLUS CULTURE AND INTACT PLANTS OF *CELOSIA* GENUS

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The genus *Celosia* belongs to the Amaranthaceae family and includes two popular ornamental plants – *C. argentea var. cristata* and *var. plumosa*. The inflorescences of some *Celosia* species contain high amounts of dopamine [1] – a catecholamine, serves as a neurotransmitter in multicellular animals. Recently, an ability of callus culture of *C.argentea* to produce dopamine has been revealed as well [2]. However, the knowledge about the content of dopamine in the vegetative parts of *Celosia* plants is rather scarce. So, the comparative study of dopamine content in callus culture and different organs of *Celosia* intact plants (both cultivated *in vitro* and *in vivo*) has been performed by us by the means of HPLC analysis. The long-term cultivated callus of *C.cristata* was obtained from the Germplasm bank of world flora of our Institute, where it is kept for more than 10 years.

Dopamine has been found in 77% samples of inflorescences, in 80% - of seedlings and in 10 - 15% - of stem, root or leaves, whereas all samples of callus (n = 80) contained this substance. The content of dopamine in air-dried callus samples was 12.2 ± 0.9 mg/g of DW, that is much higher compare to investigated samples of inflorescences (5.8 ± 0.8 mg/g of DW) or seedlings (1.9 ± 1 mg/g of DW).

Thus, this is a rather unusual example of production of individual secondary metabolite by undifferentiated cells when such production is a) so high (about 1 - 1.5% of biomass dry weight); b) more stable and several-fold higher than in differentiated cells and organs.

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PbC 9

BIOACTIVE CONSTITUENTS AND *IN VITRO* ANTIOXIDANT ACTIVITIES OF TWO ECOTYPES OF SOME SAUDI MEDICINAL HERBS

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The bioactive phytoconstituents of endemic and acclimated plants have been used for the bioprospecting of novel compounds. However, the constituents of bioactive phytochemicals and the antioxidant activity are influenced to a great extent by several variables such as altitude, sunlight, soils, season and region of cultivation. We collected *Retama raetam*, *Salsola inermis*, *Hyoscyamus albus* and *Fagonia arabica* plants from two different regions; Jabal-al-Lawaz (JAE) and Wadi-e-Dissa (WDE) of Tabuk, Saudi Arabia. Phytochemical analysis of Methanolic leaf extracts of all the plants revealed the existence of alkaloids, phenols, flavonoids, terpenoids, tannins and carbohydrate. All the screened phytochemicals were higher in content in the JAE plants than WDE, except flavonoids in *S. inermis* (WDE). Results validate that these plants from Jabal-al-Lawz have considerable amount of bioactive constituents. Methanolic extracts of *H. albus* exhibited maximum DPPH antiradical, nitric oxide scavenging and metal chelating activities; however H_2O_2 scavenging activity was highest in *R. raetam*.

It was concluded that the plants collected from Jabal-al-Lawz are rich sources of bioactive phytochemicals and antioxidants and they could be used in the treatment of oxidative-stress induced degenerative diseases and disorders.

PbC 10

SIGNIFICANCE OF SULFUR IN HEAT STRESSED CLUSTER BEAN (*CYMOPSIS TETRAGONOLOBA* L. TAUB) GENOTYPES: RESPONSES OF GROWTH, SUGAR AND ANTIOXIDATIVE METABOLISM

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A sand culture experiment was carried out to study the effects of sulfur deprivation on heat stress tolerance of two cluster bean (*Cymopsis tetragonoloba* L. Taub) cultivars (GC-1 and Pusa Nau Bahar (PNB)). Three weeks old sulfur-starved and sulfur-supplemented plants were subjected to heat stress (45°C/35°C) treatment for 24 h. Total dry weight, chlorophyll content, Chlorophyll a : b ratio, electrolyte leakage, malondialdehyde (MDA) accumulation, H2O2 content, sugar, glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), ascorbate and glutathione concentrations and antioxidant enzyme activity (superoxide dismutase (SOD) and catalase (CAT)) were monitored, at the end of the heat stress treatment. Heat stress enhanced and sulfur starvation depleted the contents of sugar metabolites, but the accumulation of sugar, G-6-P and F-6-P were not related with heat stress tolerance. Antioxidant enzyme activities of SOD and CAT were influenced significantly more by sulfur starvation than heat stress. The results showed that under heat stress, the addition of sulfur helps to mitigate the oxidative damage in both the cultivars.

However, GC-1 was more heat tolerant as it was characterized by significantly higher total dry weight, chlorophyll content, ascorbate and glutathione content and lower H2O2, MDA, electrolyte leakage than PNB.

PbC 11

IN VITRO EFFECTS OF *JUNIPERUS COMMUNIS* L. EXTRACT ON NORMAL AND MALIGNANT MURINE CELL LINES

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Background: Natural products obtained from plants have been shown to exert a wide range of biological activities. Many studies suggest that *Juniperus* genus could represent a source of bioactive compounds with potential anticancer activity.

Aim of the study: In the present work, the antiproliferative properties and the cytotoxicity of ethanolic *Juniperus communis* L. (*Juniperi pseudo-fructus*) extract (JcE) were determined on B16-F12 (murine melanoma) and L-929 (murine fibroblasts) cell lines.

Methods: The JcE was obtained by classical methods and analyzed by GC-MS (gas chromatography- mass spectrometry). The cell proliferation/viability of B16-F12 (ATCC-CRL-6475) and L-929 (ATCC-CCL-1) cells exposed to JcE were determined using a colorimetric assay (MTS test). Cell cycle distribution was analysed by flow cytometry, using propidium iodide staining.

Results: We found that treatment of B16-F12 cells significantly reduced cells proliferation and induced cell death in a dose-dependent manner compared with that in the untreated control, while the MTS test made on L-929 normal cells has proved a low toxicity of JcE. Cell cycle analysis indicated that exposure of B16-F10 cells to JcE resulted in cell cycle arrest at G2/M phase.

Conclusions: Our data demonstrate that JcE reduced viability of murine melanoma cells and modulates cell cycle progression, suggesting being a promising chemotherapeutic candidate. Our future aim will be to investigate the activity of the identified major constituents of the JcE, both in murine and human melanoma cell lines.

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PbC 12

EFFECT OF EXTRACTION PROCEDURE, SOLVENT AND DRUG-TO-SOLVENT RATIO ON PHENOLIC CONTENT FROM URTICAE DIOICA FOLIUM

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Introduction. The growing interest for polyphenols is explain by the fact that they have been associated with the inhibition of oxidative stress found in diseases like cancer, stroke and neurodegenerative pathologies [1]. *Urtica dioica* L. (stinging nettle) is a rich source of polyphenols, chiefly phenolic acids, and the therapeutic activity of the stinging nettle leaves seems to be linked to the presence of the phenolic compounds [2].

The aim of this paper is to select the optimal extraction parameters (solvent, extraction procedure, drug-to-solvent ration, CT) for phenolic compounds, using an experimental design.

Material and methods: The stinging nettle's leaves were harvested from Racari Town, Dâmbovița County, in Aprilie 2015. The extraction parameters were: solvents [water, 20% ethanol (v/v), 50% ethanol 50/50 (v/v), etanol 70% (v/v)], extraction procedures (maceration, refluxation, infusion) and the drug-to-solvent ratio (1 : 10, 1 : 20 and 1 : 30). The content of flavonoids, phenol-carboxylic, tannins and proanthocyanidins were determined using spectrophotometric assays [2]. The selection of the optimal parameters was done using an experimental design type D-optimal, Experimental-Design, type 10.0 Trial.

Results: The statistical equations are fitted more than 60% for all active principles. The statistical models described best the phenolic acids behaviour (R^2_{adj} = 89.5%) and least proanthocyandins (R^2adj = 63.60%). The statistical importance of the extraction parameters decrease in the following order: drug-to solvent ratio > type of extraction> type of solvent.

Conclusion. The methods are fitted for the active principles optimization. The selected parameters were: refluxation, 50% ethanol (v/v), drug-to-solvent ratio 1 : 30.

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PbC 13

EFFECT OF STORAGE CONDITION ON PHENOLIC AND ASCORBIC ACID CONTENT FROM *PETROSELINUM CRISPUM* FOLIUM

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Introduction. *Petroselinum crispum*, parsley, an important source of ascorbic acid and phenols, is used for culinary purposes, both fresh and dried. It is well known the sensibility of these compounds to storage conditions (loss of ascorbic acid, degradation of polyphenols) that may affect the overall quality of the raw material [1].

The aim of this paper is the comparison between three storage methods (lyophilisation, freezing and drying) of parsley leaves, *Petroselini folium*, in terms of ascorbic content and polyphenolic content.

Material and methods: The parsley leaves were bought from a local market, in Bucharest, in May, 2016. For the phytochemical exam, hydroethanolic solutions [drug-to-solvent ratio = 1 : 20, refluxation for 30 minutes with 50% ethanol (v/v)] were obtained from fresh product, lyophilised material, dried and frozen materials. The evaluation of polyphenolic compounds and ascorbic acid was assessed using spectrophotometric (Folin-Ciocalteu's method, for total phenolic compounds) and chromatographic methods (ascorbic acid) [2]. The results were expressed as g% tannic acid for phenolic compounds and mg ascorbic acid/100 g raw material.

Results: The content of ascorbic acid of the fresh leaves (100 mg) is similar to other research [3]. Loss of ascorbic acid is found in all the storage methods (lyophilisation: 9.505 mg; freezing: 8.66 mg; air-drying 0.695 mg). The polyphenolic content has a slitghly decrease during storage (fresh leaves: 2.78 g; lyophilisation: 2.49 g; freezing: 2.24 g; air-drying: 1.98 g).

Conclusion: Storage methods has an important influence concerning the quality of the raw material. Lyophilisation is the best method for conservation for parsley.

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PbC 14

THE TREATMENT OF EXTERNAL HEMORRHOIDS WITH NEW BIOAPIFIT® HERBAL ANTI-HEMHORROIDAL OINTMENT

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The efficacy of new multi-herbal ointment for the treatment of external hemorrhoids was evaluated. 50 patients ranging from 42 to 67 years with external hemorrhoids were treated 10 days (three times a day) with the herbal ointment consisted of the following ingredients: the macerates of the plants *Symphytum officinale*, *Plantago major*, *Calendula officinalis*, *Salvia officinalis*, *Hypericum perforatum*, *Achillea millefolium*, *Polygonum aviculare*, *Bellis perennis*, *Quercus robur*, *Olea europaea*, *Urtica dioica*, *Capsella bursa-pastoris*, *Matricaria chamomilla*; essential oils *Melaleuca alternifolia*, *Cympobogon martini*, *Origanum vulgare*, *Eugenia caryophyllata*, *Thymus vulgaris* ct. thymol, *Cinnamomum camphora* ct. cineol, *Cera alba*, honey and glycerol. The evaluation of the patients before and following the therapy was done in terms of pain (0 - 10), defecation discomfort (0 - 10), bleeding severity (0 - 4), anal itching severity (0 - 4) and overall subjective discomfort (0 - 10). Before the therapy the mean values and standard deviations for pain, defecation discomfort, bleeding, itching and overall subjective discomfort were 7.2 ± 0.8, 7.6 ± 0.5, 2.7 ± 0.7, 2.6 ± 0.8 and 7.7 ± 0.5, respectively. A significant decrease of all five parameters was obtained after only three days of the treatment with mean values of 3.2, 3.4, 0.7, 0.9 and 2.9 for pain, defecation discomfort, bleeding, itching and overall subjective discomfort, respectively. After five days of the therapy, there was no pain or bleeding while the mean value for overall discomfort was 0.2. At 10th day of the treatment all five parameters were graded 0 by all 50 patients.

Obtained results could be linked with the herbal composition with proven haemostatic, vasoconstrictor, astringent, anti-inflammatory and wound healing potential.

PbC 15

THE TREATMENT OF OSTEOARTHRITIS WITH NEW BIOAPIFIT® PAIN-RELIEVING HERBAL OINTMENT

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The efficacy of new multi-herbal ointment for the treatment of osteoarthritis was evaluated. 100 patients (50 males and 50 females of similar age and health status) ranging from 37 to 81 (57 ± 11.8) years with confirmed osteoarthritis of knee were treated 30 days (three times a day) with the herbal ointment consisted of the following ingredients: the macerates of the plants: *Salix alba*, *Spiraea ulmaria*, *Symphytum officinale, Mentha piperita, Juniperus communis, Pinus nigra, Rosmarinus officinalis, Viola odorata, Bellis perennis, Hedera helix, Juglans regia, Vitis vinifera, Calendula officinalis, Matricaria chamomilla, Lavandula officinalis, Achillea millefolium, Thymus serpyllum*; essential oils: *Eucalyptus globulus, Boswellia carterii, Cymbopogon citratus, Cedrus atlantica, Cinnamomum camphora, Cupressus sempervirens, Citrus paradisi*, *Cera alba*, honey and glycerol. The evaluation of the patients before and following the treatment was done by The Western Ontario and McMaster Universities Osteoarthritis (WOMAC) Index with maximum score of 96 which consists of three sub-categories: pain (0 - 20), stiffness (0 - 8) and physical function (0 - 68). Before the treatment the mean values and standard deviations for pain, stiffness, physical function and total score were 14.4 ± 3.4, 6.1 ± 1.7, 41.1 ± 13.4 and 61.5 ± 18.2, respectively. A significant decrease was obtained for all four parameters following the treatment with mean values and standard deviations of 5.3 ± 3.9, 2.6 ± 1.7, 19.5 ± 10.9 and 27.3 ± 16.1 for pain, stiffness, physical function and total score, respectively. There was no significant difference between males and females concerning the outcome of the treatment.

Multi-herbal composition with strong anti-inflammatory, analgesic and soothing effect was responsible for significant decrease of the symptoms and could be considered as possible alternative to standard osteoarthritis treatment.

PbC 16

CONCENTRATION-DEPENDENT DUAL PROOXIDANT/ANTI-OXIDANT ACTIVITY OF A PRENYL FLAVONOID ON BIOFILMS

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Candida albicans is a fungal species commonly associated with biofilm formation in human diseases. The present study investigated the prooxidant and antioxidant effects of 2', 4'-dihydroxy-5'-(1‴,1‴-dimethylallyl)-8-prenylpinocembrin (8PP), a natural prenylflavonoid, on *C. albicans* biofilms, by studying cellular stress and antioxidant response.

Biofilms of azole-sensitive (SCa) and azole-resistant (RCa) *C. albicans* strains were treated with different concentrations of 8PP (1.5 µM to 1000 µM). Biofilms were studied by crystal violet and confocal scanning laser microscopy (CSLM). Analysis of biofilmstructures was performed by COMSTAT software. Reactive Oxygen Species (ROS) were detected by the reduction of NBT, and Reactive Nitrogen Intermediates (RNI) by the Griess assay. ROS accumulation was also detected inside biofilms by using 2', 7'-dichlorodihydrofluorescein diacetate probe and visualized by CSLM. Superoxide dismutase (SOD) activity and the total antioxidant capacity of biofilms were measured by spectrophotometric methods. This work remarks the oxidative stress achieved by 8PP at lower concentrations (25 µM to 100 µM); however, at higher concentrations (200 µM to 1000 µM) an antioxidant effect was observed, with increased antioxidant defenses. The mayor changes in redox balance were observed in SCa biofilms, showing increased SOD activity values and the highest levels of nitrosative stress at 200µM of 8PP, compared to non-treated controls and RCa biofilms. Analyses carried out by COMSTAT, showed that biofilms treated with higher concentrations of 8PP showed different diffusion distances in their structures when were compared to controls.

The present study shows for the first time, a concentration-dependent dual prooxidant-antioxidant action of 8PP that can alter the biofilm formation.

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PbC 17

HEAVY METAL OCCURENCE IN EDIBLE PLANTS - HEALTH RISK ASSESSMENT

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Climate, environmental pollution, nature of the soil on which the plant is grown, and the degree of maturity of the plant at the time of harvesting are main factors influencing the concentration of heavy metals in plants. Heavy metal occurrence in edible plants cannot be underestimated as these foodstuffs are important components of human nutrition. Intake of heavy metal-contaminated edible plants may pose a risk to the human health. The presence of heavy metals such as As, Cd, Pb, Zn, Co, Fe and Se in the samples of edible plants: *Cichorium intybus* L. var. foliosum Hegi, *Allium schoenoprasum* L., *Allium porrum*, *Plantago major*, *Cucurbita maxima*, *Asparagus officinalis*, *Brassica oleracea* var. Sabauda, *Beta vulgaris*, *Brassica oleracea* var. *Acephala*, *Solanum lycopersicum* var. Cerasiforme, *Cynara scolymus*, *Brassica rapa* subsp. Rapa, *Plantago lanceolata*, *Raphanus sativus*, *Eruca sativa*, *Valerianella locusta*, *Beta vulgaris* subsp. Vulgaris, *Atriplex hortensis*, *Anthyllis vulneraria*, *Brassica oleracea* Gongylodes, *Brassica oleracea* var. Italica, *Physalis peruviana*, *Phoenix dactylifera* from Bosnian market was determined by using an atomic absorption spectrophotometer. Furthermore, health risks associated with the intake of these metals were estimated by hazard quotient (HQ) and hazard index (HI). The HQ of all metals were under 1, suggesting no health hazards of consuming investigated edible plants, for adult population. These results suggest that monitoring of heavy metals in edible plants should be performed in order to prevent excessive buildup of these heavy metals in the human food chain cause of long term consumption.

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PbC 18

EVALUTION OF PYROLLIZIDINE ALKALOIDS IN CERTAIN MEDICINAL PLANTS CORRELATED WITH THEIR TOXICITY

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Pyrrolizidine alkaloids (PAs) are a widespread class of heterocyclic organic compounds found in approximately 3% of the world flora, known for their hepatotoxicity [1]. The aim of the study was to assay the PAs of four dry extracts obtained from medicinal plants (*Senecio vernalis*, *Symphytum officinale*, *Petasites hybridus* and *Tussilago farfara*), in correlation with their toxicity effects tested in two invertebrate models: *Artemia salina* and *Daphnia magna*. A new GC-MS method, using a TR-5MS type capillary column was developed, the identification of the PAs was performed using using the Kovats retention indexes and the quantification was performed using senecionine as standard [2, 3]. The main identified PAs components were senecivernine, senecionine, seneciphylline, integerrimine, senkirkine, intermedine, symphytine, lasiocarpine, symveridine. The total PAs content found was of 494.86 mg/100 g dry matter in *Senecio*, 157.56 mg/100 g dm in *Symphytum*, 3.17 mg/100 g dm in *Tussilago* and 3.18 mg/100 g dm *Petasites* extract. All tested extracts were found to be toxic when tested on the said invertebrates, with the highest toxicity given by *Senecio* and lowest – by *Symphytum*. The results can be used to develop a GC-MS validated method for the assay of PAs in medicinal plants with a further development in the risk assessment study of PAs toxicity in humans.

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PbC 19

ANTITUMOR ACTIVITY OF FOUR PLANT EXTRACTS AND POTENTIATION OF IRINOTECAN EFFECTS AGAINST NEUROBLASTOM HUMAN CELLS LINES

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In the last years drug combination strategy was largely adopted in cancer therapy. Some studies have suggested that for cancer treatment, combination chemotherapeutics-phytochemicals may exhibit enhanced efficacy with diminished side effects [1, 2].

The aim of this study was to evaluate the chemotherapeutic potential of four plant extracts and its co-effects as a combination with irinotecan.

Antiproliferative activity was confirmed by RTCA, through impedance-based technology. The system evaluated cellular response in real time, without exogenous labels, in presence of plant extract and plant extract-chemotherapeutic cocktail at different concentrations and exhibited a high anticancer effect by determining dose-response curves through xCELLigence measurements against SYHY-5Y cell lines for 24, 48 and 72 h exposure.

The 4 plant extracts whose polyphenols content was expressed as gallic acid equivalents [GAE], *per* 1 mL sample, were: GE (*Geranium robertianum herba*), EP (*Epilobium hirsutum herba*), FG (*Fagus sylvatica folium*) and JG (*Juglans regia folium*).

The extracts of 0.5, 5 µg/mL showed no antiproliferative effect, while at higher concentrations (10, 20, 30, 40 µg/mL) of extracts, the cellular growth decreased rapidly. The concentration of 10 µg/mL was used to evaluate the antitumor effect of extracts-irinotecan combinations.

Cell index measurements for SHSY5Y cells have shown that the irinotecan-plant extract mixture has a greater inhibitory effect on cell proliferation than irinotecan alone.

Also, at the minimal concentration of irinotecan (5 µg/mL), the antiproliferative effect increases in the order of FG < EP < GE < JG.

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PbC 20

LIPID VESICLES CONTAING INDIGENOUS PLANT EXTRACTS WITH INCREASED ANTIOXIDANT ACTIVITY

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Objective: Intensive research is directed on incorporation of plant extracts in innovative drug delivery systems, like lipid vesicles [1, 2]. The aim of the paper was the incorporation of three plant extracts, *Echinacea purpurea*, *Lycium barbarum, Armoracia rusticana*, with the purpose to use such systems in biomedical or cosmetic field.

Materials and methods: Lipid vesicles with plant extracts were prepared by thin-film hydration method. The properties of loaded lipid vesicles such as: entrapment efficiency, mean particle size, polydispersity index, stability were analyzed. *In vitro* release of phytocompounds from lipid vesicles was investigated using dialysis bags and diverse kinetic models were applied to describe the release mechanism. The spectrophotometric method with DDPH was applied to antioxidant capacity determination. Also, it was investigated the cell viability of loaded lipid vesicles through MTS technique on mouse fibroblasts L-929 cells.

Results: Evaluation of loaded lipid vesicles showed good entrapment efficiency (higher than 60%), small sizes (138-200 nm), low polydispersity index and good stability over 60 days. *In vitro* drug release study showed the ability of lipid vesicles to provide slow release of phytocompounds. The release kinetics were best explained by Higuchi model. Also, the DPPH method revealed significant antioxidant activity.

Conclusions: These preliminary findings suggest that lipid vesicles could be exploited as carriers for herbal drugs in biomedical or in cosmetic applications, although further investigations are needed.

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PbC 21

ANTIBACTERIAL ACTIVITY OF COUMARINS ISOLATED FROM *SESELI DEVENYENSE* SIMOKAI FRUITS

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Coumarins are large group of natural compounds with different interesting activity e.g. antibacterial, inhibition of acetylcholinesterase or anticonvulsant. Genus *Seseli* is rich source of these compounds with interesting structure. The main problem of research concerning coumarins is isolation process, to obtain compounds with high purity.

Thus a preparative high-performance counter-current chromatography (HPCCC) method was successfully applied for isolation of natural coumarin derivatives. Compounds were obtained from methanolic extract of fruits and their identification was performed with NMR and MS methods. New coumarin aglycone devenyol and its glycosides, (+)-cis-khellactone, laserpitin as well as esters of lomatin were obtained in the pure form in single step separations in less than 40 minutes.

The antimicrobial activities of tested compounds were determined using the diffusion and dilution techniques and measuring their MIC's against two Gram+ bacteria: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228) and five Gram- : *Pseudomonas aeruginosa* (ATCC 27853), *E.coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883) and *Proteus mirabilis* (ATCC).

Majority of tested compounds appeard to be significantly active against tested microorganisms (MIC values 0.05 - 3.5 mg/ml).

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RARE COUMARINS ISOLATED FROM *PEUCEDANUM LUXURIANS* AS A POTENT ANTIBACTERIAL AGENTS

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Plants of Apiaceae family usually contain coumarins, which are used worldwide in traditional medicine as well as modern therapeutics. *Peucedanum luxurians* endemic plant from Armenia appears as a rich source of rare coumarins with interesting structure and activity, especially as antibacterial agents.

Preparative high-performance counter-current chromatography (HPCCC) method was successfully applied for isolation of natural coumarin derivatives. Compounds were obtained from methanolic extract of fruits and their identification was performed with NMR and MS methods. Stenocarpin, officinalin, officinalin isobutyrate, peucedanin and 8-metoxypeucedanin were obtained in the pure form in single step separations in less than 40 minutes.

The antimicrobial activities of tested compounds were determined using the diffusion and dilution techniques and measuring their MIC's against two Gram+ bacteria: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228) and five Gram- bacteria: *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883) and *Proteus mirabilis* (ATCC).

The results of the assay show that coumarins with rare structure isolated from *Peucedanum luxurians* have a strong antibacterial activity (0.08 - 3.5 mg/ml).

Acknowledgements: The work was financed from grant No 4/POLTUR-1/2016. The authors would like to express their garditude to Director of Botanical Garden of UAM in Poznań and specialists from this Garden for plant material.

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