4th INTERNATIONAL CONFERENCE ON NATURAL PRODUCTS UTILIZATION

FROM PLANTS TO PHARMACY SHELF

Albena Resort
BULGARIA

4th International Conference on Natural Products Utilization
13 CNPU

29 May
01 June
2019

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BOOK OF ABSTRACTS

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The 4th International Conference on Natural Products Utilization: from Plants to Pharmacy Shelf (May 29-01 June, 2019), Albena (Bulgaria), is organized with the kind support of the following organizations and entities

Illustration: Cover photo (*Centaurea trinervia* Willd.) provided courtesy of Prof. Dr. Svetlana Bancheva (IBEI-BAS)
Editor: Dr. Milen I. Georgiev

4th INTERNATIONAL CONFERENCE ON NATURAL PRODUCTS UTILIZATION: FROM PLANTS TO PHARMACY SHELF

Dear ICNPU Participants,

On behalf of the Organizing Committee, it is an outstanding pleasure and honor to welcome you to the **4th International Conference on Natural Products Utilization: from Plants to Pharmacy Shelf (ICNPU-2019). 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 the recent trends in (ethno)pharmacology, toxicology, molecular biology and biotechnology.

350+ experts from 50 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 will take place in Albena, one of the pearls of Bulgaria's North Black Sea coast. It is located in a picturesque bay 30 km away from the city of Varna. 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-2019**!

Milen I. Georgiev, PhD
Chair of the Organizing Committee

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(TR)

PROGRAMME

Tuesday, May 28, 2019

15:30 – 18:30	Registration
16:00 – 18:30	ICNPU-2019 workshop

Wednesday, May 29, 2019

08:30 – 18:30	Registration
08:30 – 18:30	Exhibition opening
09:00 – 09:30	**Official opening ceremony of ICNPU-2019**
09:30 – 10:15	**KL 1: Robert Verpoorte** (NL)
10:15 – 10:45	*Coffee break*
Session H	**Chairs: Efferth & Diederich**
10:45 – 11:20	**PL 1: Young-Joon Surh** (KR)
11:20 – 11:45	**IL 1: Albena Dinkova-Kostova** (GB/US)
11:45 – 12:00	**SL 1: Cristiana Tanase** (RO)
12:00 – 12:15	**SL 2: Piotr Michel** (PL)
12:15 – 12:30	**SL 3: Magdalena Kondeva-Burdina** (BG)
12:30 – 12:45	**SL 4: Milena N. Leseva** (BG)
12:45 – 13:00	**SL 5: Ricardo Lagoa** (PT)
13:00 – 13:10	ICNPU-2019 group photo
13:10 – 14:10	*Lunch*
Session He	**Chairs: Surh & Berger**
14:10 – 14:45	**PL 2: Thomas Efferth** (DE)
14:45 – 15:10	**IL 2: Marc Diederich** (KR)
15:10 – 15:25	**SL 6: Spiro M. Konstantinov** (BG)
15:25 – 15:40	**SL 7: Andrzej Stepulak** (PL)
15:40 – 15:55	**SL 8: Gian Luigi Russo** (IT)
15:55 – 16:10	**SL 9: Reneta Gevrenova** (BG)
16:10 – 16:25	**SL 10: Corina Danciu** (RO)
16:25 – 16:40	**SL 11: Jamal Mahajna** (IL)
16:40 – 17:10	*Coffee break*
Session Li	**Chairs: Dinkova-Kostova & Konstantinov**
17:10 – 17:35	**IL 3: Andrey Y. Abramov** (GB)
17:35 – 18:00	**IL 4: Atanas G. Atanasov** (PL/AU)
18:00 – 18:15	**SL 12: Joseph M. Hayes** (GB)
18:15 – 18:30	**SL 13: Rumyana Simeonova** (BG)
18:30 – 20:30	**Poster session I**
20:30 – 22:30	***Get-together party***

Thursday, May 30, 2019

08:00 – 18:30	Registration
08:30 – 18:30	Exhibition opening
Session Be	**Chairs: Popova & Macias**
08:00 – 08:25	**IL 5: Richard J. Robins** (FR)
08:25 – 08:50	**IL 6: Vassya Bankova** (BG)
08:50 – 09:05	**SL 14: Michael Spiteller** (DE)
09:05 – 09:20	**SL 15: Petras Rimantas Venskutonis** (LT)
09:20 – 09:35	**SL 16: Mingquan Guo** (CN)
09:35 – 09:50	**SL 17: Sorin Avramescu** (RO)
09:50 – 10:05	**SL 18: Vessela Balabanova** (BG)
10:05 – 10:20	**SL 19: Nokwanda P. Makunga** (ZA)
10:20 – 10:35	**SL 20: Ahmed Elkhateeb** (EG)
10:35 – 11:05	*Coffee break*
Session B	**Chairs: Verpoorte & Najdenski**
11:05 – 11:50	**KL 2: Rolf Müller** (DE)
11:50 – 12:15	**IL 7: Franz Bucar** (AT)
12:15 – 12:30	**SL 21: Sónia A.O. Santos** (PT)
12:30 – 12:45	**SL 22: Dunja Šamec** (HR)
12:45 – 13:00	**SL 23: Anna Szakiel** (PL)
13:00 – 13:15	**SL 24: Camelia P. Stefanache** (RO)
13:15 – 14:15	*Lunch*
Session C	**Chairs: Bankova & Robins**
14:15 – 14:50	**PL 3: Maurizio Battino** (IT)
14:50 – 15:15	**IL 8: Ioanna Chinou** (GR)
15:15 – 15:30	**SL 25: Gertrud Morlock** (DE)
15:30 – 15:45	**SL 26: Syed Ghulam Musharraf** (PK)
15:45 – 16:00	**SL 27: Raphaël Grougnet** (FR)
16:00 – 16:15	**SL 28: Lucie Cahlíková** (CZ)

16:15 – 16:30	**SL 29: Monika E. Czerwińska** (PL)
16:30 – 17:00	*Coffee break*
Session N	**Chairs: Dajic Stevanovic & Battino**
17:00 – 17:25	**IL 9: Francisco A. Macias** (ES)
17:25 – 17:40	**SL 30: Elwira Sieniawska** (PL)
17:40 – 17:55	**SL 31: Hélène Greige** (LB)
17:55 – 18:10	**SL 32: Dimitrina Zheleva-Dimitrova** (BG)
18:10 – 18:25	**SL 33: Andrei Zbuchea** (RO)
18:25 – 18:40	**SL 34: Hannah R Vasanthi** (IN)
18:40 – 18:55	**SL 35: Daniela Rigano** (IT)
18:55 – 19:10	**SL 36: H.P. Vasantha Rupasinghe** (CA)
19:10 – 19:25	**SL 37: Kanchana Usuwanthim** (TH)
19:30 – 21:30	**Poster session II**

Friday, May 31, 2019

08:00 – 18:00	Registration
08:30 – 18:30	Exhibition opening
Session O	**Chairs: Alipieva & Atanasov**
08:00 – 08:25	**IL 10: Valtcho D. Jeliazkov** (US)
08:25 – 08:40	**SL 38: Guillermo Moreno-Sanz** (ES)
08:40 – 08:55	**SL 39: Simone Carradori** (IT)
08:55 – 09:10	**SL 40: Claudio Ferrante** (IT)
09:10 – 09:25	**SL 41: Giustino Orlando** (IT)
09:25 – 09:40	**SL 42: Lucia Recinella** (IT)
09:40 – 09:55	**SL 43: Alev Onder** (TR)
09:55 – 10:10	**SL 44: Stefka Valcheva-Kuzmanova** (BG)
10:10 – 10:25	**SL 45: Rungnapa Sranujit** (TH)
10:25 – 10:40	**SL 46: Shivraj Hariram Nile** (CN)
10:40 – 11:05	*Coffee break*
Session F	**Chairs: Sieniawska & Müller**
11:05 – 11:30	**IL 11: Jianbo Xiao** (MO)
11:30 – 11:45	**SL 47: Weibin Bai** (CN)
11:45 – 12:00	**SL 48: Ashok Kumar Srivastava** (IN)
12:00 – 12:15	**SL 49: Adam Matkowski** (PL)
12:15 – 12:30	**SL 50: Smita Srivastava** (IN)
12:30 – 12:45	**SL 51: Evgeniya S. Mardanova** (RU)
12:45 – 13:00	**SL 52: Guoyin Kai** (CN)
13:00 – 13:15	**SL 53: Chao Zhao** (CN)
13:15 – 14:15	*Lunch*
Session Ne	**Chairs: Chiunou & Matkowski**
14:15 – 14:40	**IL 12: Zora Dajic Stevanovic** (RS)
14:40 – 14:55	**SL 54: Ekaterina Kozuharova** (BG)
14:55 – 15:10	**SL 55: Ivan Salamon** (SK)
15:10 – 15:25	**SL 56: Maria de Fátima Agra** (BR)
15:25 – 15:40	**SL 57: Carlos L. Cespedes** (CL)
15:40 – 15:55	**SL 58: Marilena Gilca** (RO)
15:55 – 16:10	**SL 59: Mehmet Zeki Haznedaroglu** (TR)
16:10 – 16:30	*Coffee break*
Session Na	**Chairs: Simova & Bucar**
16:30 – 16:45	**SL 60: Aleksandar Shkondrov** (BG)
16:45 – 17:00	**SL 61: Simon Vlad Luca** (RO/PL)
17:00 – 17:15	**SL 62: Magdalena Maciejewska-Turska** (PL)
17:15 – 17:30	**SL 63: Xinwei Jiang** (CN)
17:30 – 17:45	**SL 64: Jolene Brooks** (ZA)
17:45 – 18:00	**SL 65: Maria João Rodrigues** (PT)
18:00 – 18:15	**SL 66: Riham Gharib** (LB)
18:15 – 18:30	**SL 67: Nargess Shahbazi** (IR/NO)
18:30 – 18:45	**SL 68: Yancho Zarev** (BG)
18:45 – 19:00	**SL 69: Abdulwadood S.M. Alsoufi** (IQ)
19:00 – 19:15	**SL 70: Milena D. Vujanović** (RS)
20:00 – 00:00	***Gala dinner with closing ceremony and awards***

Saturday, June 01, 2019

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

KL – key-note lecture
PL – plenary lecture
IL – invited lecture + 5 min Q&A to invited oral slots
SL – short lecture (+3 min Q&A)

INVITED LECTURES

KL 1

SUGAR + SUGAR = LIQUID?

Robert Verpoorte[1], Young Hae Choi[1], Geert-Jan Witkamp[2]

[1] Natural Products Laboratory, IBL, Leiden University, The Netherlands
[2] Kluyver Laboratory, Delft University of Technology, The Netherlands

Through NMR-base metabolomics we found that in all kind of extracts of microbial, mammalian and plant cells certain organic bases, organic acids, amino acids, sugars and sugar alcohol occur in relatively large amounts. Much larger in fact than expected on the basis of these compounds being intermediates in primary metabolism. Their ubiquitous occurrence gave us the thought that they must have a function. Our first hypothesis was the possibility of ionic liquids formed by the organic bases like choline and betaine with organic acid like malic acid. The first experiments confirmed this. Further studies resulted in finding that various combinations of the mentioned compounds do give deep eutectic solvents, i.e. mixing these solid compounds in certain molar ratios results in liquids at room temperature. More than 150 combinations now have been characterized. We named them Natural Deep Eutectic Solvents (NADES). They can be divided into the following groups:

- Organic bases with organic acids: ionic liquids
- Organic bases with neutral compounds like poly alcohols and sugars
- Organic acids with neutral compounds like poly alcohols and sugars
- Amino acids with neutral compounds like poly alcohols and sugars
- Mixtures of neutral compounds like poly alcohols and sugars

By ^{1}HNMR it was shown that the NADES components are strongly bound via H-bonding, in some cases H_2O is part of these liquid crystal-like structures. The NMR also shows that with dilution with water gradually the interaction between the compounds disappears. NADES are excellent solvents for medium polar compounds, such as most secondary metabolites. In our view biosynthesis of poorly water soluble compounds occurs in NADES, e.g. attached to cellular membranes, where the polar charged head groups of the membrane lipids act as anchors for NADES in which enzymes and intermediates are dissolved. Also the ER and vesicles could be formed by metastable systems of lipids and NADES. All ingredients for NADES are found in resurrection plants, lichen etc. Also in drought or cold resistant plants typical NADES ingredients are found to be present. In terms of application the NADES are excellent solvents for both small molecules and macromolecules (proteins, polysaccharides, DNA, etc.) in which the compounds show better solubil-

ity and stability than in the currently used organic solvents. NADES thus are of interest as non-toxic, non-explosive, sustainable green solvents for many industrial applications. The first commercial NADES extracts are now on the market as ingredients for cosmetics. There are still many things to be learned from Nature!

References:

[1] Choi YH, van Spronsen, J, Dai J, Verberne M, Hollmann F, Arends IWCE, Witkamp GJ, Verpoorte R (2011) Plant Physiology 156: 1701-1715.
[2] Dai Y, Witkamp GJ, Verpoorte R, Choi YH (2013) Analytical Chemistry 85: 6272-6278.
[3] Dai Y, van Spronsen J, Witkamp GJ, Verpoorte R, Choi YH (2013) Analytica Chimica Acta 766: 61-68.

KL 2

BASIC MICROBIOLOGY, CHEMISTRY AND SYNTHETIC BIOTECHNOLOGY TO IDENTIFY AND CHARACTERIZE ANTIBIOTICS FROM MICROBES

Rolf Müller

Helmholtz-Institute for Pharmaceutical Research Saarland, University Campus E8.1, 66123 Saarbrücken, e-mail: rolf.mueller@helmholtz-hzi.de

Amongst the well-established bacterial producers myxobacteria have a great track record for the discovery of entirely new natural product scaffolds exhibiting promsing bioactivities [1]. This is at least in part due to the fact that they have been much less studied in the past in comparison to other traditional sources such as actinomycetes and bacilli. Nevertheless, the issue of rediscovery is a major hurdle for myxobacterial extracts as well. I will disucss recent results from our efforts to culture previously uncultured myxobacteria and to connect phylogentically distant clades to novel metabolites by metabolome and genome mining [2]. Examples of novel and genetically engineered natural products in preclinical development as broad spectrum antibiotics exhibiting novel mode of action(s) will be shown [3-6].

In addition, I will show examples of heterologous expression of myxobacterial compounds yielding producer strains making production of lead compounds for pharmaceutical development feasible [7].

References:

[1] Herrmann J, Abou Fayad A, Müller R (2017) Natural Product Reports 34(2): 135-160.
[2] Hoffmann T, et al. (2018) Nature Communications 9(1): 803.
[3] Baumann S, et al. (2014) Angewandte Chemie International Edition 53(52): 14605-14609.
[4] Kling A, et al. (2015) Science 348(6239): 1106-1112.
[5] Lesnik U, et al. (2015) Angewandte Chemie International Edition 54(13): 3937-3940.
[6] Hüttel S, et al. (2017) Angewandte Chemie International Edition 56(41): 12760-12764.
[7] Sucipto H, Pogorevc D, Luxenburger E, Wenzel SC, Müller R (2017) Metabolic Engineering 44: 160-170.

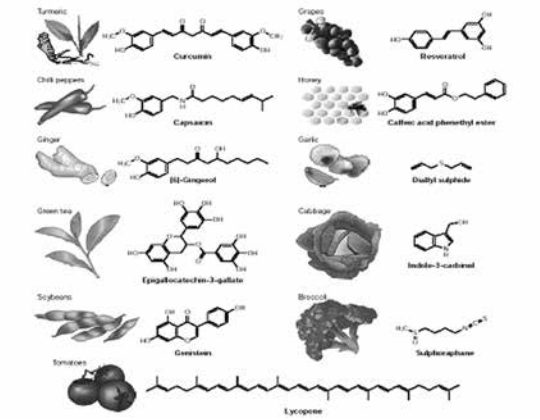
PL 1

CANCER CHEMOPREVENTION WITH NATURAL PRODUCTS: FROM OBSERVATIONAL TO INTERVENTION RESEARCH

Young-Joon Surh

Tumor Microenvironment Research Center, College of Pharmacy, Seoul National University, Seoul 08826, South Korea

The low risk of chronic diseases, such as coronary heart disease, neurodegenerative disorders, diabetes and certain cancers, results from consumption of relatively large amounts of plant-based diets including fruits, vegetables, spices, and nuts. Some marine products also contain distinct bioactive substances that exert diverse physiologic and pharmacologic effects. Health benefits associated with consumption of functional foods and beverages have been extensively investigated and well-documented. As oxidative stress and inflammatory tissue injury are implicated in a wide array of human disorders, antioxidant and anti-inflammatory naturally occurring substances have also been frequently included as active ingredients of many functional foods and nutraceuticals. In conjunction with the current concern about the practice of complementary and alternative medicine (CAM) in the management of many human disorders, the health beneficial effects of a wide array of bioactive substances in our diet as well as certain medicinal plants have been scientifically proven. Well-designed and controlled human intervention trials will help start the new era of 'precision nutrition'.



Scheme: Some natural chemopreventives [Surh Y-J (2003) Nature Reviews Cancer].

PL 2

NETWORK PHARMACOLOGY OF NATURAL PRODUCTS FOR CANCER THERAPY

Thomas Efferth

Johannes Gutenberg University, Mainz, Germany, e-mail: efferth@uni-mainz.de

To combat complex systemic diseases that harbour robust biological networks such as cancer, single target intervention is proved to be ineffective. In such cases, network pharmacology approaches are highly useful, because they differ from conventional drug discovery by addressing the ability of drugs to target numerous proteins or networks involved in a disease. Pleiotropic natural products are one of the promising strategies due to their multi-targeting and due to lower side effects. In this review, we discuss the application of network pharmacology for cancer drug discovery. We provide an overview of the current state of knowledge on network pharmacology, focus on different technical approaches and implications for cancer therapy (e.g. polypharmacology and synthetic lethality), and illustrate the therapeutic potential with selected examples from herbal mixtures, medicinal herbs and isolated phytochemicals. Finally, we present future perspectives on their plausible applications for diagnosis and therapy of cancer.

References:

[1] Kadioglu O, et al. (2014) Biochemical Pharmacology 87: 399-409.
[2] Zhao Q, Kretschmer N, Bauer R, Efferth T (2015) International Journal of Cancer 137(6): 1446-1456.
[3] Panossian A, Seo E-J, Wikman G, Efferth T (2015) Phytomedicine 22: 981-992.
[4] Saeed M, Kadioglu O, Khalid H, Sugimoto Y, Efferth T (2015) Journal of Nutritional Biochemistry 26: 44-56.
[5] Kadioglu O, Fu YJ, Wiench B, Zu YG, Efferth T (2016) Archives of Toxicology 90: 575-588.
[6] Poornima P, Kumar JD, Zhao Q, Blunder M, Efferth T (2016) Pharmacological Research 111: 290-302.
[7] Efferth T, et al. (2017) Oncotarget 8: 50284-50304.
[8] Hong C, et al. (2017) Scientific Reports 7: 11551.
[9] Hamdoun S, Fleischer E, Klinger A, Efferth T (2017) Biochemical Pharmacology 146: 63-73.

PL 3

THE ROLES OF STRAWBERRY AND HONEY PHYTOCHEMICALS ON HUMAN HEALTH: A POSSIBLE CLUE ON THE MOLECULAR MECHANISMS INVOLVED IN THE PREVENTION OF HUMAN CHRONIC DISEASES

Maurizio Battino, Francesca Giampieri, Danila Cianciosi, Johura Ansary, Tamara Forbes Hernandez

Nutrition and Food Science Group, Dept. of Analytical and Food Chemistry, CITACA, CACTI, University of Vigo - Vigo Campus, Vigo (Spain) and Department of Clinical Sciences, UnivPM, Ancona, Italy

Epidemiological studies have already established a close association between a fruit/vegetable-enriched diet and a significantly reduced incidence of chronic diseases. Bioactive phytochemicals are of increasing interest for their roles both in preventive strategies and as adjuvants in the treatment of different pathologies. During the past decade, our group has deeply evaluated the protective effects of natural compounds present in different food matrices against different types of stressors, both on *in vitro* and *in vivo* experimental models.

Our results showed that dietary bioactive compounds from strawberry and honey are able to protect stressed HDF and macrophages *in vitro*, by counteracting intracellular ROS production and apoptosis rate, increasing cell viability, promoting wound healing, reducing oxidative damage on membrane lipid, protein and DNA, attenuating inflammation as well as improving mitochondrial functionality and glycolysis activities. Importantly, we highlighted the capacity of these compounds of modulating, at molecular levels, the expression of several genes and proteins involved in apoptosis (i.e., caspase 3, p-p38 and p-Erk1/2 proteins), inflammation (i.e., NF-κB, iNOS, TNF-α, IL-1β), antioxidant defense (i.e., Nrf2, SOD, Catalase, HO-1) and mitochondrial biogenesis (i.e., AMPK, SIRT1, PGC1α).

Regarding *in vivo* studies, rats stressed with ethanol intake and fed with strawberry showed an increase in antioxidant enzyme activities (SOD and catalase), a decrease in gastric lipid peroxidation and a concomitantly inhibition of the development of ethanol-induced gastric lesions. Similarly, in rats stressed with Doxorubicin injection, strawberry consumption significantly inhibited ROS production and oxidative damage biomarkers in plasma and liver and ameliorated liver mitochondrial antioxidant levels and functionality.

References:

[1] Gasparrini M, et al. (2017) Food and Chemical Toxicology 102: 1-10.
[2] Gasparrini M, et al. (2018) Food and Chemical Toxicology 120: 578-587.
[3] Afrin S, et al. (2018) Food and Chemical Toxicology 121: 203-213.

IL 1

CYANOENONE NRF2 ACTIVATORS FOR THE PREVENTION AND TREATMENT OF CHRONIC DEGENERATIVE DISEASE

Albena T. Dinkova-Kostova[1,2]

[1] Jacqui Wood Cancer Centre, Division of Cellular Medicine, University of Dundee School of Medicine, Dundee, United Kingdom
[2] Departments of Medicine and Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, USA

Disrupted redox and protein homeostasis and chronic inflammation are characteristic features of most human pathologies. The transcription factor Nrf2 regulates the expression of large networks of genes encoding proteins that provide powerful and long-lasting protection against damage by oxidants and pro-inflammatory agents. The most potent Nrf2 activators (termed inducers) known to date are the pentacyclic cyanoenone triterpenoids, synthetic analogues of the natural product oleanolic acid, and their tricyclic and monocyclic derivatives. Irrespective of their molecular size or shape, the primary intracellular target of these electrophilic compounds is the highly reactive sensor cysteine, C151, of Kelch-like ECH-associated protein 1 (Keap1), the main negative regulator of Nrf2. The reversible covalent binding of cyanoenones to Keap1 impairs its repressor function, allowing for Nrf2 accumulation and enhanced transcription of Nrf2-target genes. The Nrf2 regulatory network includes drug metabolizing, antioxidant, anti-inflammatory and metabolic enzymes, proteasomal subunits and autophagy-related proteins, and thus plays a critical role in the maintenance of the cellular redox and protein homeostasis, and in the resolution of inflammation. The cyanoenone Nrf2 activators have shown multiple beneficial effects in cell culture and animal models of cancer, epilepsy, and Huntington's disease. Two cyanoenones bearing the triterpenoid oleanane skeleton are currently in advanced clinical trials.

IL 2

NATURAL COMPOUNDS INDUCE IMMUNOGENIC CELL DEATH IN CANCER

Marc Diederich

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

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 epigenetic treatments. It can be hypothesized that especially necrosis-related phenotypes triggered by various treatments or evolving from apoptotic or autophagic mechanisms, provide a more efficient therapeutic outcome depending on cancer type and genetic phenotype of the patient. In fact, the recent discovery of multiple regulated forms of necrosis 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.

This presentation will cover the effect of epigenetically active compounds and highlight their activity leading to non-canonical or immunogenic cell death.

IL 3

PLANT TERPENOIDS AS MOLECULAR TOOLS FOR UNRAVELLING ENERGY METABOLISM AND CELL SIGNALLING

Andrey Y. Abramov

Department of Clinical and Movement Neurosciences, UCL Institute of Neurology, Queens Square, London, UK WC1N 3BG

Mitochondrial dysfunction and deregulation of intracellular calcium signalling is proven to be one of the major triggers in mechanisms of neurodegeneration, development of cancer and heart diseases. Study of mitochondrial metabolism and calcium signalling is important for understanding basic mechanisms of cell physiology but also in cell pathology for finding the ways for treatment of disease. To study this, number of molecular tools are using by researchers for inhibition of the different enzymes, activation pathways and by using specific compounds which can modify ion transport.

Terpenoids, an abundant class of biologically active compounds which occurs in all living organisms and can display high biological activity. In a systematic investigation of 40 Central Asian species of *Ferula*, more than 100 new terpenoids have been isolated and structurally characterized. Terpenoids isolated from the plants of *Ferula* genus possess diverse biological activities. For several of them specific calcium ionophorecic activity was demonstrated. These compounds, and specifically ferutinin, are unique electrogenic calcium ionophores and they can stimulate intracellular calcium signalling and deliver calcium to mitochondria. Depending on concentrations, mitochondrial calcium can stimulate ATP production and be cell protective, high calcium in mitochondria trigger Permeability Transition pore opening and cell death. Consider these properties these compounds used by cell biologists for investigation mechanisms of cell physiology and pathology.

IL 4

INTERACTOMICS FOR NATURAL PRODUCT MECHANISM OF ACTION ELUCIDATION

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Metabolic and cardiovascular disorders are major contributors to human disease burden worldwide. To combat these disorders, the identification of pharmacologically active compounds, as well as the identification of new molecular targets, is highly relevant. Natural products have been continuing source of new drugs over the centuries and still are of high therapeutic relevance today. Moreover, natural products possess highly diverse chemical scaffolds, and are evolutionary optimized to serve diverse biological functions [1]. Interactomics is an experimental approach aiming to study the interacting partners of a certain molecule, and it emerges as a powerful technique to identify the direct molecular targets of pharmacologically active natural products. Presented in detail will be the use of novel interactomics approach (NPOT) for the identification of the molecular target underling the macrophage cholesterol efflux-enhancing action of the natural product evodiamine [2]. Our data illustrate the power of interactomics in elucidation of molecular mechanisms of action of small molecules, and outline evodiamine as a promising candidate to be further studied in the context of macrophage cholesterol efflux, a physiological process with relevance to cardiovascular and metabolic disorders.

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IL 5

NATURAL TRAMADOL – FACT OR FICTION?

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In 2013, we reported the discovery of Tramadol, previously only known as a synthetic analgesic, as a natural product in the root bark of the African medicinal tree *Nauclea latifolia* [1]. This surprising discovery arose as a result of a bio-activity guided isolation of the analgesic principle from the root bark. The claim cause considerable excitement in the community of natural product chemists, as well as substantial controversy [2, 3]. While synthetic tramadol appeared to be wide-spread in the environment in trace quantities (≤0.0001466% w/ w) due to abuse, it was hard to envisage how its occurrence at 0.4% w/w in the root sample collected was due to any other source than a natural origin.

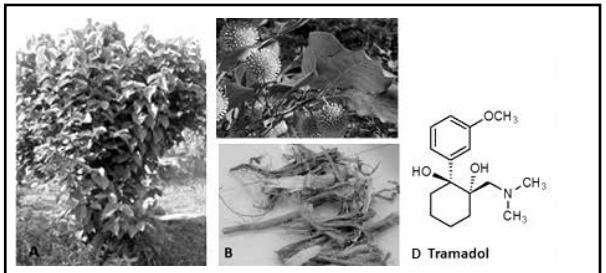

Figure 1. *Nauclea latifolia* A) tree; B) roots; C) flowers; D) tramadol.

Investigation of this problem was delayed by civil unrest in the north of Cameroon and Nigeria, access being too dangerous until mid-2017. Subsequently, a large number of samples have been collected of *N. latifolia* and various plant species growing close-by and these are currently being investigated.

Analysis by Orbitrap MS of the root tissue of a number of samples has consistently detected Tramadol but in very variable levels. However, in a number of samples, Tramadol is present at levels incompatible with contamination from a synthetic source. Furthermore, in some samples potential precursors/ metabolites are present.

In this presentation, the arguments in favour of and against the existence of Tramadol as a natural product will be advanced, and the latest results obtained with the objective of resolving the controversy will be presented.

Acknowledgements: We are grateful to a large number of other researchers who have played a role in one or more aspects of this investigation.

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IL 6

PROPOLIS OF STINGLESS BEES: A PHYTOCHEMIST'S GUIDE THROUGH THE JUNGLE OF TROPICAL BIODIVERSITY

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Stingless bees (family Apidae, subfamily Meliponini) are by far the largest group of eusocial bees on Earth. At least 600 species (61 genera) are found in tropical regions worldwide, with their highest abundance and diversity in the Neotropics (South and Central America), but also distributed in tropical Africa, Southeast Asia and Australia. They live in large colonies with one queen and between a few dozen and up to several thousand workers. Nowadays, meliponiculture is gaining popularity for honey production. Stingless bees also collect plant resins to produce propolis and use it to construct, maintain and defend their nests from infections, parasites and invaders. Some meliponines mix the propolis with an extra material: clay or soil, the resulting material is called geopropolis, it displays similar functions in the hive. Stingless bees propolis is used in the traditional medicines of indigenous peoples for treating wounds, gastrointestinal disorders, etc, and possesses antimicrobial activity. Of course the chemical composition of stingless bee propolis depends on the local flora, but numerous studies, including our own, have revealed that honeybees and stingless bees rarely use the same resin sources. Thus, studying stingless bees propolis gives the opportunity to find out new bioactive compounds and new sources of such compounds.

We have identified potent antimicrobial diterpenes, triterpenes, xanthones and new tocotrienol derivatives in stingless bees propolis from different Meliponini species and different tropical regions.

IL 7

PLANT SECONDARY METABOLITES AS NEW ANTIBACTERIALS

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Multidrug- and extensively drug-resistant bacteria challenge the global health system forcing to urgent development of new antimicrobials and development of new concepts of prevention and control of bacterial infections. In traditional medicine, treatment of bacterial infections is a major issue, yet medicinal plants still represent an insufficiently explored source of new anti-infectives with the potential of tackling multiple targets, and consequently lowering the risk of drug resistance development.

In our current research we explore one feasible way to combat this increase of resistance rates by application of efflux pump inhibitors (EPI), targeting ubiquitously expressed membrane transport proteins of bacterial cell walls which are able to extrude xenobiotics from the cytosol. In a study of naturally occurring and synthetic capsaicins and capsinoids, capsaicin and dihydrocapsaicin proved to be superior efflux pump inhibitors compared to the standard verapamil. On the other hand, the less pungent capsinoids qualified for further investigation as antibacterials against mycobacterial infections [1]. In an ongoing study of *Iris sp.* [2] several isoflavonoids proved to be resistance modulators of bacterial resistance in a *Mycobacterium smegmatis* model. Extracts from juniper (*Juniperus communis*) were able to act via a different mechanism, i.e. exerting antiadhesive properties as quantified by real-time PCR [3].

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IL 8

SELECTED ROYAL JELLY AND POLLEN SAMPLES FROM GREECE – CHEMICAL ANALYSES AND BIOLOGICAL ACTIVITIES

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Royal Jelly (RJ) is a milky secretion of hypopharengeal glands of worker bees *Apis mellifera* and is a sole food of the queen-bee during her life, while Bee pollen is a raw material produced by flowering plants pollen, mixed with nectar and bee secretions by the bees [1, 2]. Both are known to possess bioactivities, therefore they have considerable commercial appeal, ranging from the pharmaceutical and food industries to the cosmetic.

The objective of our studies was the characterization of the quality of more than 30 marketed RJ samples from Greece, as well as six pollen samples (3 multi and three monofloral from *Cistus creticus* and *Pinus* species) through different analytical techniques. Several secondary metabolites, including the main fatty acids (decanoic, decenoic acid derivatives) and flavonoids from RJ and pollen respectively, were isolated and structurally determined by modern spectral means.

The RJ samples, were further subjected i) in a clinical study at postmenopausal healthy women, showing a significant decrease in LDL and increase in HDL- cholesterol ii) in an *in vivo* study on Wistar male rats in Water-maze test, where RJ exerted an improvement of the spatial memory. Pollen samples' total phenolic and flavonoid contents were estimated, while antioxidant activities were determined by DPPH, ABTS assays, as well as by inducing highly proteasome activity demonstrating clearly its beneficial effect towards enhancement of cellular antioxidant mechanisms. Finally *Cistus* pollen by bioautography approaches was used in screening for anticholinesterase potential activity, together with antimicrobial activities against a panel of Gram (±) bacteria.

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IL 9

NANOENCAPSULATIONS OF POTENTIAL NATURAL PRODUCTS DRUGS

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Encapsulation techniques applied to natural products provide a method that improves their physicochemical properties, such as solubility and bioavailability, which are very important in biological applications. Strigolactone mimics and APO analogs are some of the natural products assayed as new natural herbicides that have been encapsulated by our research group. Nevertheless, anticancer drugs and provitamins natural compounds have been also tested as core compounds in the new core/shell studies.

Inside the methods recently applied, some of the most remarkable are: supramolecular polymer micelles with cyclodextrin polyrotaxanes and acetogenins encapsulated, showing pH responsiveness [1] and anti-melanoma activity. Polymer nanoparticles with phthalimide-lactones (strigolactones mimics) encapsulated with anti-parasitic plant applications [2]. Organic nanotubes and metal-organic frameworks as encapsulators of disubstituted disulfides (APO analogs) to be applied in weed control and on anti-cancer activities. Furthermore, we will show recent advances in new natural supports with supramolecular structure to be used in nanoencapsulation.

Acknowledgements: This research was supported by the Ministerio de Economía, Industria y Competitividad (MINEICO), Spain, Project AGL2017-88-083-R.

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IL 10

THE OLD NEW CROP: OVERVIEW OF HEMP RESEARCH AND DEVELOPMENT IN THE UNITED STATES

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Hemp (*Canabis sativa* L.) is a new-old crop, one of the most promising due to its multiple utilizations and the content of a whole array of biologically active substances synthesized and accumulated in different plant parts. The numerous economic products of *C. sativa* are the basis for grouping hemp into four categories: (1) fiber hemp, (2) oilseed hemp, (3) medical cannabis, and (4) recreational cannabis. The fiber and oilseed/grain hemp are collectively known as industrial hemp. Industrial hemp has been grown as a commodity fiber crop in the United States since the 1750s until 1930s. As in many other countries, *C. sativa* was banned and was considered an illegal crop in the United States for several decades. The 2014 "Farm Bill", (Section 7606 of the US Congress Agricultural Act of 2014), authorized the cultivation of industrial hemp, specified as "the plant *Cannabis sativa* L. and any part of such plant, whether growing or not, with a delta-9 tetrahydrocannabinol [THC] concentration of not more than 0.3 percent on a dry weight basis". The 2014 Farm Bill allowed "Institutions of higher education" and state departments of agriculture to grow hemp under a pilot program, provided state laws legalized it. The 2018 Farm Bill decriminalized *C. sativa* at federal level and legalized the production of hemp as an agricultural commodity. However, in some states *C. sativa* plant is still illegal, while other states allowed the production and utilization of all groups of hemp. In addition, 'agricultural hemp' became legally accepted name in one state, which fostered significant production of CBD, making hemp the largest crop commodity.

The talk will review current legal environment, the economic products of hemp, botany and ecology, phytochemical characterization, including, terpenes, essential oil and seed fatty acids.

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IL 11

METABOLITES OF POLYPHENOLS IN CELLS

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We have comprehensively explored the structure-stability relationship of natural polyphenols in DMEM medium without cells. Polyphenols with catechol or pyrogallol structure were evidently instable in DMEM medium without cells. We further investigated the metabolites of quercetin and luteolin when incubated with several cells (cancer and normal) at 37 °C in 5% CO_2 for 48 h. There are no any original compounds but their metabolites in the cell culture. However, we cannot detect any original compounds and metabolites inside the cells. The metabolites of quercetin were found to be the glucuronide, sulfate and methylate of its oxidized quinone. The metabolites of luteolin were found to be its glucuronide, sulfate and methylated form. The metabolites were quite different from different cells. Quercetin was found to enter into A549 cells and be glucuronidated within 30 min. Then, these quercetin glucuronides were quickly oxidized to quercetin quinones inside the A549 cells.

Acknowledgements: This work was financially supported by Multi-Year Research Grant of University of Macau (MYRG2018-00169-ICMS).

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IL 12

ENCAPSULATION FOR INNOVATIVE AND FUNCTIONAL PRODUCTS IN FOOD AND AGRICULTURE – WHERE NEXT?

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Added value of food and feed products is achieved by addition of various bioactive molecules. Most of components with prominent biological effects exhibit undesirable features upon inclusion into the food matrix, referring stability, bioavailability, effects on food sensory characteristics shelf life and interaction with food ingredients. Encapsulation of individual bioactive molecules or their mixtures is among best biotechnologies to maintain the biological, functional, and physicochemical properties of materials which are used as pharmaceuticals, nutraceuticals and agrochemicals. Encapsulation is a technology of entrapping an active agent within a shell or carrier material which enables designing of release and delivery of bioactive molecules and living cells into the food, feed, textile, cosmetic and different agricultural matrixes [1, 2]. The most used materials for outer wall are polysaccharides, such as starch and cellulose and their derivates, and plant exudates and extracts (gums, galactomannans, pectins, etc.), in addition to marine (carrageenans and alginates) and animal polysaccharides (e.g. dextran, chitosan, xanthan and gellan) [3]. Proteins and lipids are also widely used as carrier materials. Depending on key features of core and capsule material, as well as characteristics of a food or agrochemical matrix, certain types of encapsulation technologies are developed, such as: emulsification, spray-drying, spray-chilling, freeze-drying, melt extrusion, coacervation, melt injection, *in situ* polymerization and fluidized-bed-coating [3]. The present paper aims to review recent achievements in application of encapsulated bioactive molecules, mainly essential oils and polyphenols in functional foods, as well as in feed and agrochemical products, such as pesticides, controlled release fertilizers and plant growth regulators.

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SHORT LECTURES

SL 1

THE ROLE OF NATURAL PRODUCTS IN CANCER PREVENTION AND THERAPY – EVALUATION BY PROTEOMIC APPROACHES

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Research into natural products has experienced a certain resurgence recently. One reason for this interest is due to the growing evidence supporting the potential application of natural products as agents for cancer prevention and treatment. Natural products can regulate cellular signaling pathways, as well as down-regulate the expression of oncogenic miRNAs and up-regulate the expression of tumor suppressive miRNAs. By modulating these key processes, natural products can inhibit cancer cell growth and cancer stem cell (CSC) renewal, therefore deterring tumor progression and development. Furthermore, by targeting and inhibiting CSC, natural products could prevent the emergence of drug-resistant tumors. However, additional *in vitro* and *in vivo* studies and clinical trials are required to achieve the true value of natural products for the prevention and/or treatment of cancer.

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SL 2

(6*S*, 9*R*)-VOMIFOLIOL FROM *GAULTHERIA PROCUMBENS* EXHIBITS ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITY IN A CELLULAR MODEL OF HUMAN NEUTROPHILS

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Aims: (6*S*, 9*R*)-vomifoliol (VO), a derivative of 3-oxo-*α*-ionol, previously isolated from *Gaultheria procumbens* chloroform leaf extract, exhibited significant inhibitory activity towards lipoxygenase and hyaluronidase [1]. Additionally, the potential use of VO in the treatment of inflammation-related ailments was suggested in the literature [2]. However, there are only few known sources of VO, and a small number of reports concerning its biological activity. Therefore, the aim of the present study was to establish the value of various plant materials obtained from *G. procumbens* as sources of VO, and to investigate the anti-inflammatory and antioxidant effects of VO in a cellular model.

Methods: The content of VO in *G. procumbens* leaf, fruit, and stem chloroform extracts was determined by GC-MS. The bioactivity of VO was investigated in a model of human neutrophils obtained *ex vivo* and stimulated by LPS and *f*MLP. The effect of VO on the cells viability, the ROS level, and the release of the pro-inflammatory cytokines and proteases was evaluated.

Results and Conclusions: Incubation of human neutrophils with VO at 25-75 μM resulted in a dose-dependent and significant reduction of the ROS levels and the secretion of IL-1*β*, IL-8, TNF-*α*, elastase, and MMP-9. The strongest effects were observed for ROS, IL-1*β*, and IL-8. Although the *Gaultheria* extracts contained at most moderate amounts of VO, the tested isoprenoid revealed strong ability to modulate the pro-inflammatory responses of human neutrophils, and might be a promising model compound for development of novel anti-inflammatory agents.

Acknowledgements: National Science Centre, Poland (Grant Project: 2015/19/N/NZ7/00959).

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SL 3

EFFECTS OF *AMANITA MUSCARIA* EXTRACT AT DIFFERENT *IN VITRO* NEUROTOXICITY MODELS ON SUB-CELLULAR AND CELLULAR LEVELS

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One of the main compounds of *Amanita muscaria* is muscimol. Some literature data found that unilateral infusions of muscimol at ventral thalamic cores of 6-th patients with essential tremor, lead to reversible suppression of the tremor, without influencing the speech and willow movements [1]. Another study show the effect of unilateral infusion of muscimol at *Substancia nigra* of two patients, suffering from Parkinson's disease, which is connected also with significant decrease of the tremor [2]. These studies also proved free radical scavenging and antioxidant activity of the mushroom [1, 2].

According to these data, we investigate the possible neuroprotective effects of *Amanita muscaria* extract (at concentrations: 10 μg/ml, 1 μg/ml and 0.1 μg/ml) on different models of neurotoxicity in rat brain microsomes, rat brain synaptosomes and neuroblastoma cell line *SH-SY5Y*. We also investigate the *Amanita muscaria* extract, for possible inhibitory activity on human recombinant Monoaminoxidase-B (hMAOB) enzyme. We found that the extract revealed statistically significant neuroprotective effects on some of the *in vitro* neurotoxicity models and possessed inhibitory activity (at concentration 1 μg/ml) on hMAOB, closer to those of Selegiline.

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SL 4

MODULATION OF SIRTUIN ACTIVITY AND ITS EFFECT ON A MURINE MODEL OF ARTHRITIS

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Sirtuins are a family of protein deacylases, which are involved in cellular stress responses and function as metabolic sensors, and transcriptional regulators. We wanted to investigate the effect of sirtuin activity modulation on disease outcome of experimentally induced arthritis in mice, and to understand how Sirt1 activation/inhibition affects the neutrophils - key players in arthritic pathology.

Arthritis was induced by i.p. injection of a cocktail of monoclonal antibodies against collagen type II (CAIA). *In vitro* analysis was performed on purified bone-marrow (BM) neutrophils. We utilized synthetic compounds which specifically activate or inhibit Sirt1 activity - SRT 2183 and EX527, respectively.

Both compounds had a beneficial effect on clinical scores in CAIA mice. Parp1 cleavage, a marker for cell death, was reduced in SRT 2183 treated BM neutrophils *in vitro*. BM neutrophils from non-arthritic mice injected with SRT 2183 up-regulated IL-1β, a major pro-inflammatory cytokine, when compared to neutrophils from vehicle-only controls and CAIA mice. Consistently, IL-1β was increased in un-stimulated neutrophils following treatment *in vitro*, but this response was masked upon LPS challenge. In contrast, EX527 did not have the same effects. Our results suggest that IL-1β signaling most likely does not proceed through the non-canonical pathway involving MAPK, such as p38.

While Sirt1 activation and inhibition both appear to have beneficial effects on clinical symptoms *in vivo*, and on cell viability *in vitro*, they most likely act through (or are involved in) different signaling pathways.

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SL 5

IS CYTOCHROME C AT THE CENTER OF ANTIAPOPTOTIC ACTION OF FLAVONOIDS?

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Flavonoids have differential capacities to reduce cytochrome c (Cc) and inhibit the cardiolipin-induced peroxidase activity, and this Cc regulating ability may underlie their cytoprotective actions, more evidenced in conditions of oxidative stress and mitochondrial dysfunction. Cc has a major role in the initial stages of the apoptotic pathway, which is linked to mitochondrial dysfunction. In the cytosol, Cc can trigger the apoptosome assembly and activation of caspases that execute the cell death program. However, this is dependent upon the redox state of Cc and efficient reductants of Cc have been found to prevent caspase activation and apoptosis in different models. In this communication, the network of oxidation-reduction processes of Cc in the cell is presented with emphasis to the implications for apoptosis induction.

Published data on the effect of flavonoids on mitochondrial apoptosis in cellular models have been collected and analyzed. We found that the antiapoptotic efficiency correlates with the differential capacity of flavonoids to regulate Cc, supporting the concept of a tight coupling between modulation of Cc functions and antiapoptotic protection. Inhibiting apoptosis at an early stage, such as Cc mitochondrial release or apoptosome formation, is an attractive strategy to block or slowdown the progression of degenerative pathologies.

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SL 6

ANTINEOPLASTIC POTENTIAL OF NON-TOXIC NATURAL PRODUCTS (BULGARIAN-GERMAN EXPERIENCE)

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The unfavorable safety of existing anticancer medications and the issue of multidrug resistance have fuelled the search for plant compounds. One of the used approaches for identifying prospective candidates is based on ethno-pharmacology. Review of own published and unpublished data with curcumin, justicidin B and cannabidiol will be presented. Our studies aimed to determine the antineoplastic activity of natural products such as curcumin, justicidin B and cannabidiol in lymphoma, leukemia and urinary bladder cancer cells. Because of its low bioavailability and water insolubility curcumin was included in nanosized drug delivery systems. Cytotoxicity was measured by the MTT assay. Quantification of the sub-G1 fraction formation and DNA fragmentation detected by a specific ELISA were also performed. Changes in the signaling proteins were analyzed by immunoblot. Curcumin incorporated in copolymeric micelles showed favorable antibacterial activity as well. Apoptosis induction in tumor cells was evidenced by up- or down-regulation of pro- and anti-apoptotic regulators, caspase activation, PARP cleavage and oligonucleosomal DNA fragmentation. Interestingly, the presence of CD13 (myeloid marker known as aminopeptidase N) is associated with faster apoptosis induction. Curcumin caused concentration-dependent GSH level increases. Mouse experiments showed protection by curcumin against cisplatin-induced chromosomal aberrations (anticlastogenic effect).

Taken together, our data indicate that all tested natural products have a wide spectrum of pleiotropic activities including antitumor effects and protection of normal bone marrow, thus rendering them to promising candidates in the complex treatment of neoplastic diseases.

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SL 7

OSTHOLE AS POTENTIAL THERAPEUTIC AGENT FOR RHABDOMYOSARCOMA AND LARYNX CANCER

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Coumarins are a group of naturally occurring compounds common in the plant world. These substances and their derivatives exhibit a broad range of biological activities. One of the naturally occurring coumarins is osthole, which can most frequently be found in plants of the *Apiaceae* family Anticancer features of osthole have been demonstrated both in vitro on different cell lines, and in vivo using animals xenografts. Our studies focused on osthole as a potential therapeutic compound for specific types of cancer – rhabdomyosarcoma and larynx cancer. Chemotherapy regimens for patients bearing these cancers are very limited and include cisplatin, which causes serious side effects. Overcoming acquired cisplatin resistance and reducing cisplatin-mediated side effects are challenges for chemotherapy. In our *in vitro* studies performed on larynx cancer and rhabdomyosarcoma cell lines, osthole inhibited proliferation, motility and invasiveness of tumor cells, which was associated with the induction of apoptosis and cell cycle slowdown. Although the exact molecular mechanism of osthole anti-cancer mode of action has not been fully elucidated, it has been linked with differentially regulated *CDKN1A* and *TP53* gene expression depending on cancer cell type. Control cells – human skin fibroblasts were not affected by osthole treatment. Additionally, osthole and cisplatin applied together augmented their anti-cancer activities and yielded an additive type of pharmacologic interaction by means of isobolographic analysis.

Thereby, combined therapy using osthole and cisplatin could be suggested as a potential chemotherapy regimen against rhabdomyosarcoma and larynx cancer.

SL 8

FLAVONOID QUERCETIN IN CHRONIC LYMPHOCYTIC LEUKEMIA: FROM PRECLINICAL TO CLINICAL STUDIES

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Quercetin is the most abundant flavonoid present in the diet and its anticancer properties have been largely investigated [1]. Among these, quercetin is able to ameliorate resistance to apoptosis in cancer cells. Our data demonstrate that quercetin synergistically sensitizes to apoptosis several leukemic cell lines and B-cells isolated from patients affected by chronic lymphocytic leukaemia (CLL), when associated with pro-apoptotic agents, including ABT-737, a BH3-mimetics. Using HG3 cells, derived from primary B-cells, we demonstrate that the combined treatment of ABT-737 plus quercetin synergistically enhances apoptosis acting on multiple targets. In fact, ABT-737 exhibits its therapeutic efficacy against pro-survival Bcl-2 factors, while quercetin inhibits the PI_3K/Akt signalling pathway leading to the reduced expression of Mcl-1, an additional member of Bcl-2 family, which is responsible for apoptotic resistance in CLL. Mcl-1 is activated by Akt, but it is weakly inhibited by ABT-737. We identified the kinases CK2 and PI_3K as direct and primary targets of quercetin. Both enzymes positively regulate PI_3K/Akt signalling pathway upstream. The inhibition of CK2 activity by quercetin occurs within one minute from the treatment. In addition, the uptake of quercetin in HG3 cells is very rapid and its toxicity against normal peripheral blood cells is very low [2].

Taken together our studies on *in vitro* and *ex vivo* models suggest a possible clinical use of quercetin in adjuvant chemotherapy against CLL. To this aim, we designed of clinical study to demonstrate the efficacy of the molecule as potential chemopreventive agent in the early phase of the disease.

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SL 9

GYPSOPHILA SAPONINS ENHANCE THE CYTOTOXICITY OF ETOPOSIDE IN HODGKIN LYMPHOMA CELLS

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Highly glycosylated saponins **G**lucuronide **O**leanane-type **T**riterpenoid **C**arboxylic **A**cid 3,28-**B**idesmosides (GOTCAB) are accumulated in *Gypsophila* L. roots. In the study we aimed at investigating the possible synergistic effects of *Gypsophila trichotoma* GOTCABs and cytostatic etoposide towards the resistant Hodgkin lymphoma cell line HD-MY-Z. Their intrinsic cytotoxicity was tested on CCL-1 (mouse fibroblasts) according to ISO 10993-5/2009. The combination effects with etoposide were evaluated on HD-MY-Z cells using the symbolic mathematical software MAPLE. The cell clonogenicity was determined by CFU assay, the induction of apoptosis and generation of ROS were measured by Cell Death ELISA and ROS kit, respectively. Liquid chromatography-mass spectrometry allowed the identification or tentative assignment of more than 20 core GOTCAB structures in the root extract. Tested gypsogenin-based saponins possessed C-28 ester-bonded chain substituted with acetyl, *cis/trans* methoxycinnamoyl and both acetyl and sulphate groups. No cytotoxic effect was observed up to 20 µg/mL. Etoposide alone exerted IC_{50} >30 µg/mL. In the presence of saponins (20 µg/mL), a strong synergism (Fa = 0.8, CI = 0.2) was observed at etoposide concentrations significantly lower than 30 µg/mL. The combination inhibited the cell clonogenicity, induced apoptosis and interfered with the generation of ROS.

The results emphasize the arabinose in the C-3 chain and acetylation pattern of the carbohydrate chain at C-28 of the aglycone as an important structural feature for cytotoxicity enhancing activity. Triterpenoid saponins are a valuable tool to improve the efficacy and tolerance of cytostaics.

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SL 10

NEW INSIGHTS INTO THE ANTICANCER ACTIVITY OF FLAVONE APIGENIN

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The flavone apigenin (4´,5,7-trihydroxyflavone; Api) is an important component of the human diet, distributed in a wide number of fruits, vegetables and herbs with the most important sources being chamomile, celery, celeriac, parsley [1]. The aim of the study was to elucidate some of the anticancer mechanism of Api using A375 human melanoma cell line. Results have shown that the flavone presents antiproliferative activity against selected cell line with an IC_{50} of 33.02 µM. The antiproliferative mechanism involves also a G2/M arrest and lactate dehydrogenase release. Incubation with Api lead to caspase 3 activation as well as positive staining for Annexin V and PI, hallmarks of apoptosis. Moreover Api interfere with the mitochondrial respiration by modulating both glycolytic and mitochondrial pathways for ATP production. Metabolic activity of human DCs under LPS-activation was clearly attenuated by stimulation with high concentrations of Api (24 h and 48 h). Il-6 and IL-10 secretion was almost completely blocked by 30 and 60 µM stimulation with Api and TNF alpha secretion was reduced by about 60%. Using the *in vivo* CAM assay, the study investigated *in ovo,* the tolerability and potential influence of Api on the normal and tumoral angiogenic process, next to the effect produced directly on the development of A375 melanoma cells. Results have shown reduced number of capillaries inside the application area. Api 30 µM and 60 µM influenced tumor cell growth and migration, inducing a limited tumor area inside the application ring, associated with low number of capillaries.

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SL 11

EVALUATION ABILITY OF NATURAL PRODUCTS IN OVERCOMING TUMOR MICRONVIROMENT (TME)-MEDIATED CHEMORESISTANCE IN OVARIAN CANCER

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Ovarian cancer (OC) ranks as the second most common type of gynecological malignancy, has poor survival rates and is frequently (>75%) diagnosed at an advanced stage. Platinum-based chemotherapy, such as carboplatin, represents the standard-of-care for OC. However, toxicity and acquired resistance to therapy have proven challenging in the treatment of patients with OC. Despite advances in OC diagnosis and treatment, approximately 85% of patients will experience relapse, mainly due to chemoresistance. Chemoresistance, a principle obstacle to durable response in OC patients, is attributed to alteration within the cancer cells and is also mediated by tumor-microenvironments.

Recently, we developed platinum (IV) prodrugs that exhibit enhanced activity and ability to overcome chemoresistance in OC cells [1]. Moreover, we developed a fluorescent-based apoptosis biosensor that measures OC cell sensitivity to platinum-based drugs in co-culture with non-cancerous cells. Exposure of OC cells to soluble factors collected from mesenchymal stem cells or adipocyte cells resulted in reduced sensitivity to platinum compounds. Moreover, co-culture of OC cells with non-cancerous cells resulted in a significant reduction in OC sensitivity to platinum drugs. Our working hypothesis argues that a variety of signaling pathways are affected by the interaction between OC cells and component(s) of the microenvironment which leads to chemoresistance in OC. We will present data showing effects of natural products in restoring sensitivity to platinum drugs and consequently to overcome chemoresistance.

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SL 12

NATURAL PRODUCT BASED DISCOVERY OF GLYCOGEN PHOSPHORYLASE INHIBITORS: A MULTIDISCIPLINARY APPROACH

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Diabetes mellitus, characterized by hyperglycemia, is a chronic severe multiform disease with considerable socio-economic consequences. Recent WHO figures reveal an increase of cases from 108 million people in 1980 to 422 million in 2014, affecting ~8.5% of the global adult population. Regulation of the glycogen metabolism is a therapeutic strategy for blood glucose control in type 2 diabetes. Glycogen phosphorylase (GP) plays a key role in the glycogenolysis pathway, hence GP has been widely used as a target for compounds that might prevent glycogen breakdown under high glucose conditions. GP is an allosteric enzyme with six different binding sites discovered to date. The majority of inhibitor design efforts to date have focused on the catalytic site and in particular the design of glucose analogue inhibitors but other natural product analogues such as flavonoids and pentacylic triterpenes have revealed considerable potential [1]. Design efforts employing a multidisciplinary approach of computation, synthesis, crystallography, *in vitro* and *ex-vivo* studies have proved particularly effective. Recent examples of *in silico* motivated discovery of GP natural product inhibitors will be presented, where docking and post-docking methods have led to some of the most potent GP inhibitors discovered to date [2, 3].

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SL 13

BONE PROTECTIVE EFFECTS OF PURIFIED EXTRACT FROM *RUSCUS ACULEATUS* ON OVARIECTOMY INDUCED OSTEOPOROSIS IN RATS

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A lot of natural compounds present in dietary and medicinal plants could be useful in the prevention and treatment of many chronic diseases including osteoporosis (OP). *Ruscus aculeatus* is a source of steroidal saponins that could mimic human hormones (as estrogen) and might alleviate the symptoms of OP. The aim of the present study was to investigate the effects of purified extract from *R. aculeatus* (ERA) on the skeletal system of rats with estrogen deficiency induced by bilateral ovariectomy (OVX). The experiments were carried out on 3-months-old Wistar rats, divided into five groups: sham-operated control (SHAM), ovariectomized (OVX) rats, OVX supplemented with two doses of ERA - 50 and 100 mg/kg/day, orally for 45 days, and OVX supplemented with the positive control dioscin (40 mg/kg). Serum and urine bone turnover markers and bone histopathological changes were studied. All the animals were examined radiologically. Bilateral ovariectomy led to disturbance in biochemical parameters and changes in the region of tibial and femoral metaphysis. SERA restored most of the affected biochemical parameters in OVX rats more significantly at the higher dose. These effects were commeasurable with dioscin, well known hormonal modulator and bone protector from plant origin.

These findings provide new insights on the effects of *R. aculeatus* against OVX-induced bone loss, which could be developed as a potential candidate for prevention of postmenopausal osteoporosis in the future.

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SL 14

PLANT-ENDOPHYTE COMMUNICATION FOR BIOSYNTHESIS OF BIOACTIVE NATURAL PRODUCTS: MAYTANSINE AS AN EXAMPLE

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Studies on microbe-host interactions in plant and animal systems aimed at understanding the role of these associations and their utility in pharmaceutical and agricultural sectors are gaining impetus. Several recent studies have lent evidence to the fact that certain so-called "plant metabolites" are actually biosynthesized by associated endophytic microorganisms [1]. We recently investigated biosynthesis of the important anticancer and cytotoxic compound maytansine in Celastraceae plants in order to elucidate its actual producer(s), which has been an open question since its discovery in the 1970s. We showed that maytansine is actually a biosynthetic product of root-associated endophytic bacterial community in *Putterlickia verrucosa* and *Putterlickia retrospinosa* plants [2]. This extremely interesting outcome provided the scientific basis to investigate the actual producer(s) responsible for maytansine biosynthesis in *Maytenus* plants. Endophytic communities harboring different tissues of *Maytenus serrata* originating from Cameroon were investigated using a combination of bioanalytical tools such as HPLC-HRMS^n and MALDI-MSI, and targeted genome mining techniques to elucidate the source and sites of maytansine biosynthesis. We proved that the biosynthesis of maytansine in *M. serrata* is shared between the endophytic bacterial community colonizing the stem and the host plant containing non-culturable cryptic endophytes [3]. Our work demonstrates that maytansine is biosynthesized in *M. serrata* only when the host plant joins forces with its selected and very eco-specific endophytic bacterial community.

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SL 15

PHYTOCHEMICAL COMPOSITION, BIOACTIVITIES AND GENOTOXIC PROPERTIES OF *PAEONIA OFFICINALIS*, *DIOSCOREA CAUCASICA*, *BERGENIA CRASSIFOLIA* AND *SOLIDAGO CANADENSIS* EXTRACTS

Aušra Adamonienė[1], Gražina Slapšytė[2], Veronika Dedonytė[2], Juozas Rimantas Lazutka[2], Jūratė Mierauskienė[2], Ona Ragažinskienė[3], Petras Rimantas Venskutonis[1]

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Medicinal herbs are important sources for searching new natural ingredients for food supplements and pharmaceuticals. Our recent studies demonstrated significant antioxidant potential of European goldenrod [1] and heartleaf bergenia [2] extracts, while screening their phytochemical composition revealed the presence of health beneficial constituents. However, scientific knowledge on phytochemicals, bioactivities and toxicity of many plants remain scarce. To fill this gap our study investigated *Paeonia officinalis* L. (Paeoniaceae), *Dioscorea caucasica* Lipsky (Dioscoreaceae), *Bergenia crassifolia* (Saxiofragaceae) and *Solidago canadensis* (Asteraceae) extracts isolated by different solvents.

Phytochemicals were analysed by chromatography with various detectors, bioactivities assessed by antioxidant capacity and enzyme inhibition assays, Ames *Salmonella*/microsome test, alkaline single-cell gel electrophoresis (comet) assay and cytokinesis-block micronucleus assay in human lymphocytes were used for evaluating genotoxicity.

B. crassifolia, *S. canadensis*, *D. caucasica* herb and *P. officinalis* root and herb extracts showed negative results in the Ames test and micronucleus assay, indicating that they do not produce reverse mutation in bacterial cells and are not genotoxic in human lymphocytes *in vitro*. All investigated extracts induced primary DNA damage evaluated by the comet assay. The determined variation in response was due to the plant extract tested and donor susceptibility. Significant genotoxicity was determined in lymphocytes for *P. officinalis* and *D. caucasica* methanol extracts.

The extracts possessed antioxidant capacity and inhibited selected enzymes, while their activity was dependent on plant species, applied solvent and extract concentration. In general, the results provide important information for further studies leading to the development of valuable substances for foods, pharmaceuticals and cosmetics.

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SL 16

MOST RECENT ADVANCES IN INTEGRATIVE STRATEGIES FOR SCREENING ENZYME INHIBITORS FROM NATURAL PRODUCTS

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Recently, it has been found that almost half of the small-molecule drugs in the market are enzyme inhibitors [1], and many commercialized inhibitor drugs have been derived from natural products which will continue to be a potential source for new inhibitors due to their rich resources, diversified chemical structures, together with lower toxic and side effects. Therefore, robust and high-throughput techniques that can effectively and efficiently screen for enzyme inhibitors from the complex natural products are highly desired for new drug discovery and development, especially for the most popular "targeted natural drugs" efforts. Towards this end, our group has made great efforts in the development of integrative strategies for screening different enzyme inhibitors from natural products of interest in the past five years. Different drug target enzymes like Topo I, Topo II, COX 1, COX 2, α-glycosidase and son on, have been selected in our efforts, and an array of natural inhibitors, such as alkaloids, flavonoids, and saponins from different plants against those enzymes above have successfully been fished out and validated [2]. For example, an alkaloid from Lycoris radiata was screened out, and exhibited good dose-dependent inhibition against Topo I with IC_{50} at 7.25 ± 0.20 μg/mL without any modification comparable to the commercial drug CPT at 6.72 ± 0.23 μg/m (already used in clinic), which could be a very promising lead compound for future studies.

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SL 17

SFME EXTRACTION OF ESSENTIAL OIL FROM THE VEGETAL MATERIAL: SURFACE-RESPONSE OPTIMIZATION AND MODELLING

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Microwave assisted extraction of essential oil from different plants is a very efficient technique due to rapidity and high yield. Moreover, solvent free microwave extraction (SFME) is an emerging method which brings an important improvement in essential oil separation. However, this approach like any other extraction process need optimization and required a design of experiments (DOE) [1-3]. In this study, petals of Rosa "Gruss an Aachen" were submitted to SFME process and response surface methodology was used to evaluate the effects of processing parameters. The selected factors of composite design are the time of hydro-distillation and the ratio of the plant material and water and the extraction yields varied in the range of 0.11-0.14% (v/w) under different conditions. The volatile oil obtained by SFME of the rose petals was analyzed by gas chromatography and gas chromatography/mass spectrometry (GC/MS). Fifty-four compounds representing 99.08% of the oil were characterized. The volatile oil was found rich in sesquiterpenes and aliphatic components.

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SL 18

A VIEW ON BIOLOGICAL ACTIVITY OF SELECTED ASTERACEAE SPECIES GROWN ON VITOSHA MT. (BULGARIA)

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The flora of Vitosha Mt. (Bulgaria) is extremely rich and diverse. "Zlatni mostove" locality is an area with glacial origin and it is a habitat of a great number of plant species. In this study we investigated selected Asteraceae species: *Tanacetum macrophyllum* (Waldst. & Kit) Schultz Bip., *Senecio nemorensis* L., *Telekia speciosa* (Schreb.) Baumg. and *Cirsium appedinculatum* Griseb. which belong to the same plant communities. They were screened for antioxidant and enzyme inhibitory activity. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazine-6-sulphonic acid) (ABTS) and ferric and cupric reducing antioxidant power (FRAP and CUPRAC), metal chelating and phosphomolybdenum methods were used for antioxidant activity evaluation. In addition, cholinesterase, α-amylase and α-glucosidase inhibitory activities of the extracts were tested by microtiter plate assays. The phytochemical profile of the plants was assessed using spectrophotometric and UHPLC-HRMS techniques. The obtained results revealed that all of the studied plants have significant antioxidant and enzyme inhibitory potential. Moreover, a variety of caffeoyl- and feruloylquinic acids as well as, flavonoid aglycones and glycosides were identified. In conclusion, the assayed Asteraceae species have a potential against oxidative stress linked diseases.

Acknowledgements: The study was supported by Grant D-222/12.12.2018 from the Medical Science Council at the Medical University of Sofia, Bulgaria.

SL 19

OPPORTUNITIES FOR THE NATURAL PRODUCTS SECTOR USING MEDICINAL CAPE FLORA GUIDED BY METABOLOMIC APPROACHES

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South Africa, home to three biodiversity hotspots, has an incredible floral heritage that has medical and psychocultural significance to local people. Diverse ethno-cultural utilisation is thus encouraging entry of new products into global markets based on South African endemics, especially those from the species-rich Cape Floristic Region. Here, I will provide the unique opportunities that are presented by interfacing various biotechnologies to Cape plants for the phytopharmaceutics trade. Leaf-spray LC-MS/MS has been useful as a qualitative tool enabling fast and accurate detection of mesembrine alkaloids in *Sceletium tortuosum* (Mesembryanthemaceae) [1]. Microplant and callus cultures produce a wide array of these alkaloids for potential production using bioreactors. By applying both non-targeted and targeted metabolomics, influence of varied microenvironments on the phytochemistry and cytotoxicity of *Sutherlandia frutescens* (Fabaceae) plants is illustrated [2]. Data on the effects of strigolactones and overexpression of the AtTAXIMIN gene on metabolism are discussed. As the final example; *in vivo* studies have shown the *Dodonaea viscosa* (Sapindaceae) extract as a potential adjuvant anticancer therapy [3]. To better understand this extract, 28 chemicals were putatively identified from three disparate populations surveyed; and, 3 were structurally characterised via NMR. Combining microsatellite and chemometric analyses confirmed biogeographical-based genetic structure amongst populations, pinpointing a chemotype that may fit domestication and industrialisation. This particular study illustrates the exciting potential of medicinal biodiversity of South Africa that still remains chemically under explored.

Acknowledgements: Financial support was provided by the National Research Foundation, Pretoria (IKS160517165326; Grant: 105154).

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SL 20

LC-MS BASED METABOLOMIC PROFILING OF *LEPIDIUM CORONOPUS* WATER EXTRACT, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES AND CHEMOSYSTEMATIC SIGNIFICANCE

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The water extract of *Lepidium coronopus* (L.); Al-Shehbaz (syn. *Coronopus squamatus* (Forrsk.) Asch); (LCWE) was subjected to phytochemical investigation using HPLC-ESI-MS analysis. Thirteen flavonoid derivatives were detected. Nine kaempferol glycoside and acyl derivatives were identified, in addition to; two quercetin and two isorhamnetin glycosides. Among the identified compounds, 8 and 12 (kaempferol-di-*O*- glucoside-sinapoyl acetate isomers) are newly identified natural products and were firstly identified in the current study using LC-MS technique. LCWE was evaluated for its anti-inflammatory potential *in vivo*. It showed significant inhibition of the carrageenan induced hind rat paw edema, showing potencies 78.5, 78.5 and 89.3% at 100, 200 and 300 mg/kg, respectively. Meanwhile, the effects of LCWE on PGE_2, TNF-α and MPO production in the inflamed paw exudate were measured. Central and peripheral analgesic activities were evaluated by hot plate and writhing techniques. LCWE protected mice against acetic acid induced writhing by 28.2, 37.0 and 54.2% at 100, 200 and 300 mg/kg. LCWE Peripheral analgesia was stronger than central effect. LCWE also inhibited RANKL stimulated TRAP activity in RAW 264 cells completely at 50 and 20 µg/mL without any significant cytotoxicity to RAW 264 macrophages. The metabolomic profile of LCWE explained its biological activities.

Furthermore, the identified flavonoid constituents have strong chemosystematic significance confirming the change of nomenclature from the genus *Coronopus* to the genus *Lepidium*.

SL 21

PROMISING ANTIBACTERIAL DITERPENES FROM MACROALGA *BIFURCARIA BIFURCATA*

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In recent years marine resources have been seen as promising sources of value-added compounds. Macroalgae, in particular, become amongst the most exploited marine resources due to their high biological and chemical diversity and fast-growing properties. In this work the lipophilic fraction and particularly the diterpenes composition, of brown macroalgae *Bifurcaria bifurcata* was characterized in detail by gas chromatography-mass spectrometry (GC-MS). The extract presented antioxidant, anti-inflammatory and antibacterial activities. A relevant and promising synergetic effect of the extract with antibiotics was also verified [1]. In order to allow the use of *B bifurcata* diterpenes rich extracts in biomedical or pharmaceutical fields a green and sustainable extraction methodology was evaluated and optimized, namely high-pressure assisted extraction [2]. The optimization of the extraction conditions, namely pressure, time of extraction and solvent content, in order to maximize the diterpenes content, was carried out. The model was validated and *B. bifurcata* extract obtained at optimum conditions presented a diterpenes content significantly higher (6395±767 mg/g of extract) than that shown by conventional dichloromethane extraction (777±16 mg/g of extract). The bioactivities of high-pressure *B. bifurcata* extract were also verified.

Acknowledgments: S.A.O. Santos thanks the project AgroForWealth: Biorefining of agricultural and forest by-products and wastes: integrated strategic for valorization of resources towards society wealth and sustainability (CENTRO-01-0145-FEDER-000001), funded by Centro2020, through FEDER and PT2020, for the financial support. This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, FCT Ref. UID/CTM/50011/2019, financed by national funds through the FCT/MCTES. Acknowledgments are also due to FCT/MEC to the QOPNA research Unit (FCT UID/QUI/00062/2013).

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SL 22

DEVELOPMENT OF MALDI IMAGING MASS SPECTROMETY TECHNIQUE FOR SPATIAL MAPPING OF SPECIALIZED METABOLITES IN WHISK FERN (*PSILOTUM NUDUM* (L.) P. BEAUV.)

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Psilotum nudum (L.) P. Beauv. (Psilotaceae) is a seedless, rootless, leafless, fern-like vascular plant whose simple architecture resembles that of the earliest vascular plants. Although the direct descendance from early ancestors is disputed, it is still a viable model system to better understand the evolution of chemical diversity in plants. Newer technologies using Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) allows investigation of the small molecules distribution within biological systems through the direct analysis of thin tissue sections. In order to identificate and determinate spatial distribution of the main compound in *P. nudum*, we optimized tissue embedding technique, type, thickness and method for matrix applying as well as ionisation parameters.

Using combination of MALDI imaging with ion mobility-mass spectrometry we successfully identified and localized predominant metabolites, biflavonoid amentoflavone and signature phenolic glycoside psilotin and 3'-hydroxypsilotin.

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SL 23

OLEORESINS OF CINNAMON, GINGER AND BLACK PEPPER AS AN ALTERNATIVE TO SYNTHETIC PRESERVATIVES IN FOOD

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Synthetic preservatives are commonly used in modern food processing. Biopreservation, based on natural antioxidants and antimicrobial compounds derived from plant resources (including spices), can become an attractive alternative. In this study, the oleoresins of cinnamon, ginger and black pepper were obtained after extraction of respective spices with acetone and ethanol, and afterwards their antimicrobial and antioxidant properties were evaluated. The yield of extraction from plant material was the highest for oleoresin of cinnamon (16.9%), lower for black pepper (9.1%) and the lowest for ginger (6.9%). Radical scavenging potential of obtained oleoresins was evaluated by DPPH test. The established IC_{50} values accounted for 3.1 µg/ml for cinnamon, 20.6 µg/ml for ginger and 154.8 µg/ml for black pepper. Antimicrobial properties against chosen microorganisms causing food contamination and poisoning, i.e. Gram-negative strains *Escherichia coli*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Salmonella enterica* sv. typhimurium, Gram-positive strains: *Bacillus cereus*, *Staphylococcus aureus*, and fungus *Candida albicans* were tested by agar well difusion method. All oleoresins showed activities against *P. aeruginosa*, *B. cereus*, *Y. enterocolitica*, *S. aureus* and *C. albicans* with MIC = 12.5 mg/ml (ginger oleoresin was less active against *Y. enterocolitica* with MIC = 50 mg/ml). Cinnamon oleoresin was the only extract active against *S. enterica* with MIC = 25 mg/ml and *E. coli* with MIC = 50 mg/ml. Spice oleoresins are often used for food colouring because they are economical, cleaner (no bacterial contamination), with easier quality control and a longer shelf life.

The results obtained in this study point to the possibility of application of some oleoresins as a possible alternative to synthetic preservatives in food.

SL 24

PHENOLIC PROFILE, *IN VITRO* ANTIOXIDANT ACTIVITY AND *IN VIVO* CYTOTOXICITY OF *ARTEMISIA* SPECIES FROM REPUBLIC OF MOLDOVA

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The study aims at assessing the phenolic profile of three *Artemisia* species and their antioxidant and cytotoxic potential. Plant material consisted in *A. annua*, *A. absinthium* and *A. lerchiana* aerial parts harvested from the *ex situ* collection of Botanical Garden, Chisinau, R. Moldova. Methanolic extracts were investigated by HPLC-DAD [1]. Total phenolic content (TPC) was determined by Folin-Ciocalteu method. The antioxidant activity was assessed by DPPH and ABTS radical scavenging assays, reducing power and ferrous ion chelating assays. Cytotoxicity was determined *in vivo* on *Triticum aestivum* L. caryopses [2, 3]. Chlorogenic acid, caffeic acid, cynarin, hyperoside and isoquercitrin were the major phenolic compounds identified in *Artemisia* extracts. The highest TPC was determined for *A. annua* (24.85 mg/g dw), followed by *A. lerchiana* (10.43 mg/g dw) and *A. absinthium* (4.89 mg/g dw). The highest DPPH radical scavenging effect was determined for *A. lerchiana* extracts (EC_{50} values ranging from 27.96 ± 0.21 to 40.82 ± 0.89 µg/mL). *A. annua* exhibited the highest ABTS scavenging effects (EC_{50} values ranging from 12.76 ± 0.35 to 16.47 ± 0.32 µg/mL) and ferric ions reducing activity (EC_{50} values range: 15.06 ± 0.01 – 21.96 ± 0.08 µg/mL). All *Artemisia* extracts exhibited low ferrous ions chelating effects. The treatment of wheat caryopses with *Artemisia* extracts did not inhibit the germination, growth and biomass accumulation.

In conclusion, the phenolic content and the antioxidant effects of *Artemisia* extracts suggest their possible use in food and pharmaceutical industries.

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SL 25

MAXIMAL INFORMATION ON PRODUCT QUALITY AND SAFETY WITH MINIMALISM IN THE ANALYTICAL EFFORT

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More than ever, consumers are getting concerned about the food or food supplements they consume. Botanical extracts play an important role in commercial food products with increasing tendency, though frauds are quite common. Botanical specifications and regulation need to be improved. Product changes are not only caused by natural variances, but also by residues and contaminations along the global product chain, the addition of illicit compounds or adulterations. Small changes do matter, if active components at the trace level have impact on the consumer`s health, especially when consumed regularly. Which analytical effort is rational? Which non-target method can point to effects? All the thousands of compounds in a botanical extract under control? Which instrumentation is affordable?

An analytical technique that could cope with this complex task should be matrix-tolerant and avoid a discrimination of ingredients. As we only see what is detectable with the given detector, different detection principles are a must. Chemical marker compounds are used to evaluate the product quality, but they cannot represent the whole sample complexity, including its activity. At least, bioprofiling or effect-directed fingerprints should be considered for routine product quality, especially to ensure the product safety.

How the image-based straightforward hyphenated HPTLC technique can contribute to this daunting challenge is explained via examples given for plant extracts and functional food ingredients.

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SL 26

MASS SPECTROMETRIC DEREPLICATION OF NATURAL PRODUCTS USING MASS SPECTROMETRY INTEGRATED APPROACH

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Plant metabolites can act as drugs for the treatment of a variety of diseases due to their unique skeletal features. A large number of plant metabolites are used as drugs for the treatment of many diseases. The structural diversity of these plant metabolites formed by complex enzymatically controlled pathways is still not fully explored. Moreover, to preserve the medicinally important endemic and non endemic plant species and their sustainability, the quantity of plant material has been limited to the analytical level. Therefore, sensitive and high-throughput dereplication methods are required for better, unambiguous and high-throughput analysis of natural products in complex mixtures.

This talk will focus on the high-throughput dereplication strategy for the unambiguous identification of different classes of natural product through LC-ESI-MS/MS in the plant extract using an integrated approach which includes several confirmatory steps such as exact masses measurement, diagnostic fragment ions, databases search, and isotopic pattern. Based on above mentioned integrated approach, we have recently investigated different classes of natural products including withanolides (steroidal lactones) [1], pregnane-type steroidal alkaloids [2] and *Buxus* steroidal alkaloids [3, 4] and indentified them in the extract of *Withania somnifera*, *Sarcococca coriacea* and *Buxus papillosa*, respectively, by using electrospray ionization quadropole time-of-flight mass spectrometry (ESI-QTOF-MS/MS) and LC-QQQ-MS analysis. Moreover, the fragmentation pathways and characteristic fragments of a new triterpenoid [5] and some diterpenoids [6] by using ESI-QqTOF-MS/MS will also be presented.

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SL 27

IMPLICATION OF XANTHINE OXIDASE INHIBITION AND ANTIOXIDANT ACTIVITY OF *KALANCHOE PINNATA* HYDROETHANOLIC EXTRACT AND POLAR COMPOUNDS IN ETHNOMEDICINAL USES

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Originated from Madagascar, *Kalanchoe pinnata* (Crassulaceae) is widely cultivated all over tropical regions. Main characterized polar metabolites are kaempferol, quercetin and myricetin glycosides, a vinyl glycoside and malic acid. Many papers have reported a wide range of ethnomedicinal indications of leaves juice or extracts. Some of them describe the treatment of pathologies associated with hyperuricemia like urolithiasis [1], or pain caused by inflammation such as knee ache [2].

Xanthine oxidase is involved in the catabolism of hypoxanthin and xanthin to form uric acid. This enzyme is responsible for the medical condition known as gout, characterized by hyperuricemia and accumulation of uric acid crystals in joints, causing oxidative damage and intense pain. Long-term treatment with allopurinol generates serious side effects such as hepatitis, nephropathy and skin rash. Thus, alternative solutions are needed. Flavonoid derivatives, along with strong antioxidant properties, were already reported as XO inhibitors [3]. This prompted us to proceed to the evaluation of *Kalanchoe pinnata* hydroethanolic extract (KPHE) for XO inhibitory activity and antioxidant activity. Then, after determination of total flavonoid content and isolation of the major ones using CPC, we performed the evaluation of each compounds for XO inhibition and DPPH assay. KPHE and some of the pure flavones demonstrated inhibitory activity. All of these data may corroborate the central role of XO inhibition in addition to the intrinsic antioxidant effects of flavones in some of the traditional uses of KPHE. Molecular modeling studies are on progress in order to determine the possible binding mode with the enzyme.

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SL 28

DERIVATIVES OF CRINANE-TYPE OF AMARYLLIDACEAE ALKALOIDS AS POTENTIAL DRUGS IN THE TREATMENT OF ALZHEIMER'S DISEASE

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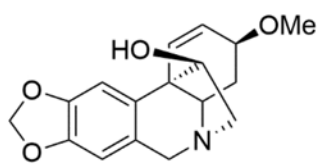
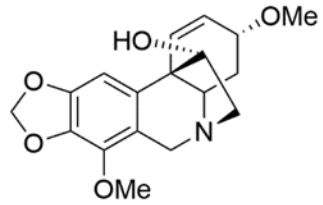
Modern research has shown that Amaryllidaceae alkaloids represent a rich reservoir of potential small molecules exhibiting several medicinal properties through various mechanisms. Among the many Amaryllidaceae alkaloids, galanthamine has been given a great amount of attention due the fact that it possesses potent acetylcholinesterase inhibition activity. Some of Amaryllidaceae alkaloids have shown remarkable cytotoxic and antiproliferative activity against diverse types of cancer cells. One of the most interesting compounds is haemanthamine (HA), β-crinine-type of Amaryllidaceae alkaloids, which displays significant in vitro cytotoxic activity against several different types of cancer cell lines (e.g. MOLT-4, HepG2, HeLa, etc.) [1]. Similar compound to haemanthamine is ambelline (AMB), which differ from HA only in orientation of 5,10*b*-ethano bridge and presence of methoxy group in position C7.



Haemanthamine



Ambelline

HA has been isolated from the bulbs of *Narcissus* cv. Dutch Master, and AMB from *Nerine bowdenii*, as a start material for the preparation of their derivatives. Twenty new derivatives of both alkaloids were developed. All derivatives were screened for their inhibitory potential against cholinesterases. The active compounds were further studied for their potency to inhibit GSK-3β, and ability to permeate through the blood-brain barrier. Some compounds, namely 11-*O*-(2-methylbenzoyl)-haemanthamine, and 11-*O*-(4-nitrobenzoyl)-haemanthamine, 11-*O*-benzoylambelline, and compounds marked as LC-70, LC-73 revealed the most intriguing profile. *In vitro* data were further corroborated by detailed inspection of their plausible binding modes in the active sites of AChE and BuChE.

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SL 29

ANTI-LIPASE AND ANTI-AMYLASE ACTIVITY OF EXTRACTS FROM SELECTED PLANT MATERIALS AFTER GASTROINTESTINAL DIGESTION *IN VITRO*

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Searching of pancreatic lipase (PL) and α-amylase inhibitors from natural products for the treatment of obesity and diabetes mellitus type 2 is justified due to a wide range of adverse effects of presently used drugs such as orlistat and acarbose.

The aim of the study was to establish α-amylase and PL inhibitory activities of the fractions obtained after an artificial metabolic transformation of selected plant extracts and an identification of active metabolites.
Fruits of *Chaenomeles japonica* (CJ), *Hippophaë rhamnoides* (HR) and flower of *Hibiscus sabdariffa* (HS) were selected for the study. The aqueous and ethanolic (60%, v/v) extracts were transformed in the artificial conditions of gastrointestinal digestion, which were split into four categories: salivary, gastric, duodenal and colon [1]. The composition of digested products (DP) was analyzed by HPLC-DAD-MS method.

The effects of the crude extracts and gastrointestinal aliquots on α-amylase and PL activity were evaluated using an *in vitro* fluorescence method [2]. Orlistat and acarbose were used as positive controls.

The aqueous and ethanolic extracts of CJ fruit and HS flower showed the most relevant inhibitory potential of both enzymes (IC_{50} range 35.8-53.6 μg/ml). The gastric DP of CJ fruit and HS flower were the most active DP of all, and at concentration of 31.25 μg/ml inhibited α-amylase by 60%. Procyanidins and hibiscus acid derivatives have been identified in the tested preparations. Thus, they might determine the extracts activity in the gastrointestinal tract.

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SL 30

EVALUATION OF SAFETY OF ESSENTIAL OILS-BASED MICROEMULSIONS

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Essential oils (EO) possess a wide range of biological activities. However their application in aqueous media is often limited due to their hydrophobicity and volatile character. Encapsulation prevents rapid evaporation of highly volatile constituents and enables mixing of larger amounts of EO with aqueous solvents without separation of phases [1]. The process of formation of ME is forward-driven, what makes these formulations inexpensive and stable during a long storage [2]. Very volatile character of EO constituents is an important issue in activity testing, as some compounds are simply lost during assays. What is more, the good solubilisation is crucial to make tested substances available for interactions with cells. ME systems containing EO were shown to improve antimicrobial properties and possess antioxidant potential. They were studied for the application in food products, infected wounds healing, acne treatment and others. However, very little is known about in vitro cytotoxicity of these formulations. ME are considered as useful carriers of EO diminishing their irritating properties [1] but although the components usually used for preparation of ME are generally recognized as safe (GRAS), the toxicity of surfactants has been reported [3]. Because ME formulations require the presence of emulsifiers at a quite high concentration [2], the evaluation of ME cytotoxicity should not be omitted during biological testing. For this reason, stable, water-dilutable microemulsions containing essential oils were evaluated for their *in vitro* antioxidant and cytotoxic properties. The comparison of cytotoxicity of EO solubilized in microemulsions and in dimethyl sulfoxide (DMSO) as well as the recovery of volatiles from cells culture medium over time was also performed.

While the enhanced antioxidant activity of EO in microemulsions (EO/ME) was observed, the analysis of cytotoxicity profiles of vehicle, EO/ME and EO in DMSO in Vero and HeLa cell lines revealed that vehicle itself strongly determines observed cytotoxic effect. At the same time ME provided good solubility of constituents of EO and diminished evaporation of volatiles from culture medium over time.

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SL 31

EFFECT OF A SERIES OF 22 ESSENTIAL OIL COMPONENTS ON THE MEMBRANE PERMEABILITY OF DPPC LIPOSOMES OF DIFFERENT CHOLESTEROL CONTENT

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Essential oils (EOs) are a mixture of natural, volatile, fragrant compounds known for their broad biological activity spectrum. Numerous studies used liposomes to highlight the effect of EO components on membrane fluidity and permeability or to protect them against environmental degradation caused by heat, light, etc. [1]. Nevertheless, the composition of liposome formulations differs between studies, which results in different outcomes regarding the effect of EO on membrane properties. One of the main lipid components included in liposomal formulation is cholesterol (chol). The latter decreases the membrane permeability in a dose-dependent manner [2]. Therefore, a series of 22 EO components of different chemical classes (phenylpropenes, monoterpenes, sesquiterpenes bearing different functional groups) was studied for their effect on the permeability of DPPC liposomes membrane of different chol content (DPPC:chol 100:10; 100:25; 100:50; 100:75 and 100:100 molar ratios). Liposomes encapsulating sulforhodamine B (SRB) were prepared by the reverse phase evaporation method and incubated in the presence of EOs at a molar ratio DPPC: EO component of 25%. Membrane permeability was assessed after 72 h by following the release of SRB from liposomes at 37 °C by fluorescence spectroscopy.

Among the 22 compounds, 14 exerted a permeabilizing effect on 10% chol membranes. This number was progressively reduced upon increasing chol content; EO components presenting a hydroxyl group were the most permeabilizing active agents and linalool, a monoterpene alcohol, was solely active at chol content 75%. These findings may help to understand EOs pharmacodynamics and could serve for the development of liposome formulations loading EO components.

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SL 32

IN VIVO SAFETY ASSESSMENT OF *CLINOPODIUM VULGARE* WATER EXTRACT CHARACTERIZED BY UHPLC-HRMS

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Clinopodium vulgare L. (Lamiaceae) was used in the traditional Bulgarian medicine for treatment of diabetes, gastric ulcers and cancer. In this study we aimed at safety assessment of *Clinopodium vulgare* lyophilized water extract (CVE) characterized by ultra high-performance liquid chromatography – Orbitrap high resolution mass spectrometry (UHPLC-HRMS). The acute and sub-acute toxicity of CVE was determined in two rodent species (mice and rats), and two routes of administration – oral (p.o.) and intraperitoneal (i.p.). $LD_{50\ (i.p.)}$ was found to be 675 mg/kg (mice) and 500 mg/kg (rats). Acute i.p. administration resulted in central nervous system toxic effects, manifested by somnolence, ataxia, and difficulty breathing. LD_{50} (p.o.) was higher than 2000 mg/kg for both species. In the sub-acute oral administration in male Wistar rats during fourteen days, CVE administered at tree doses 50, 100, and 200 mg/kg/day did not exert any toxic effect on hematology, blood and urine biochemistry, and histomorphology in pancreas, liver, spleen and kidney. In addition, on the basis of the accurate mass measurements, fragmentation patterns in MS/MS analyses and comparison with standards, a variety of clinopodic, salvianolic, yunnaneic and caffeoylquinic acids, *O*- and *C*-glycosides of flavones and flavanones were identified or tentatively elucidated in CVE for the first time. Rosmarinic acid was the major compound. In conclusion, CVE did not cause hematological, biochemical and histopathological changes after oral administration and it is safe for internal use.

The obtained UHPLC-HRMS profile revealed the studied species as a new rich source of water soluble dimer and trimer phenylpropanoid derivatives.

SL 33

EXPERIMENTAL STUDY OF AN INNOVATIVE OINTMENT WITH INCREASED WOUND HEALING ACTIVITY, MADE FROM MEDICINAL PLANTS EXTRACTS AND OTHER NATURAL INGREDIENTS (DERMAPLANT)

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Aim: The objective of this research is the experimental study of an original complex formula, made solely from medicinal plants and natural ingredients. The ointment formula comprises olive oil extract from a mixture of nine medicinal plants with well known wound healing activity, together with sea buckthorn oil, lavender essential oil, coconut oil, beeswax and conifer resin.

Methods: The safety and efficacy of the ointment were assessed through pharmacodynamic and toxicological studies. The research has been carried out by applying a number of experimental models.

Results:

- There are no toxic or irritant effects on the experimental models.
- It does not contain pyrogenic impurities. Safety tests have revealed that the product is free of endotoxin contaminants.
- Microbial load is within the pharmacopoeial range, without pathogenic microorganisms
- The test in vitro on 3T3 fibroblasts demonstrated a remarkable prolifera tion-stimulating capacity (+ 18% increase in proliferation rate).
- The evaluation of the *in vivo* repair effect has revealed stimulation of repair of dermal injured tissue on a model of unilateral thermal injury in rat, the 6-day repair process being accelerated by 69%.

Conclusions: The pharmacodynamic and toxicological studies highlighted noteworthy proliferation-stimulating and wound healing activities of the product DERMAPLANT, without toxic, adverse or irritant effects. The ointment proposes a topical composition for burns and wounds, which is effective and well tollerated.

SL 34

SYNERGESTIC EFFECT OF MANGIFERIN WITH ORAL HYPOGLYCEMIC AGENTS IN DIABETIC CONDITIONS

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Among the worldwide health threats, diabetes is the most prevalent non-communicable endocrine disorder among the population. Combination drug therapy with phytomolecules improves the treatment efficiency and also provides a platform to minimalize the side effects of modern drugs for long term treatment. The present study aims to investigate the interaction between a natural molecule mangiferin with commercially available oral hypoglycemic drugs metformin and gliclazide during diabetic condition. Initially *in-vitro* cytotoxicity and glucose uptake studies were performed in HepG2 cells. COMPUSYN software was used to predict the combination effect of metformin and gliclazide in combination with different dosage of mangiferin. *In vivo* studies were performed in STZ induced diabetic male Sprague Dawley rats. Serum parameters (ALT, AST, ALP, LDH and γGT), Enzyme markers (Hexokinase D, Fructose 1,6- bisphosphatase, Glucose 6-phosphatase, Glucose 6- phosphatase dehydrogenase), Hepatic oxidative stress markers (TBARS, GSH, SOD, CAT,GPx, GST), Gene (Glut 2, AMPK, Akt, ACCβ, GAPDH) and protein (PPARα, PPARγ, βactin) expression studies and histopathological studies were performed in rat liver. The *in vitro* studies on HepG2 cells suggest a positive interaction at certain doses in terms of the anti-diabetic efficacy as evidenced by glucose uptake. The hepatic enzymes, oxidative stress markers, carbohydrate metabolizing enzymes and protein expression studies confirms that the combinations of mangiferin with metformin and mangiferin with gliclazide presented desirable outcomes. The molecular pathways involved in insulin dependent Akt, and insulin independent AMPK signaling for cellular glucose uptake exhibits synergism to mitigate diabetic conditions and can be used as add on therapy.

SL 35

ANTIOXIDANT AND GENOPROTECTIVE EFFECTS ON CACO-2 CELLS OF (POLY)PHENOL-RICH LIQUOR OF *HYSSOPUS OFFICINALIS*

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Hyssopus officinalis aerial parts, well-known for bearing essential oil-producing glands, are also rich in phenol and polyphenol compounds [1]. These latter, still rarely studied in hyssop, markedly differ based on the plant collection site as their biosynthesis, maximized in the wild, is finely regulated by multiple abiotic and biotic factors. In this context, in order to achieve new insights into the polyphenol constitution of a homemade-like preparation (HOEE) from hyssop growing wild in Southern Italy, UHPLC HRMS analyses were carried out. HOEE mainly consisted of hydroxycinnamoyl derivatives (57.2%), among which some different and never reported coumarin and lignan compounds. Dicaffeoyl quinic acids were the main chlorogenic compounds, whereas flavonoid glycosides and methoxyflavonoids were the less abundant compounds. The preliminary assessment of HOEE antiradical properties through different test tube assays provided a solid basis for originally exploring its antioxidant activity towards differentiated Caco-2 cells. Experimental evidence on genoprotective activity of hyssop aerial parts, herein testified for the first time, raised the potential of their using as a functional food for oxidative stress-induced diseases prevention.

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SL 36

FLAVONOIDS AND THEIR DERIVATIVES AS POTENTIAL ANTI-CANCER AGENTS

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To enhance the bioavailability and biological activities of flavonoids, we have synthesized a series of novel flavonoid derivatives with higher hydrophobicity. The lipase-catalyzed acylation of phloridzin (PZ) and isoquercitrin with six long-chain fatty acids was optimized [1]. We have identified an effective and safe flavonoid derivative with potential for treating hepatocellular carcinoma, acute monocytic leukemia, and breast cancer [2]. The antiproliferative potency of fatty esters of PZ was comparable to the potency of the chemotherapeutic drugs. The fatty acid esters of PZ inhibited DNA topoisomerases IIα activity that might induce G0/G1 phase arrest, induced apoptosis via activation of caspase-3, and decreased ATP level and mitochondrial membrane potential in HepG2 cells. Antiproliferative efficacy of one of the derivatives, docosahexaenoic acid acylated PZ (PZ-DHA) was investigated using a triple negative breast cancer cell line (MDA-MB-231) [3]. PZ-DHA, but not PZ, caused the death of MDA-MB-231 in vitro which was both time- and dose-dependent. PZ-DHA-induced apoptosis was indicated by DNA fragmentation and activation of caspase 3/7. However, reactive oxygen species production was not required for PZ-DHA-mediated cell death. PZ-DHA showed low cytotoxicity toward human mammary epithelial cells. Moreover, in vivo tumor suppressor effect of PZ-DHA was observed when MDA-MB-231 cells xenografted non-obese diabetic severe combined immune-deficient (NOD-SCID) mice were subjected to intratumoral injections of PZ-DHA. Anticancer efficacy observed in cell cultures and mice suggest PZ-DHA as a potential treatment for breast cancer.

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SL 37

THE EFFECTS OF *MORINGA OLEIFERA* LAM. LEAVES EXTRACT ON HUMAN T-CELL AND DNA DAMAGE INDUCED BY OXIDATIVE STRESS

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The hall mark of chronic inflammatory disease environments is the generation of excessive reactive oxygen species, which are targets for nutrient antioxidants. At least one of the consequences of prolonged exposure to reactive oxygen species is the inhibition of T-cell responses, differential regulation of T_H1 versus T_H2 and inhibition of T regulatory cell responses. *Moringa oleifera* extract while reported to have anti-inflammatory properties in experimental inflammatory models, little is known on how it brings about the effects. We have examined the effects of *Moringa oleifera* Lam. on human T-cell responses, (IL-2 and IL-2 mRNA) and DNA damage (quantifying the oxidized derivative of deoxyguanosine, 8-OHdG) under oxidative stress. T-cells treated with oxidative substances, uric acid, aluminum chloride, hydrogen peroxide, and ultraviolet irradiation showed marked reduction in IL-2 production, which was prevented by pre-treating with Moringa extract. These effects correlated with prevention of the down-regulation of *IL2* gene expression and a reduction in the DNA damage caused by oxidative stress. Post treatment with the extract had little effect. Thus one of the benefits of *Moringa* extract to reduce inflammation is likely to be its ability to prevent T-cell hyporesponsiveness experienced in inflammatory diseases, through an action at the pre-transcriptional level.

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SL 38

CRITICAL EFFECTS OF SOLVENT SELECTION AND PLANT DESCARBOXILATION ON THE CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF *CANNABIS* EXTRACTS

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Cannabinoids are terpenophenolic secondary plant metabolites uniquely found in *Cannabis sativa* L. Althought more than 104 cannabinoids have been identified [1], the two most abundant are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). However, cannabis cultivars yielding significant amounts of other minor non-psychoactive cannabinoids such as cannabigerol (CBG), Δ^9-tetrahydrocannabivarin (THCV), and cannabivarin (CBDV), are being increasingly sought after for their potential medical value [2]. Cannabinoids are present in the plant as their acidic precursors, which readily decarboxilate into their neutral forms upon heating. *Cannabis* extracts are usually prepared by running organic solvents, such as alcohols, alkanes or pressurized CO_2, through cannabis plant material (flowers and leaves) and then drying the solvent away.

In the present study, a library of cannabis extracts was prepared from 10 distinct cultivars, internally developed by Phytoplant Research and registered under the Community Plant Variety Office (CPVO). Both decarboxilated (120-150 °C, 2h) and non-decarboxilated dry plant material was extracted using three different solvents (ethanol, butane and hexane). The resulting extracts were analyzed by GC-MS, including a derivatization step for the simultaneous quantification of neutral and acidic cannabinoids, and 10 cannabinoids were reported. The biological activity of the different extracts (cell toxicity, anti-inflammatory and anti-angiogenic potential) was also screened *in vitro*. Interestingly, significant changes in the pharmacological activity of cannabis extracts were found to be not only variety-specific but, also, solvent-dependent. Our results indicate that solvent selection and proper decarboxilation of plant material represent key aspects for the standardized production of cannabis extracts with reproducible pharmacological activity and potency.

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SL 39

CHROMATOGRAPHIC ANALYSES, *IN VITRO* BIOLOGICAL ACTIVITIES AND CYTOTOXICITY OF *CANNABIS SATIVA* L. ESSENTIAL OIL: A MULTIDISCIPLINARY STUDY

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Due to renewed interest in the cultivation and production of Italian *Cannabis sativa* L., we proposed a multi-methodological approach to explore chemically and biologically the essential oil and the aromatic water of this plant. After the analysis of the EO yield, we studied the chemical composition in terms of cannabinoid content, volatile component, phenolic and flavonoid pattern and colour characteristics. Then, we demonstrated the ethnopharmacological relevance of this plant cultivated in Italy as a source of antioxidant compounds, towards a large panel of enzymes (pancreatic lipase, α-amylase, α-glucosidase, cholinesterases) and selected clinically relevant, multidrug-sensible and -resistant microbial strains (*S. aureus*, *H. pylori*, *Candida* and *Malassezia* spp.), and evaluating the cytotoxic effects in normal and malignant cell lines (MCF-7, MDA-MB-468, Caco-2, Mz-ChA-1 and H69). Preliminary *in vivo* cytotoxicity was also performed on *G. mellonella* larvae (Figure 1). The results corroborate the use of this natural product as a rich source of important biologically active molecules and their role in the eradication of *S. aureus* biofilm [1].

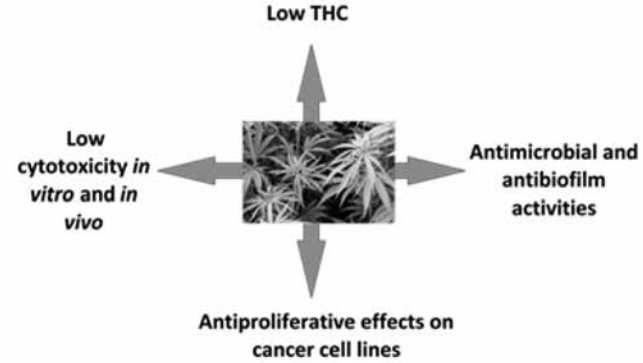

Figure 1

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SL 40

MULTIPLE PHARMACO-TOXICOLOGICAL CHARACTERIZATION ON HEMP COMMERCIAL CULTIVARS: FOCUS ON BIOLOGICAL ACTIVITY OF FUTURA 75 CULTIVAR

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One of the most promising economic perspectives of hemp production chain is female inflorescence valorization. By contrast, scientific literature lacks on chemical composition or biological activity data from aqueous fraction obtained from industrial hemp flowers, which have long been considered as waste products. In this context, the main focus of the following study is the evaluation of protective effects related to aqueous flower extracts from four commercial hemp cultivars (Futura 75, Kc virtus, Carmagnola Cs and Villanova). We evaluated the extract phytochemical profile. Then, we studied the water extracts both in vitro and ex vivo in order to assay protective effects in an experimental model of ulcerative colitis, constituted by isolated LPS-stimulated colon. All cultivar extracts displayed similar total phenol and flavonoid content. On the other hand, Futura 75 cultivar extract displayed a better antioxidant and anti-inflammatory profile. Considering this, Futura 75 extract has been subsequently assayed to evaluate its effect on pathogen bacterial and fungal species involved in ulcerative colitis, finding a significant inhibition on the growth of *C. albicans* and selected Gram positive and negative bacterial strains.

Taken together, our results support the potential efficacy of Futura 75 water extracts in managing the clinical symptoms related to ulcerative colitis.

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SL 41

MULTIPLE PHARMACOGNOSTIC EVALUATION OF *CROCUS SATIVUS* L BYPRODUCTS AS INNOVATIVE SOURCES OF ACTIVE PRINCIPLES: FOCUS ON ANTHER BIOLOGICAL ACTIVITY

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Multiple studies revealed the potential application of high quality saffron byproducts as cheap sources of bioactive compounds endowed with antioxidant activity. In the present study, we analyzed the total fatty acids of the anthers, and explored the pharmacological and toxicological potential of anthers, by evaluating genotoxic and protective effects in multiple cell lines, brine shrimps and isolated rat tissues.

The phytochemical analyses showed that anthers are rich in long chain fatty acids most of which are unsaturated (80.51%). Particularly, anther water extract revealed to be well tolerated by multiple cell lines, and able to modulate reactive oxygen species (ROS) levels, without exerting either genotoxic or cytotoxic effects. The same extract was also able to blunt lipopolysaccharide (LPS)-induced nitrite and malondialdehyde (MDA) in isolated rat tissues. On the other hand, considering the concomitant null effect on HCT116 cell migration, in wound healing experimental paradigm, our findings suggest the efficacy of water anther extract as protective agent without any direct reverting effects on lesioned tissues.

Concluding, the promising results, deriving from the pharmacological and toxicological evaluations, support the valorization of saffron anthers as a strategy to optimize and develop the productive chain of Abruzzo saffron (Italy).

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SL 42

MULTIPLE PHARMACOLOGICAL APPROACH TO EVALUATE PROTECTIVE EFFECTS OF *COTONEASTER INTEGERRIMUS* ON *EX VIVO* AND *IN VITRO* MODELS OF COLON INFLAMMATION

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The present study evaluated the biological potential of methanol and aqueous extracts of the twigs and fruits of *Cotoneaster integerrimus* Medik. Lethality bioassays performed on *Artemia salina* showed that aqueous and methanol *C. integerrimus* extracts were non-toxic in the concentration range (0.1-20 mg/mL), with a $LC_{50} \geq 2.5$ mg/mL, for each single extract. The protective effect of the extracts was assessed *in vitro* against hydrogen peroxide-induced lactate dehydrogenase activity and tumor necrosis factor (TNF)-α gene expression in colon cancer HCT116 cell line. All the extracts down-regulated (H_2O_2)-induced TNFα gene expression, in HCT116. By contrast, it was observed that the LPS-induced increase in colon nitrite, prostaglandin E_2, and 8-iso-$PGF_{2\alpha}$ levels were counteracted mostly by the methanol twig extract. The present study showed protective effects induced by *C. integerrimus in vitro* and *ex vivo*. Particularly, results amassed herein advocates for further bioprospection of this species that could open new avenues for the development of nutraceuticals and functional foods geared towards the management of chronic inflammatory diseases.

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SL 43

MECHANISMS OF RELAXATION INDUCED BY *VACCINIUM MACROCARPON* (CRANBERRY) EXTRACT ON RAT CORPUS CAVERNOSUM

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Vaccinium macrocarpon (Cranberry; Ericaceae) grows in Eastern North America. Cranberry has been used due to several important biological activities. Various forms of cranberry have been used for the management of urinary tract infections (UTIs) [1, 2]. Although the effects of cranberry products may have a beneficial effect on the erectile dysfunction (ED) [3], the action mechanisms are not clearly defined yet. Male Sprague-Dawley rats (n = 8) were used in this study. The aim of the present study was to investigate the effect of concentrated the extract of cranberry fruit (E, one of the dietary supplements present in the market) on rat corpus cavernosum (CC) tension and its underlying mechanisms. Experiments were performed in rat CC using the organ bath system. After phenylephrine (Phe, 10 μM) contraction, cranberry-E (0.01 - 100 μg/ml) caused a concentration-dependent relaxation, which was not dependent on a functional endothelium, and were not significantly attenuated by inhibitors of endothelial NO synthase [e.g. the nitric oxide synthesis inhibitor N(omega)-nitro-L-arginine methyl ester (L-NAME; 10 μM), the soluble guanylate cyclase inhibitor ODQ (10 μM)], the relaxant responses were determined in the presence of some inhibitors. Cranberry-E induced relaxation of rat CC (maximum response: 76.1±3.6%) after Phe-contraction. Cranberry-E evoked long-lasting relaxations. The relaxant responses to acetylcholine (10 μM), electrical field stimulation (10 Hz), and sodium nitroprusside (0.01 μM) in rat CC were increased after incubation with cranberry-E. The underlying mechanism of cranberry products is likely independent of the nitric oxide-cyclic guanosine monophosphate pathway. It is suggested that Cranberry-E -induced activation can modulate CC cells through the cholinergic receptors nitrergic neurons and cavernosal smooth muscle activity. Further, these *in vitro* studies suggest that consumption of cranberry-E may be strongly efficient and represent an exciting new strategy to prevent and diminish ED in men.

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SL 44

PROTECTIVE EFFECTS OF *ARONIA MELANOCARPA* FRUIT JUICES AND *ARONIA MELANOCARPA*-BASED JUICES IN A RAT MODEL OF INDOMETHACIN-INDUCED GASTRIC ULCERS

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Aronia melanocarpa fruit juice has been demonstrated to exert a protective effect against indomethacin-induced gastric ulcers in rats [1].

The aim of the present study was to investigate the effects of two *Aronia melanocarpa* fruit juices and two *Aronia melanocarpa*-based fruit juices on indomethacin-induced gastric mucosal damage in rats.

The juices used were: AM1 and AM2 (produced from homogenized *Aronia melanocarpa* fruits at 20 °C and 60 °C, respectively), AMRC (a mixture of AM2 with *Rosa canina* fruit extract) and AMAV (AM2 with *Alchemilla vulgaris* herb added). Male Wistar rats were used. Each of the juices (10 ml/kg) was administered as a pretreatment for 10 days. Indomethacin (30 mg/kg) was given orally and after 4 hours, the effects were estimated by morphometrical, histopathological, biochemical and immunohistochemical investigations.

Indomethacin caused heavy destructions of the gastric mucosa, evaluated morphometrically and histopathologically. The pretreatment with the four juices reduced the number and area of indomethacin-induced gastric lesions, as well as the gastric gland destruction and gastric erosions, the highest being the effect of AMAV. The biochemical and immunohistochemical investigations showed that indomethacin increased oxidative stress in gastric mucosa, decreased PGE_2, increased TNFalpha and Bax, decreased Bcl-2 and NF-kB. The juices antagonized these effects of indomethacin and the most pronounced was the effect of AMAV.

The protective effect of *Aronia melanocarpa* fruit juices and *Aronia melanocarpa*-based fruit juices against indomethacin-induced gastric lesions could be attributed to their polyphenolic contents.

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SL 45

PHYTOCHEMICALS AND IMMUNOMODULATORY EFFECT OF *NELUMBO NUCIFERA* FLOWER EXTRACTS ON HUMAN MACROPHAGES

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Immunosenescence, a declined immune function with advanced age, contributes to low-grade inflammation [1]. *Nelumbo nucifera* or sacred lotus is widely used in Ayurveda and traditional medicines [2, 3]. This research characterizes phytochemicals inherent in lotus flower and investigates the antioxidant and immunomodulatory activity of ethyl acetate (EA) and ethyl alcohol (ET) lotus petal extracts. In the experiment, human monocytes-derived macrophages were stimulated by lipopolysaccharide to mimic bacteria-induced inflammation. The results showed that ferulic acid, coumarin, and chlorogenic acid were three dominant polyphenols. The EA and ET lotus petal extracts also possessed high antioxidant capability. Furthermore, the extracts exhibited immunomodulatory properties by suppressing TNF-α secretion in inflammatory-induced human macrophages by inhibiting NF-κB-dependent inflammatory response. In essence, the lotus petal extracts possess remedial attributes beneficial to individuals afflicted with inflamm-aging due to declined immune functions.

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SL 46

UTILISATION OF BIOACTIVE COMPONENTS FROM PLANT BIOWASTE AS AN ANTIOXIDANTS AND ENZYME INHIBITORS FOR FUNCTIONAL FOOD AND DRUG DEVELOPMENT

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The food processing industry generates large volumes of biological waste that could be used to produce value-added products and their utilization becomes an important issue for the development of the food industry and bio-economy. This food processing results in tremendous losses of valuable non-nutritional, nutritional, and functional bioactive components, including proteins, fibers, carbohydrates, phenolics, carotenoids, and other secondary metabolites. However, it is possible to recover important bioactive components from waste generated during the industrial processing of various foods and food products by applying various extraction and analytical methods. In most cases, the wasted by-products can present similar or even higher contents of bioactive compounds than the final product. The aim of this project is to promote the production and processing of different fruits including citrus, apple, onion, and soybean waste highlighting the possibility of the integral exploitation of by-products rich in bioactive compounds and having health benefits. Finally, the importance of extraction techniques of bioactive compounds designated as food additives is also included. The extraction of secondary metabolites from fruit skin, peel and seed wastes using various extractions methods with aid of solvents like n-hexane, ethanol, chloroform, and methanol is a challenging task due to their chemical diversity and complex structures. Therefore, it's very important to explode novel extraction technologies having low cost, short time consumption, easy operation processes, and utilizing eco-friendly solvents. Thus, this study is aimed to determine the effect and feasibility of these green methods for extraction of bioactive components from different fruit waste for possible analysis of bioactive compounds with health benefits.

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SL 47

THE BENEFICIAL EFFECTS OF ANTHOCYANINS AND ITS PRODUCTS DEVELOPMENT

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Anthocyanins are a group of polyphenols, widely existed in dark colored fruits, vegetables and grains. Epidemiological investigation and nutritional intervention of anthocyanins have exhibited broad-spectrum biological effects that benefit patients with chronic diseases including cardiovascular disease, diabetes and fatty liver disease [1]. Our recent studies also revealed that anthocyanins could ameliorate the male reproductive damage and skin photodamage [2]. Anthocyanins as natural edible pigments that are adopted diffusely in food industry, such as wine, berries beverage, cakes and ice cream. However, the beneficial effects of anthocyanins as functional food have not been fully developed. Only several anthocyanins-rich extracts about cranberry, blue berry products are popular in some district, but the number and variety of anthocyanin products are limited, which can not meet the market requirement. Additionally, the poor stability and the bioavailability of anthocyanins restrains the development of anthocyanins rich products, which are vulnerable to be degraded by sunlight, heat and oxygen. Now days, we have also been trying to encapsulate the anthocyanins use β-cyclodextrin and chitosan for a better physical stability. Interestingly, cyanidin-3-*O*-glucoside (C3G) as an anthocyanins monomer is more efficient in the capsule than the bare form on the protection of mice skin acute photodamage. All in all, anthocyanins have great potential to be developed as food additives and functional foods, however more work are needed to explore the underlying mechanism of diseases prevention and the stability improvement of anthocyanins. After all, the procedure of industrialized production of anthocyanins should be accelerated.

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SL 48

AJMALICINE PRODUCTION BY MASS PROPAGATION OF HAIRY ROOTS OF *C. ROSEUS* IN BIOREACTOR

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Ajmalicine is one of the popular alkaloid of *Catharanthus roseus* which is used for circulatory disorders. The major production protocol this drug features solvent extraction from different plant parts of *C. roseus* which invariably have significantly low content of the drug. The high demand of ajmalicine necessitates the development of alternative production protocols. Hairy roots are particularly suitable for production of bioactive compounds due to relatively high growth rates and secondary metabolite content compared to other organ cultures and serve as a stable parent cell line for mass propagation.

Successful attempt was made to optimize the media recipe for cultivation of hairy roots of *C. roseus* in shake flask cultivation conditions wherein a biomass 5 g/L and ajmalicine accumulation of 25 mg/L was obtained. Mathematical models were then developed & extensively used to understand the culture behaviour of hairy roots under different cultivation conditions & optimize ajmalicine production.

The suitability of the different bioreactor configuration & cultivation strategies were then assessed by doing the mass cultivation of hairy roots of *C. roseus* in conventional bubble column reactor, rotating drum reactor, modified bubble column reactor (stirred tank with impeller removed) with propylene mesh & polyurethane (PUF) support. It was possible to accumulate the biomass and ajmalicine concentration of 7.69 g/L and 34 mg/L respectively with the modified bubble column reactor with PUF support. The relative merits and limitations of the different cultivation strategies will be highlighted.

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SL 49

MANIPULATING BENZYLISOQUINOLINE ALKALOID PROFILE FOR ANTIMICROBIAL ACTIVITY OF SELECTED PAPAVERACEAE PLANTS AND TISSUE CULTURES

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Several isoquinoline alkaloids have been long considered as potent antimicrobial agents. Some of them, such as berberine and coptisine are made commercially using plant tissue culture-based biotechnological approach. However, much less is known about other compounds that are abundant for example in some traditional medicinal plants of Papaveraceae (encompassing Fumarioideae). Here, we use plant tissue and organ culture for obtaining extracts and individual alkaloids and test them against pathogenic bacteria, candida yeast and HSV-2 viruses. The focus is on *Chelidonium majus* L., a well-known herb used externally against infectious diseases. In addition, we include tissue culture of several related species from genus *Corydalis*, *Fumaria*, and *Pseudofumaria*.

In case of Gram-positive pathogens - *Staphylococcus aureus* and *Enterococcus faecalis,* a strong reduction of bacterial growth in comparison to untreated bacteria was observed. Candida growth was inhibited by 70 to 80%, depending on the fungal strain with *C. glabrat*a being the most sensitive. However, Gram-negative bacteria were resistant to all extracts and alkaloids except berberine. HSV-2 viruses were only moderately (by 33%) inhibited by root culture extracts.

These results prove a high potential of *in vitro* grown organs and dedifferentiated callus tissue for production of significant amounts of pharmacologically relevant alkaloids. The various proportions in the alkaloid profile depend on the culture conditions such as supplementation with growth substances and sugars as well as on the illumination with light of different spectrum.

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SL 50

RATIONAL METABOLIC ENGINEERING OF *HELIANTHUS ANNUUS* FOR ENHANCED PRODUCTION OF ALPHA-TOCOPHEROL

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Alpha-tocopherol, an essential dietary supplement, synthesized by photosynthetic organisms is the most biologically active antioxidant component of vitamin E in humans. Attempts to improve the yield of alpha-tocopherol using plant cell cultures for sustainable production has gained significance in recent years. Here, we developed a high alpha-tocopherol yielding cell line of *Helianthus annuus* using a model based metabolic engineering approach. To this end, we employed constraint-based analyses on an adapted metabolic model to identify and rank overexpression targets. Experimental validation of the top-ranked strategy (HPPD overexpression) resulted in a high alpha-tocopherol yield in the transformed cell line (up to ~240 µg/g), which was ~10 fold more than in the untransformed cell line. A cell suspension was developed from this high yielding transformed cell line for *in vitro* production of alpha-tocopherol. Implementation of various yield (elicitation and precursor addition) and productivity enhancement strategies (optimization of media and culture conditions) on this transformed cell suspension led to further enhancement in the alpha-tocopherol yield up to 412.2 µg/g and titre up to 6.4 mg/L in comparison to that in the un-transformed and un-optimized cell suspension culture of *H. annuus* (with a yield of 24 µg/g and productivity of 498 µg/L). Thus, a multi-fold productivity enhancement could be achieved by integrating metabolic engineering with bioprocess optimization which can further be scaled-up in bioreactors for sustainable large-scale production of alpha-tocopherol.

SL 51

PLANT-PRODUCED RECOMBINANT PROTEINS COMPRISING CONSERVATIVE ANTIGENS OF INFLUENZA A VIRUS

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The effective control of influenza A infection may be achieved through the development of a broad-spectrum recombinant vaccine based on conserved viral antigens. In this work we used the extracellular domain of the M2 protein (M2e) and the conservative stalk region of hemagglutinin (HA2). In order to increase immunogenicity of these antigens, they were genetically fused to adjuvants. The first adjuvant was bacterial flagellin, the ligand for Toll-like receptor 5. Two variants of candidate vaccine were developed. The first recombinant protein consisted of flagellin fused to four tandem copies of the M2e peptide. In the second protein we included a conservative fragment of HA2 between the flagellin and M2e. Recombinant proteins were produced in *Escherichia coli* and in *Nicotiana benthamiana* plants using the recently developed self-replicating potato virus X (PVX) based vector, pEff. The recombinant fusion proteins carrying an N-terminal 6-histidine tag were purified using affinity chromatography on Ni-NTA resin and used for animal experiments. Immunization of mice with purified proteins induced high levels of M2e and HA-specific serum antibodies and provided protection against lethal challenge with influenza A virus. Within the second approach we fused M2e peptide to the capsid protein of the hepatitis E virus, which is able to self-assemble into virus-like particles. The M2e-HEV fusion proteins were successfully expressed in *Nicotiana benthamiana* plants using the pEff expression system. Purification of plant-produced proteins is underway.

This study confirms the feasibility of production of candidate influenza vaccines in plants.

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BIOSYNTHESIS AND REGULATION OF BIOACTIVE CONSTITUENTS IN *SALVIA MILTIORRHIZA*

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Salvia miltiorrhiza Bunge (Danshen) is a famous traditional Chinse medicine widely used for the clinical treatment of cardiovascular and cerebrovascular diseases. Tanshinones and phenolic acids are two main groups of bioactive constituents in *S. miltiorrhiza* with good biological activities such as antitumor, anti-ischemic antioxidant properties. In order to improve the contents of tanshinones and phenolic acids to satisfy the clinical demand, a series of metabolic engineering strategy have been successfully utilized. A series of genes associated with the biosynthesis of tanshinones and phenolic acids including transcription factors (WRKY, AP2/ERF, MYB, etc) were discovered. Overexpression of *SmWRKY1* classified into subgroup III could significantly elevate the transcripts of genes coding for enzymes in the MEP pathway especially *SmDXSII* and *SmDXR*. Also, a novel AP2/ERF transcription factor *SmERF115* responsive to MeJA is found to have pushing effect on the phenolic acid production by regulating *SmRAS1*.

Together, the presented work provided new insights to further understand regulation mechanism of tanshinones or phenolic acids biosynthesis and metabolic engineering in the future.

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SL 53

POLYSACCHARIDES FROM MARINE MACROALGA *ENTEROMORPHA* AND THEIR PHARMACEUTICAL APPLICATIONS

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Macroalga *Enteromorpha* polysaccharides have presented various physiological and biological activities based on their specific chemical structures such as monosaccharides and glycosidic linkages. Its bioactivities and chemical compositions have been widely studied. The modified *Enteromorpha* polysaccharides had powerful bioactive functions. The sulfated EPPs with groups exhibited excellent immune and antioxidant activities with lower molecular weights. The carboxymethylated, hydroxamated, phthaloyl, and acetylated derivatives also significantly enhanced antioxidant and antibacterial activities as well as moisture absorption. *Enteromorpha* polysaccharides were treated as novel natural agents, exhibiting outstanding effects compared with ordinary drugs. However, their structure-activity relationships of *Enteromorpha* polysaccharides have been not well established.

The purpose of this paper is to review the current advances of macroalgae *Enteromorpha* polysaccharides on their structural characterization, biological activities and mechanisms of action.

SL 54

TRADITIONAL USE OF THE INVASIVE PLANT *XANTHIUM STRUMARIUM* L. (*ASTERACAE*) IN THE FOLK MEDICINE

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Xanthium strumarium L. (Asteraceae) is an annual herb which reproduces solely by seed. So far its centre of origin was considered Central or South America. Recent archeological research revealed that the burs of *X. strumarium* were used in Yuergou site (400–200 cal BC) in the Turpan Basin of northwestern China. This adventive (not native) to Europe plant reduces germination of various crops and behaves like and aggressive invasive species. *X. strumarium* is the most frequently recorded plant in the field borders between the crop land and adjacent territories the agricultural areas in Bulgaria.

The aim of this study is to reveal the potential of *Xanthium strumarium* as a cheap sources of compounds with valuable pharmacological activities. Here we analyse: 1) the traditional ethnobotanical data from its native habitats; 2) the modern investigations of pharmacological activity and essential secondary compounds.

Native Americans used the plants as febrifuge drug and an immunostimulant. In India *X. strumarium* is known to have powerful diaphoretic properties and is recommended in chronic malaria, diuretic, leucorrhoea and urinary diseases, eczema and skin disease, bleeding, insect bite. In Napal it is used to treat boils and pimples. In Pakistan it is reported as a diuretic, astringent, sedative, analgesic, smallpox and dysentry cure, against stomach diseases, earache and strumous disease, leprosy, headache, fever, etc.

Xanthium strumarium contains sesquiterpene lactones and possesses anticancer, antitussive, antifungal, antiinflammatory, antinociceptive, hypoglycaemic, antioxidant, antitrypanosomal, and antidepressant-like activity, diuretic effects, insecticidal and herbicidal activities as well as antitrypanosomal effect.

SL 55

COMMON JUNIPER (*JUNIPERUS COMMUNIS* L.) IN SLOVAKIA

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Common Juniper (*Juniperus communis* L.) is shrub or small tree; 2-20 ft. Bark reddish brown, shredding off in papery peels. Leaves (needle) taper to a spiny tip, in whorls of 3´s with 2 white bands above (or 1 white band sometimes divided by a green midrib, broader than green margin). Flowering time is April to June. Fruits on short stalk; round to broadly oval, bluish black, usually with 3 seeds. The fruit is a berry-like cone which is green the first year and ripens to a bluish-black or dark purple color in the second year.

The harvest of juniper fruits had a long tradition in Slovakia. Juniper was used for the production of the spirit called "Borovička" in many former historical regions of the Slovakia. Juniper could also be used for jams, herbal teas and sometimes it was consumed fresh. In the kitchen, it was used as a spice. To know chemical composition of the raw material of juniper berries is very important for industrial production of the "Borovička" – alcoholic beverage. In regard to the final quality of this Slovakian national liquor, distiller companies need to prefer the juniper fruits with the high pinene contents, as donors of aroma, odor, and lower essential oil quantity.

Samples of juniper fruits were collected from different places (20) in Slovakia and the contents of essential oil and their GC profiles were determined.

The content of essential oils ranged between 0.50±0.10% and 1.80±0.10%. The qualitative-quantitative characteristics of the juniper essential oil were presented four main conponents: α-pinene (29.0±0.68 – 61.0±0.60%), sabinene (8.00±0.30 – 22.0±0.98%), myrcene (7.5±0.10 – 16.2±0.66%) and caryophyllene (3.5±0.30 – 17.5±0.33%). Ecological diversity of sites, where juniper occurs, its geological age tertiary resulted in great variability, that is, adaptation to the specific conditions (ecotypes, chemo types, varieties, etc.).

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SL 56

APPLICATION OF FTIR SPECTROPHOTOMETRY AND MULTIVARIATE ANALYSIS TO CHARACTERIZATION OF THE SPECIES OF *SOLANUM* OF THE ERYTHROTRICHUM CLADE

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Solanum L. (Solanaceae), with approximately 1400 species, which has economically important species such as tomato (S. *lycopersicum* L.), eggplant (S. *melongena* L.), and potato (S. *tuberosum* L.). Many species of *Solanum* are known in Brazil as "jurubeba" and used in folk medicine for the treatment of various diseases, such as hepatic and renal dysfunctions, inflammations, syphilis, external ulcers, tuberculosis and anemias [1]. The objective of this work was to obtain a kind of fast yet informative fingerprinting for species of *Solanum* of the Erythrotrichum clade [2], which are erroneously identified. Nine species of *Solanum* were examined for their affinities. The samples were prepared directly on a KBr pellet and analyzed on the Cary 630 FTIR Spectrometer. The baseline procedure for all spectra was done using the software Spectra Gryph 1.2.10 by processing the spectra with an adaptive coarseness of 15 and 0 offset. Then, the spectral data were normalized (absorbance 0 and 1) and processed in Past 3.22 Software for principal component analysis (PCA). These fingerprints were then analyzed by the PCA, revealing the affinities of the species and suggesting two groups: one with species of the section *Erythrotrichum* and, the other, with species of the section *Polytrichum*, sensu [3]. What is found particularly worthy of interest here is the fast and informative method used for fingerprinting. While the method is not new, the present application appears to be an important technique for distinguishing among complex species of *Solanum*.

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SL 57

NEW ADVANCES IN THE RESEARCH ON MAQUI BERRY, *ARISTOTELIA CHILENSIS*

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Anthocyanins, flavonoids and organic acids widely occurring in extracts of the fruits of Aristotelia chilensis ("Maqui"), concertedly acts on the expression of cyclooxygenase-2 (COX-2), NF-κB, HT-29 and Caco-2 colon cancer cell growth inhibition and on the production of inflammatory mediators. To assess the anti-inflammatory effects of extracts from fruits of "Maqui Berry", on the HT-29 and Caco-2 colorectal cancer cell lines by measuring COX-2 and NF-κB as well as to analyze their antioxidant activities effects . Methanol/water extracts and its partitions (acetone and ethyl acetate) from three varieties of "Maqui" were used to access their effects on growth of HT-29 and Caco-2 colon cancer cells, COX-2, NF-κB, NO formation, oxidations by DPPH, TBARS, FRAP and ORAC. Fractions rich in anthocyanins, exhibited potent chemoprotective abilities on decreasing growth of HT-29 and Caco-2 colon cancer cells. The results indicated that the extracts suppressed the production of nitric oxide (NO), through the down-regulation of inducible nitric oxide synthases (iNOS). Growth of HT-29 and Caco-2 cells was suppressed. Their structural features showed inhibition of NF-κB and COX-2 protein expressions and a potent antioxidant activity by assays of DPPH, FRAP, TBARS and ORAC. The inhibition of growth cells and NO production by selected extracts was dose-dependent with significant effects seen at concentration as low as 25.0 and 10.0 ppm, respectively. The phenolics (anthocyanins, flavonoids, and organic acids) that occur in these samples may provide therapeutic potential against colon cancer.

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SL 58

BITTER TASTE AND ASTRINGENCY – PREDICTORS OF ANTIINFLAMMATORY ACTIVITY?

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Background: Herbal taste has been considered in traditional medicine as an ethnopharmacological descriptor indicative of therapeutic activities [1]. In a previous study we found that bitter phytomolecular taste of Indian herbals is associated with ayurvedic anti-inflammatory activity (sankr. *sothahara*) [2].

Aim: To establish whether there are correlations between phytochemical tastes/ orosensations and evidence-based anti-inflammatory activity.

Methods: A database was constructed by integrating the taste of 466 phytochemicals (listed in the PhytoMolecularTasteDB [3]) and the eventual evidence based- anti-inflammatory activity. A literature search was performed using the following phrase "specific phytochemical name AND antiinflammatory", (e.g. azadirachtin AND antiinflammatory) in PubMed, Elsevier databases and Google Scholar. The relevant studies were collected regardless of study design, language, year of publication or publication status. Standardized criteria were utilized for selection. Antiinflammatory activity was considered to be evidence- based if supported by at least one *in vitro*, animal or human study. Potential associations between taste of the phytoconstituents and anti-inflammatory activity were statistically analyzed using chi square test.

Results: The anti-inflammatory activity was found to be significantly associated with bitter taste ($p = 0.006$) and astringent orosensation ($p = 0.02$), but not with pungent orosensation or with sweet or salty tastes. Interestingly, lack of sour taste seems also to be associated with anti-inflammatory activity ($p = 0.03$).

Conclusions: Both bitterness and astringency appear to be associated with anti-inflammatory activity. Further experimental studies are required to confirm and understand the biological mechanism of this linkage.

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SL 59

NEUROPROTECTIVE EFFECTS OF OPTIMIZED SYSTEM EXTRACTS OF *MORUS NIGRA* L. FRUITS

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Plants have been used for treatment throughout humanity. Recent investigations focus on optimizing the extraction processes of commonly used plants for pharmacy shelf. This study aimed to investigate the neuroprotective effect of optimized system extracts of *Morus nigra* L. fruits that had been previously reported with anti-inflammatory, antiviral, antidiabetic activities. Extraction was performed with supercritical carbon dioxide, subcritical water and microwave assisted extraction systems as advanced extraction technologies; following conventional methods as orbital shaker and sonification. Optimization of the extraction was performed with Box-Behnken Design regarding tyrosinase inhibitory activity; quantitative determination of total phenol, flavonoid and anthocyanin contents carried out with spectrophotometer; the evaluation of anthocyanin content analysis as cyanidin-3-glycoside, cyanidin-3-rutinoside, pelargonidin-3-glycoside and pelargonidin-3-rutinoside were determined by UPLC-DAD-ESI-MS/MS [1]. Selected extracts were evaluated for neuroprotective effects by analysing viability in SH-SY5Y neuroblastoma cells by using WST-1 assay [2]. Remarkable number of the extracts at indicated concentrations (10 and 100 µg/ml) were found to protect SH-SY5Y cells from 6-OHDA-induced toxicity by increasing cell viability. Maximum protection was observed following pretreatment of orbital shaker extract with 10 µg/ml and cell survival was significantly increased from 68.21 ± 13.73% (6-OHDA only treated cells) to 141.72 ± 49.28% ($p<0.05$). Among the extracts obtained by advanced techniques the microwave assisted extract with 10 µg/ml increased the cell to 129.97 ± 28.96% ($p<0.05$) with promising neuroprotective activity.

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SL 60

CYTOTOXIC ACTIVITY OF SAPONINS, OBTAINED FROM *IN VITRO* CULTURES OF *ASTRAGALUS GLYCYPHYLLOS*

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In the last decade the need of plant-derived cytotoxic compounds exceeds the possibilities for obtaining them from naturally-grown sources. *In vitro* plant biotechnology offers a unique and often invaluable alternative of production of complex biologically active substances without harming the flora [1]. *Astragalus glycyphyllos* L. (Fabaceae) is a plant native to Bulgaria that has been reported to contain triterpenoid saponins and flavonoids. It is used in folk medicine as an antihypertensive, diuretic, anti-inflammatory, anti-tumour, laxative, expectorant, etc. [2, 3].

The aim was to investigate the cytotoxicity of *in vitro* cell cultures, obtained from *A. glycyphyllos*.

Three types of *in vitro* cultures were developed – callus, shoot and suspension cultures. Murashige and Skoog's medium, supplemented with various concentrations and combinations of plant growth regulators and different photoperiods were used.

In all cultures the saponin content was determined by a LC-MS method. Compared to the wild grown species, *in vitro* shoot cultures accumulated double the amount of the main saponin (225.00 ng/mg dw) found in the plant. Saponin-rich fractions obtained from different cultures were tested for cytotoxicity on a panel of various malignant human cells and IC_{50} values were determined.

In vitro cultures of *A. glycyphyllos* could serve as an alternative way for production of saponins, with promising cytotoxic activity.

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SL 61

IRIDOID DIGLYCOSIDES FROM *VERBASCUM* SPECIES AND THEIR BIOLOGICAL ACTIVITIES

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Verbascum species (mulleins) are very well-known herbs used since ancient times in traditional folk medicine of many European countries as oral or topic remedies for coughs, bronchitis, asthma, hemorrhoids, migraine, wounds and eczema [1]. More than 200 secondary metabolites (iridoids, phenylethanoids, flavonoids, saponins, phenolic acids and polysaccharides) have been previously reported in *Verbascum* species [2].

This work was aimed at isolating acylated catalpol-type iridoid diglycosides from *V. ovalifolium* Donn ex Sims and *V. blattaria* L. and investigating their inhibitory potential on interleukin 8 (IL-8) and tumor necrosis alpha (TNF-α) production in lipopolysaccharide-stimulated neutrophils. Application of high-performance countercurrent chromatography (HPCCC) with various two-phase solvent systems composed of *n*-hexane-ethyl acetate-*n*-butanol/methanol-water afforded premnacorymbosides A and B, saccatoside, scorodioside and 6-*O*-(3″,4″-di-*O*-*trans*-cinnamoyl)-α-L-rhamnopyranosylcatalpol from *V. ovalifolium*, as well as 6-*O*-(4″-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosylcatalpol, scrophuloside A3 and gmelinoside L from *V. blattaria*. At 50 μM, scorodioside and premnacorymboside A showed the most promising IL-8 inhibitory effects (inhibitions of 64.66% and 61.23%, respectively), whereas 6-*O*-(3″,4″-di-*O*-*trans*-cinnamoyl)-α-L-rhamnopyranosylcatalpol was the most active compound against TNF-α production (inhibition of 81.03%). Acylated catalpol-iridoid diglycosides with a cinnamoyl group linked at C2″ were more potent than those substituted at C3″ or C4″. Acetylation significantly enhanced the activity of cinnamoyl-substituted derivatives. Furthermore, an additional cinnamic acid residue clearly potentiated the TNF-α inhibitory effects. This is the first time these iridoids diglycosides were isolated from *Verbascum* genus and their cytokine down-regulatory properties were proven.

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SL 62

PHYTOCHEMICAL STANDARDIZATION OF A VACCUUM-DRIED EXTRACT OBTAINED FROM *TRIFOLIUM MEDIUM* L. (FABACEAE) AND EVALUATION OF ITS INHIBITORY EFFECTS IN TWO HUMAN BREAST CANCER CELL LINES

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Phytochemical standardization has been developed to qualify and determine the content of phytoestrogenic isoflavones in the dry herbal formulation obtained from the green, aerial parts of *Trifolium medium* L. (zigzag clover). The efficient extraction technique assisted by the operation of ultrasounds (UAE) was performed for obtaining a series of 50% (v/v) aqueous-ethanolic extracts that were further combined, condensed to dryness, diluted with water and, finally, dried in vacuo. These processes led to obtaining the lyophilisate (TML), that was further subjected to the standardization procedure using the coupled RP-LC/PDA method. A gradient chromatographic system, with acetonitrile and 0.001 M phosphoric acid, and an Aquasil C18 stainless-steel column (250 x 4.6 mm i.d.; dp = 5 um), were used, as the mobile and stationary phase, respectively. Detection of all isoflavones was performed at 260 nm. Both glycosidic (ononin, sissotrin, daidzin, genistin) and aglycone (formononetin, biochanin A, daidzein and genistein) forms have been identified and quantified in TML as the predominant compounds. The total isoflavone content in TML exceeded 12% of dry weight. Cytotoxic effects of TML and reference substances (formononetin and ononin) were examined on MCF-7 (progesterone and estrogen receptor-positive human breast epithelial adenocarcinoma) and MDA-MB-231 (a triple negative human breast adenocarcinoma) cell lines (obtained from American Type Culture Collection) using MTT assay.

Satisfactory results have been obtained, especially as regards to inhibiting a viability of TML-treated MDA-MB-231 cells, compared to control, therefore not only estrogenic but also non-estrogenic model of TML cytostatic activity may be suspected, including other types of polyphenolic compounds.

SL 63

FOLIC ACID SUPPLEMENTATION REPRESSED HYPOXIA-INDUCED INFLAMMATORY RESPONSE IN THP-1 CELLS

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Hypoxia is positively correlated with inflammation and various chronic diseases. Folic acid as a dietary compound has been shown to ameliorate inflammatory reactions, but the metabolism of folic acid protecting against hypoxia-induced injury is still unclear. In our study, we examined the inflammatory signal transduction pathway in folic acid treated human pro-myelomonocytic cells (THP-1 cells) under hypoxic culture conditions. Our results indicated that the supplementation with folic acid significantly reduced the levels of interleukin 1β, interleukin 8, tumor necrosis factor and increased levels of interleukin 10 in cells under hypoxic conditions. Treating THP-1 cells with folic acid suppressed oxidative stress, hypoxia-inducible factor 1α (HIF-1α), and upregulated PHD1 in a dose-dependent manner. Folic acid suppressed hypoxia-induced Akt phosphorylation and NF-κB translocation [1]. Additionally, folic acid targeted the activation of Janus kinase 2 (JAK2), down-regulated the phosphorylation of activators of transcription 3 (STAT3) in cells. However, the absence of folic acid did not make cells more vulnerable under hypoxic conditions. In conclusion, folic acid efficiently inhibited the inflammatory response of THP-1 cells under hypoxic conditions by mediating PI3K/Akt/HIF-1α and JAK2/STAT3 signaling pathway. Our study supports a basis for treatment with folic acid for chronic inflammation, which is correlated with hypoxia [2].

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SL 64

ASSESSING THE PHYLOGEOGRAPHY AND METABOLOMIC SIGNATURES OF WILD ROOIBOS (*ASPALATHUS LINEARIS*, (BURM. F.) DAHLG.) ECOLOGICAL POPULATIONS

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Aspalathus linearis, (Burm. f.) Dahlg., Fabaceae, is a commercially important South African legume endemic to the Cape Floristic Region. It is globally recognized for its production of rooibos tea. It occurs naturally in the Cederberg region of the Western Cape and in the south-western parts of the Northern Cape. This plant exhibits a plethora of health benefits and extracts are used as active ingredients in cosmetics, dietary supplements, alcoholic beverages as well as food products that are exported across the globe. Rooibos research often focuses on its powerful antioxidant properties due to the abundance of flavonoids and other phenolic compounds found throughout the plant. The focus of this study was to discriminate wild rooibos ecotypes from various geographical regions and assess relatedness and genetic diversity between these wild populations using the concepts of phylogeography. Phylogeographical analysis of both chloroplast and newly developed species-specific nuclear markers (microsatellites), reveal population structure, genetic differentiation and genetic diversity of these wild rooibos populations. We investigated the intrapopulation and interpopulation differences of the metabolomic profiles of the wild ecotypes using an LC-MS/MS approach. The phenolic chemistry of the samples revealed three main clusters, yet each population showed distinct chemical profiles. Novel phenolic compounds were also detected in these wild rooibos populations. The biochemical data was then integrated to the genetic data to better understand the metabolomic-genetic networks at play linked to rooibos ecological types. Lastly, this study aimed to establish a method of conservation for these threatened legume species utilizing micropropagation protocols.

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SL 65

GREENHOUSE SALINE CULTIVATION OF TWO SELECTED MEDICINAL HALOPHYTE SPECIES: AGRONOMIC STUDIES AND FUNCTIONAL PROPERTIES OF OBTAINED BIOMASS

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Halophytes can withstand saline soils containing up to 1.7 M NaCl, and other stressful abiotic constraints, such as drought and high light intensity. Several halophytes have ethnomedicinal applications (e.g. for the treatment of dermatologic disorders), bioactive properties (e.g. antioxidant) and are edible (e.g. *Salicornia*). They can be cultivated in conditions where glycophytes cannot (e.g. saline irrigation systems including brackish water, or aquaculture effluents) and the levels of bioactive components may be manipulated by agrotechnical practices (e.g. irrigation water salinity and harvest regime). In our previous work the halophytes *Limonium algarvense* Erben and *Polygonum maritimum* L. were identified as promising sources of ingredients for the cosmetic, food, pharmaceutical and veterinarian industries. In this work these species were produced in a greenhouse under irrigation with artificial saltwater (100/200 mM), and aquaculture effluents (whole water/1:1 dilution). Plant growth performance was evaluated, and extracts from obtained biomass were evaluated for *in vitro* antioxidant, anti-inflammatory, toxicological properties, and for chemical composition. Increased irrigation salinity reduced plant growth and yields. Extracts maintained *in vitro* biological properties and interesting chemical profile, dependent on the salinity and harvest regime. These data suggest that the biological and chemical properties of both species are maintained under saline irrigation, being promising candidates for production in saline agriculture.

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SL 66

ESSENTIAL OIL ENCAPSULATION IN LIPOSOMES AND DRUG-IN CYCLODEXTRIN-IN-LIPOSOMES: EFFECT OF DRUG CHARACTERISTICS ON DRUG ENCAPSULATION AND RELEASE KINETICS

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Liposomes and drug-in-cyclodextrin-in-liposome (DCLs) were recognized as effective drug delivery systems applied to preserve essential oil components enlarging their applications in pharmaceutical industries [1, 2]. We investigated the effect of chemical structure, aqueous solubility, octanol/ water partition coefficient (log P), and Henry's law constant on the encapsulation and release of essential oil components from liposomes and drug-in-hydroxypropyl-β-cyclodextrin-in-liposomes (DCLs) constituted of lipoid S100 and cholesterol. Using the ethanol injection method, the essential oil components estragole, eucalyptol, isoeugenol, pulegone, terpineol, and thymol were encapsulated into conventional liposomes and DCLs. The formulations were characterized for their particle size, drug encapsulation efficiency and loading rate, phospholipids and cholesterol incorporation rates, drug release, and storage stability. The features of essential oil components promoting their incorporation in conventional liposomes were the presence of a hydroxyl group in their structure and exhibiting a low Henry's law constant as well as a low aqueous solubility value. Also, the drug release from liposomes was controlled by the liposome size and composition. Regarding DCLs, the drugs manifesting high log P values were better loaded into vesicles. Furthermore, DCLs exhibiting a high encapsulation efficiency value presented delayed drug release compared to the corresponding inclusion complex. In conclusion, several parameters control the incorporation and release of essential oils from liposomes and DCLs and should be considered in future studies.

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SL 67

TOWARDS THE IDENTIFICATION OF BACTERIAL SECONDARY METABOLITES WITH PANCREATIC BETA CELL REGENERATION-PROMOTING ACTIVITY USING A MICROSCALE *IN VIVO* DIABETES MODEL IN ZEBRAFISH

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Diabetes is a condition of multifactorial origin, and has recently been recognized as being influenced by the intestinal microbiota. Loss of pancreatic beta cell mass can be observed in both type 1 (T1DM) and advanced type 2 diabetes mellitus. Research in both animal models and humans has revealed beta cell regeneration-associated signaling pathways that are influenced by probiotics and their metabolites, triggering proliferation and activity of pancreatic beta cells directly and indirectly by production/fermentation of SCFAs and GABA [1]. In our ongoing biodiscovery program to identify bacterial secondary metabolites with pancreatic beta cell regeneration-promoting activity, we are using a transgenic zebrafish model of T1DM with pharmacologically inducible cell-specific ablation of pancreatic beta cells, Tg(*ins:cfp-nfsb*). This microscale *in vivo* bioassay enables the rapid assessment of the bioactivity of whole extracts, chromatographic fractions, and pure metabolites [2, 3] with regard to their potential to promote pancreatic beta cell regeneration. Using this bioassay, we are screening probiotic strains and their primary metabolites, e.g. short-chain fatty acids (SCFAs), for their ability to increase beta cell mass. Active strains and metabolites will next be analyzed using qRT-PCR of beta cell markers, immunohistochemistry analysis and a free glucose assay. Additional tests in higher animal models will help determine which of these probiotics and their metabolites have therapeutic potential for T1DM.

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SL 68

THE USE OF KENDRICK MASS DEFECT PLOTS FOR SCREENING NATURAL MATRIXES FOR POTENTIAL ANTICANCER AGENTS SUCH AS COLCHICINE BINDING SITE TUBULIN INHIBITORS

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Metabolite detection in complex biological matrixes is always a great challenge because of the multiple types of endogenous components. The current study is focused on Kendrick Mass Defect (KMD) analysis as a powerful data mining tool which could be used in understanding complex mass spectra such as those of plant extracts [1]. Building Kendrick plots allows easy differentiation of small structural differences as methylation, respectively demethylation of molecular ions of certain group of compounds falling in the same homologous order. Thus, the trimethoxyphenyl group of colchicine binding site tubulin inhibitors (CBSIs), which exerts the most dominant effects on modulating the pharmacological properties, is easy to be defined by high resolution accurate mass analysis [2].

Herein we have demonstrated a complex approach of LC/MS data interpretation of different plant sources, which resulted in tentatively identification of several CBSIs. Comparative analysis of different *in vitro* cultures and native sources of *Gloriosa superba* showed the presence of various tropolone type alkaloids such as colchiceine, gloriosine, demecolcine, gloriosamine D, cornigerine, etc. Glycosylated colchicinoids, which may have more beneficial effects and a better toxicity profile in comparison to aglycones, were also observed [3]. Analysis of *in vitro* cultures and native sources of different *Linum* species resulted in the identification of several aryl-tetralin-type lignans, such as podophyllotoxin, 6-methoxy podophyllotoxin, acetyl podophyllotoxin, etc., known to be strong tubulin inhibitors.

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SL 69

ALTERATIONS IN FREE STEROL LEVEL IN MARIGOLD (*CALENDULA OFFICINALIS*) HAIRY ROOT CULTURE IN RESPONSE TO STIMULATION BY SELECTED PHYTOHORMONES

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Hairy root cultures represent a convenient tool in plant biotechnology due to many advantages such as high growth rate without external supply of phytohormones, particularly auxins and cytokinins. Therefore, the influence of these hormones has not been investigated so far with respect to metabolic modulations or elicitiation of production of valuable metabolites by hairy roots. This study was aimed to investigate the effect of selected plant hormones on the synthesis of sterols in marigold (*Calendula officinalis*) hairy root culture (CC16 line), established by transformation with the use of wild-type *Agrobacterium rhizogenes* strain ATCC 15834 and grown in liquid ½ MS medium. Two types of phytohormones: cytokines (naturally occurring 6-furfurylaminopurine, i.e. kinetin, and synthetic 6-benzylaminopurine, BAP) and auxins (naturally occurring indole-3-acetic acid, IAA, and synthetic 1-naphthaleneacetic acid, NAA) were applied in concentration of 0.75 mg/l during 7 days. Air-dried samples of hairy roots were extracted with diethyl ether in Soxhlet apparatus. Obtained extracts were separated by preparative TLC on SiO_2 in a solvent system $CHCl_3$/MeOH (97:3 v/v) and fractions containing free sterols were directly analyzed by GC-MS/FID. The main sterol profile was composed of cholesterol, campesterol, isofucosterol, sitosterol, sitostanol and stigmasterol. The total content of sterols in CC16 line accounted for approx. 440 µg/g d.w. Phytohormones markedly influenced the sterol metabolism in hairy roots, e.g. kinetin increased the content of these compounds by 30%, particularly the level of predominating stigmasterol (by 32%), campesterol (31%), sitosterol (by 7%) and isofucosterol (3-fold), whereas the amounts of cholesterol and sitostanol remained unchanged.

SL 70

FRESH FRUITS OF ELDERBERRY (*SAMBUCUS NIGRA* L.) AS UNIMPROVED POTENTIAL OF BIOLOGICALLY ACTIVE COMPOUNDS

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Nowadays, medicinal plants have an important role in preventing and treating many diseases. Consumption of berry fruits in their frozen, fresh, or dried forms have increased. Elderberry fruit belongs to the wild berry category and is grown in Europe and North America. After collecting, removing impurities, fresh fruits of elderberry crushed in the blender and used to make extracts. Extracts were obtained using traditional (maceration) and modern extraction techniques MAE and UAE (microwave - assisted and ultrasound assisted extraction), and water and 50% ethanol were used as solvents. The content of total phenols (TP) and flavonoids (TF) were analyzed spectrophotometrically. Antioxidant potential was analyzed using *in vitro* antioxidant assays: ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) and CUPRAC (cupric reducing antioxidant capacity). Enzyme inhibitory effects were tested against cholinesterases and tyrosinase. The results are showed that the water and 50% ethanol extracts acquired by MAE and maceration had a higher content of TP and TF, than the extracts obtained by the UAE. The most effective antioxidant assay was CUPRAC ranged from 153.13±2.25 to 216.99±3.82 mg TE/g E. MAE water and 50% ethanol extracts was showed stronger antioxidant activity than UAE and maceration extracts. The examined extracts were demonstrated a very strong enzyme inhibitory activity, according to all investigated enzymes. The best enzyme inhibitory activity was realized with MAE ethanol extract according to the tyrosinase enzyme 243.70±2.26 mg KAE/g E.

Thanks to the great biological activity and high content of secondary metabolites, fresh fruits of elderberry could be potential phytochemicals, which could prevent the development of certain diseases.

POSTER PRESENTATIONS

PP 1

GENETIC EFFECTS OF LASER LIGHT ON PATHOGENIC *ESCHERICHIA COLI*

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The present work aimed to explore effects of irradiation by two laser types on three samples of *Escherichia coli* recovered form wound injury patients besides investigating some of their virulence factors and compare them with the untreated control samples. Laser types used in this study were: neodymium-doped yttrium aluminum garnet pulse Nd: YAG (energy: 200 mJ, distance: 20 cm, time: 10 sec. with 532 nm wave length) and diode (energy: 5 mj, distance: 20 cm, time: 10 sec. with 650 nm wave length). The genomic assessment of the bacteria was performed via Random Ampliphied Polymorphic DNA (RAPD PCR) technique through using five oligomeric primers. Results revealed appearance of new bands and disappearance of others with certain molecular weights of treated samples comparing with the controls. Nd: YAG laser scored high effects than the diode. Thermal propagation caused more changes of DNA. Laser light has antibacterial activity and toxigenecity against the studied bacteria.

PP 2

MEDICINAL FLORA FROM THE MOUNTAINS OF KURDISTAN: *ALLIUM SICULUM* SSP. LEAVES AND THE EFFECTS ON DIURESIS

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Iraqi Kurdistan contains a large number of medicinal herbs that have been used for centuries but have not been discovered. Moreover, Kurds have lived in these mountainous regions continuously for more than 4000 years. This has allowed traditional healers to gain outstanding knowledge and experience with nature and the natural herbs and remedies around them. Regretfully, due to rapid urbanization, this ancient knowledge is threatened and, if not researched and understood, will be lost.

Allium siculum ssp. leaves are one such plant. This beautiful flowering plant (known as *Handresha* in Kurdish) has long been used in villages in Kurdistan. If a traditional healer is approached by someone who is having difficulty breathing or difficulty with urination, they encourage the person to take these leaves. The result seems to be a decrease in urinating and/or breathing difficulty. They also advise the shepherds to not bring the sheep to eat these plants because it will lead to excessive urination.

In this presentation, Mr. Serageldin briefly will present examples of Kurdish medicinal flora and will explain the process he took in identifying the pharmacological and diuretic properties of this plant. The conclusion of his study showed that *Allium siculum* ssp. significantly and safely increased urine flow, sodium and potassium excretion rate and GFR, and safely reduced blood urea and serum creatine. While pharmaceutical treatments have the unwanted side effects of hypokalemia and hyponatremia, this natural plant did not. Further studies are needed, but the preliminary results of Mr. Serageldin's work are very promising.

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PP 3

LEAF EPIDERMAL CHARACTERS OF NINE SPECIES OF BRAZILIAN *BIGNONIACEAE* KNOWN AS MEDICINAL, AS A SUPPORT FOR THEIR QUALITY CONTROL

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Bignoniaceae is a most important lianas family, which has about 82 genera and 827 species centered in the neotropics [1], which includes the largest group of neotropical lianas [2]. This work was performed with the objective to carry out a morphoanatomical study of epidermis of nine species of Bignoniaceae known as medicinal: *Amphilophium crucigerum* (L.) L.G. Lohmann, *Anemopaegma citrinum* Mart. ex DC., *Bignonia ramentacea* (Mart. ex DC.) L.G.Lohmann, *Bignonia sciuripabulum* (K.Schum.) L.G.Lohmann, *Dolichandra unguis-cati* (L.) A.H.Gentry, *Fridericia dichotoma* (Jacq.) L.G.Lohmann, *Fridericia parviflora* (Mart. ex DC.) L.G.Lohmann, *Handroanthus impetiginosus* (Mart. ex DC.) Mattos and *Xylophragma* sp. Paradermic 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. The anticlinal walls of epidermal cells were sinuous on the abaxial surfaces of eight species, except in *B. ramentacea;* the types straight, curved and sinuous also were observed on the adaxial surfaces. All species showed hypostomatic leaves and anomocytic stomata at the level of epidermis, except in *F. parviflora* with the anisocytic type. Most species showed more than one type of stomata, especially anomocytic and anisocytic; the cyclocitic and paracytic types also were observed in *B. ramentacea* and *F. parviflora,* respectively. Idioblasts of crystal prismatic were observed in three species. All species showed glandular and eglandular trichomes, and seven species also showed peltate trichomes. The set of epidermal characters and their annexes, mainly the trichomes morphology, were distinctive parameters to separate the species.

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PP 4

TARGETED BIOTECHNOLOGICAL PRODUCTION OF CYTOTOXIC FLAVONOIDS IN BALCAN ENDEMIC *SIDERITIS SCARDICA* GRIESB

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Shoot cultures of Balkan endemic *Sideritis scardica* were initiated and treatments with plant growth regulators (PGR) and activated charcoal (AC) were performed. Cytotoxicity of the ethyl acetate and butanol fractions of the methanolic extract of commercial samples of *S. scardica* was tested on MCF7 (human breast adenocarcinoma cell line) and compared with the same preparations of the *in vitro* cultured plant in PGR-free medium. Chemical characterization of the samples was performed by LC/MS analysis.

While the ethyl acetate fraction exhibited marked cytotoxic activity, no negative effect on cell growth, as compared with the control non-treated cells, was recorded for the butanol preparation of the commercial sample. All *in vitro* samples, cultivated in the control medium, showed weak cytotoxicity against the tested adenocarcinoma cell line.

Chemical analysis demonstrated that cytotoxicity of the most active ethyl acetate fraction of the commercial sample was related to its enrichment in flavone derivatives and especially with the predominance of apigenin, hypolaetin and isoscutellarein glycosides, combined with significant drop of phenylethanoids. On the contrary, the butanol fraction was highly enriched in phenylethanoids, present together with the flavones.

Interestingly, AC treatments stimulated significantly biomass and flavonoids (luteolin, apigenin and hypolaetin derivatives) as compared with PGR application.

The results demonstrate the high cytotoxicity of flavones, and are indicative of the possible cytoprotective effect of phenylethanoids in *Sideritis scardica* extract. The conducted biotechnological experiment led to the achievement of differential stimulation of phenolics productivity *in vitro,* which could be a promising approach in obtaining plant biomass with desired properties.

PP 5

COMPARISON OF EFFICIENCY OF SELECTED ABIOTIC AND BIOTIC ELICITORS IN ENHANCEMENT OF SAPONIN PRODUCTION IN MARIGOLD (*CALENDULA OFFICINALIS*) HAIRY ROOT CULTURES

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Elicitation is widely used to increase the production of valuable metabolites in plant *in vitro* cultures. One of a class of such valuable metabolites are saponins, e.g., oleanolic acid glycosides produced by marigold (*Calendula officinalis* L.). Hairy root cultures, established by transformation with the use of wild-type *Agrobacterium rhizogenes* strain ATCC 15834, has the capacity of the synthesis of these compounds and their secretion into the surrounding liquid medium. The aim of the study was to investigate the influence of selected abiotic and biotic elicitors, i.e. jasmonic acid (JA) and salicylic acid (SA), chitosan, heavy metals (silver and cadmium ions), UV-C radiation and ultrasound treatment (50 kHz) on saponin production and secretion. JA (100 µM) was the most effective, increasing the accumulation of saponins in roots (20-fold) and their secretion to the medium (113-fold). Low concentrations of SA (50 and 100 µM) increased slightly (by 43%) saponin accumulation and more markedly (5-fold) their secretion, however, higher SA concentration (500 µM) reduced slightly accumulation and secretion of saponins. Chitosan was less efficient in enhancement of saponin production (up to 3-fold) than both hormones, JA and SA. Heavy metals increased the saponin secretion (up to 12-fold) but they had harmful influence on hairy roots growth. UV-C increased the saponin accumulation up to 2.4-fold and their secretion up to 8.5-fold. Ultrasound (20 min exposure) did not change markedly saponin accumulation but increased their secretion up to 11-fold. The obtained results showed significant differences in efficiency of various elicitors in enhancement of saponin production.

PP 6

ANTIOXIDANT PROTECTION OF NEW PROPOLIS MICELLAR FORMULATION IN OXIDATIVE STRESS AND LIPID PEROXIDATION MODELS *IN VITRO*

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An increasing interest for development of new propolis formulations exists approaching to improve the aqueous solubility of its biologically active constituents, thus enhancing their oral or parenteral delivery and bioavailability. Recently, a new formulation of micellar propolis was developed, based on its encapsulation in poly(ethylene oxide)-block-poly(n-butyl acrylate) (PEO-b-PnBA) copolymers. The obtained stable aqueous colloid systems were characterized by complete encapsulation of all bioactive lipophilic propolis constituents and sustained release profile. The performed *in vitro* safety assessment of cytotoxicity in HepG2 (human hepatocellular carcinoma), L929 (fibroblastoma) and SH-SY5Y (human neuroblastoma) cells indicate beneficial profile of blank micellar carrier and propolis-loaded PEO-b-PnBA micelles.

We found a promising antioxidant protection by the newly developed propolis-loaded PEO-b-PnBA micelles in different *in vitro* experimental models: H_2O_2 – induced oxidative stress in SH-SY5Y cells and in Fe^{2+}/Ascorbic acid induced lipid peroxidation in rat liver microsomes. Cell viability and malondialdehyde (MDA) content were determined as markers of cytotoxicity and lipid peroxidation damage, respectively. The protective antioxidant effects of propolis loaded in polymeric micelles were superior as compared to those of free propolis in both *in vitro* models, especially in the highest tested propolis concentration (500 μg/ml).

In conclusion, the high stability and sustained release provided by PEO-b-PnBA micellar propolis formulation contribute to its favorable protective effects. Therefore, PEO-b-PnBA micellar propolis formulation might be considered as a promising delivery system against oxidative stress conditions in different cells.

PP 7

TRITERPENOID NRF2-ACTIVATORS REGULATE NEUTROPHIL SENESCENCE IN MURINE MODEL OF JOINT DAMAGE

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The triterpenoids ursolic and oleanolic acids, as well as, the synthetic compound 2-cyano-3,12-dioxo-oleana-1,9(11)-dien-28-oic acid methyl ester (CDDO-ME) act as strong activators of the leucine-zipper transcription factor NRF2, which regulates the cellular redox homeostasis [1]. Herein we studied the effect of these triterpenoids on neutrophil homing and functions in murine model of joint damage.

Collagenase-induced osteoarthritis (CIOA) was induced Balb/c mice. CDDO-ME was injected intraarticularly twice per week starting at day 7 post CIOA at 50 μg/knee. Control sham-group received 0.01% DMSO/PBS. At day 14 the histological damage of the knee joints was evaluated. *In vitro* CDDO-ME at 100 nM elevated NRF2 and pp38 levels in unstimulated and LPS-stimulated neutrophils. NRF2 activation resulted in restricted PARP cleavage in unstimulated neutrophils but not in LPS activated cells. CDDO-ME, ursolic and oleanolic acids (1-10 μM) inhibited CXCR4 expression in both neutrophil populations. *In vivo* CDDO-ME improved the histological score of joint damage, decreased MMP-9 and TNF-α levels in synovial fluid, reduced the number and frequency of blood neutrophils and increased the NRF2 expression. Moreover, CDDO-ME down-regulated CXCR4 expression and decreased the frequency of apoptotic Annexin V^+ neutrophils indicating a suppression of neutrophil senescence. The higher frequency of mature $CXCR4^+CD11b$ neutrophils in bone marrow of

CDDO-ME-treated mice showed that the triterpenoid stimulated the homing of senescent neutrophils from blood to bone marrow.

In conclusion, our data suggest that triterpenoids may regulate neutrophils senescence *via* NRF2 activation. These findings are important particularly because CDDO-ME is currently in a number of advanced clinical trials [2].

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PP 8

METABOLITE PROFILE AND BIOLOGICAL ACTIVITY OF *SALVIA FORSSKAOLEI*

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Salvia forsskaolei L. occurs in southeastern part of Balkan Peninsula and Northern Anatolia in Asia Minor. The species is often cultivated in the gardens as ornamental because of its beautiful violet-blue 2-lipped flowers. So far the phytochemical studies of *S. forsskaolei* have been restricted to the roots, while the information about the composition and activity of the aerial parts is lacking. The aim of present study was the analyze metabolite profile and biological effects of extracts obtained from the aerial parts of the species. The plant material was sampled in a natural population situated at the edge of Oriental Beech forest in Strandzha Mts (Southeastern Bulgaria). Acetone exudate and methanolic extract of S. forsskaolei were analyzed for their composition by GC/MS and TLC. Free radical scavenging activity was evaluated by DPPH method. The insecticidal effect was evaluated by dip leaf test method on *Myzus persicae* Sulz.

Triterpene was found as the main compound in the acetone exudate, forming about 24% of all detected compounds. Additionally, fatty acids and alcohols, alkanes and mono-, di- and tri-saccharides were identified. Scutellarein 6,7,4'-trimethyl ether (salvigenin) was identified as the main flavonoid aglycone by co-TLC with authentic sample. Phenolic, fatty and organic acids, and a variety of mono-, di- and tri-saccharides were identified *i*n the methanolic extract and its fractions. Significant free radical scavenging activity was established for methanolic extract of the species - IC_{50} = 25 µg/mL. Also, moderate inhibitory effect on *Myzus persicae* was found for methanolic extract. The information reported is new for *Salvia forsskaolei*.

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PP 9

CURRENT STATE OF KNOWLEDGE ABOUT METABOLITE PROFILE AND BIOLOGICAL ACTIVITIES OF *THYMUS* SPP. IN EUROPE: A REVIEW

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Genus *Thymus* comprises more than 250 species of perennial herbaceous or fruticose plants, classified into 8 sections. Total 66 species with numerous subspecies and varieties are listed in Flora Europaea. Due to the taxonomic complexity and difficult identification of the species the studies focused on a relatively small part of the existing diversity. Usually samples of different species are bulked together for the purposes of the pharmaceutical industry, and they are designated as *Thymus* spp., which approach does not take into account the peculiarities in the phytochemical composition of the different species. The objective of the review is to make a survey on the phytochemical investigations and biological activities of the thymes occurring naturally in Europe. The major part of the studies focused on the determining of essential oil composition of the species. Very typical phenomenon for the genus is the chemical polymorphism, which is due both to the ecological factors, and to genetic variation. Phenolic compounds were studied to a lesser extent. A correlation was established between the phenolic content and antioxidant activity of extracts with different polarity. Besides antioxidant activity, antibacterial activity has received particular attention in many experimental designs.

The most frequently investigated species are relatively few, while the majority of thyme species in Europe still remain poorly studied, or not studied at all. This concern especially the endemic species distributed in remote and hardly accessible areas.

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PP 10

MONITORING OF THE ETHANOL CONTENT IN BULGARIAN ROSE OIL SAMPLES BY NIR SPECTROSCOPY

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Bulgarian rose oil (*Rosa damascena* Mill.) is one of the world most sought-after products for its fine aroma and has a status of a protected geographic indication since 2014. Geographic and botanical origin, environmental conditions and production technology [1] are among the factors, affecting the chemical composition and quality of essential oil.

According to the international standard (*ISO 9842*), only fresh rose flowers are used for oil production. However, at the peak of the harvesting season, some rose flowers undergo various degrees of fermentation prior to distillation. Both, the amount and the quality of essential oil are affected significantly. Although ethanol is a natural occurring component of the rose oil, its content increases due to the fermentation process and, therefore, could be used as a marker for this undesired process. The quantitation of ethanol in the rose oil is usually performed by GC-FID, which brings substantial equipment/maintenance costs and time for analysis. At the same time the spectroscopic methods, based on near infrared (NIR), mid infrared (MIR) and Raman spectroscopy can provide a fast and non-destructive alternative [2, 3].

The aim of the current study is to offer an alternative approach applying a simple, fast and non-destructive NIRS method for quantification of ethanol. For this purpose, quantitative analysis of ethanol content in various rose oil samples from Bulgaria was performed by means of GC-FID followed by NIR-spectral measurements and suitable model was developed by calibration of NIR spectra with partial least squares algorithm (PLS).

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PP 11

EXPLORING OF ROSE AND LAVENDER ESSENTIAL OILS CHIRAL COMPONENTS BY ENANTIOSELECTIVE GAS CHROMATOGRAPHY

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Essential oils (EO) have been widely used all over the world and their use is constantly increasing because of the strong demand for pure natural ingredients in many fields (in fine perfumery, as natural cosmetics and cosmeceuticals, as food supplements and in clinical aromatherapy). EO are of high-added value and therefore, they are often subject of adulteration by adding non-volatile ingredients, synthetic compounds, or cheaper essential oils. Bearing in mind the extremely complex chemical composition of the EO, sophisticated analytical techniques are required ensuring quality control and consumer safety.

The development of stable chiral phases for GC, has allowed detailed study of enantiomeric composition of the volatile compounds. ES-GC has become an important technique for detection of adulterants, essential oil profiling and quality control, based on the fact that plants produce metabolites mainly as chiral molecules. Thus, diastereomer and enantiomer composition, which is characteristic for the geographic and botanical origin, can allow detection of accidental or deliberate addition of a synthetic products or even the discrimination of different geographic or botanical origin.

Therefore, the aim of the current study is to explore composition of some chiral components, which are important quality and authenticity markers in Bulgarian lavender and rose oil samples by means of ES-GC/MSD/FID. In this respect linalool, rose oxides and citronellol in rose oil and linalool, camphor and linalyl acetate in lavender oil samples have been studied and an analytical protocol for detection of synthetic citronellol, linalool and linalyl acetate was developed.

PP 12

HESPERETIN BASED ALTERNATIVE APPROACHES TO DIABETES CONVENTIONAL THERAPIES

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Diabetes mellitus is a metabolic disease that affects an increasing percent of the global population. In recent years phytochemistry based alternative approaches to the conventional treatment are establishing themselves as valuable tools in the management of diabetes [1]. Hesperetin, the aglycone of the naturally occurring flavanone-glycoside hesperidin, possesses anti-oxidantion, anti-hypercholesterolemia, anti-hypertension, anti-inflammatory and anti-anaphylaxis properties [2] but also proved beneficial effects in diabetes experimental models [3]. The aim of the study was to investigate the degree of protection on the diabetes onset and treatment outcome of a hesperetin based enriched diet, in a murine diabetes model. The healthy Wistar rats diet was enriched with hesperetin [0.02 mmols/kg body weight (bw)] or hesperetin - vanadyl sulphate (0.02 mmols/kg bw – 0.1 mmols/kg bw) for 30 days. In the 22nd day of the experiment the animals were exposed to alloxan (130 mg/kg bw i.p.) in order to induce diabetes. At the end of the experiment glycaemia and lipid profile was evaluated. The results pointed out an improvement of the glycaemia, triglycerides and my abstract cholesterol levels for both hesperetin and hesperetin - vanadyl sulphate groups compared to the control groups.

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PP 13

MENTHA GATTEFOSSEI MAIRE, AN UNEXPLORED ENDEMIC SPECIES: HPLC-DAD-ESI-Q-TOF-MS/MS POLYPHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY

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Mentha gattefossei Maire (Lamiaceae) is a perennial and aromatic plant, endemic to Morocco [1]. Its aerial parts are used traditionally to alleviate symptoms of the digestive disorders, bronchitis, cold, cough and migraine. Also, the plant is used as food and for flavoring purposes [1, 2]. This study assessed the polyphenolic profile and antioxidant activity of an alcoholic extract obtained from aerial parts of *M. gattefossei* species cultivated in Republic of Moldova within *ex situ* conservation programmes at international level. The extract of *M. gattefossei* was subjected to HPLC-DAD-ESI-Q-TOF-MS/MS analysis and separated on a Phenomenex Gemini C18 (100 × 2 mm, 3 µm) column, with a gradient obtained from 0.1% formic in water and 0.1% formic acid in acetonitrile. The levels of total phenolics and flavonoids were also estimated. The extract showed potent reducing capacity and free radical scavenging properties and also good iron-chelating activity. The metabolite profiling revealed the presence of several phenolic acids (hydroxybenzoic acid, chlorogenic acid, rosmarinic acid, salvianic acid A, syringic acid) and flavonoids (diosmin, isoquercitrin, acacetin rutinoside). Of these, rosmarinic acid was by far the major phenolic compound, its content reaching 12.42 ± 0.23 mg/g extract. This investigation provided an insight on polyphenols from *Mentha gattefossei* and their bioactivity.

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SYNERGISM OF MORUSIN AND KUWANON G WITH CONVENTIONAL ANTIBIOTICS AGAINST *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS*

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The aim of this work was to evaluate the antibacterial effectiveness of morusin and kuwanon G, prenylated constituents of mulberry tree, both alone and in combination with conventional antibiotics against methicillin-susceptible (MSSA) and -resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* strains. Minimum inhibitory concentrations (MICs) were determined by the broth microdilution assay [1] whereas potential synergistic interactions were assessed by the checkerboard and time-kill assays [1, 2]. Data from the latter two assays were analysed and interpreted using the fractional inhibitory concentration index (FICI) and the difference in bacterial killing induced by the combination prenylated compound - antibiotic in comparison to the most active component of the combination tested alone, after 24 h incubation (ΔLC_{24} expressed as $\log_{10}$ CFU/mL). Morusin and kuwanon G inhibited the bacterial growth with MIC values of 6.25 and 12.5 mg/mL, respectively. Morusin and kuwanon G showed different extent of synergism with antibiotics (oxacillin, gentamicin, tetracycline, ciprofloxacin) against *Staphylococcus* strains (FICI = 0.12-0.38) with significant enhancements of antibiotic activity (4-512 fold). Moreover, morusin showed antibiotic resistance reversal effects: it reversed oxacillin resistance of MRSA and tetracycline resistance of *S. epidermidis*. Two combinations, namely morusin-oxacillin and morusin-gentamicin, at ½MIC, showed bactericidal synergy against MRSA ($\Delta LC_{24} > 2 \log_{10}$ CFU/mL). Combinations of morusin or kuwanon G with antibiotics seem to be a promising approach in the treatment of staphylococcal infections.

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PP 15

A COMPARISON BETWEEN MODERN EXTRACTION METHODS OF *ECHINACEA PURPUREEA* FRESH AND PLANT WASTE

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Medicinal and aromatic plants (MAP) have always been an important element in maintaining and improving human health and vitality. The study focused on *Echinacea purpureea* - a medicinal and aromatic plant - which was selected for its high content of total phenols and antioxidant activity. Improvement of extraction methods is the main concern of the researchers for an efficient material exploitation and reduction of quantities of plant waste resulted from the extraction processes. Therefore, we have opted in this study for a comparison between three MAP extraction methods using modern techniques, two of them being green methods: microwave hydrodiffusion and gravity, solvent-free microwave extraction and accelerated solvent extraction. The optimization study consisted in the usage of experimental design statistical techniques based on polynomial equations. In this study, favorable conclusions can be drawn regarding the selection of the optimal extraction parameters for each method, following an optimal ratio between the extraction results and material resources used in the process. Also, the efficiency of the accelerated solvent extraction (ASE) method was assessed. Optimal operational conditions with regard to extraction temperature (50-130 °C) and solvent concentration (methanol : water 70 : 30, v/v) were identified in order to maximize the extraction of useful compounds from Echinacea (*Echinacea purpureea*).

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IMPROVED TRITERPENOID PRODUCTION IN HAIRY ROOT CULTURES BY ELICITATION AND PRECURSOR FEEDING OF *CENTELLA ASIATICA* (L.) URBAN

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Centella asiatica, which belongs to the Apiaceae family, is a tropical and subtropical medicinal plant [1]. It contains a variety of triterpenoids, including madecassoside, asiaticoside, madecassic acid, and asiatic acid [2]. In the present study, *C. asiatica* hairy root (HR) cultures were established from petiole and leaf segments via *Agrobacterium rhizogenes*-mediated transformation for the triterpenoid production. In total, 36 lines were confirmed using PCR amplification of *rol* B genes, and eight HR lines were selected for further study. Line HP4, derived from the petiole, displayed the highest growth index, whereas the highest triterpenoid saponin accumulation (46.5 mg/g DW) was noted in line HP2. Accumulation of triterpenoid saponins was 1.4-fold higher in hairy root cultures derived from the petiole than those derived from leaves. Furthermore, to increase secondary metabolite accumulation, the hairy root cultures were subjected to treatment with precursors (squalene and pyruvic acid) and elicitors. Treatment with squalene at 2.5 mM and pyruvic acid at 5 mM led to a greater degree of triterpenoid synthesis (57.53 and 29.13 mg/g DW, respectively) than did the other treatments. However, the highest triterpenoid content was obtained following treatment with 400 μM methyl jasmonate (60.25 mg/g DW). The results indicated that *C. asiatica* hairy root cultures could rapidly accumulate biomass and could be scaled up to enable growth in bioreactors for the production of triterpenoids for the pharmaceutical and cosmetic industries.

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PP 17

STUDY OF THE PREVENTIVE EFFECTS OF A COFFEE EXTRACT IN SPINAL CORD INJURY-INDUCED NEUROPATHIC PAIN DEVELOPMENT IN MICE

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Aims: Considering the preclinical evidences showing that polyphenolic compounds may exert antinociceptive effects, the present work aimed to study preventive effects of a coffee polyphenolic extract (CPE) in spinal cord-induced neuropathic pain development in mice.

Methods: Female CD1 mice were subjected to mild SCI and daily treated with CPE (10 and 15 mg/kg; i.p.) during the first week post-surgery. Thermal hyperalgesia and mechanical allodynia were weekly evaluated up to 21 days post-injury (dpi), by means of Hargreaves test and Von Frey filaments respectively. The locomotor functional recovery after SCI was assessed by Basso mouse scale (BMS). In addition, at 21 dpi, histological analysis was carried out to study the levels of astrogliosis (GFAP) and microgliosis (Iba1). To obtain the polyphenolic extract, the vegetal material was boiled with saline solution. The extract polyphenols quantification was performed by Folin-Ciocalteu method, using gallic acid as standard.

Results: The concentration of total polyphenols in the CPE was 1985 mg/L. Mechanical allodynia and thermal hyperalgesia development was significant attenuated in treated SCI-animals up to 21 dpi. Both doses reduced the reactivity of astrocytes but only the lowest dose (10 mg/kg) reduced the reactivity of microglia. No major impairment in locomotor function was detected at any experimental group according to BMS test results.

Conclusion: CPE treatment may be a suitable therapeutic strategy to prevent development of spinal cord injury-induced neuropathic pain.

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INVESTIGATION AND CONSERVATION OF THE BALKAN ENDEMIC *CENTAUREA MANNAGETTAE* PODP. (ASTERACEAE) IN BULGARIA

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Centaurea mannagettae Podp. is a species of high conservation importance distributed in Bulgaria and R Macedonia. It is included in the *IUCN Red List of Threatened Plants*, the *Bulgarian Biodiversity Act* and the *Red Data Book*. This taxon is found in dry, stony places on limestone in the mountains of Pirin, Rila and Slavyanka, as well as in two hilly regions - Besaparski Hills and Sakar. The species has wonderful decorative qualities, participate in the composition of habitats of European importance. Its metabolic profile is also to be studied. The aim of the present work is to study the status of *C. mannagettae*'s populations in Bulgaria, to identify the threatening factors and to formulate appropriate measures for conservation. Observations were made on 5 populations. They are small and highly fragmented. The chromosome numbers of 2 of them were studied and 2 ploidy levels were established. The main threat is due to the destruction/ degradation of the specific habitat as a result of the use of land for wine production and obtaining of inert materials in Besapara hills; the construction of ski runs in Pirin Mt.; erosion; low reproductive and migration potential of the species, etc. It is necessary to preserve the species in all its populations, because the presence of different ploidy levels suggests different morphological and genetic variability. At this stage, it is sufficient to observe compliance with the conservation legislation and to monitor populations annually.

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A REVIEW OF DIFFERENT EXTRACTION METHODS OF *SILYBUM MARIANUM*

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Milk thistle, *Silybum marianum* is a medicinal annual or biennial plant that has its origins in the Mediterranean basin. Extracts from milk thistle seeds have been used as a form of treatment for various liver ailments for over 2000 years. Recent research has supported its efficiency as hepatoprotector [1], and its use for medication in cases of liver disease, hepatitis and damage to the liver that was caused by the abuse of alcohol and drugs. The latest research has shown that silymarin, the main extract from milk thistle is helpful against skin and prostate cancer [2]. Milk thistle seeds contain between 1.5 and 3% silymarin. Silymarin is a mix of flavonolignans which include of silychristin, silydiadin, silybin, and silybinin. Given its high medicinal potential, there has been increasing interest in refining extraction methods in order to obtain the highest yields while minimizing time expenditure and solvent use. This paper is a review of three extraction methods for silymarins: ASE (Accelerated Solvent Extraction) - a form of PSE (Pressurized Solvent Extraction), boiling water extraction, as well as boiling solvents extraction. Taxifolin and silychristin - two important components of the complex flavonoid mixture silymarin - were extracted best using boiling water, whereas silybinin A and silybinin B - the main components of sylimarin - had the highest yield when methanol was used. The most efficient extraction method for silybinin A and silybinin B was ACE using methanol.

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PP 20

VALORIZATION OF GRAPE POMACE THROUGH ENCAPSULATION OF POLYPHENOLS EXTRACT INTO MESOPOROUS SILICA-TYPE MATRICES

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Grape pomace is a well-known wine industry by-product and an important source of polyphenols which presents antioxidant, anti-inflammatory and anti-cancer properties [1].

Herein, we report the encapsulation of polyphenol grape pomace ethanolic extracts into three mesoporous MCM-41-type silica matrices in order to reduce plant extract sensitivity to light and to improve its stability, while preserving its antioxidant capacity. For this, pristine MCM-41 silica and heteroatom-modified silica materials were prepared according to previously reported procedure that involves an ion-exchange process between surfactant ions linked on the inner pores surface of silica precursors and the corresponding heteroatom ion in ethanolic solution, followed by the metal oxide formation on silica surface during the calcination step [2].

Three grapes variety (Cabernet Saugvinon, Feteasca Neagra from the Black Sea region and commercially available mixed grapes) were used to prepare grape pomace ethanolic extracts by a conventional extraction [3] or microwave assisted procedure (at 75W MW power).

The polyphenol extracts composition was analyzed by HPLC, and the antioxidant capacity (DPPH and ABTS assays), total polyphenolic and ascorbic acid content, total flavonoid, as well as total anthocyanin pigment content were also determined. The encapsulation of polyphenolic extract in pristine mesoporous silica (MCM-41), silica material modified with ceria (Ce-MCM-41), which proved radical scavenger properties, or mesoporous silica decorated with MgO (Mg-MCM-41) was performed through incipient wetness impregnation method and the resulted composite materials show similar antioxidant activity as the corresponding extract.

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PROPERTIES OF *SALVIA OFFICINALIS* LEAVES EXTRACT INCORPORATED IN MESOPOROUS INORGANIC SUPPORTS

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Extracts obtained from various medicinal herbs present antioxidant, antimicrobial, anti-inflammatory or even antitumoral properties and their benefits for human health are well known [1]. The aim of this work was to study the antioxidant capacity and antimicrobial properties of *Salvia officinalis* (hill area of Transylvania, Romania) extract encapsulated in mesoporous inorganic matrices, MCM-41 silica, 3-aminopropyl functionalized silica (MCM-APTES) and titania. The sage leaf extracts at different plant/absolute ethanol ratios were obtained at reflux either by conventional method or by microwaves irradiation. For sage ethanolic extracts, the polyphenols content (Folin Ciocalteu method) and radical scavenging effect (DPPH and ABTS assays) were determined by UV-vis spectroscopy [2] and their composition through HPLC analysis. Enhanced polyphenols content and antioxidant capacity were obtained for the sage alcoholic extract prepared by microwave irradiation. Selected extracts were incorporated in mesopores of pristine and functionalized inorganic matrices by incipient wetness impregnation procedure followed by vacuum solvent evaporation and the total content of natural compounds was determined by thermogravimetric analysis. The antimicrobial activity of common sage extracts and materials resulted through its incorporation in mesoporous inorganic matrices was tested against *Escherichia coli* and *Staphylococcus aureus* strains. It was proved that the encapsulated sage extract samples presented good antioxidant capacity and antimicrobial activity.

Acknowledgements: This research was supported by the Romanian project PCCDI no. 85/2018.

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ANTI-*CAMPYLOBACTER* ACTIVITY OF PHYTOCHEMICAL FORMU-LATIONS FROM ALPINE AND KARST PLANTS, TRADITIONALLY USED AGAINST INTESTINAL DISORDERS

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Due to high prevalence of infections with resistant *Campylobacter jejuni*, the investigation of novel anti-*Campylobacter* agents is much needed. New control strategies are increasingly focusing on natural antimicrobials. We prepared ethanol and/or hexane extracts and their fractions from the alpine and karst plants, traditionally used against intestinal disorders, as are *Anaphalis margaritacea*, *Juniperus communis*, *Peucedanum ostruthium*, *Leontopodium nivale* ssp. *alpinum*, *Origanum vulgare*, *Thymus vulgaris* and *Satureja montana*. We investigated their influence on growth inhibition, activity of key efflux pumps (CmeABC, CmeDEF and CmeGH) and/or on their motility, adhesion, and quorum sensing (QS) activity. After plant material preparation, using an accelerated solvent extraction with different solvents, we analyzed their chemical composition by TLC, GC-MS and HPLC-MS/MS. We determined minimal inhibitory concentrations (MICs) with broth microdilution after dying with resazurin. Further, we investigated their influence on: (i) efflux pumps with the ethidium bromide accumulation assay, (ii) bacterial membrane integrity using LIVE/DEAD BacLight assay, (iii) sensitivity of mutants lacking activity in CmeABC, CmeDEF and CmeGH efflux pumps, (iv) bioluminescence measurement of the reporter strain *V. harveyi* BB170. The results exposed the influence of all tested extracts on growth, membrane integrity and/or their efflux pumps. MICs varied from 125 mg/L - 2000 mg/L. The most promising ethanol extracts of A. *margaritacea,* and *S. montana* showed good antimicrobial activity by influencing bacterial efflux pumps. *J. communis* had the strongest anti-QS activity and anti-adhesion activity. Studies of the mechanism of bioactivity are going on as they are essential for efficient application of identified anti-*Campylobacter* compounds.

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BIOACTIVE COMPOUNDS AND QUALITY PARAMETERS IN DIFFERENT ORGANIC APPLE VARIETIES

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The interest on organic plant-based foods is constantly growing due to their health benefits and ecological importance along with increasing demands of the consumers for quality foods produced sustainably. Organic apples were known to present high content in polyphenols, compounds which are recognized to have multiple biological activities [1].

The aim of this work is to evaluate the variations in quality parameters (firmness, total soluble solids and titratable acidity) and bioactive compounds (anthocyanins and vitamin C) of three organic apple varieties.

Organic apple varieties (Gala, Golden and Red Prince) harvested at maturity from an organic farm in August and September 2018 were used for this study. Firmness, total soluble solids and titratable acidity were performed using instrumental analyses [2]. The total anthocyanin content was determined in acidified methanolic extracts by a spectrophotometric method whereas the vitamin C was monitored by HPLC. The firmness and titratable acidity were both significantly higher in Golden variety. The Red Prince variety showed higher total soluble solids and total anthocyanin content than Gala and Golden varieties, what recommends their use for processing immediately after harvesting or in the first few weeks, while the Golden variety can be stored under controlled conditions for further processing. The data also pointed that the Golden variety have high vitamin C content as well as higher firmness and titratable acidity values compared to the red apple varieties.

From these results it can be concluded that quality parameters and bioactive compounds of organic apples are generally influenced by the variety.

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PHENOLIC PROFILE AND CONTENT OF AERIAL PARTS OF LINGONBERRY (*VACCINIUM VITIS-IDAEA L.*)

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Leaves and stems of lingonberry constitute natural sources of food and beverage and are consumed as dietary supplements or pharmaceutical products for health benefits. In plants, the quality and quantity of phenolic compounds are influenced by the parts of the plant to be used.

The aim of this study is to compare the dynamic accumulation of phenolic compounds in leaves and stems of lingonberry.

For this work leaves and stems of wild lingonberry were collected from Borca (Neamt, Romania) during the year 2017. The total phenolic content of the ethanolic extract solutions was determined by the Folin-Ciocalteu method and expressed as gallic acid equivalents, whereas the characterization of the respective phenolic compounds were monitored by using Ultra Performance Liquid Chromatography (UPLC).

Qualitative analysis showed the predominant presence of quercetin glycosides and monomers and oligomers of catechin and epicatechin, in both leaves and stems. The total phenolic content in leaves, either by Folin method or UPLC, was higher than in stems. Stems were found to contain predominantly flavanol oligomers as the more abundant class of the total weight of phenolic compounds. In leaf extracts, arbutin appears as the major constituent of phenolic compounds.

Results from this study indicated that all aerial parts of lingonberry are suitable for valorization as sources of natural phenolic compounds as well as to be valuable feedstocks for the production of herbal supplements.

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ANTIOXIDANT EFFECTS OF *RUSCUS ACULEATUS* ON THE BONES AND LIVERS OF OVARIECTOMISED RATS

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Oxidative stress is a key mechanism involved inmany chronic diseases including OP. Improving antioxidant status could help the prevention of menopause induced OP. The purpose of this study was to investigate the antioxidant properties of extract from *Ruscus aculeatus* (ERA) on the bones from ovariectomized (OVX) rats. The experiments were carried out on 3-months-old Wistar rats, divided into six groups: Group 1 was sham-operated control (SHAM). Rats with bilateral ovaeiectomy (OVX) were Group 2. Groups 3, 4 and 5 were OVXrats and giventree doses of ERA – 50, 100 and 200 mg/kg/day, orally for 45 days. OVX rats supplemented withestradiol (40 μg/kg/day, for 45 days were Group 6. Markers of oxidative status as follows: level of reduced glutathione (GSH), malondialdehyde (MDA) quantity, superoxidedismutase (SOD) and catalase (CAT) activitieswere measured in bones and livers from euthanized rats. Ovariectomy and subsequent estrogen deficiency led to statistically significant oxidative stress discerned by decreased activity of antioxidant enzymes and level of GSH and increased MDA quantity in the liver and bones of experimental animals. Administration of ERA at higher doses100 and 200 mg/kg amelioratedabove mentioned parameters more significantly in the livers of the experimental animals. The antioxidant effect of ERA is comparable to the estradiol protective effect. Thus, ERA supplementations at both doses, 100 and 200 mg/kg that showed the best results, may reduce oxidative stress in OVX rats which may prevent bone loss and increase bone health via its anti-oxidative property.

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A TRADITIONAL THAI ANTIHYPERTENSIVE HERBAL RECIPE LOWERING BLOOD PRESSURE IN L-NAME-INDUCED HYPERTENSIVE RATS

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A traditional Thai antihypertensive herbal recipe (TTAH) has been used for treatment of hypertension in southern Thailand, in particular Nakhonsithammarat province [1]. There is no scientific evidence proving its efficacy even if the quality control and the safety of TTAH was previously studied in animals [2]. This study investigated effect of TTAH on blood pressure of L-NAME-induced hypertensive Wistar rats. Rats orally received L-NAME (50 mg/kg/day) for 4 weeks and blood pressure was monitored by tail cuff method every week. Administration of L-NAME significantly increased systolic blood pressure while parallel treatment with TTAH (400, 200, and 100 mg/kg/day) resulted in reducing blood pressure. Detection using LC-MS in positive electrospray ionization mode revealed the main constituent, piperine, which may contribute to antihypertensive activity.

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PP 27

INVESTIGATION ON THE ANTI-OBESITY EFFECT OF A TRITERPENOID-ENRICHED EXTRACT OF *CYNOMORIUM SONGARICUM* AND ITS POSSIBLE BIOCHEMICAL MECHANISM IN HIGH FAT DIET-INDUCED OBESE MICE

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The stem of *Cynomorium songaricum Rupr.* (Cynomorii Herba) or Suo-Yang in Chinese (the literal translation being "locking the Yang") is a "Yang-invigorating" tonic herb commonly used in the practice of traditional Chinese medicine (TCM). This herb is mainly used for the treatment of sexual impotence and fatigue. HCY2, an ursolic acid-enriched extract of Cynomorii Herba, has been shown to significantly reduce body weight gain and the weight of white fat pads in various adipose tissues as well as attenuate indices of the associated metabolic syndrome, namely, hyperglycemia, glucose intolerance, dyslipidemia and fatty liver in high-fat diet (HFD)-fed mice. The current study demonstrated that the weight reduction produced by HCY2 is associated with activation of the AMPK signalling pathway with resultant increases in mitochondrial biogenesis and mitochondrial uncoupling in skeletal muscle *in vivo*. A recoupler, ketocholestanol, was used to explore the possible role of mitochondrial uncoupling in the anti-obesity effect afforded by HCY2 in HFD-fed mice.

Taken together, our results suggest that HCY2 may be proved to be useful for the prevention and/or treatment of obesity and related metabolic disorders.

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SCREENING OF ALCOHOLIC AND AQUEOUS EXTRACTS OF *MUSSAENDA ERYTHROPHYLLA* (RUBIACEA) IN ALBINO RATS; FOR ITS DIURTIC ACTIVITY

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The traditional systems of treatment such as Ayurveda, Unani, and Siddha, western herbal medicine, traditional Chinese medicine and homeopathy use herbs for the treatment. Many researchers have prescribed about the importance of herbal medicine in the treatment of various diseases and because of the accessibility and cost effectiveness herbal treatment is still in practice by large number of practitioners. *Mussaenda erythrophylla* (Rubiacea) plant was reported to possess a number of medicinal properties including used for cough, jaundice, showed hepatoprotective effects, anti-helminthic activity, appetite stimulant activity and also has anti-oxidant. The present study was investigated the diuretic activity of plant alcoholic and aqueous extracts of *M. erythrophylla*. It was carried out by using method at the dose of 200 and 400 mg/kg and compared with Furosemide (standard) at 20 mg/kg. Alcoholic and aqueous plant extracts were showed significant 3.69±0.12 and 5.37±0.20 ($P < 0.01$) increase in the urine at 400 mg/kg, respectively when compared with standard group i.e. Furosemide 3.95±0.26 and 7.91±0.38 at 20 mg/kg at 5 hr and 24 hr. However alcoholic and aqueous plant extracts were showed significant increased urinary concentration of sodium, potassium and chloride 106.18±5.28, 74.33±2.14 and 119.74±4.49 ($P < 0.01$) at 400 mg/kg 7.917±0.38 at 24 hr when compared with that of Furosemide (20 mg/kg) 127.92±2.11, 88.01±1.69 and 149.06±7.65 respectively. This result revealed that alcoholic plant extract showing more significant diuretic activity when compared to aqueous plant extract. It may be possessing triterpenoids, glycosides, and flavonoids and sugars may play role in diuretic activity.

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GINKGO BILOBA ACTIVE COMPOUNDS, EMERGING RESOURCE FOR PLANT PROTECTION

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Ginkgo (*Ginkgo biloba* L.), a living fossil native to China, the first to green again after Hiroshima bombing, is also one of the most known and used traditional medicine on Earth, with numerous associated pharmacological activities. Standardized ginkgo leaf extracts are one of the best-selling herbal products and countless studies looked into this species phytocompounds, methods of extractions, roles on human health etc. While the main bioactive constituents are terpene trilactones and flavonoid glycosides, many secondary metabolites, as amino acids, allyl phenols, carbohydrates, inorganic salts, lipids, organic acids, polyphenols, terpenoids have been isolated from the plant. Recent studies have been analysed the possibility to use ginkgo componds in agriculture and plant health. For instance, aqueous gingko extract repelled snails from eating lettuce leaves coated with the extract; an aqueous methanol leaf extract inhibited the growth of roots and shoots of garden cress, lettuce, timothy and ryegrass seedlings; a substance extracted from the external ginkgo seed coat proved acaricidal activity on citrus red mite, *Panonychus citri*, with no phytotoxicity to plants; the antifeedant activity of ginkgo secondary metabolites against *Hyphantria cunea* larvae launched the development of new applications of *G. biloba*; in the USA, a patent for a pesticide against cotton pests, including ginkgo bilobalide and ginkgolide A, B, C, J, and M was issued since 2015. In this paper available advances about the usage of ginkgo derived phytoactive components in agriculture are discussed, including the challenges and perspectives on using ginkgo as a resource for new effective pest management methods.

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EFECTIVE PHYTOCHEMICALS AGAINST THE TOMATO LEAFMINER *TUTA ABSOLUTA* (MEYRICK) (LEPIDOPTERA: GELECHIIDAE). A REVIEW

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The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), a terrifying invasive pest threatening the tomato production worldwide, quickly develops insecticide resistance. The multiple negative impacts of intensive use of synthetic pesticides on environment, humans and other non-target organisms and *T. absoluta* high biotic potential forced researchers and practicians to quickly develop alternative methods of control and integrated IPM programs for this pest. Phytochemicals have been used for a long time in crop protection, as home-made preparations. Recently, antifeedant, growth inhibiting, repellent, and insecticide effect have been demonstrated for various plant-derived compounds, used as isolated substances or mixtures. Botanical pesticides based on azadirachtin, carvacrol, cinnamaldehyde, citronellal, eugenol, linalool, nicotine, pyrethrin, rotenone, thujone, thymol, α-terpineol, 1,8-cineol, etc are already commercially available. Numerous new phytochemicals have been tested: black pepper ethanolic leaf extracts were found promising for larvae control; basil oil, turmeric oil, rosemary oil, and thyme oil lead to satisfactory results; basil, geranium, chinaberry, onion and garlic aqueous extracts shown promising efficiency on second instar larvae of *T. absoluta* etc. In the same time, folk medicine also offers recipes for plant health. Answers to an online questionnaire revealed that Romanian farmers are using macerates of hot pepper, basil, marigold, nettle, and garlic as repellents, ash and garlic extracts for seeds treatments, nettle and seaweed maceration for plant enhancing resistance and hot pepper and garlic infusions to control the tomato leaf miner. The current paper summarizes the results of different phytochemical preparations or plant presence on *T. absoluta* and synthesizes the main future research needs.

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ANTIMICROBIAL ACTIVITY OF *ALOE ARBORESCENCE* LYOPHILIZED LEAF GEL

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Introduction: Current research results indicate activity of *Aloe arborescens* in relation to infections with viral and fungal etiology. Therefore the aim of the study was to evaluate its antibacterial activity.

Methods: Fresh whole leaves of *A. arborescens* were supplied by Phytopharm Klęka S.A., they came from controlled crops. Extracts were obtained from the leaves of one-, two-, three- and four-old and they were analyzed by RP-HPLC-DAD method. The standardization was conducted in regards to aloenin and aloin as marker compounds. The antibacterial properties were tested against selected G(+) and G(-) bacteria.

Results: Content of aloenin and aloin were different depending on the age of the leaves. Leaves from different years shown different activities on G(+) and G(-) bacteria. Extract from *A. arborescens* has the highest activity against *Bacillus subtilis* among bacteria G(+) and to *Escherichia coli* and *Pseudomonas aereuginosa* among bacteria G(-).

Conclusions: The antibacterial activity is correlated with the age of the leaves from *A. arboscescens*. There was no relationship between the content of marker compounds and microbiological activity.

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MUCOADHESIVE VAGINAL DRUG DELIVERY SYSTEM CONTAING *CHELIDONII HERBA* LYOPHILIZED EXTRACT WITH CHITOSAN

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Introduction: *Chelidonium majus* L. (Greater celandine, Papaveraceae) contains isoquinoline alkaloids (e.g. chelidnonine, sanguinarine), flavonoids, saponins, carotenoids, organic acids, vitamins and mineral elements. Due to presence of isoquinoline alkaloids *Chelidonii herba* extracts are widely used as an antibacterial, antifungal, antiviral (including HSV-1 and HIV-1) and anti-inflammatory agent in the treatment of skin diseases. While chitosan is biocompatible and biodegradable carrier about especially valuable properties for preparation of mucoadhesive formulations. The purpose of the present study was to formulate vaginal tablet based on chitosan loaded with *Chelidonii herba* lyophilized extract. For this aim physicochemical (physical and chemical stability, rheological and dissolution properties) and microbiolgical characterisitcs will performed.

Methods: Dry hydroalcoholic extract of *C. majus* was lyophilized to obtain powder and then it was standardized in regards to isoquinoline alkaloids (chelidnonine, sanguinarine) content, according to European Pharmacopoeia 9th Edition (*C. herba* monograph). Chitosan nanoparticles containing *Chelidonii herba* lyophilized extract were obtained in gelation form, through direct compression. Quality control of mucoadhesive vavinal drug delivery with Chelidonii extract involved studies of hardness, porosity, mucoadhesive, disintegration and dissolution. The changes of dissolved isoquinoline alkaloids concentrations were measured by using the UPLC-DAD method.

Results: An increase in chitosan in suitable concentration in the mucoadhesive vavinal drug delivery caused increased mucoadhesive properties and prolonged release of active compounds. *C. herba* lyophilized extract and showed interesting microbiological activity.

Conclusions: Based on obtained results mucoadhesive drug delivery, containing *C. herba* lyophilized extract with chitosan, seems to be a promising strategy for preparation of vaginal drug delivery system.

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CHEMICAL AND BIOLOGICAL PROFILE OF TWO *AJUGA* SPECIES CULTIVATED IN NORTHERN ROMANIA

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Ajuga genevensis and *Ajuga reptans* are two medicinal plants used in Romanian traditional medicine for their anti-inflammatory properties for rheumatism and gout. They are also credited with astringent, tonic and diuretic activities. Although these species are common for the wild flora in our country, our investigation was conducted on specimens harvested from the experimental fields of "Stejarul" Biological Research Centre, Piatra Neamt, Romania. Their introduction in culture as a source of bioactive compounds is intended for pharmaceutical purposes. Therefore, the investigation of their characteristics and benefits is of extreme importance for future use in pharmaceutical preparations and/or food supplements. Their chemical profile was assessed on hydro-alcoholic extracts by spectrophotometry and thin layer chromatography and HPLC techniques. First, the enzymatic activities of the samples were investigated against lipoxygenase, butyryl and acetylcholinesterase. For the i*n vivo* biological activities the extracts (25 and 75 mg/kg bw) were administered in a 6-hydroxidopamine Parkinsonian rat model. The results confirmed the taxonomic and chemical variability between the investigated samples. In terms of compound profile, the polyphenols (flavones and polyphenolic acids) were richer in *A. reptans* (168.3 mg % and 230.4 mg % respectively), whereas *A. genevensis* contained higher quantities of iridoids (1860 mg % as compared to 1250 mg %). The biological activity confirmed the antioxidant and protective properties of both extracts. Moreover, the anti-amnesic activity was significantly increased for *A. reptans* as compared to *A. genevensis.*

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ZEBRAFISH-BASED PHENOTYPIC SCREENING IDENTIFIES NEW BIOACTIVE SECONDARY METABOLITES FROM AN ANTARCTIC TUNICATE

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Secondary metabolites from marine organisms are structurally diverse small molecules with high levels of bioactivity, and represent an underutilized resource for modern drug discovery. To facilitate the identification of drug-like marine metabolites, the significant potential of *in vivo* models of human disease – in particular those suitable for medium-throughput screening and bioassay-guided fractionation – should be more fully explored in ongoing and future marine biodiscovery efforts [1, 2]. Here, we report the application of a microscale, phenotypic zebrafish bioassay for marine biodiscovery. We screened over 400 crude extracts of marine invertebrates and algae on basis of their ability to cause morphological changes to developing zebrafish embryos. Our focus was on organisms native to Antarctica. The Antarctic Circumpolar Font has isolated this ecosystem, resulting in an endemic flora and fauna with concomitant diversity of the chemistry of the Antarctic organisms. Our large-scale *in vivo* phenotypic screen identified an Antarctic 'yellow top' tunicate, known as *Synoicum* sp., with significant bioactivity in this assay. Indole alkaloids, known as meridianins, have been isolated from this organism in the past [3]. Reverse-phase MPLC and HPLC was used to fractionate MeOH : DCM extracts, and 1D and 2D NMR and high-resolution electrospray mass spectrometry was used to identify compounds, of which two were new. The bioactivity of isolated compounds was tested in a series of phenotypic zebrafish assays, revealing some of them to elicit profound changes to embryonic patterning that are currently the subject of further analysis to elucidate the signaling pathways involved.

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EXPLORING THE FRUITS OF THE EXTREMOPHILE *CARPOBROTUS EDULIS* L. AS A POTENTIAL SOURCE OF HEALTH PROMOTING PRODUCTS

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Carpobrotus edulis (L.) N.E. Br, (syn. *Mesembryanthemum edule* L.) (hottentot-fig, sour fig), is an edible perennial plant native to the Cape Coast region of South Africa, where it is used in traditional medicine for the treatment of several illness, including tuberculosis, laryngitis, mouth infections and HIV/AIDS infection. Different plant organs, namely leaves, stems and roots, display multiple biological activities relevant for human health improvement (e.g. antioxidant, anti-bacterial, neuroprotective and antiglycation) and several chemicals were already identified in this species (e.g. procyanidins B2, (–)-epicatechin). However, to the best of our knowledge, nothing is known about the chemical profile or biological activities of *C. edulis* edible fruits. In this work food grade extracts (ethanol, acetone and water) were prepared from fruit pulps and fruit peels from *C. edulis*, and evaluated for *in vitro* antioxidant properties, and capacity to inhibit key enzymes for neurodegeneration (acetyl- and butyrylcholinesterase), diabetes (α-glucosidase and amylase), and hiperpigmentation (tyrosinase). Extracts were evaluated by spectrophotometric methods and by LC-MS. In general, the peel extracts displayed the highest antioxidant activity. The extracts also displayed relevant enzymatic inhibitory properties, and varied phenolic contents. Results suggest that fruits from *C. edulis* could be sources of active ingredients for human health improvement.

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HALOPHYTE PLANTS AND ITS POTENTIAL USE IN ANIMAL NUTRITION AND HEALTH: FROM ETHNOVETERINARY USES TO BIOACTIVE PROPERTIES

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More than 2600 species of halophyte plants occur in the Mediterranean region, thriving in saline environments such as coastal saltmarshes, sand dunes and salt lakes. Halophytes contain several bioactive metabolites and have been increasingly studied for its chemical profile and bioactive properties, such as antioxidant, anti-inflammatory and antiparasitic. These plants are widely used for human nutrition, human health improvement, and for veterinary purposes, and recent review papers described their chemical composition, biological activities, functional properties and traditional medicinal uses [1-3]. However, there is no available comprehensive information describing their traditional veterinarian applications. The aim of this work was to summarize the ethnoveterinary uses of halophytes in the Mediterranean area, discuss their potential usage in animal nutrition and explore their possible applications as nutraceuticals or as prophylactic/therapeutic agents (phytotherapeuticals). Several species were described for uses as nutraceuticals, fodder, feed or feed supplement in animal nutrition, and the main veterinary pharmacological traditional applications included the treatment of gastrointestinal and inflammatory disorders, parasitic diseases and those of the reproductive system. Plantaginaceae is the most cited plant family, regarding the number of ethnoveterinary uses described. This work is an initial approach to explore the potential use of halophytes species in veterinary sciences by taking advantage of their traditional uses.

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PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT, ANTIMICROBIAL AND ANTICANCER POTENTIAL OF *OENOTHERA BIENNIS* L. HYDROALCOHOLIC EXTRACT AGAINST A375 HUMAN MELANOMA CELL LINE

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Oenothera biennis L. or evening primrose has the best studied biological activity from all the members of the *Onagraceae* family and possess a wide range of medicinal properties including antioxidant, anti-inflammatory, anti-diabetic, anti-bacterial, anti-fungal, anti-neuropathic, anti-cancer activity [1]. The aim of the study was the phytochemical characterization of *O. biennis* L. hydroalcoholic extract (OB) as well as the evaluation of the antimicrobial and anticancer potential against A375 human melanoma cell line. The ethanolic extracts obtained from the aerial part of *O. biennis* L. have shown total phenolic content of 6314.96 mg GAE/g extract and an antioxidant activity of 7258.67 µmol Trolox/g extract. Individual polyphenols determined by LC-MS include: gallic acid (1064.61 µg/g), caffeic acid (11525.75 µg/g), epicatechine (78406.93 µg/g), coumaric acid (268.77 µg/g), ferulic acid (1250.06 µg/g), rutin (3528.73 µg/g), and rosmarinic acid (1601.58 µg/g). OB had a bacteriostatic effect against tested bacterial strains, but it was bactericidal only against *Candida* spp. and *S. aureus*. In the set experimental conditions OB manifested significant antiproliferative activity against A375 human melanoma cell line only at the highest tested concentration, namely 60 µg/ml. Annexin-PI staining have shown a weak number of events corresponding to early apoptosis, late apoptosis or necrosis. On the other hand OB elicited antiangiogenic potential on the CAM model.

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SECONDARY METABOLITES PRODUCTIVITY IN *INULA BRITANICA* IS RELATED TO BIOMASS FORMATION AND PHYSIOLOGICAL ADAPTATION IN TISSUE CULTURE CONDITIONS

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Inula britannica is widely distributed throughout Western Europe and Turkey, reaching China through Iran and Pakistan. The species is of great importance for Traditional Chinese Medicine as well as Kampo medicine in Japan. Decoction of aerials or blossoms is used for treating asthma, and as an expectorant. Flowers are used as antibacterial, carminative, diuretic, laxative, stomach, tonic, rapid-healer, for hepatitis and tumors.

Shoot cultures of the plant were developed from material collected in Bulgaria. An experiment for elucidation of the combined effect of vitamins (Murashige and Skoog *vs.* Gamborg) and auxin and cytokinin treatments on secondary metabolite productivity, biomass formation and physiological status of shoot cultures was conducted.

Gamborg vitamins considerably stimulated plant growth as compared with Murashige and Skoog ones. Further on, the addition of benzyl adenine as cytokinin stimulated biomass formation in terms of axillary rosettes formation, however it suppressed aerial and root length and caused callusogenesis at explant base, this effect being slightly alleviated by 2-Naphthylacetic acid addition. Gamborg vitamins supplementation stimulated polyphenolics and total sesquiterpene lactones content *in* vitro. Interestingly, plant growth regulators affected polyphenolics and sesquiterpene lactones britannin and gaillardin ratio in a different way depending on the vitamins added. Biomass formation and secondary metabolite productivity were also found to be dependent on oxidative stress and photosynthetic pigments content of the cultures. The results are indicative of the relations between growth, development and secondary metabolite production in the plant organism.

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PHOTOSTIMULATION OF POLYPHENOLICS PRODUCTIVITY IN *ARTEMISIA ALBA* CELL AGGREGATES IN LIQUID MEDIA

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As a part of a broader program for biotechnological development of *Artemisia alba*, non-differentiated cell aggregates were developed and grown in liquid media. Lines were initiated from sterile leaf explants and root cuttings, the first ones characterized by significantly higher lipid peroxidation and oxidative stress. Lines were maintained in liquid media supplemented with N^6-benzyladenine (BA) in combination with either indole-3-butyric (IBA) acid or 2-Naphthylacetic acid (NAA).

Light treatment stimulated polyphenolics production and increased stress hormones salicylic, abscisic and jasmonic acid. Interestingly, light affected differentially zeatin levels in relation to the initial explants from which they were initiated. Thus, while *trans*-zeatin content was generally enhanced by light treatment in the leaf-initiated lines, the *cis*-zeatin content was lowered in the same samples. This effect was media-dependent for root-initiated lines.

The initial physiological status also affected the morphology and biosynthetic capacity of the resulting lines. Thus, leaf initiated cell aggregates were heterogenous and to a higher degree non-differentiated, as compared with the ones initiated from root cuttings. This was accompanied by a higher polyphenolics productivity of the root-initiated lines. In addition NAA grown lines were more compact and with larger size as compared with IBA grown ones. This was also related to higher biosynthetic capacity of the NAA-grown lines. Qualitative characterization of the polyphenolics is in process in order to assess the potential of the obtained system as a prospective source of phytotherapeuticals.

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ARONIA MELANOCARPA JUICE IMPROVES COGNITIVE AND LOCOMOTOR FUNCTIONS OF AGED RATS

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Aging is associated with decreasing cognitive function that affects life quality. The quest for healthy ageing has led to the extensive study of antiaging properties of plant polyphenols. The aim of the study was to assess the effect of polyphenol-rich *Aronia melanocarpa* juice (AMJ*)* on some behavioural functions (learning and memory) and brain morphology of aged rats. Male Wistar rats (n = 24) were divided in 3 groups: young controls (YC), age - 2 months, without AMJ supplementation; old controls (OC), age 24 months, without AMJ supplementation; and AMJ group - 24 months aged animals, supplemented orally with AMJ (10 ml/kg for 105 days). Activity cage and shuttle box tests were used to examine locomotor functions and memory of animals, whereas fragments of hippocampus and frontal cortex were used for morphometric analysis and acetylcholinesterase activity determination. The activity cage test showed that OC decreased the number of horizontal movements compared to YC ($p<0.05$), whereas AMJ supplemented rats increased the number of vertical movements compared to both young and old controls ($p<0.05$). In the active avoidance test, supplemented rats increased the number of avoidances on 3rd, 4th and 5th days of learning session, compared to the respective day of old controls ($p<0.05$). AMJ supplementation did not change the acetylcholinesterase activity of test animals.

These results indicate that AMJ supplementation induces ameliorating changes in the ability of old rats to learn tasks and improves their locomotor functions.

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EFFECT OF GAMMA IRRADIATION ON CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF DRIED ROSE HIP FRUITS (*ROSA CANINA* L.)

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Gamma irradiation is among the most widely used methods for decontamination of dried fruits, herbs, spices and nuts. However, its effect on the bioactive substances of treated materials is not fully clarified. Rose hips (*Rosa canina* L.) are a very rich source of biologically active substances, such as vitamins C, A, E and B; minerals, essential fatty acids, polyphenols and polysaccharides, and reveal high antioxidant activity. With over 1,000 tons of annual raw material export, *R. canina* holds the first place of export among Bulgarian herbs. Therefore, it is of particular interest to assess the effect of gamma irradiation on the chemical composition and antioxidant activity of dried rose hips, including the seed oil, which is the aim of the current study. Freeze-dried fruits were irradiated at ^{60}Co source with 8200 Ci activity with doses of 10 kGy and 25 kGy. Then, the content of carbohydrates, organic acids, polyphenols, flavonoids, uronic acids, fatty acids, carotenoids, tocopherols and antioxidant activity of the irradiated and untreated samples were compared. The obtained results are important from a practical point of view, because they reveal the changes in chemical composition of rose hip fruits after gamma irradiation.

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ANTIDIABETIC AND ANTIHYPERTENSIVE EFFECT OF 3.5-DICAFFEOYLQUINIC ACID ISOLATED FROM *GEIGERIA ALATA*

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Introduction: *Geigeria alata* (Asteraceae) is an annual herb used in Sudanese folk medicine for treatment of diabetes, epilepsy, pneumonia, and rheumatism [1]. The study aimed to investigate *in vivo* antidiabetic and antihypertensive effects of trans-3,5-diCQA, a major acylquinic acid isolated from *G. alata* roots.

Materials and Methods: The studied CQA was isolated using methods of ultrasound assisted extraction and low pressure liquid chromatography. The structures of 3,5-diCQA was established by nuclear magnetic resonance and high resolution mass spectra. Male normotensive (NTR) and spontaneously hypertensive (SHR) rats were used for *in vivo* experiment. Type 2 diabetes was induced by intraperitoneal (i.p.) injection of streptozotocin (STZ, 45 mg/kg BW), 15 min after the i.p. administration of nicotineamide (NA) (110 mg/kg BW). 3,5-diCQA was administered at dose 5 mg/kg by oral gavage for 28 days in diabetic NTRs and SHRs. The changes of blood glucose level, systolic blood pressure (SBP) and some biochemical serum parameters were measured.

Results: 3,5-diCQA administration resulted in statistically significant: reduction of blood glucose levels by 35% and 42% in diabetic NTRs and SHRs, respectively, decrease of SBP by approximately 22% in both non-diabetic and diabetic SHRs, and attenuation of increased cholesterol, triglycerides, ASAT and creatinine serum level in 3,5-diCQA treated diabetic SHR, compared to the diabetic SHRs.

Conclusions: 3,5-diCQA prevents the blood biochemical changes related to diabetes and hypertension. Its administration to diabetic rats from both strains improved their glycemic status and exerts antihypertensive effect.

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PP 43

MICELLAR CURCUMIN POTENTIATES THE ANTIBACTERIAL EFFECT OF MILTEFOSINE AND ERUFOSINE AGAINST PATHOGENIC *STAPHYLOCOCCUS AUREUS* STRAINS

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In the light of the emerging bacterial resistance to broad-spectrum antibiotics, the search for new antibacterial therapeutics and drug combinations is nowadays one of the most challenging topics. In the present study, we investigated for first time the antibacterial, biofilm and quorum sensing inhibitory effects of the third generation anticancer alkylphosphocholine (APC) erufosine against pathogenic Gram-positive bacterial strains in comparison to the prototype of this pharmacological class of drugs - miltefosine. We searched also for synergistic anti-staphylococcal combinations between both APCs and curcumin incorporated in co-polymeric micelles based on a mixture between Pluronic® P123 and Pluronic® F127 (P123/F127). The quantitative redox-activity experimental data were evaluated with the Lambert-Pearson model in the MAPLE software, whereas the drug-drug interactions were calculated using the fractional inhibitory concentration methodology. Similar to miltefosine, erufosine showed a moderate bacteriostatic effect in clinically relevant concentrations (<60 μM) and inhibited the bacterial redox activity up to 90% at minimal inhibitory concentrations [1]. Combinations with P123/F127 micellar CRM at a ratio 1:1 enhanced the effect of both APCs towards methicillin-resistant staphylococci. Erufosine achieved median biofilm inhibition at lower concentrations ($MBIC_{50}$ = 1.87 μM) than miltefosine ($MBIC_{50}$ = 6.0 μM) and curcumin ($MBIC_{50}$ = 24.84 μM) and inhibited the bacterial motility. In conclusion, the estimated antibacterial potential of erufosine widens the spectrum of its useful pharmacological effects. The established synergistic combinations could be beneficial for the application

of both APCs in cancer therapy, since numerous malignancies are accompanied by bacterial infections.

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ANTI-STAPHYLOCOCCAL, ANTINEOPLASTIC AND SKIN IRRITATION EFFECTS OF ETHYL ACETATE EXTRACT FROM AERIAL PARTS OF *GEUM URBANUM* L.

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Aim of the present study was to investigate the anti-staphylococcal biofilm effect of the ethyl acetate (EtOAc) extract from the medicinal plant *Geum urbanum* L., as well as its *in vitro* and *in vivo* toxicity regarding normal and malignant keratinocytes.

We used phytochemical methods to obtain 10 fractions from EtOAc extract after performing a fingerprint analysis. By thin-layer chromatography-direct bioautography was selected the most active fraction. We calculated their minimal inhibitory and bactericidal concentrations (MIC/MBC) [ISO 20776-1:2006(E)], determined the dehydrogenase activity in several pathogenic *Staphylococcus aureus* strains by redox-activity test and studied the anti-biofilm potential by microbiological and molecular-biological methods. The *in vitro* cytotoxicity was tested on the cell lines HaCaT (normal human keratinocytes), A-375 (malignant melanoma) and A-431 (epidermoid squamous carcinoma) by MTT [ISO 10993-5] and CFU assays. The antineoplastic effect of the extract was studied following cell clonogenicity, induction of apoptosis

(caspase-3 activity), glutathione levels and modulation of the cell cycle (flow cytometry). The skin irritation potential of the extract was investigated on albino rabbits [ISO 10993-10].

The EtOAc solved compounds were proved as effective anti-staphylococcal agents with favorable skin tolerance, as evidenced by a primary irritation index equal to zero. The extract showed strong antineoplastic (IC_{50} = 7÷20 µg/mL) and anti-biofilm activity by sub-MICs which outlines new perspectives for its development as natural product for specific skin applications.

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LAVENDER ESSENTIAL OILS FOR INHIBITING OF *TOMATO SPOTTED WILT VIRUS* ON GARDEN SAGE (*SALVIA OFFICINALIS*)

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The effect of *Lavandula angustifolia* Mill. oils with different chemical composition on the viral concentration of *Tomato spotted wilt virus* (TSWV) in *Salvia officinalis* plants was tested. Standard oil, Hemus variety and D11 sample oil (high in terpene-4-ol 12.32 %) were used.

Essential oils were diluted in 1000 ppm, 3000 ppm, 5000 ppm and 10000 ppm and applied three times for three consecutive months by spraying small parcels of garden sage. The experiment was carried out to track the antiviral inhibitory effect on the plant pathogenicity of tomato bronze virus (TSWV) and influence the concentration of the oils used. Such an effect would contribute to limiting the disease caused by TSWV, as well as to controlling its thrips vectors. The ELISA (DAS-ELISA) serological method was used. The effect is reported by the extinction values for TSWV in the different samples.

The results showed that both oils have an antivirus inhibitory effect. Lavender essential oil D11 at concentrations of 1000 and 3000 ppm exhibits a 4 to 6-fold higher antiviral inhibitory effect than that of the Hemus variety oil (0.313 OD and 0.262 OD versus 1.398 OD and 1.662 OD respectively). For standard lavender, the same effect is reached at 5000 ppm and 10000 ppm - 0.224 OD and 0.232 OD respectively.

These annual and initial results revealed that lavender oils have potential as a tool of inhibiting *Tomato spotted wilt virus* in sage. The effectiveness of their application depends on the chemicals, particularly of terpinen-4-ol quantity.

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COMPARATIVE STUDY OF THE EFFECTS OF *ROSA ALBA* L. ESSENTIAL OILS OBTAINED BY WATER-STEAM DISTILLATION AND SUBCRITICAL FLUID EXTRACTION ON THE VIABILITY AND PROLIFERATION OF BURKITT LYMPHOMA CELLS

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The present study was directed to the investigation of concentration and time-dependent cytotoxicity of *Rosa alba* L. essential oil, obtained by steam distillation and subcritical fluid extraction on Raji cell line, derived from patients with Burkitt's lymphoma.

As a raw material fresh flowers of *R. alba* L., from plantation of the Institute of Rose and Aromatic Plants, in Kazanlak were used. Rose oils were extracted of semi-industrial processing line in Institute. The following incubation conditions for cell cytotoxicity were used: $1x10^6$ cells/ml, plant extract – 50-1000 µg/ml, incubation time – 24-72 h, humidified atmosphere (37 ^{0}C, 5% CO_2). The analyzing parameters were: cell survival (trypan blue staining), intracellular superoxide (hidydroethidium test), hydroperoxides (OxySelectTM In Vitro ROS Assay), total antioxidant capacity (OxySelect^{TM} TAC Assay), induction of apoptosis (FITC-Annexin V test).

It was found that essential oils obtained by water-steam distillation in a concentration of 750 µg/ml and over, inhibited cell growth by 40%, and oil by subcritical fluid extraction at same concentration by 50% after 48 h incubation. Water distillated oil of *R. alba* L. increased the level of apoptosis ~4 times, do not influence total antioxidant capacity of lymphoma cells, decreased the value of hydroperoxides by 50% versus controls, non-treated cells. Essential oil obtained by subcritical fluid extraction decreased the level of endogenous superoxide by 60%, and cause not significant changes on the other tested parameters.

Data obtained suggest that both of essential oils obtained by classical water steam distillation as well subcritical fluid extraction of *R. alba* L. have a promising anti-cancer activity towards Burkitt's lymphoma B-cell line (Raji) *in vitro.*

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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF MYRTENAL IN RODENTS

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Search for new pharmacological agents is an important factor in delivering better therapy. Inflammation and pain are common phenomena associated with a number of diseases. Many plants active ingredients exhibit analgesic and anti-inflammatory activity. The bicyclic monoterpenoid Myrtenal (M) is found in many plants essential oils. Researches on total plant extracts as well as on essential oils reveal a wide range of biological effects with various mechanisms. But there is no data in the literature about Myrtenal effects in pain and inflammation.

Aim of study was to investigate M effects in models of pain and inflammation in laboratory rodents.

Materials and methods: Anti-nociceptive activity of M (30 mg/kg bw, i.p.) was tested in male ICR mice after single and repeated administration on two experimental pain models - Acetic acid writhing test (antipyretic type analgesia) and Hot plate test (narcotic type analgesia). Anti-inflammatory activity of M (40 mg/kg bw, i. p.) was evaluated on the 24th hour from the last treatment after 5-days administration via carrageenan-induced inflammation model on rat paw in comparison to NSAID referent.

Results: In our experiments on Wistar rats and ICR mice M demonstrated significant anti-inflammatory and anti-nociceptive properties (toward both peripheral and thermal pain). These effects were combined with the observed in our previous studies anti-oxidant effect of Myrtenal [1].

Conclusions: Possible mechanisms of action of Myrtenal are complex and they probably include its antioxidant properties.

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EVALUATION OF REGENERATIVE AND ANTIINFLAMMATORY EFFECT OF SEA-BUCKTHORN DERIVED FATTY ACIDS ON SKIN CELL TYPES

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Sea-buckthorn oil is a natural compound that elicited a lot of interest due to its antioxidant, antiangiogenic and anti-tumor effects. Due to antiinflammatory effects, this natural product has also been used as food supplement or natural adjuvant to conventional drugs. Although the chemical composition of the oil is rather well described, less is known about the molecular effects of its components. **Methods:** Fatty acid fractions were purified by HPLC. Cytotoxicity profile of purified fatty acids was assessed by end-point assays (MTS, LDH). Cell regeneration was tested by real-time assays – impedance readings and videomicroscopy and inflammatory effect by quantitative assessment of cytokines by multiplexing assay. **Results:** Purified fatty acids demonstrated a better regenerative effect than the commercially available oil. In terms of antiinflammatory effect, in basal state, no significant effect was detected for either purified fatty acids or oil, but with prior stimulation with LPS, a significant effect was noted for fatty acids. **In conclusion**, purified fractions are a better choice than the whole natural product, in terms of alternative therapy.

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CHEMICAL PROFILING AND BIOACTIVITY OF *ORIGANUM SIPYLEUM* EXTRACTS: EXPLORING FOR NOVEL SOURCES FOR POTENTIAL PHARMACEUTICAL, FOOD, AND COSMETIC APPLICATIONS

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Origanum sipyleum L., an endemic plant of Western Anatolia has been used as a medicinal tea, food additive, and for the production of essential oil. In this study, the biological potential of three extracts (ethyl acetate, methanol, and aqueous) of *O. sipyleum* was assessed based on antioxidant activity against key enzymes of clinical relavance. The chemical profile of the plant was assessed using spectrophotometric and LC-MS techniques. Additionally, we explored potential antioxidant and anti-inflammatory effects induced by the extracts in an experimental model of ulcerative colitis induced by LPS challenging. LC-MS analysis revealed that the extracts contained different classes of phenolics, such as rosmarinic acid, phlorizin and gallic acid. We found that the aqueous extract was the most effective antioxidant, displaying the highest DPPH and ABTS scavenging, FRAP, CUPRAC, molybdenum(VI) reducing, and metal chelating effect. The aqueous extract howed the strongest acetylcholinesterase (AChE) inhibition; the methanol extract showed the highest α-glucosidase inhibition, while the ethyl acetate extract was the most effective on butyrylcholinesterase (BChE), tyrosinase, and α-amylase. The total flavonoid content was highest in the aqueous and ethyl acetate extract, respectively. Finally, we found that all extracts were effective in reducing LPS-induced activity of pro-oxidant and pro-inflammatory biomarkers including nitrites, LDH, PGE2 and 5-HT, in rat colon, with the best activity showed by ethyl acetate extract. Our results indicated that the three solvent extracts varied in their chemical and biological profiles, but overall, *O. sipyleum* showed promising therapeutic properties, nonetheless, need to be further validated in *in vivo* models.

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INNOVATIVE APPROACHES TO OBTAIN "GOOD CHEMICALS" FROM EDIBLE PLANTS WASTE

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Nowadays, large quantities of wastes are obtained in different industries, which can be can transformed into "green products"; thus, the interest of the scientific community shifted towards the recovery of chemical compounds from (edible) plants wastes. With an increasing number of people in this century, there is an increase of global production of edible plants, so a normal increase of wastes. In this category of edible plants, we consider agricultural production of plants for food production and medicinal plants which have a valuable and important role in economic, ecological aspects of local communities all over the world. For instance, in the last five years, the production of plants rich in different biological active compounds, like grapes, apples, and other fruits, increased with approximatively 25%, based on the FAO statistics [1]. All over the world, production of bio-products leads to the generation of large quantities of plants waste, mostly unexploited. It is expected that in 2020 the total available land for medicinal and aromatic plants cultivation will be increased to approx. 20.5 Mha. The significant quantities of biological active compounds with beneficial properties can be obtained from these plants' wastes.

Herein, different types of medicinal and aromatic plants which processed yield wastes rich in "good chemicals" are discussed. The modern extraction techniques applied to obtain biological active compounds are also included.

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PHYTOSYNTHESIZED AND RADIOLOGICAL SYNTHESIZED SILVER NANOPARTICLES: OBTAINING AND BIOLOGICAL ACTIVITIES

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Phytosynthesis of noble metal nanoparticles represents a very important area of research both for scientists working in the area of materials science, as well as for researchers in the natural products compounds, as the size and shape of nanoparticles (and thus their biological properties) is in a very strong correlation with the composition of the extracts used for the synthesis [1].

Another green alternative for the synthesis of nanoparticles is represented by their radiological synthesis [2]. The present paper studies the differences between phytosynthesised nanoparticles (obtained using *Asplenium scolopendrium* L. extracts) and nanoparticles obtained using γ-rays, in terms of morphology (evaluated using transmission electron microscopy, X-ray diffraction, UV-Vis spectrometry) and biological properties, evaluated by antimicrobial, cytotoxic (*Allium* test) and phytotoxic (*Triticum* test) assays. The obtained results showed a concentration-dependent effect, with major differences recorded between the two types of nanoparticles.

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INFLUENCE OF IONIZING RADIATION ON THE BIOLOGICAL PROPERTIES OF NATURAL EXTRACTS

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Natural extracts possess several important biological activities (such as antioxidant, antimicrobial or anti-inflammatory properties) [1, 2]. In order to be used as herbal formulation, several methods were proposed for the hygienization and shelf-life extension, including ionizing radiation [3].

However, when applying such a strategy, the effect of the treatment on the products properties should be evaluated. The present paper evaluates the influence of γ-irradiation (accomplished with Co-60 isotope, irradiation dose rate 1.2 kGy/h) on the composition of *Asplenium scolopendrium* L. extracts (using HPLC) and on their biological properties, evaluated by antimicrobial, cytotoxic (*Allium* test) and phytotoxic (*Triticum* test) assays. The obtained results suggest a dose-dependent effect, suggesting that relatively small radiation doses could prove to be an effective treatment, in the same time increasing the biological properties of the crude extracts.

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RESPONSE OF BIOMASS DEVELOPMENT AND ESSENTIAL OIL COMPOSITION OF *SALVIA OFFICINALIS* L. TO IRRIGATION FREQUENCY AND HARVEST TIME

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Salvia officinalis L. (sage), an aromatic plant from Lamiaceae family, can be found worldwide and its leaves are commonly used as ingredient in food and cosmetic industry. Sage essential oil is applied in the treatment of a range of diseases and has been shown to possess antimicrobial, viricidal, cytotoxic and antifungal activities. Generally, essential oils and their composition are strongly affected by environmental conditions including the growing region, climate, and harvesting time [1, 2]. Water shortage is the major factor that affects plant growth and is a serious problem in many areas of the world causing considerable losses in agricultural production. Water deficiency induces various physiological and metabolic responses such as stomatal closure, decline in growth rate, secondary metabolites and expression of stress specific genes. So, the objective of our research was to study the effects of irrigation frequency and harvest time on quantity and quality of *S. officinalis* essential oils. Sage was cultivated at six experimental sites in south-central Italy in different growing environments. The essential oils (S1 - S6), extracted by hydrodistillation according to the European Pharmacopoeia, were analyzed by GC and CG/MS: peak identification was accomplished by comparison of their mass spectra with NIST 02 and Wiley 275 libraries, as well as by comparison of their retention indices with literature values. Our data show that fresh weight and essential oils yields decreased with increasing irrigation intervals, whereas essential oil content was stimulated by water stress and increased as the irrigation interval increased. α-Thujone, β-thujone, camphor and borneol were the major compounds found in the oils and their quantity varied at different irrigation intervals and harvest times. Determining the best condition for cultivation environment which can results in producing plants with the highest percentage of essential oil compound is regarded as the most important purpose of the studies in the field of medicinal plant cultivation.

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PP 54

NMR-BASED METABOLOMICS OF *VERONICA* SPP

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The *Veronica* (common name speedwell; Plantaginaceae) is a genus of flowering plants, widely distributed in Europe. *Veronica* ssp. are known to be rich in iridoid glycosides and hence widely used in traditional medicine [1]. In our previous studies a comprehensive platform for NMR-based metabolomics and metabolite profiling has been developed and applied to several medicinal plants species [2, 3]. The aim of our ongoing project is an investigation on the anti-inflammatory potential of plants from *Veronica* genus along with the isolation of their bioactive principles. For this purpose NMR-based metabolomics of 6 *Veronica* species (i.e. *V. rhodopaea*, *V. austriaca*, *V. urticifolia*, *V. barrelieri*, *V. officinalis* and *V. montana*) has been performed.

The NMR spectra (1D, 2D-TOCSY, -HSQC, -JRes) revealed the presence of abundant primary metabolites (amino acids and sugars), besides the typical fingerprint compounds in *Veronica* – iridoid glycosides. Among the other *Veronica* species, *V. austriaca* was found to be rich in arbutin – a natural hydroquinone, known to inhibit tyrosinase. Further, in order to determine the chemical differences/similarities between the six *Veronica* species, chemometrics tools (SIMCA-P software package) were applied and the obtained results will be discussed accordingly.

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PP 55

DEVELOPMENT OF A NOVEL PLATFORM FOR PLANT-BASED NATURAL PRODUCTS TO TARGET DISEASES

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Fibrosis accounts for approximately 67% of deaths in the developed world. Many of the different types of fibrosis currently have no drugs on the market for their treatment. One such type is liver fibrosis, the incidence of which is expected to increase due to modern western lifestyles. For this reason, treatments urgently need to be developed and, therefore, there is a demand for novel sources of compounds with potential therapeutic activity. Plants are a huge, largely untapped resource of novel compounds with potential for biopharmaceutical activity. They have been used for centuries in traditional medicines across the world. There have been numerous studies reporting successful treatments with herbal medicines, however, it often appears that purified extracts or phytochemicals do not have the same beneficial properties as whole extracts can have. This is because it does not take into consideration the interplay between the phytochemicals present in the real plant matrix. In this project, plants will be directly incubated with in vitro plate based models of liver fibrosis and their abilities to modulate fibrosis progression will be determined. Following this, they will be put through a bioassay that is able to pinpoint single effector compounds as well as identify synergic effects between phytochemicals with the aim of identifying novel active compounds with anti-fibrotic activity. Different plants will be tested which will allow direct comparison of known medicinal plants with novel ones of unknown effect.

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ANTISEIZURE AND ANALGESIC ACTIVITY OF NEWLY SYNTHESIZED MELATONIN DERIVATIVES BEARING AROYLHYDRAZONE MOIETY

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Despite the plethora of new antiepileptic drugs, one-third of patients with epilepsy remain treatment-resistant. The unmet needs of those patients force the researchers to test new neurologically active compounds. When some substances show a neuropharmacological activity, it is a common practice to extend the research for other promising pharmacological activities.

Series of several aroylhydrazone based molecular hybrids bearing melatonin scaffold were synthesized and tested for antiseizure and antinociceptive activity. Male ICR mice were injected intraperitoneally with the tested substances or with the vehicle only (controls). We performed maximal electroshock (MES) test, 6Hz test, hot plate test, formalin test, and rotarod test.

We identified four substances, namely (1) 4-chloro-N'-[(E)-(5-methoxy-1H-indol-3-yl)methylidene]benzohydrazide; (2) N'-[(E)-(5-methoxy-1H-indol-3-yl)methylidene]furan-2-carbo-hydrazide; (3) N'-[(E)-(5-methoxy-1H-indol-3-yl)methylidene]thiophene-2-carbohydrazide; and (4) N'-[(E)-(5-methoxy-1H-indol-3-yl)methylidene]-4-methyl-1,2,3-thiadiazole-5-carbohydra-zide to be with both antiseizure and antinociceptive activities. Neither was show to impair the performance of mice in the rotarod test.

Further testing is needed to elucidate the mechanism of the potentially beneficial effects of the tested substances and to evaluate the toxicity and efficacy of the proposed molecules.

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PP 57

SUPERFIFTY (SF), A COMMERCIALLY AVAILABLE EXTRACT OF THE SEAWEED *ASCOPHYLLUM NODOSUM*, PROTECTS CROP PLANTS FROM OXIDATIVE STRESS

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Oxidative stress is a consequence of many abiotic stresses, including drought, salinity, extreme temperatures, and pollutants. We show that SuperFifty (SF), a commercially available extract from the seaweed *Ascophyllum nodosum*, can protect *Arabidopsis thaliana*, tomato, and pepper from paraquat (PQ)-induced oxidative stress. SF-treated plants do not show any damage after subsequent PQ application, whereas plants that were not treated with SF developed substantial necrotic lesions after the PQ treatment. Measurements of stress and cell death parameters such as conductivity and trypan blue staining confirmed the protective effect of SF. Gene expression studies further supported this notion, as reactive oxygen species-sensitive genes, used for markers for oxidative stress, were induced only in the damaged PQ treated plants that were not pre-treated with SF. The molecular priming effect of SF was further investigated by GC-MS metabolomics. Metabolites and biochemical pathways regulated in the three species were identified and their possible role in stress protection discussed. The results demonstrate that SF offers an effective and broadly applicable technology for crop protection through molecular priming.

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PP 58

BEHAVIORAL EFFECTS OF CHLOROGENIC ACID IN OVARIECTOMIZED RATS

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Chlorogenic acid (CGA) is abundant in coffee, tea, berries, etc. It decreases locomotor activity, exerts anxiolytic effect and improves learning and memory processes in young/healthy rats [1-3].

This study aimed to investigate the effects of CGA on locomotor activity, anxiety and depressive behavior in ovariectomized rats.

Female Wistar rats were divided into 3 groups: SO (sham-operated), OV (ovariectomized) and OV+CGA. The oral treatment started 15 days after the operation. SO and OV groups received distilled water. OV+CGA group was treated with CGA (20 mg/kg). After 10 weeks of treatment, the animal behaviors were recorded in the open field test (OFT), social interaction test (SIT) and forced swim test (FST).

In the OFT, the horizontal and vertical movements of OV rats were slightly decreased in comparison with SO rats. CGA further decreased them to values that were not significantly different from those of OV rats but were significantly lower ($p<0.05$) than those of SO rats. The social interaction time of OV rats was significantly lower ($p<0.001$ vs. SO group) while this index for OV+CGA group was not significantly different from that of SO rats. In the FST, the immobility time of OV group was significantly increased ($p<0.01$ vs. SO group) while for OV+CGA group it was lower than that of OV group but still significantly higher ($p<0.05$) than that of SO group.

In conclusion, CGA slightly decreased the locomotor activity, antagonized anxiety and did not affect depressive behavior in ovariectomized rats.

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PP 59

FIRST RESULTS OF METABOLITE PROFILE AND ANTIOXIDANT ACTIVITY OF FOUR ENDEMIC *THYMUS* SPECIES OF THE BULGARIAN FLORA

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The paper presents first results of a study on the chemical composition of four rare and endemic species of genus *Thymus*. *T. perinicus* is a Bulgarian endemic growing only on Pirin Mts., *T. comptus* and *T. longedentatus* are endemics to Balkan Peninsula, and *T. atticus* is a sub-endemic, occurring also in Asia Minor. Due to the limited distribution range, the information about metabolite profile and antioxidant activity of the species is scarce and the present study aims at filling in this gap. Standard methods of investigation were applied – GC/MS and TLC approach to describe the chemical composition in acetone exudate and methanol extract, and DPPH method for determining of free radical scavenging activity. The results revealed that the main constituents of the extracts were mono- and di-saccharides, accompanied by organic and fatty acids, alcohols and alkanes. Nine phenolic acids were identified, with quinone acid being the most common one, represented in all studied samples and in the largest quantity. All species demonstrated antioxidant activity comparable to that of the other *Thymus* species.

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METABOLITE PROFILE AND ANTIOXIDANT ACTIVITY OF *THYMUS AZNAVOURII* VELEN., A RECENTLY DISCOVERED NEW SPECIES FOR BULGARIA

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Thymus aznavourii Velen. is a species with high conservation value, due to its limited distribution range and small population size. Until very recently it was known only from Turkey-in-Europe, but was found also in Southeastern Bulgaria in 2018. Metabolite profile and antioxidant activity of the newly discovered species were investigated within a large-scale study on the Bulgarian representatives of the genus. The composition *of* acetone exudate and methanol extract of *T. aznavourii* was analyzed by GC/MS and TLC. Free radical scavenging activity was evaluated by DPPH method. The main components of the chemical composition of the extracts were mono- and di-saccharides. Other constituents identified included organic and fatty acids, alcohols and alkanes. Particularly rich was the composition of free phenolic acids, the most dominating of them being quinone acid. The results revealed that the species demonstrates high level of antioxidant activity.

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EVALUATION OF LAKTERA NATURE® POTENTIAL TO MODULATE THE FUNCTION OF CYP3A4 ISOENZYME

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CYP3A4 is the most common enzyme involved in the metabolism of nearly 50% of the drugs used in the clinical practice. Modulation of its function, inhibition or induction, may result in clinically relevant drug interactions. Thus, the test for activity on the individual cytochrome enzymes involved in drug metabolism, especially CYP3A4, is a part of each drug development. Laktera Nature® contains unique strains of probiotics - *Lactobacillus bulgaricus DWT1*, *Lactobacillus helveticus DWT2*, *Lactobacillus lactis DWT3* and *Streptococcus thermophilus DWT 4, 5, 6, 7, 8,* isolated from spring water in Bulgaria. Many beneficial effects are described of the probiotics, including anti-tumor activity [1]. Based on previous studies demonstrating the impact of the probiotics on CYP1A2 [2], the purpose of the present study is to evaluate the potential of Laktera Nature® to modulate the function of CYP3A4 isoenzyme. The activity of human *CYP3A4* was analyzed using Vivid P450 screening kit. The results have shown that Laktera Nature®, used in concentration range from 10 to 0.625 mg/ml, didn't influence the activity of CYP3A4. Therefore, the usage of Laktera Nature® is not associated with potential risk of drug interactions related to CYP3A4 isoenzyme.

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PP 62

A RESEARCH OF THE SEDATIVE AND ANXIOLYTIC ACTIVITIES OF THE NATURAL MONOTERPENE MYRTENAL IN EXPERIMENTAL ANIMALS

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Introduction: Myrtenal is a bicyclic monoterpene of natural origin, which has a variety of biological effects.

Aim: To investigate the anxiolytic and sedative properties of Myrtenal (M) in experimental animals.

Materials and methods: The modulation of narcotic effect of two barbiturates (hexobarbital - metabolized by hepatic monooxygenases and barbital excreted by kidney unchanged) by M in single dose (30 mg/kg bw, i.p.) on male Wistar rats was observed. The acute effects of M on sedation duration of Diazepam in ICR mice were also studied. It was used Flumazenil for the interruption of sedation. An anxiolytic effect of M was evaluated after a single and repeated treatment (7- and 14-days) of mice using Marble burying test- MBT using Diazepam as a referent. Statistical processing of the results was performed with ANOVA and GraphPad Prism.

Results: M demonstrated a significant impact on sleeping time of barbital and did not affect the hexobarbital sleep duration. Probably this mechanism is central rather than metabolic. The combined application of M and Diazepam produced narcosis (for average 16 minutes), while Diazepam (without M) produced only sedation. The administration of Flumazenil led to a rapid recovery of the animals, which supports our hypothesis for a central mechanism of action of M. The performed MBT test M shown a remarkable short-term anxiolytic effect ($p < 0.01$) after acute treatment, which decreased after 7- and 14-days application.

Conclusions: Myrtenal is a promising pharmacological agent, which has sedative and anxiolytic activities.

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PP 63

NMR-BASED METABOLOMICS FOR DISCRIMINATION OF OAK HONEYDEW HONEY FROM MACEDONIA AND BULGARIA

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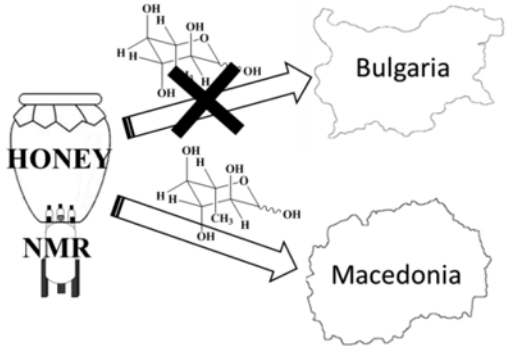
Honey has been used as a nutrient, an ointment and a drug since ancient times. Many studies have shown that honeys possess antioxidant, antibacterial, anti-inflammatory, antimutagenic, anticancer, antiproliferative, immunomodulatory and wound healing effects [1]. The medicinal properties of honey severely depend on its botanical and geographical origin. Honeydew honey has higher level of antibacterial and antioxidant activity compared to nectar honey that is why usually its price is higher [2].



15 samples Macedonian honey (7 oak honeydew honeys and 8 forest honeys) and 15 samples Bulgarian honey (7 oak honeydew honeys and 8 polyfloral honeys) from different geographical regions were analyzed using 1D (^{1}H, ^{13}C, TOCSY) and 2D (HSQC, TOCSY) NMR experiments. Intensities of signals in ^{13}C spectra were used for semi-quantitative determination of 35 components - carbohydrates, butane diol, proline, quercitol and other. Statistical analysis of the proton and carbon data was performed using ANOVA, PLS-DA,

Nightingale`s diagram and box-plots. Chemometrics allows unambiguous discrimination of honeydew honeys from both countries - Macedonia and Bulgaria, based on the differences in concentration of 11 components. 11 of the Macedonian samples contained quinovose – reducing monosaccharide that has not yet been found in any honey.

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PP 64

FIRST STUDY ON THE RELEASE OF A NATURAL ANTIMICROBIAL AGENT, ESTRAGOLE, FROM FREEZE-DRIED DELIVERY SYSTEMS BASED ON CYCLODEXTRINS AND LIPOSOMES

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Estragole is considered as a promising candidate for food preservation due to its antioxidant and antimicrobial properties. However, its sensitivity to light and oxygen, its volatility and hydrophobicity prevent its large application. In order to increase the stability of estragole, conventional liposomes (CL) and drug-in-cyclodextrin-in-liposomes (DCL) were prepared by the ethanol-injection method using Phospholipon 90H in combination with cholesterol and then freeze-dried using hydroxypropyl-ß-cyclodextrin (HP-ß-CD) as a cryoprotectant. Fresh and reconstituted vesicles demonstrated nanometric vesicles size, spherical shape and negative surface charge. Moreover, all suspensions were homogeneous. Compared to CL, DCL improved estragole encapsulation efficiency, and conserved the same loading rate during freeze-drying. Freeze-dried CL and DCL retained estragole more efficiently compared to the reconstituted ones, as evidenced by release study performed by multiple headspace extraction.

Finally, we demonstrated, for the first time, that HP-ß-CD did not affect the membrane fluidity of fresh and reconstituted Phospholipon 90H/cholesterol CL and DCL.

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PRELIMINARY BIOACTIVITY ASSESSMENT OF SEVERAL *SCIRPOIDES HOLOSCHOENUS* (L.) SOJÁK AND *HELICHRYSUM ARENARIUM* (L.) MOENCH EXTRACTS AND DERIVED FOOD SUPPLEMENT FORMULAS

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Several types of extracts and food supplements formulas (capsules developed in laboratory phase) derived from *Scirpoides holoschoenus* (L.) Sojak and *Helichrysum arenarium* (L.) Moench) were tested on *Triticum aestivum* L. (wheat) caryopses in order to obtain preliminary data on the biological action (support of liver function) and cytotoxic potential [1]. In this regard, the wheat caryopses were treated with several dilutions of the extracts and food supplement formulations (based on the dried extracts in different proportions) [2]. Prior to the treatments, the extracts and formulations were phytochemically analyzed in order to evaluate the bioactive compounds content and composition [3]. Caryopses germination ratio, plant growth, biomass accumulation were the main parameters determined within the phytobiological tests. Phytochemical analysis highlighted a higher content in total polyphenols, polyphenolcarboxylic acids and flavonoids in *H. arenarium* fluid extract and in *S. holoschoenus* dried extract excepting the flavonoids. None of the experimental variants showed a negative influence on germination and physiological processes in wheat plants, thus providing some insights into the evaluation of cytotoxicity, important aspect in establishing the administration doses. The lack of cytotoxic effects suggests their safety on administration for their possible use in phytotherapy.

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CONTRIBUTIONS TO THE BIOMETRIC AND PHYTOCHEMICAL STUDY OF SOME *VERBASCUM* SPECIES IN EASTERN ROMANIA

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Verbasci flos is the common name for the flowers of *Verbascum thapsus*, *V. densiflorum* and *V. phlomoides*. *V. thapsus* is used in ethnomedicine for the treatment of inflammatory diseases, asthma, cough [1]. *V. phlomoides* and *V. nigrum* are used in European traditional medicine for their antiinflammatory action in the respiratory tract [2]. Our study aims at assessing the phytochemical features of *V. nigrum*, *V. phlomoides*, *V. thapsus*, *V. densiflorum* species from Eastern Romania. *Verbascum* extracts were phytochemicaly analyzed by means of spectrophomometry, TLC and HPLC, to determine the content and composition compounds of polyphenolic and flavonoid type. In addition, the morphological traits of individual plants were evaluated. TLC chromatograms showed distinct fingerprints for each species. The flavonoid luteolin was identified in all samples, while quercetin was identified only in *V. nigrum*, apigenin in *V. densiflorum*. In *V. phlomoides,* apigenin, luteolin and rutoside were identified. Among polyphenolcarboxilic acids, caffeic and ferulic acids were present in all samples and chlorogenic acid was found only in *V. phlomoides*. Quantitative determinations highlighted a moderate interspecific variation of the phenolic content (e.g. 1.030 – 1.338 g GAE/100 d.w. for *V. nigrum* and 1.245 – 1.387 g GAE/100 d.w. for *V. densiflorum*). The morphologic assessment of individual plants showed high inter- and intraspecific variations.

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GC-MS BASED METABOLOMICS STUDY OF THE RESURRECTION PLANT *RAMONDA SERBICA*

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Ramonda serbica represents the remnant of the Tertiary tropical and subtropical flora in Europe and is the rare resurrection plants of Northern Hemisphere temperate zone. The plant remains well-hydrated during spring, late autumn and in winter. In summer and early autumn when plants are subjected to drought, their desiccation tolerance comes into operation and they spend it in anabiosis [1]. In this study, metabolic responses to dehydration and rehydration of *R. serbica*, were investigated. For this purpose, GC-MS/FID based metabolomics method was performed. Leaves from the control (well-watered), dehydrated and partially rehydrated plants were sampled. Each leaf was powdered using liquid nitrogen and then freeze-dried. The internal standard (10-undecenoic acid) was added to the dry plant material and then extracted in methanol/water using ultra-sonication. After centrifugation of the mixture, the supernatant was dried and derivatized using two-step procedure involving oximation and silylation. GC-MS/FID analysis of each sample was then performed. The metabolites were identified using EI-MS spectra. The areas from FID chromatograms were used for multivariate data analysis. Two OPLS-DA models were applied to investigate dehydration and rehydration process. According to high VIP scores, sugars, such as fructose, glucose, galactinol, sucrose, together with glyceric acid, xylonic acid δ-lactone and aspartic acid were found to be the most influential in the OPLS-DA models.

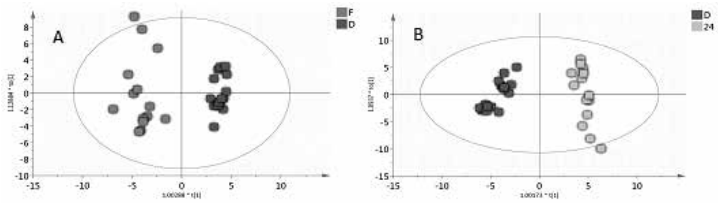

Figure 1. Score plots of the OPLS-DA models of dehydration (A) and rehydration (B) process of *R. serbica*. Each point corresponds to one leaf sample - green F (control plant), blue D (dehydrated plant), and yellow 24 (partially rehydrated plant).

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CALLOGENESIS FROM *CISTUS LADANIFER* SUBSP. *LADANIFER*

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Callus culture is a plant biotechnology technique, which may be used for plant strain maintenance and propagation as well as for secondary metabolite production [1]. *Cistus ladanifer* L. secretes a resin (labdanum), which is rich in compounds such as methylated flavones, labdanum-type diterpenes and volatile mono- and sesquiterpenes. These are associated to several biological activities with potential application in pharmaceutical, agrichemical and food additive industries [2, 3]. Therefore, this species genome has potential for important secondary metabolite production. Two types of explants (internode stem segments and leaves) were collected from *C. ladanifer* subsp. *ladanifer* plants in a natural shrubland in Castelo Branco (PT). The explants were established in MS + sucrose solid medium supplemented with different combinations of growth regulators. Based on response percentage and callus growth index (CGI) assessment after 1-month culture in diffuse light, stem segments with the combination between 2,4-D (0.5 mg/L) and BAP (0.5 mg/L) were found to yield the best results. Although morphologically different, all the *calli* obtained in the first culture were sub-cultivated in three different media varying the ratio of 2,4-D and BAP combination. *Calli* development was morphologically evaluated and CGI was assessed. At the end of 5-week culture, with the best ratio for *callus* growth was found to be 0.5 mg/L 2,4-D and 0.5 mg/L BAP. All *calli* tended to stabilize in greenish friable callus. This exploratory study of *callus* culture of rockrose highlights the advantages of using the combination between 2,4-D and BAP to trigger and enhance the growth of friable *calli* for future studies on secondary metabolites production which are undergoing.

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AN ETHNOBOTANICAL STUDY OF *PTEROSPARTUM TRIDENTATUM* (L.) WILLK IN PORTUGAL BEIRA INTERIOR REGION

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The objective of the present study was to increase the knowledge of this species in center region of Portugal as well as to try to recover and register the traditional knowledge, in the attempt to valorize its use and to encourage the conservation of this biological resource, which could help in the management of the natural patrimony and sustainable rural development.

The methodology used in this study follows the recommendations of some authors [1, 2] and the information was collected through an personal interview with the characterization of the informant, knowledge of the plant and its uses and was carried out in several places in the Beira Interior region, where traditionally prickled broom is still widely used, confirming that its use is still very widespread and that traditional knowledge about this plant has been transmitted from generation in generation. The plant is not cultivated, but the preferential harvest time in natural habitats occurs in the spring and early summer months, which corresponds to the flowering period of the wild plant, since the flower is the most used part. For its different uses, the plant is initially dry in the shade and airy placed, being consumed mainly in the form of tea/tisane. This plant continues to be widely used in this region, mainly as a medicinal plant, used for problems of stomach, diabetes, kidneys and bladder, liver, intestines and rheumatism.

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ANTIPROLIFERATIVE AND ANTICLONOGENIC ACTIVITY OF *COTINUS COGGYGRIA* ON MDA-MB-231 TRIPLE NEGATIVE BREAST CANCER CELLS

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Cotinus coggygria is a highly perspective in a pharmacological aspect medicinal plant that, in the last years, has provoked the scientific community interest in regard to its antimicrobial, immunomodulatory, anti-inflammatory, antioxidant and anticancer potential. Our previous investigations indicated that *C. coggygria* aqueous ethanolic leaves extract possesses significant anticancer activity on MCF7 and T47D human breast cancer cell lines and a minor effect on the proliferation of non-cancerous breast epithelial cell line MCF10A [1]. The both cell lines, MCF7 and T47D, are molecularly classified as Luminal A breast cancer subtype and are characterized as estrogen and progesterone receptor-positive and human epidermal growth factor receptor 2-negative. The aim of the present study was to assess the effect of *C. coggygria* extract on cell growth of hormone-independent triple negative breast cancer cell line MDA-MB-231. The influence on cell proliferation was explored by MTT assay after 72 h treatment period in a range of extract concentrations in parallel with light microscopy examination of cell morphological characteristics and single cell proliferative capacity and capability to form a colony was investigated using colony formation assay after 7 days treatment duration. The obtained results showed that extract exposure inhibited considerable cell growth of MDA-MB-231 cells but in a slighter degree when compared to both hormone-sensitive cell lines MCF7 and T47D. Induction of MDA-MB-231 cell morphology alterations and reduced cell clonogenicity was also observed. Further studies will include in-depth research on the molecular targets of the extract action.

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SOLUBILIZATION OF AROMA AND ESSENTIAL OILS USING DEEP EUTECTIC SOLVENTS

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Essential oils (EO) are widely known for their numerous properties such as antioxidant and antibacterial properties. However, these oily liquids and their constituents present a high volatility, low stability and water solubility that limit their wide applications. Therefore, different encapsulation systems are considered to enhance their solubility, improve their stability and control their release. Deep eutectic solvents (DES) have recently attracted researchers' attention due to their green character and the ease of their preparation. In fact, DES can be prepared by simply combining two or more compounds that are able to interact via hydrogen bonds, at a certain molar ratio.

In this study, the ability of 10 DES to solubilize different EO compounds was tested. The solubilization effect of DES was also determined in the presence of cyclodextrins demonstrated as being an effective encapsulation system for EOs.

The solubilization and the release of the natural compounds in DESs were evaluated by static headspace-gas chromatography and multiple headspace extraction, respectively. All the tested DES presented high retention ability of EOs, as well as a controlled release of *trans*-anethole, used as a model of essential oil components.

Furthermore, the addition of different types of cyclodextrins (CD) to choline chloride : urea DES improved its solubilizing effect towards *trans*-anethole, probably due to the formation of an inclusion complex between CD and *trans*-anethole in the DES. Moreover, novel DESs were synthesized, using CD as one of the solvent's components.

These results show that DESs are promising solvents for the solubilization and controlled release of EO constituents.

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ASSESSMENT OF BIOACTIVE COMPOUNDS CONTENT VARIABILITY OF *ROSA CANINA* L. WILD POPULATIONS IN DOBROGEA REGION, ROMANIA

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The aim of the study is to assess the variability of the bioactive compounds content of some *Rosa canina* L. wild populations in Dobrogea Region, Romania for their prospective cultivation. In this respect, the plant material consisting in ripe fruits, was harvested from 5 wild populations belonging to 3 varieties. Selection of the varieties was based on phenotypic criteria: fruit color, shape and size, presence or absence of thorns on the branches. The different types of fruit extracts were analysed in order to determine the content and composition of antioxidant compounds of the polyphenolic and flavonoid type. Phytochemical analysis was performed by means of spectrophomometry, TLC and HPLC. Main antioxidant compounds of the flavonoid type are represented by the derivatives of apigenin and luteolin, and the polyphenols caffeic acid, chlorogenic, ferulic and p-coumaric acids [1, 2]. Phytochemical analysis of the extracts showed a high quantitative variation of the polyphenolcarboxilic acids and flavonoid content even within the same variety, harvested from the same area. The study on these wild populations has been conducted for a period of 4 years, and the multiannual results obtained will constitute the basis for the selection of individuals with high productivity for the establishment of cultures in degraded areas.

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ANTI-INFLAMMATORY POTENTIAL OF EXTRACTS AND ISOLATED COMPOUNDS FROM *ARTEMISIA COPA* AND *FABIANA DENUDATA* USED IN THE TRADITIONAL MEDICINE OF ATACAMA PEOPLE (CALAMA, CHILE)

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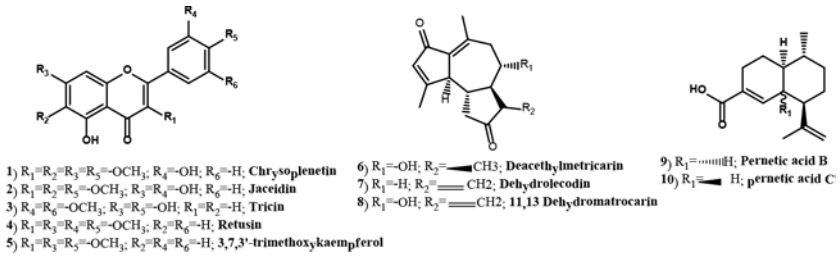
North Andean part of Chile is composed of different areas located between 2400 and 4500 m of altitude, with alternation of arid plains, oasis, mountains and valleys. Colonial and economic mutations have led to co-existence of several ethnic groups with intercultural relationships, mainly characterized by Aymara culture. In previous ethnobotanical surveys in this region [1, 2], treatment of respiratory, gastro-intestinal and urinary disorders as well as pain and inflammation were among the most cited indications. A total of 18 plants were selected regarding their therapeutic uses and extracted with methanol and ethyl acetate.

The anti-inflammatory activity was evaluated for extracts from plants with reports dealing with inflammatory disorders. Inhibition of cytokines (IL-6 and TNFα) production was performed with RAW 264.7 mouse macrophage cell line stimulated with LPS incubated with extracts at 100, 50 and 1 µg/mL. From all tested samples, ethyl acetate extracts from *F. denudata* and *A. copa* showed the strongest inhibition of pro-inflammatory cytokines production (98-70%) at 100 and 50 µg/mL. Moreover, *F. denudata* inhibited the mTNFα production of 70% at 1µg/mL.

Both extracts were subjected to fractionation resulting so far in the purification of polymethoxyflavones (**1**-**3** from *A. copa* and **4**-**5** from *F. denudata*) and sesquiterpene derivatives (**6**-**8** from *A. copa* and **9**-**10** from *F. denudata*). Anti-inflammatory activity and effects of isolated compounds in several inflammatory signalization pathways are on progress.



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CHEMICAL PROFILING OF RARE *VERBASCUM ORIENTALE* AND *VERBASCUM RUPESTRE*

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The *Verbascum* genus (common name mullein; Scrophulariaceae) attracts attention because mullein plants accumulate several groups of bioactive compounds. The aim of our ongoing project is investigation on the healing potential of plants from this genus and their bioactive molecules. In our previous study from methanol extract of the endemic *V. nobile* two novel iridoid glycosides were identified and it was shown that both compounds might have a potential to modulate T cell-related pathologies [1].

In order to establish individual compounds of rare *V. orientale* and *V. rupestre*, dried and powdered aerial parts of both species were extracted with methanol. Obtained extracts were in small amounts and only few secondary metabolites from *V. orientale* were isolated and identified: aucubin, catalpol, luteolin-7-*O*-rutinoside, quercetin-7-*O*-rutinoside and dehydrodi-coniferyl alcohol-9'-β-glucoside. Therefore, NMR metabolic approach was applied to study differentiations of investigated species. Extracts from aerial parts and roots were analysed using several NMR experiments (^{1}H, ^{13}C, 2D TOCSY, HSQC, JRes). Differences between the two species were determined by chemometrics (ANOVA, PLS-DA, Venn diagram).

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CHARACTERIZATION OF PHYTOCHEMICAL COMPONENTS AND ANTI-PROLIFERATIVE ACTIVITIES OF *HAGENIA ABYSSINICA*

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The *Hagenia abyssinica* (Bruce) J. F. Gmel., also known as kosso in East Africa, is an important medicinal plant used to treat various ailments especially intestinal parasites [1, 2]. However, its phytochemical components and their corresponding biological activity have not been well explored. In this context, we strived to conduct both phyto-chemical and bioactivity studies, aiming to reveal its underlying mechanisms of action, especially for its anticancer and antioxidant activity. The barks of *H. abyssinica* were firstly extracted with alcohol, and partitioned with four different organic solvents. Then, five different fractions were tested with MTT, and antioxidant activity, and those fractions with better activity were further separated with various chromatographic techniques, resulting in the isolation of at least 10 compounds, and their subsequent activity tests are still on-going. As a result, the MTT and antioxidant tests showed that N-hexane and ethyl acetate fractions had better anti-proliferative activity with inhibition rates to Hep G2 cells at 82.25%, 72.45%, respectively; and the N-hexane and dichloromethane fractions with better antioxidant activity than the three other fractions using DPPH and ABTS tests. Furthermore, 8 out of 10 compounds isolated were identified from *H. abyssinica* for the first time. This study revealed good anti-proliferative and antioxidant activity of *H. abyssinica*, and the further phytochemical study and activity tests on those isolated compounds will help to unravel its mechanisms of action.

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AGROBACTERIUM-MEDIATED TRANSFORMATION AND OPTIMIZATION OF TRANSGENIC CARROT CELL CULTURE FOR THE PRODUCTION OF RECOMBINANT SWEET-TASTING PROTEIN BRAZZEIN

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Recently the importance of low calorie sweeteners is increasing for persons who care about their health, especially the persons affected by diseases linked to the consumption of sugar [1]. Brazzein (molecular mass of 6.5 kDa) is the smallest naturally occurring sweet-tasting protein [2, 3]. In this study was conducted to optimize the culture conditions of transgenic carrot cell lines as a part of study to establish stable expression system of brazzein protein from carrot cell cultures. The induced carrot cell line was transformed with *Agrobacterium* and transgenic cell suspension culture was induced. Brazzein expression was analyzed every 3 days during the culture period of cell suspension culture. In order to investigate the optimal light condition of Brazzein expression, five different kinds of light quality were examined: white, red, blue, mixed light. By *Agrobacterium*-mediated transformation, three cell lines (TL1, 11, 12) were induced, and among them TL12 showed the highest cell proliferation, so the line was used for the further experiments. Fresh and dry weight of carrot explants were significantly increased in the mixed treatment of CH (0.25 g/L) and BA (0.22 mg/L). After 4 weeks of culture under different light quality, carrot cell line showed the excellent cell biomass in all light condition. Total protein content was the highest at 1.1 mg/g FW in cell cultures grown under blue light. The yield of brazzein protein by stress factor will be analyzed. Based on the results obtained in this study, we intend to establish a stable brazzein protein production system using air-lift bioreactor.

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EFFICIENT CULTURE SYSTEM OF BULBLET AND CALLUS USING AN AIR-LIFT BIOREACTOR AND THE PHENOLIC COMPOUND PRODUCTION IN *LILIUM DAURICUM* KER GAWL, AN ENDANGERED SPECIES

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Lilium dauricum, an endangered species in Korea, contains various bioactive compounds, and it is used as a functional food and medicinal agent in Northeast Asia [1, 2]. The aims of this study were: (1) to establish an in vitro bulblet culture by using an air-lift bioreactor and callus culture for the species conservation and use its bioactive compounds; (2) to investigate their phenolic compounds, and efficacy from both cultures system. The MS medium supplemented with 4.44 µM BA and 3% sucrose, had the highest bulblet production (12.5-fold in growth rate). Addition of 7% sucrose was suitable for bulblet enlargement, with an approximate 2.5-, and 7-fold increase in diameter and FW, respectively. The highest rate of callus (100%) was obtained with a combination of 4.14 µM picloram and 2.32 µM Kinetin. The callus proliferation occurred on MS medium supplemented with 4.52 µM 2,4-D, 1.11 µM BA, 5.37 µM NAA and 0.25 g/L casein hydrolysate. A significant difference was observed in the total phenolic compound content of callus, which was 1.5-fold higher than in bulblet. As a result of the cytotoxicity test on the Hacat human keratinocyte (DK-Hacat) cell line, it was confirmed that the extracts of both bulblet and callus were not cytotoxic. In skin keratinocyte inflammation inhibitory activity, the bulblet extract was found to have the most effective anti-inflammatory effect in a concentration-dependent. These results suggest a suitable system for optimizing bulblet and callus culture of *L. dauricum*, in order to provide a useful bio-material for industrial purposes, and the conservation of this species.

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AGATHISFLAVONE ISOLATED FROM *SCHINUS POLYGAMUS* (CAV.) CABRERA LEAVES PREVENTS SCOPOLAMINE-INDUCED MEMORY IMPAIRMENT AND BRAIN OXIDATIVE STRESS IN ZEBRAFISH (DANIO RERIO)

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Agathisflavone, a biflavonoid isolated from *Schinus polygamus* (Cav.) Cabrera leaves has been reported to promote various biological activities such as anti-inflammatory properties, promoting cognition and preventing cancer, anti-oxidant and antiapoptotic activities. Here, we tested the hypothesis whether anxiety, amnesia, and brain oxidative stress induced by scopolamine could be counteracted in zebrafish model by agathisflavone and tried to ascertain the underlying mechanism. Agathisflavone (1, 3 and 5 µg/l) was administered by immersion to zebrafish once daily for 8 days period. Anxiety and memory impairment was induced with scopolamine (100 µM) and measured with the novel tank diving test (NTT) and the Y-maze test. The identification of the agathisflavone was done by spectroscopy, and the structure of the compound was confirmed by (-) Electrospray Ionisation Mass Spectrometry (ESI-MS). The brain oxidative status and acetylcholinesterase (AChE) activity were also investigated. Agathisflavone from *S. polygamus* (Cav.) Cabrera leaves was identified. Also, we demonstrated that agathisflavone significantly reversed scopolamine-induced behavioral score alteration in the NTT and Y-maze tests. Consequently, agathisflavone promoted inhibition of AChE activity and restored the brain antioxidant status. Our results demonstrate that agathisflavone promotes brain antioxidant action and ameliorates scopolamine-induced anxiety and memory deficits in zebrafish.

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EARLY DETECTION OF TOXIC CYANOBACTERIA BY REAL-TIME PCR IN BULGARIAN DAM WATERS, AND EVALUATION *IN VITRO* EFFECTS OF SAPONINS, ISOLATED FROM *ASTRAGALUS GLYCYPHYLLOS* AND *ASTRAGALUS GLYCYPHYLLOIDES*, ON CYANOTOXIN (ANATOXIN)-INDUCED NEUROTOXICITY

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As a result of blooming, some cyanobacteria (*Microcystis, Anabaena, Planktothrix*, etc.) produce toxins at concentrations that are high enough to poison and even kill animals and humans. According to Annex II of the Framework Directive 2000/60/EC, transformed into the Bulgarian legislation, for the characterization of surface water, at this stage there are no acceptable limit values for cyanotoxins. Methods for the investigation and monitoring of cyanobacteria and their toxins are under development.

The aim of the study is to selection and application of a constellation of molecular-genetic markers for the early detection of toxic microalgae. Samples from eight Bulgarian dams, which are used for drinking purposes, were tested by RealTime PCR. To assess the presence of bacterial DNA, specific primers were selected to demonstrate *Cyanobactria*.

Some of these *Cyanobacteria* produced cyanotoxins, which are neurotoxic. On rat brain microsomes, rat brain synaptosomes and neuroblastoma cell line SH-SY5Y, we investigate the possible neuroprotective effects of saponins, isolated from *Astragalus glycyphyllos* and *A. glycyphylloides,* in a model of cyanotoxin (anatoxin)-induced neurotoxicity.

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OPTIMIZATION OF PHYSIOLOGICAL CONDITIONS FOR PRODUCTION OF BIOLOGICALLY-ACTIVE EXTRACELLULAR POLYSACCHARIDE FROM RED MICROALGA *PORPHYRIDIUM*

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The red algae (Rhodophyta) contain several classes of well-known polysaccharides, which have wide application in microbiology, biotechnology and medicine. The extracellular polysaccharide of the microalga *Porphyridium cruentum,* synthesized and released into the surrounding medium, showed promising properties as an anticancer agent. **The aim:** To increase the production of a desired algal polysaccharide, optimization of the conditions is needed.

Materials and methods: For this study, the unicellular red microalga *Porphyridium cruentum* (Rhodophyta), strain VISCHER 1935/107 was investigated. Algae were grown intensively at 22 °C and light intensity of 132 µmol photons/(m^2s). For our purposes, 4 variants of combinations of nutrients and carbon dioxide were tested. Two types of broth medium were studied (M1; M2). Algal culture constantly supplied either with 2% CO_2 or with atmospheric air (0.04% CO_2). Cells were removed with centrifugation and the supernatant was measured with viscometer B3, DDR.

Results showed that the highest viscosity (n = 6.33 m Pa.sec) was obtained by applying M1 in combination with 2% CO_2. It was 0.25 times greater than those in M1 with 0.04% CO_2 and twice as much compared to the viscosity of M2 with 2% CO_2.

In conclusion, we established that the most suitable conditions for obtaining higher quantities of polysaccharide is medium 1 with 2% CO_2 as the increase in viscosity of the medium is directly related to the amount of extracellular polysaccharide.

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CYTOLOGICAL CHARACTERISTICS OF DIFFERENT ORIGIN DERIVED CALLI AND ADVENTITIOUS ROOTS, AND THEIR PEHNOLIC PROFILING IN *CAMELLIA JAPONICA*

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Camellia japonica is an evergreen tree belonging to the family Theaceae, and enriched in physiologically bioactive compounds [1]. In this study, in order to establish an in vitro culture system [callus and adventitious root (AR)] for producing useful phenolic compounds, were compared by investigating biomass and phenolic compounds from 4 different origin-derived calli and ARs in *C. japonica*. The effect of light quality on the accumulation of phenolic compounds was also investigated. Callus and AR were induced from leaf, root, petal, and ovary tissue. Among the 4 calli and 4 AR lines, the highest biomass was achieved in ovary-derived callus (CO), and in leaf-derived adventitious root (AdL). Between the two culture systems, callus culture was more suitable for producing phenolic compounds when considering the culture period and biomass, while the phenolic content per gram DW was higher in the adventitious root cultures. There was no significant difference in callus growth of red light (R) and red and blue mixuture (RB) light compared to that of the control (dark). The highest content of total phenolics and flavonoids was obtained in the callus cultured under RB light, and the DPPH radical scavenging activity was also higher in this treatment than others. The results of HPLC analysis also showed the highest content of phenolics in callus cultured under RB treatment. The results obtained in this study could be used for the large-scale production of biomass and phenolic compounds in *C. japonica* for industrial use.

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ENCAPSULATION OF ANTHOCYANIN-RICH EXTRACTS BY FREEZE DRYING USING DIFFERENT WALL MATERIALS

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The objective of this study was to investigate characteristics of anthocyanin-rich extracts freeze dried with different wall materials: maltodextrin, gum Arabic and skim milk powder. The anthocyanin-rich extracts were obtained from grapeskin and black soybean coat as winery and soybean processing byproducts, respectively.

The morphology of encapsulates, their chemical and physical properties as well as anthocyanin release profiles in different pH conditions (1.2, 6.8, 7.4 and water) were analyzed. Antioxidant capacity, total anthocyanin content and total phenolic content were determined by spectrophotometric methods while individual anthocyanins were determined by HPLC method. The colour of the samples was measured before and after 6-month storage.

Freeze drying of the extracts along with these three wall materials resulted in formation of porous and irregularly shaped encapsulates with low water activity (0.19-0.26), high yields (85-93%), and solubility from 65 to 92%. All encapsulates exhibited significant antioxidant capacity, good colour stability after storage period (ΔE = 0.8-5.2) and antimicrobial activity against six pathogen bacteria. High content of total anthocyanins (1.7-3.9 mg/g) were achieved, with malvidin-3-glucoside and cyanidin-3-glucoside as predominant in grapeskin and soybean coat extracts. The release profiles indicated prolonged release of anthocyanins in different media, especially from gum Arabic-based encapsulates.

These results have shown that freeze dried encapsulates of grapeskin and soybean coat as byproducts of agri-food processing could be used as a source of natural colourants and bioactive compounds with prolonged release and improved stability.

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BIOSYNTHESIS AND CONTENT OF *NICOTIANA* GENUS ALKALOIDS

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It has been more than 25 years since two comprehensive panels of alkaloid composition in 60 *Nicotiana* species were performed [1, 2]. Even then, comparison of species-specific results was complicated by the use of different growing conditions and potentially different cultivars. To obtain consistent metabolomic/transcriptomic results, we grew 60 species of the *Nicotiana* genus and performed a combined liquid chromatography–mass spectrometry metabolomics analysis associated with comprehensive gene expression sequencing (RNAseq) in leaf and root tissues.

The major purpose of this project is to understand the variability of alkaloid biosynthetic routes in *Nicotiana* species. Additionally, global transcriptomic analysis may help to improve the accuracy of the *Nicotiana* genus classification by refining genetic distance information.

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PP 84

OPTIMIZATION OF MICROWAVE ASSISTED EXTRACTION OF ARONIA BERRIES BY ARTIFICIAL NEURAL NETWORK

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The aim of this study was to simulate and optimize microwave-assisted extraction of aronia berries using an artificial neural network (ANN) coupled with genetic algorithms. The ANN architecture had an input layer consisted of three neurons (ethanol concentration, microwave power, and extraction time), eight neurons as the hidden layer, and three neurons (total extraction yield-TEY, a total content of polyphenolic-TPC and flavonoid-TFC compounds) as the output layer. The 70% of the experimental data were used for training, 15% for testing and the rest of the data (15%) were used for network validating. Total content of polyphenolic and flavonoid compounds in obtained extracts were determined by routine spectrophotometric methods [1].

The ANN-GA model provides a set of optimal conditions for each of the responses. For all responses, optimal value of the extraction time was about 15 min, the concentration of ethanol was in the range of 51-55%, while the optimum value of the microwave power differentiated the most for individual cases of responses (600, 571 and 441 W for the optimization of TPC, TFC and TEY, respectively). The consistency of experimental and predicted values were experimentally verified and confirmed by high R^2 (>0.929) and low mean relative percent deviation (lower than ±4.027%) values.

Thus, ANN methodology deserves great attention as the effective tool for the simulation and optimization of microwave assisted extraction of aronia berries.

Acknowledgements: The Ministry of Education, Science and Technological Development of the Republic of Serbia supported this study through the project OI 172047.

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HIBISCUS SYRIACUS ANTHOCYANIN-RICH EXTRACT INHIBITS MELANOGENESIS BY ACTIVATING THE ERK SIGNAL PATHWAY: MOLECULAR DOCKING OF ANTHOCYANINS WITH ERK, ADENYLNYL CYCLASE, AND PKA

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Hibiscus syriacus is a garden shrub and grows in a wide climatic range from mild to tropical temperature. The bark and root of *H. syriacus* have been used to cure diarrhea and bacterial infection, and its seed are effective to fever and cold. Recently, native flowers of *H. syriacus* possess promising potential as a new edible source and colorants with various anthocyanins. However, the function of anthocyanins isolated from *H. syriacus* has been poorly determined. In the current study, we, for the first time, evaluated whether anthocyanin-rich extract from *H. syriacus* Pulsae and Paetanshim flower (PS and PTS, respectively) inhibits melanogenesis *in vitro* and *in vivo*. Our results showed that PS and PTS did not directly downregulate mushroom tyrosinase activity *in vitro*; but significantly decreased the extracellular and intracellular melanin content in B16F10 cells compare with untreated control accompanied by the inhibition of α-MSH-induced MITF and tyrosinase expression. We also found that PS and PTS attenuated pigmentation of zebrafish larve under both normal and α-MSH-stimulation with no change of heart rate. Furthermore, PS and PTS activated the phosphorylation of extracellular signal-regulated kinase (ERK) and a specific ERK inhibitor, PD98059, resulted in the recovery of intracellular and extracellular melanin downregulated by PT and PTS in B16F10 cells and of melanogenesis in zebrafish larva. PT and PTS possess similar pattern of 17 specific anthocyanins, which also target adenylyl cyclase and protein kinase A. These findings suggest that anthocyanins from PS and PTS inhibit melanogenesis by activating the ERK signal pathway.

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ENHANCEMENT OF GINSENOSIDE BIOSYNTHESIS IN *PANAX GINSENG* ADVENTITIOUS ROOT CULTURE BY FEEDING OF *CENTELLA ASIATICA* EXTRACT

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Panax ginseng C.A. Meyer (ginseng) is a well-known medicinal plant that has been traditionally used for centuries [1-3]. *Centella asiatica* shares upstream components of the ginsenoside biosynthesis pathway with *P. ginseng*, as this species also produce triterpenoid. Therefore, the present study was conducted to investigate the effects of *C. asiatica* extract feeding in ginseng adventitious root cultures for increase of ginsenoside production. The cultures were fed with 0 - 1,000 mg/L of *C. asiatica* extract. After one week of treatment, dry matter was highest (14.97%) with *C. asiatica* extract feeding at 1,000 mg/L. *β*-Glucosidase activity increased as feeding increased from 0 to 100 mg/L. The content of the major ginsenosides was highest with the 100 mg/L treatment (1.61 mg/g DW), whereas that of the minor ginsenosides was higher by about 64% with the 100 mg/L treatment (0.33 mg/g DW) than in the control. Ginsenoside content and productivity were highest with the 10 and 100 mg/L treatments. Protopanaxatriol (PPT)-type ginsenoside content was significantly lower with the 1 000 mg/L treatment than in the control, but no significant difference was observed between content with the 0 and 100 mg/L treatments. Protopanaxadiol (PPD)-type ginsenoside content was highest with the 100 mg/L treatment (0.96 mg/g DW). The results of this study confirmed that *C. asiatica* extract feeding was effective in improving ginsenoside content in ginseng adventitious root cultures.

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CAMPTOTHECIN POSITIVELY REGULATES G_2/M PHASE ARREST THROUGH ROS GENERATION: IMPLICATIONS OF ER STRESS-MEDIATED AUTOPHAGY

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In the present study, we report that camptothecin (CPT) caused irreversible cell cycle arrest at the G_2/M phase, and was associated with decreased levels of cell division cycle 25C (Cdc25C) and increased levels of cyclin B1, p21, and phospho-H3. Interestingly, the reactive oxygen species (ROS) inhibitor, glutathione, decreased CPT-induced G_2/M phase arrest and moderately induced S phase arrest, indicating that the ROS is required for the regulation of CPT-induced G_2/M phase arrest. Furthermore, transient knockdown of nuclear factor-erythroid 2-related factor 2 (Nrf2), in the presence of CPT, increased the ROS' level and further shifted the cell cycle from early S phase to the G_2/M phase, indicating that Nrf2 delayed the S phase in response to CPT. We also found that CPT-induced G_2/M phase arrest increased, along with the ataxia telangiectasia-mutated (ATM)-checkpoint kinase 2 (Chk2)-Cdc25C axis. Additionally, the proteasome inhibitor, MG132, restored the decrease in Cdc25C levels in response to CPT, and significantly downregulated CPT-induced G_2/M phase arrest, suggesting that CPT enhances G_2/M phase arrest through proteasome-mediated Cdc25C degradation. Our data also indicated that inhibition of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) inhibited CPT-induced p21 and cyclin B1 levels; however, inhibition of ERK blocked CPT-induced G_2/M phase arrest, and inhibition of JNK enhanced apoptosis in response to CPT. Finally, we found that CPT-induced G_2/M phase arrest circumvented apoptosis by activating autophagy through ATM activation. These findings suggest that CPT-induced G_2/M phase arrest through the ROS-ATM-Chk2-Cdc25C axis is accompanied by the activation of autophagy.

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ISOLATION OF HIGHER ADDED VALUE COMPONENTS FROM MARAL ROOTS (*RHAPONTICUM CARTHAMOIDES* L.) BY SUPERCRITICAL CARBON DIOXIDE AND ULTRASOUND-ASSISTED EXTRACTIONS

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Maral root (*Rhaponticum carthamoides* L*)* is well-known medicinal plant in folk medicine, possessing a broad spectrum of beneficial pharmacological effects on brain, blood, cardiovascular and nervous systems. In addition, various malar root-based preparations and extracts exert antioxidant, immunomodulatory, anticancerogenic, antimicrobial, antiparasitic and insect antifeedant activities, indicating its potential as an effective adaptogenic herbal remedy [1]. Innovative extraction techniques have been suggested as sustainable, green alternatives to conventional techniques for effective isolation of higher-added value bioactive ingredients from various plant materials [2, 3]. This study investigates multistep biorefining of maral roots into valuable fractions consecutively applying supercritical carbon dioxide (SFE-CO_2) and ultrasound-assisted (EAE) extractions. The obtained results showed that SFE-CO_2 (pressure 46.2 MPa, temperature 55 °C, time 420 min) yielded 0.74 g of lipophilic constituents from 100 g of maral roots. UAE was optimized for the effective isolation of ethanol and water-soluble fraction from the residue after SFE-CO_2 by response surface methodology (RSM) based on central composite design (CCD). Under different experimental conditions (time 5-30 min, amplitude 20-100%), 2.1-5.5 g/100 g and 14.9-19.1 g/100 g of polar constituents were recovered with ethanol and water, respectively. Phenolic acid and flavonoid containing ethanol and water extracts demonstrated strong *in vitro* antioxidant capacity and inhibiting properties towards the physiologically important enzymes The combined SFE-CO_2 and UAE significantly reduced antioxidant capacity of starting plant material, showing that suggested biorefining process is efficient to remove the considerable portion of the antioxidatively active constituents from maral roots.

Acknowledgements: This study was supported by JSC "Endobiotech" (Kaunas, Lithuania).

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CHEMICAL COMPOSITION OF SUPERCRITICAL CO_2 EXTRACTS FROM JUNIPER BERRY

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Juniperus communis L. is a coniferous shrub distributed in Europe, South Asia, and North America. In view of an increasing use of its extracts and fractions in medicinal, pharmaceutical, and perfumery products, the application of efficient and environmentally friendly technologies for the plant processing is of crusial importance.

The aim of this work was to obtain extracts from Juniper berries by supercritical CO_2 (SCF) extraction, and to compare their chemical composition with the one of extract obtained by steam distilation. SFC extraction under various conditions (pressure and temperature) was conducted, resulting in separation of two fractions - liquid and semiliquid.

The main constituents of the liquid fraction (2nd separator 40 °C, 50 bar) were mono- and sesquiterpenes: *α*-pinene (33.3%), germacrene-D (13.4%), myrcene (8,6%) and *β*-caryophillene (4.2%), while the semiliquid one (1st separator 25 °C, 100 bar) was characterised by presence of diterpenes such as sandaracopimaric acid (44.3%), pimaric acid (18.6%), communic acid (8.0 %) and imbricatolic acid (3.8%). Similar chemical composition was found for the liquid fraction from SCF extraction and the steam distilled oil.

Acknowledgements: The work was supported from the Foundation Information and Nature Conservation through a project "Municipal Model for Medicinal and Aromatic Plants Conservation and Sustainable Use (www.herbvaluebg.org)" funded by TFCSP of the Bulgarian-Swiss Cooperation Programme, and by the Project BG161PO003-1.2.04-0007-C0001 "Upgrading of the IOCCP equipment for utilization of medicinal and aromatic plants through green technologies.", OP "Development of the Competitiveness of the Bulgarian Economy", was gratefully acknowledged.

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COMPARATIVE STUDY OF FRANKINCENSE ESSENTIAL OIL EXTRACTED BY HYDRO-DISTILLATION (HD) AND MICROWAVE-ASSISTED HYDRO-DISTILLATION (MAHD) EXTRACTION METHODS AND ITS DEVELOPMENT IN SOLID LIPID NANO-FORMULATION FOR TOPICAL APPLICATION

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Frankincense is an oleogum resin obtained from *Boswellia* trees family Burseraceae [1]. It is used as a traditional medicine in cosmetics, in the treatment of inflammation, wound healing, skin diseases, urinary tract infections, and gynaecological disorders, as an immunostimulant and for the treatment of respiratory infections [2]. It contains essential oil 5-9%, 65-85% resin, and 21-22% gum [3]. The oil of the frankincense gum resin is one of the most important commercial essential oils available on the international market [2]. So in this study the extraction of frankincense essential oil was done using two different methods (Hydro-distillation and Microwave-Assisted Hydro-distillation), analyzed by GC-MS and we found that the major component in both methods was n-octyl acetate which was 78% in HD and 86.97% in MAHD. To improve its bioavailability and topical efficacy, Frankincense oil was loaded into solid lipid nanoparticles (SLNs). SLNs were prepared via emulsification followed by ultrasonication technique using compritol 888 ATO as a solid lipid and tween 80 as a surfactant. The anti-aging activity of the essential oil and the nanoformulation (SLNs) were investigated using antioxidant assays, the determination of rabbit collagen type 1 alpha 2 (COL1A2) in skin homogenate was done by enzyme-linked immune-sorbent quantitative assay, *in vitro* determination of anti-elastase and anti-collagenase activities were achieved as well as histopathological examination of the skin tissue using Hematoxylin and eosin (H&E) staining and Masson's Trichrome Staining. The results indicated that Nanoparticles formulation was significantly than the essential oil.

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EFFECTS OF PURIFIED EXTRACT OF *AMORPHA FRUTICOSA* L. SEED AGAINST TYPE 2 DIABETES IN SPONTANEOUSLY HYPERTENSIVE RATS

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Metabolic syndrome is defined as a condition characterized by a variety of diagnostic criteria, most important of which are obesity, dyslipidaemia, type 2 diabetes and arterial hypertension. All of them contribute to an elevated risk of cardiovascular morbidity and mortality.

The potential of *Amorpha fruticosa* L. (Fabaceae) against diabetes and metabolic disease is promising based on the *in vitro* tests. This deserves further investigation. Additionally, the toxicity review in relation to safety revealed that until now there are no published data about human toxicity of *A. fruticosa*. Due to the fact that *A. fruticosa* is an aggressive invasive plant species growing almost unrestrictedly, it can provide abundant and cheap resources. The objective of this study was to evaluate the acute toxicity of a purified extract of *A. fruticosa* and its effects in experimental model of streptozotocin-induced type 2 diabetes in male spontaneously hypertensive rats (SHR).

Additional benefit to the human health through environmental management based on vast use of *A. fruticosa* for medicinal purposes might contribute to resolve problems associated with this aggressive invasive species in the natural habitats in many European countries.

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IN VITRO ANTIPROLIFERATIVE ACTIVITY OF PURIFIED SAPONINS' MIXTURE FROM *ASTRAGALUS GLYCYPHYLLOS* ON GRAFFI TUMOUR CELLS

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Astragalus glycyphyllos L. (Fabaceae) has been extensively used in Bulgarian folk medicine as an antihypertensive, diuretic, anti-inflammatory, anti-tumour, etc. [1]. The species contains predominatly cycloartane-type saponins, which exhibit various pharmacological action, incl. cytotoxicity [2, 3].

The aim was to evaluate the possible anti-tumour/anti-proliferative activity of purified saponins' mixture, from *A. glycyphyllos*, on *in vitro* cell culture from Graffi tumour.

Viability and proliferative activity of the Graffi myeloid tumour cells was assessed by MTT test. The morphological alterations were visualized and analysed by fluorescent microscopy after double staining with acridine orange/ethidium bromide, 4´,6-diamidino-2-phenylindole and Annexin V- fluorescein isothiocyanate and propidium iodide.

Graffi tumour cells were sensitive to purified saponins' mixture after 24 and 48 h treatment. A decrease of the viability/proliferation of the Graffi tumour cells was induced. The effects observed were concentration- and time-dependent. Fluorescent microscopy investigation showed that these antiproliferative/anti-tumour effects were connected to the induction of apoptosis.

The results indicate that purified saponins' mixture induced significant antineoplastic effects *in vitro*.

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PHARMACOLOGICAL ACTIVITY OF PHYTOMEDICINES TAKEN FROM *PULMONARIA RUBRA* AND TWO VARIETIES OF *PULMONARIA MOLLIS*

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The plants of *Pulmonaria* genus having medicinal properties are used in folk medicine from for ages. Earlier we showed [1] that the extracts taken from the generative shoots of *P. mollis* Wulfen ex Hornem. *P. obscura* Dumort and *P. officinalis* L. have an evident antianemic activity. Investigated plants are the most widespread species of *Pulmonaria* genus. At the same time indicated above plants have very close morphological structure of floral shoots. Both this and close chemical compositions can explain a similar pharmacological action of their phytoremedies.

The three plants have the most discriminating floral shoots - *P. rubra* Schott, *P. mollis* Wulfen ex Hornem and *P. mollis* Wulfen ex Hornem var. *albiflora* which have red, violet or blue and white flowers in maturity correspondingly.

The aim of work was a comparative research of antianemic activity of phytomedicines made on basis floral shoots of *P. rubra*, *P. mollis* and *P. mollis* var. *albiflora*.

The floral shoots collected in ending of flowering were used as the research objects. Investigation of pharmacological activity was done with using rats with iron-dificience anemia [2].

It was established that the extracts from all of floral shoots possess near antianemic action, which is equal to the action of a modern synthetic medicine on a basis of Fe^{3+}-hydroxide polymaltose complex. The cure for experimental rats with iron-deficiency anemia (initial level of hemoglobin was less than 65 g/l) by this extract has led to the restoration of the hemoglobin level for 8-10 days, but rats without treatment have the restoration of the hemoglobin level occurring by the 26th day).

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FLAVONOID COMPOUNDS OF SOME SPECIES OF *FILIPENDULA* GENUS

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The plants of *Filipendula* genus are well-known as the sources of essential oils, which are used in folk and scientific medicine. At the same time the considered plants are sources of various phenolic compounds which also affect pharmacological effect of the phytoremedies taken from these plants. The purpose of the real work was comparative research of flavonoid structure of *Filipendula* genus plants.

The species of the meadowsweets (*F. denudate* Juz., *F. ulmaria* Maxim. and *F. camtschatica* Maxim.) which are characteristic of the Eurasia growth area (from the Baltic Sea to the Pacific Ocean) were chosen as objects of the research. Determination of content of flavonoids was carried out by the TLC and HPLC methods [1].

As a result, there was an establishment the maintenance two aglycones - quercetin and kempferol and their derivates, which are represented as their 3-*O*- and 4´-*O*-glycosides. The absence of an ulmaroside (quercetin-4´-*O*-rutinoside which first was detected in [1]) in chemical composition of *F. camtschatica* should be noticed.

Further the taken extraction was subjected to acid hydrolysis. After hydrolysis ethanol liquor $AlCl_3$ was added to obtained solution. Formed chromogene complex has a characteristic maximum of absorption of 428 nanometers that there corresponds to a complex quercetin-$AlCl_3$. Quantitative determination of content of flavonoids was carried out on measured optical density of investigated solutions with using the known extinction coefficient. The content of flavonoids in *F. camtschatica* - 0.015% (calculated as quercetin) was esteblished what is less than their maintenance in *F. denudata* and *F. ulmaria* contain 1.2 and 1.4%, respectively.

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PP 95

MOLECULAR MECHANISMS LINKING ENVIRONMENTAL TOXICANTS TO CANCER DEVELOPMENT: SIGNIFICANCE FOR PROTECTIVE INTERVENTIONS WITH POLYPHENOLS

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Human exposure to environmental toxicants with diverse mechanisms of action is increasingly concerning. In addition to well-recognized carcinogens, different chemicals in environmental and occupational settings have been suggested to impact health, adding to cancer susceptibility by genetic and epigenetic changes. Epidemiological evidences for this association have been difficult to consolidate, but many experimental studies show that compounds, such as polycyclic aromatic hydrocarbons, pesticides and heavy metals, trigger cellular and molecular alterations that can ease cancer initiation, promotion and progression. Herein, recent insights into the pathological mechanisms of these chemicals, namely effects on cell redox and calcium homeostasis, mitochondria, microRNAs expression and inflammatory signaling, are discussed with a focus on the possible implications for multi-stage carcinogenesis and its reversal by polyphenols.

Plant-derived polyphenols, such as curcumin, resveratrol, epigallocatechin-gallate, quercetin, and others, reduce cancer incidence, and can be useful nutraceuticals for alleviating detrimental outcomes of environmental toxicants and pollutants. Polyphenols can block malignant transformation and control cancer progression through direct action on tumor-intrinsic factors and interplay with whole-body effects. However, development of polyphenol therapies requires more studies to validate the biological efficacy of the phytochemical, identify effective doses and mode of action. Merging current understanding of multifactorial actions of specific polyphenols and chief environmental toxicants in this work aims to potentiate the delivery of useful phytochemical-based protective treatments to high-risk individuals due to environmental exposure.

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SCREENING FOR ANTI-INFLAMMATORY ACTIVITY IN SELECTED SPECIES FROM FAMILY BLECHNACEAE AND POLYPODIACEAE

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The aim of present study was selection of potential sources of anti-inflammatory agents among fern species. In our previous study, we tested 54 species form 11 Pteridophyte families for antioxidant capacity. We found a potential bioactive extracts in some Blechnaceae and Polypodiaceae species. The most significant antioxidant capacity was measured in *Blechnum appendiculatum*. The IC_{50} value (7.07 µg/mL) in DPPH assay was comparable with standard antioxidant Trolox with IC_{50} value 7.01 µg/mL. In ORAC assay, we found strong antioxidant capacity in *Polypodium vulgare* (1.013 g of Trolox equivalent/g dry weight).

Further, we tested inhibitory activity toward pro-inflammatory enzymes (cyclooxygenase 1 and 2) using cell free system with an enzyme immunoassay (EIA) screening ELISA kit. Our results revealed a potential source of selective cyclooxygenase-1 (COX-1) inhibitor in both *Blechnum* species. Selective COX-1 inhibitors were found effective in prevention of cardiovascular diseases [1].

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PHARMACOLOGICAL ACTIVITY OF SECONDARY METABOLITES ISOLATED FROM *XANTHOPARMELIA CONSPERSA* (EHRH. EX ACH.) HALE

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The genus *Xanthoparmelia* (family Parmeliaceae) produces about 92 secondary metabolites, which are made by the mycobiont and accumulate in the lichen cortex or in medullary layer on the outer surfaces of the hyphae. In this work, the phytochemical investigation using LC-ESI-QTOF-MS and GC-MS techniques was done for chemical characterization of extracts of *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale. In the course of our studies on medicinal plants, we evaluated the extracts of *X. conspersa* for their biological activities as antimicrobial, antimalarial, anti-leishmanial and antitrypanosomal agents and we investigated their cytotoxicity effects on the viability of human fibroblasts cell line (MTT assay).

LC-ESI-QTOF-MS and GC-MS analyses confirmed the presence of usnic acid, sticitc and norstictic acids and new secondary metabolite, atraric acid which is the first report from *X. conspersa*. Secondary metabolites in lichens have several biological activities, including antioxidant, antipyretic, antiproliferative, antitumor, anti-viral (e.g., HIV) or antibacterial. In the present study, the anti-leishmanial activity of extract of *X. conspersa* against *Leishmania donovani* with an IC_{50} of 2.64 ug/mL (amastigoty) and 6.17 ug/mL (amastigoty + THP1) and antitrypanosomal activity with an IC_{50} of 7.18 µg/mL were reported. The ability of the extract to induce the cytotoxic effect in fibroblasts was observed in dose-dependent manner. The cytotoxic effect of the extracts in the cells was observed at concentrations significantly exceeding concentrations toxic for *Leishmania* (over 100 µg/mL).

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CYTOTOXICITY AND BIOLOGICAL ACTIVITIES OF *PULSATILLA* SAPONIN D ISOLATED FROM *PULSATILLA SPECIES*

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Pulsatilla species contain triterpenes and saponins, mainly the oleanane and lupane-type. Toxic ranunculin, anemonin and protoanemonin characteristic for Rannunculaceae family are also present. Because of production of cell poison exerting mutagenic activity, *Pulsatilla* species are of toxicological importance. In this work the phytochemical investigation using LC-ESI-QTOF-MS technique was done for chemical characterization of extracts of rare polish plant species: *Pulsatilla patens* subsp. *patens* and the *Pulsatilla vulgaris* subsp. *vulgaris*. In the next step, extracts and the isolated *Pulsatilla* saponin D were investigated for their cytotoxicity against human fibroblasts cell line (MTT assay). In the course of the studies on medicinal plants, the extracts of *P. patens* and the *P. vulgaris* were additionally evaluated for their biological activities as antimicrobial, antimalarial, anti-leishmanial and antitrypanosomal agents. LC-ESI-QTOF-MS analysis of the crude methanolic extracts of the roots of *P. patens* and *P. vulgaris* confirmed the presence of *Pulsatilla* saponin D (hederagenin 3-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-glucopyra-nosyl(1→4)]-α-L-arabinopyranoside). The anti-fungal activity against *Candida glabrata* was noticed for the extract of *P. patens and P. vulgaris* with an IC_{50} value of 9.37 µg/mL and 11 µg/mL, respectively. The ability of the *Pulsatilla* saponin D to induce the cytotoxic effect was evaluated applying MTT test in human dermal fibroblasts culture. Concentrations of the compound exceeding 1 µg/mL induced the significant cytotoxicity in the cell culture.

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ELLAGIC ACID ALLEVIATES PROGRESSION OF PARKINSON'S DISEASE IN RATS WITH 6-HYDROXYDOPAMINE MODEL

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Parkinson's disease (PD) is a progressive neurodegenerative disease related to selective loss of dopaminergic neurons in substantia nigra pars compacta resulting in multiple symptoms including motor and cognitive disturbances. One of the underlying mechanisms for neuronal death is oxidative damage. Ellagic acid (EA) was found to be a potentially strong antioxidant in different kinds of oxidative stress conditions. Preventive effects on cognition in experimental models of Alzheimer's type dementia have also been reported. The aim of this study was to assess the preventive effects of EA in rats with 6-OHDA experimental models of PD. Male Wistar rats were divided into the following groups: i) sham-operated (SO); ii) striatal 6-OHDA (10 µg/2 µl)-lesioned control group; iii) 6-OHDA-lesioned rats pretreated for 5 days with EA (50 mg/kg, i.p.). On the 2nd and 3th week post lesion animals were subjected to behavioral tests (apomorphine-induced rotations, rotarod**,** passive avoidance test), neurochemical evaluation (DA brain levels) and evaluation of oxidative stress indexes (lipid peroxidation, total glutathione (tGSH), antioxidant enzymes) in brain homogenates. The results showed that in 6-OHDA group apomorphine-induced rotations and number of falls were increased, latency time in passive avoidance test and DA levels were decreased, lipid peroxidation levels were increased and tGSH decreased as compared to SO group. In conclusion EA treatment significantly decreased apomorphine-induced rotations, improved cognitive functions, motor performance and oxidative damages induced by 6-OHDA by decreasing the tGSH level and restoring the catalase activities. This clearly demonstrated neuroprotective effect of EA may be due to its antioxidant capacity.

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ANTIOXIDANT AND ANTIMIROBIAL ACTIVITY OF THE WINE MADE FROM THE GRAPE VARIETY 'PROKUPAC' WITH ADDITION OF AROMATIC HERBS

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Aim of this study is to analyse influence of addition of different aromatic herbs on the antioxidant and antimicrobial activity of the wine, made from a grape variety of Prokupec using standard technological procedures. The alcoholic fermentation was carried out at 25 °C with the addition of *Saccharomyces cerevisiae* Lalvin V1116 and lasted 6 days. Anise seeds (*Pimpinella anisum* L.), cinnamon bark (*Cinnamomum zeylanicum*), wormwood leaf (*Artemisia absinthium*) and licorice root (*Glycyrrhiza glabra*) were added to the grape pomace at the beginning of fermentation, in the quantity of 1% (w/w). The analysis of wine samples was performed after 6 months of storage. Antioxidant activity was determined using DPPH assays. Antimicrobial activity was determined using disk-diffusion and microdilution method. The highest antioxidant activity was noticed in 'Prokupac' with cinnamon (EC_{50} = 0.005±0.0005 mL/mL), while lowest antioxidant activity was found in 'Prokupac' without aromatic herbs (EC_{50} = 0.022±0.0016 mg/ml). Antimicrobial assays confirmed that „Prokupac"wine with aromatic herbs has antimicrobial effect against several bacteria: *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Enterococcus faecalis*, *Sarcina lutea*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, with minimal inhibitory concentration in the range 15-500 µL/mL. It can be concluded that addition of aromatic herbs has a positive effect on antioxidant and antimicrobial activity of wine.

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ANTI-DIABETIC POLYACETYLENES FROM THE TISSUE CULTURES OF *CENTELLA ASIATICA*

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Centella asiatica (L.) Urban, which has many common names including gotu kola, hydrocotyle, marsh penny and white rot, has been widely cultivated as a vegetable or spice in China, Southeast Asia, Sri Lanka and India. Previous reports showed that the main constituents are triterpenoid including asiatic acid, asiaticoside, madecassic acid and madecassoside. Their biological activities include anti-inflammatory, antioxidant, cytotoxic, antimicrobial, neuroprotective and other activities have been widely claimed in many reports [1]. Due to its beneficial effects, sufficient production is required for the development as products. Plant tissue culture technology is suggested as a powerful tool for obtaining natural substances. It provides not only improved biomass and bioactive metabolites accumulation but also new secondary metabolites for better pharmacological activity [2]. In this study, tissue cultures of *C. asiatica* were prepared and the constituents were compared with those of *C. asiatica* herbs. HPLC and TLC analysis showed the characteristic major compounds in cultured *C. asiatica*. The purification of methanol extract of cultured roots of *C. asiatica* using chromatographic techniques yielded 8-acetoxy-1,9-pentadecadiene-4,6-diyn-3-ol (1) and araliadiol (2), as determined by various spectroscopic methods including 1D-NMR (^{1}H and ^{13}C) and 2D-NMR (HSQC and HMBC). These compounds increased glucose uptake in L6 skeletal muscle cells. Therefore, tissue cultures of *C. asiatica* can be useful sources for bioactive polyacetylenic compounds.

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SYNTHESIS AND BIOACTIVITY EVALUATION OF ARYLNAPHTHALENE LIGNANS

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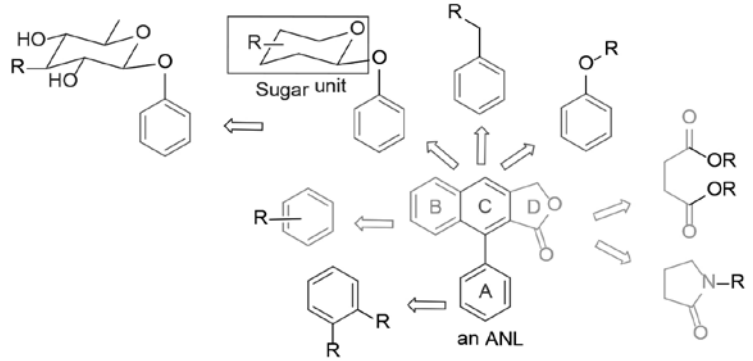
A recent study has demonstrated the nano-molar range anti-HIV potential of the arylnaphthalene lignans (ANLs) isolated from a *Justicia* medicinal plant [1, 2]. To understand the structure-activity relationship (SAR), we have carried out the total synthesis of ANL analogues, which was designed based on the modification of the four ANL rings (rings A-D), followed by attachment of different sugar units (Scheme 1). More than 80 novel ANL analogues have been synthesized and their antiviral activities against HIV, avian flu and Ebola have been evaluated using a "One-Stone-Two-Birds" evaluation system [1]. Cytotoxicity of these compounds was determined using human cancer cell lines. A number of the synthesized ANL demonstrated potent antiviral activities.



Scheme 1. Structural modification of arylnaphthalene lignans (ANLs). Rings A-D have been constructed based on total synthesis.

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PANCREATIC LIPASE INHIBITORS

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The dysregulated lipid homeostasis due to various genetic, environmental and lifestyle factors has been considered a critical putative pathways mediating obesity [1]. The scientific advancements unleashing the molecular dynamics of lipid metabolism, utilization and transport have provided deeper insights on the emerging roles of lipid hydrolyzing enzymes, including pancreatic lipase (PL) [2]. The PL is secreted from pancreatic acinar cells, a critical lipase responsible for the digestion of dietary triglycerides (TG) in the small intestine. We hypothesized that inhibiting PL would prevent the breakdown of TG and prevent the absorption of fatty acids into the systemic circulation and adipocytes.

Our study examined the PL inhibitory activity of selected medicinal plants. The plants *A. galanga* and *C. grandis* has been reported for PL inhibitory activity where used as a control. The PL enzyme inhibition showed potent activity against *M. uniflorum* and *C. paradisi* when compared with the reported plants, where *C. tetragonoloba* showed lesser inhibitory activity, all these plants where compared with standard orlistat which showed the most potent PL inhibitory activity.

The bioassay-guided fractionation and elucidation of isolated phytocompounds indicated that certain plant flavonoids from the plants, which have been previously reported to elicit anti-obesity effect have shown inhibitory effects on PL. Of note, the inhibitory effect of flavonoids on PL depends on the number and position of phenolic hydroxyl groups and size of the molecule [3]. Whilst, our findings suggest the potential roles of PL inhibitors in the development of novel therapeutic strategies to combat obesity, the precise mechanisms by which plant-derived PL inhibitors combat obesity remain to be fully characterized.

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NETWORK PHARMACOLOGY BASED APPROACH TO UNDERSTAND THE MECHANISM OF *LAGENARIA SICERARIA*: A CORRELATIVE STUDY FOR THE TREATMENT OF OBESITY

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Herbal medicines are becoming more mainstream in clinical practice and show value in treating and preventing diseases. However, due to its extreme complexity both in chemical components and mechanisms of action, deeper understanding of herbal drugs is difficult. Thus, a comprehensive systems pharmacological approach which could identify the active ingredients and their targets in the crude drugs and more importantly, to understand the mechanism of action for the pharmacological properties. In this present study, a novel system pharmacology approaches that integrates the oral bioavailability screening, drug-likeness prediction, target and pathway identification and network analysis has been established to investigate the herbal medicines. *Lagenaria siceraria* was also experimentally subjected for pancreatic lipase assay and *in-vivo* hyperlipidemic model, both were compared for a correlative approach. The comprehensive systems approach effectively identified 18 bioactive components and 66 potential targets. These 66 targets were closely associated with aseries of diseases of Alzheimer's disease, Obesity and Diabetes mellitus. These targets were mapped to drug-target and drug-target-pathway-disease networks to find out the target proteins and to elucidate the mechanism. In the experimental part, the aqueous extract showed potent inhibition on lipase, which showed less potent compared with orlistat and it also showed significant ($P < 0.001$) increase in serum lipid profiles. This work provides a novel *in silico* strategy for the investigation of herbal drugs, which has been demonstrated and well-studied lagenaria. This attempt should be helpful for

understanding the definite mechanisms of action for herbal medicines and discovery of new drugs from medicinal plants.

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CARDIOVASCULAR ACTIVITY AND PHYTOCHEMICAL PROFILE OF *VIBURNUM OPULUS*, *CORNUS MAS* AND *SORBUS AUCUPARIA* FRUIT EXTRACTS

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Viburnum opulus, *Cornus mas* and *Sorbus aucuparia* are not only known for the nutritional value of their edible fruits, but also for their traditional use in cardiovascular diseases [1-3]. The aim of this study was to evaluate the phenolic profile and evaluate the capacity of the acetonic extracts to increase NO production and induce aortic rings' vasorelaxation.

Arginase inhibitory activity was spectrophotometrically assessed. The vascular reactivity was performed on thoracic aorta rings from Sprague-Dawley rats; the mechanisms involved in vasorelaxation were studied, including eNOS pathway, K^+ and Ca^{2+} channels involvement. Phytochemical constituents were identified by HPLC-DAD-ESI-Q-TOF-MS/MS in negative and positive ion mode; the separation was achieved with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) following a gradient elution.

The highest inhibition of arginase was exhibited by *V. opulus* extract (IC_{50} = 91.18 µg/mL), while the other extracts showed a weaker activity. The most potent vasodilatory effect was attributed to *V. opulus* extract (EC_{50} = 5.58 µg/mL), followed by *S. aucuparia* (EC_{50} = 72.07 µg/mL) and *C. mas* (EC_{50} = 100.27 µg/mL) extracts. *V. opulus* extract exerted the vasorelaxant effect on phenylephrine-precontracted aortic rings through the stimulation of eNOS, inhibition of extracellular Ca^{2+} influx and activation of voltage-dependent K^+ channels. Flavonoids and iridoids were the most important classes of compounds identified in our extracts. Further studies are needed to clarify the compounds responsible for the biological activity.

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BIOAVAILABILITY OF PHENYLETHANOID GLYCOSIDES: A SYSTEMATIC REVIEW

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Phenylethanoid glycosides (PhGs) are naturally occurring water-soluble compounds widely distributed in traditional Chinese medicines and other medicinal plants. These compounds are structurally characterized with a hydroxyphenylethyl moiety to which a glucopyranose is linked through a glycosidic bond. To date over 400 PhGs compounds have been isolated and identified from medicinal plants. Pharmacological studies have shown that PhGs possess a wide range of biological activities such as antibacterial, antiviral, antitumor, anti-inflammatory, antioxidant, neuro-protective, immunomodulatory, hepatoprotective, tyrosinase inhibitory actions, etc. Recently, interest in PhGs has been growing, with a significantly increasing volume of articles describing PhGs novel structures, diverse bioactivities, as well as their pharmacokinetics having been published. Despite of the promising biological effects, PhGs failed to fulfill its therapeutic potential due to poor intestinal absorption, fast metabolism and poor bioavailability. In order to improve the efficacy of PhGs, attempts to understand their metabolic process and ways to improve their bioavailability are being studied [1, 2]. This article aims to review some concepts regarding PhGs' bioavailability. It summarizes the latest advances on the stability, ingestion, absorption, bioavailability, and biotransformation of PhGs through different approaches. Attention is also given to effects of interaction between PhGs and macromolecules on the bioavailability and bioactivity of PhGs. Recent advances of methods such as chemical modification and nanotechnology to improve the bioavailability of PhGs are also summarized. We also outline the future perspectives for their potential application.

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EVOLUTION OF THE PLANT ANTIOXIDANT NETWORK AND REGULATION OF SECONDARY METABOLITE PRODUCTION BY REACTIVE OXYGEN SPECIES

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Reactive oxygen species (ROS) are versatile signals that regulate plant growth, development, and responses to environmental stimuli. Plants have the most elaborate antioxidant network among all kingdoms, enabling them to fine-tune the levels of ROS according to cellular needs. This has allowed plants to co-opt ROS as regulators of diverse plant secondary metabolites, including molecules with useful commercial and medicinal properties.

This review summarizes the current knowledge about the ROS network, its evolution in the plant Kingdom, and the role of ROS in regulating plant-specific pathways. Focus is given to the regulation of secondary metabolite production by ROS, with emphasis on secondary metabolites with valuable pharmacological applications in humans and other species.

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DYNAMICS OF DOPAMINE CONTENT DURING THE CALLUSOGENESIS OF *CELOSIA CRISTATA* LEAVES

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Dopamine is a catecholamine, an animal neurotransmitter which had been found also in some plant species. The callus culture of *Celosia cristata* contains high amounts of dopamine [1], and this amount is significantly higher than those in the leaves. This is not quite common for cell cultures because the ability of biosynthesis of many secondary metabolites decrease during the process of cell de-differentiation. So it is interesting to investigate an influence of callusogenesis on dopamine biosynthesis.

The leaves of *in vitro* plants were used; they have been cultivated on callusogenic medium during 49 days. A number (n = 15-20) of the explants were taken from the medium at regular intervals, air-dried and used for HPLC-based determination of dopamine content.

It has been shown that during the cultivation the average amount of dopamine increases during the first two weeks (an average in 5.5 times); moreover, a sharp increase of the substance amount for the first three days was observed, following by some decrease at the end of the first week. This level kept relatively stable for 2-4th weeks of cultivation and was constantly declining for the next two weeks, decreasing to 49th day to an initial level.

This data demonstrates that the content of dopamine is an easily variable value, it quickly responds on external influences. The sharp initial increase in dopamine content occurs, probably, as a response to stress caused by mechanical damage and/or components of cultivation medium. A callusogenesis itself, as a process of de-differentiation of cells, does not lead to significant changes of dopamine content.

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PROTECTIVE FUNCTION OF DOPAMINE IN PLANTS: TWO FACTS IN FAVOR

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Plants of different families can produce the animal neurotransmitter dopamine. Sites of its biosynthesis and accumulation, role and distribution in plants remain poorly understood. Our investigations concern a determination of the presence/amount of this catecholamine in plants of different families (mainly betalain-synthesizing) and studying the influence of various external factors on its biosynthesis. One of such factors is elicitor methyl jasmonate, which is known as one of the main modulators of the expression of plant secondary metabolism genes. Airborne methyl jasmonate is used by plants as a starting signal for defence system of themselves and neighbouring plants.

During these investigations, two facts have been observed that corroborate the protective role of dopamine for plant organisms:

1. The study of the dopamine content in peel and pulp of red beet showed that the peel contains dopamine in significantly higher amounts.

2. It was shown that gaseous methyl jasmonate causes the significant (from 20% to 2-3 times) increase of dopamine content in aboveground part of all five investigated plant species of three families: *Talinum paniculatum* (Portulacaceae), *Calandrinia eremaea* (Montiaceae), *Beta vulgaris* (Amaranthaceae), *Celosia cristata* (Amaranthaceae), *Cistanthe grandiflora* (Portulacaceae).

Acknowledgements: We are grateful for Leipzig Botanical Garden (Leipzig, Germany) for providing the initial seeds *Cistanthe grandiflora*; Potsdam Botanical Garden (Potsdam, Germany) – for seeds of *Calandrinia eremaea*; Germplasm bank of world flora of Institute of Cell Biology and Genetic Engineering of NAS of Ukraine (Kyiv, Ukraine) - for *in vitro* plants of *Talinum paniculatum* and *Celosia cristata*.

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COMPARATIVE DETERMINATION OF ESSENTIAL OIL COMPOSITION IN THE BALKAN ENDEMIC PLANT *STACHYS THRACICA* DAVIDOV DURING THE PROCESS OF *EX SITU* CONSERVATION

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Stachys thracica Davidov. (Thracian woundwort) is a Balkan endemic species included in the Red Data Book of Bulgaria with the national conservation status: "rare" (R). The plants from genus *Stachys* have long history of use in ethnomedicine under the form of extracts, decoctions, ointments for treatment of genital tumors, sclerosis of the spleen, inflammatory diseases, cough, ulcers and infected wounds. In the present work we aimed to access effective protocol for *ex situ* conservation of the Thracian woundwort and comparative determination of the essential oil composition in *in situ*, *in vitro* cultivated and *ex vitro* adapted plants. Successful micropropagation was achieved on basal MS medium with 30 g/L sucrose and 7 g/L agar. *Ex vitro* adaptation was accomplished in growth camera with 83% survival. GC-MS analysis of the essential oils resulted in the identification of 41, 37 and 38 compounds in *in situ* grown, *in vitro* cultivated and *ex vitro* adapted plants respectively, constituting 80.1%, 85.2% and 86% of the total oils. Germacrene D, β-elemene, α-cadinol, α-limonene and (Z)-β-ocimene were the principal components representing 65.3%, 71.1% and 70.3% of the oil of *in situ*, *in vitro* cultivated and *ex vitro* adapted plants respectively. All three groups of plants seem to store predominantly sesquiterpenes at the expense of monoterpenes. Different growth conditions did not affect significantly the composition of essential oils. A collection from *in vitro* tissue and *ex vitro* cultures, which is an approach for preservation of *S. thracica* has been established.

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A VALIDATED HPLC-PDA METHOD FOR QUANTIFICATION OF BIOACTIVE CONSTITUENTS IN BLACKTHORN FLOWERS AND DRY EXTRACTS PREPARED THEREOF

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Aims: Blackthorn (*Prunus spinosa* L.) flower is a polyphenol-rich herbal medicine valued as vasoprotective, anti-inflammatory, diuretic, detoxifying, and spasmolytic agent. The qualitative LC-MS studies proved huge complexity of the phenolic matrix (59 components, including flavonoids, A-type proanthocyanidins and phenolic acids) [1]. As detailed standardisation is required to assure the therapeutic effectiveness and safety of plant medicines, the aim of the present study was to develop, optimise and validate the first HPLC method for determination of individual blackthorn constituents in the flower preparations.

Methods: The analyses were performed on Prominence-i LC-2030C chromatograph (Shimadzu) and a C18 Ascentis Express fused-core column (2.7 µm, 150 mm × 4.6 mm; Supelco). As close structural similarity of the blackthorn polyphenols present a significant challenge in terms of HPLC separation, multiple parameters were optimised for method development with the use of a standard mixture of 31 model analytes.

Results and Conclusions: The separation was accomplished with an acetonitrile-tetrahydrofuran gradient at a flow rate of 1.09 mL/min and column temperature of 28 °C. The validation showed good precision, accuracy, linearity, and sensitivity of the measurements. The method allowed to effectively separate matrix peaks within 35 min, and it was successfully employed for determination of 31 dominant constituents in dry extracts and plant materials purchased from European manufacturers. The relevant variability in quantitative composition of commercial samples was observed, which suggests the need to introduce quality control studies for the flowers, and the developed method was proved suitable for this purpose.

Acknowledgements: National Science Centre, Poland, grant No 2017/27/N/NZ7/02074.

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PP 112

INFLUENCE OF *HIPPOCASTANI CORTEX* ON BLOOD HEMOSTASIS AND ITS PROTECTIVE EFFECTS ON PLASMA CONSTITUENTS UNDER OXIDATIVE STRESS *IN VITRO*

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Aims: Chestnut bark (*Hippocastani cortex,* HC) is a traditional herbal medicine applied in conditions connected with vascular damage and defective blood clotting, such as cutaneous capillary fragility, venous insufficiency, and hemorrhoids [1]. Not much is, however, known about its potential mechanisms of action. The antioxidant activity of HC, which is listed among the mechanisms of its vasoprotective effects, has been studied so far only in a limited number of models [2, 3] and there is hardly any information on the impact of HC on blood hemostasis. Thus, the aim of this *in vitro* study was to evaluate the protective effects of HC and its main constituents on human plasma components (including fibrinogen) under oxidative stress conditions and to determine their influence on some of the hemostasis parameters.

Methods: The methanolic extract, prepared from a commercial HC sample, was characterized by LC-MS/MS and LC-PDA. The biological tests were performed in blood samples and fractions obtained from healthy volunteers.

Results and Conclusions: All investigated analytes efficiently attenuated the negative impact of peroxynitrite-induced oxidative stress in human plasma: increased the non-enzymatic antioxidant status of plasma, reduced the protein nitration, thiol oxidation and lipid peroxidation. Moreover, some statistically significant effects on hemostasis parameters were demonstrated, including inhibition of ADP-mediated platelet aggregation, decrease of activated partial thromboplastin time (APTT) and prothrombin time (PT) as well as increase of thrombin time (TT). A moderate anticoagulant activity of the analytes was suggested, however more detailed studies are still required.

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THE EFFECT OF *RHODIOLA ROSEA* EXTRACT ON HIGHLY METASTATIC BREAST CANCER

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Breast cancer is one of the most common types of cancer worldwide and major cause of mortality. Approximately 20% of breast cancer express epidermal growth factor receptor and are classified as triplenegative breast cancer (TNBC). MDA-MB-231 is a highly aggressive, invasive and poorly differentiated epithelial TNBC cell line as it lacks estrogen receptor (ER), progesterone receptor and human epidermal growth factor receptor 2 amplification [1].

Rhodiola rosea is widely distributed in high altitude areas, predominantly in Northeast Asia and Eastern Europe. *R. rosea* extracts, as well, as its constituents have undergone numerous studies underlying their anticancer and immune stimulating effect [2].

The aim of this study was to investigate the cytotoxic effect of 50% methanolic extract of *R. rosea* rhizomes on TNBC MDA-MB-231 and normal ER positive MCF-10A cell line as a control. The phytochemical profile of the investigated extract has been analyzed through NMR-based metabolomics.

The thiazolyl blue tetrazolium bromide (MTT) assay revealed that the cell viability of MDA-MB-231 has decreased to 67% (12.5 µg/mL) and 48% (200 µg/mL). The IC_{50} value was calculated to be 161 µg/mL. The cell viability of MCF-10A cell line was slightly decreased at the highest concentration of the extract and varied from 81 to 100% in the range of 200 to 12.5 µg/mL.

The obtained results revealed the potential of the extract as a source of bioactive molecules with eventual cytotoxic activity on TNBC. Further mechanistic studies of the observed effect should be performed.

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ANTIPROLIFERATIVE AND APOPTOTIC ACTIVITY OF *RHODIOLA ROSEA* L. AGAINST U87 MG CELL LINE

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Gliomas are the most common and lethal primary brain tumours, with glioblastoma (GBM) being the most invasive and malignant form. The GBM is a complex disease with very poor prognosis arising from changes in multiple biological networks and hence require complex therapeutic approaches [1]. For that reason many investigations have been focused on multi-target botanical agents such as *R. rosea*, considering them as the nature's answer and simultaneous impact over multiple functional networks during disease treatment with fewer side effects over the human body [2].

This study aimed to identify and quantify the major secondary metabolites in *R. rosea* wild-grown in Bulgaria by NMR-based metabolomics and HPLC. The anti-proliferative and apoptotic activity of 70% methanolic extract of *R. rosea* rhizomes, as well as, its major molecules were evaluated towards human glioblastoma cell line U87 MG.

The NMR spectra revealed salidroside, p-tyrosol, rosarin, rosavin and rosin as dominant molecules in the extract, which estimated amounts by HPLC were 2.67, 0.02, 0.37, 1.92 and 0.04%, respectively. For evaluation of the cytotoxic activity (MTT assay), the extract and the pure compounds were applied at concentrations in the range from 25 to 1 000 µg/mL. The estimated IC_{50} values for the extract and salidroside were 616 and 960 µg/mL, respectively. The analysis of the cell apoptosis showed that incubation with the extract and the pure molecules influenced in a non-dose dependent manner this process.

Further a combined formulation of the identified molecules in a ratio corresponding to that in the rhizomes could be developed and evaluate its effect on U87 MG glioblastoma cell line.

Acknowledgements: This study has been supported by a grant from National Science Fund of Bulgaria (contract number DM11/3)

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NATURAL COMPOUNDS TARGETING CALCIUM SIGNALLING IN CANCER

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Calcium signalling is one of the major regulated processes in cell physiology and the role of calcium control mechanisms in cancer pathogenesis is gaining increasing attention. Recent studies connect changes in cell calcium homeostasis to different carcinogenesis steps, angiogenesis and metastasis. Therefore, calcium signalling regulation is an emerging therapeutic target in cancer [1].

Altered expression or function of calcium channels, namely ORAI isoforms and STIM1 participating at Store-Operated Calcium Entry (SOCE), were closely associated to cancer cell phenotype and disease outcome [2], and the downstream calcium signalling AMPK and AKT pathways were linked to autophagy and metabolic remodelling. In this work, we have used different text and data mining tools aiming to unveil natural compounds able to target cell calcium signalling related to cancer hallmarks. The analysis of the available data point to epigallocatechin-3-gallate, resveratrol, di-indolyl methane derivative, proscillaridin A and stemphol, as candidate calcium-targeting natural occurring drugs. Modes of action more supported are by decreasing calcium entry via SOCE, promoting Endoplasmic Reticulum stress, acting on calcium-induced signalling pathways and calcium dysregulation-induced cell death. Nevertheless, identification of the direct molecular targets and evaluation of the *in vivo* efficacies for these strategies requires more research.

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DEVELOPMENT AND ACCUMULATION OF BİOACTİVE METABOLİTES IN MEDICINAL PLANTS *IN VITRO* CULTURES UNDER LED ILLUMINATION

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Cell, tissue, and organ cultures of medicinal plants proved to be essential for the discovery of biosynthetic pathways and their regulation, providing means for influencing pharmacologically relevant natural products. In the present study, we used *in vitro* cultures of several medicinal plants (*Agastache rugosa*, *Salvia yangii*, *Moluccella laevis*, *Chelidonium majus*, *Stevia rebaudiana*) to test the influence of different illumination regimes and media composition on morphogenesis and bioactive metabolite contents.

Tissue and organ cultures were initiated from explants from various organs of aseptically germinated seedlings. Callus tissue was induced on media supplemented with a combination of auxins and cytokinins. Microshoot cultures were obtained from excised apices. The cultures were maintained in different illumination regimes - white fluorescent and LED of various spectra. Phytochemical screening was performed using spectrophotometry and HPLC.

The morphogenic response of explants differed between white and photosynthetically active radiation (PAR) light. Callus induction percent, number and length of axillary shoots, rooting and biomass increase were determined. Conditions of culture, including illumination spectrum, influenced content of rosmarinic acid and isoquinoline alkaloids significantly. However, response to each of the tested factor differed between species. Precursor (amino acids) feeding influenced developmental processes but did not increase level of the respective metabolites. Surprisingly, feeding resulted in marked decrease of alkaloid production *in vitro* in *Chelidonium*.

The spectrum of light illuminating plant *in vitro* cultures can influence morphogenic processes and accumulation of specialized metabolites that are important for pharmacological properties. However, each species reacts differently and to improve accumulation of desired substances, the conditions of culture must be established experimentally.

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BIOLOGICALLY ACTIVE COMPOUNDS ACCUMULATION IN "HAIRY" ROOT CULTURES OF MEDICINAL PLANTS

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It is known that "hairy" root cultures obtained using *Agrobacterium rhisogenes*-mediated transformation can synthesize biologically active compounds (BAC) native for the mother plants. We used our collection of "hairy" roots of *Artemisia annua*, *A. vulgaris*, *A. dracunculus*, *A. tilesii*, *Bidens pilosa*, *Cichorium intybus*, and *Althaea officinalis* to study the accumulation of flavonoids, artemisinin, sugars, antioxidant and reducing activity, and the capacity of the extracts to synthesize the silver nanoparticles (AgNps).

Transgenic roots were cultivated on the Murashige and Skoog medium, collected in 20 – 30 days and lyophilized. HPLC chromatography method was used to study the BAC accumulation. Antioxidant activity was evaluated by scavenging of DPPH radical. AgNps were obtained by the addition of ethanolic extracts to 1 mM $AgNO_3$ solution and studied by the TEM and UV-Vis spectrophotometric analysis.

Flavonoids, artemisinin, sugars content as well as antioxidant and reducing activity were greater in some root lines compared to the control. The extracts differed in their capacity to synthesize AgNps, and this parameter correlated with the flavonoids content and reducing activity of the extracts.

So, "hairy" root cultures of studied medicinal plants can produce complex of biologically active compounds and can be also used for the synthesis of the silver nanoparticles.

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PROOXIDATIVE PROPERTIES OF THE EXTRACT FROM *ARTEMISIA TILESII* LEDEB "HAIRY" ROOTS

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Extracts from "hairy" roots of unexplored plants *Artemisia tilesii* is a new potential source of active pharmaceutical ingredients (AFI).

The aim of the work was to determine the influence of the extract on the formation of free radicals in the model chemical system. Extracts demonstrated the activity can be used as an AFI of drugs with potential antibacterial action.

Plant biomass was obtained by the method [1] from the dried material by 70% ethanol extraction. The effect of the dried extract on free radical processes was investigated in the model reaction of autooxification of adrenaline by the modified method [2]. This express method of analysis differs in accuracy, repeatability and reproducibility. The dry extract contained flavonoid-rich compounds of 33.6% in routine equivalent (RE).

The evaluation of the effect of the extract on the process of autooxification of adrenaline by the value of the first-order rate constant k_1 indicates that the extract acts as an agonist of free radical processes. The extract in the 2 - 8 mkM RE accelerated the autooxification of adrenaline in 2.3 - 3.6 times. The value of the rate constant of the first order of formation of free radicals was $k_1 = 26.30 \pm 0.80$ 1/s and $k_1 = 93.80 \pm 1.26$ 1/s for the concentrations of 0 and 8 mkM RE flavonoids in the system.

The ethanol-water extract from *A. tilesii* "hairy" roots reveals its prooxidative properties under conditions of the model reaction of autooxification of adrenaline. This effect requires further research.

Acknowledgements: The authors express their gratitude to Iryna Pashchenko and Oleksandr Danilenko for their help in extract preparation.

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EVIDENCES FOR THE INTERACTION BETWEEN *CITRUS BERGAMIA* FLAVANONES AND THE AMPK/SIRT-1 AXIS: A KEYSTONE OF SEVERAL DISEASES

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A previous report [1] indicated that the flavonoid-rich extract of bergamot juice (BJe) exerts an anti-inflammatory effect through the activation of SIRT1 in leukemic monocytes THP-1 exposed to lipopolysaccharide (LPS). In this study we deeply investigate the mode of action of BJe, along with its major flavonoids on SIRT1 through *cell-free, in silico* and *in vitro* experimental models. In the *cell-free* assay, all the tested compounds as well as the whole BJe inhibited the deacetylase activity of SIRT1. This finding was reinforced by the results of *in silico* study. In THP-1 cells exposed to LPS, a reduction of SIRT1 activity was observed, effect that was reverted by the pre-incubation with either BJe or its major flavonoids. This effect was also observed at gene level. Employing an activator and an inhibitor of AMPK (AICAR and dorsomorphin, respectively), we discovered its involvement in the activation of SIRT1 elicited by BJe or its major flavonoids in whole cell.

Our study indicates the dual role of BJe and its components, depending on the employed experimental model, as well as reveals their mode of action on the AMPK/SIRT1 axis, suggesting their role as promising candidates in pathologies in which this axis is implied.

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PHENOLIC CONSTITUENTS OF *GAULTHERIA PROCUMBENS* LEAVES MODULATE THE PRO-INFLAMMATORY AND PRO-OXIDANT RESPONSE OF HUMAN NEUTROPHILS

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Aims: *Gaultheria procumbens* L. (Ericaceae) is a traditional medicinal plant, the leaves of which are recommended in the treatment of inflammation-related disorders [1]. Their main components are salicylates, but also other polyphenols: flavonoids, proanthocyanidins and caffeoylquinic acids [2, 3]. However, the impact of non-salicylate phenolics on the anti-inflammatory activity of the leaves has not been investigated so far. Therefore the aim of the present work was to compare the anti-inflammatory and antioxidant effects of the dominant leaf constituents in a model of human neutrophils.

Methods: Chlorogenic acid (CHA), cinnamtanin B1 (CB1), gaultherin (GT), and miquelianin (MQ), four main leaf components, were isolated from hydromethanolic extract by column chromatography, preparative HPLC, and flash chromatography. Their structures were confirmed by LC-ESI-MS/MS, IR, CD, and NMR studies. Next, the isolates were investigated for their effects on the viability and pro-inflammatory functions of neutrophils obtained *ex vivo* and stimulated by LPS and *f*MLP. In particular, their impact on the ROS level and the release of IL-1*β*, IL-8, TNF-*α*, elastase, and MMP-9 was evaluated.

Results and Conclusions: The analytes did not influence the cells viability. CHA was the most efficient modulator of the production of ROS, IL-1*β* and TNF-*α*; GT of the MMP-9 release; MQ of the IL-8 secretion; and CB1 of the elastase release. All analytes might be considered significant contributors to the anti-inflammatory activity of the leaves. They might be also recommended as standardization markers suitable for quality control of the plant material and extracts thereof.

Acknowledgements: National Science Centre, Poland (Grant Project: 2015/19/N/NZ7/00959).

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ASSESSMENT OF THE ANTIOXIDANT, CYTOTOXIC AND ANTIMIGRATORY EFFECTS OF *OLEA EUROPAEA* L. EXTRACTS ON MCF7 HUMAN BREAST CANCER CELL LINE

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Olea europaeae L. is a species belonging to the *Oleaceae* family reported to possess various beneficial effects, including anti-inflammatory, antihypertensive, antioxidant, antitumor and hypoglycemic properties [1-3]. The present study was aimed to evaluate two types of *O. europaea* L. extracts concerning their antioxidant capacity and cytotoxic and antiproliferative effects on MCF7 human breast adenocarcinoma cell line. The ethanolic extracts were obtained from the leaves of the species. For the first extract (O.f. 1) the leaves were harvested from Spain, and for the second extract (O.f. 2), from Greece. DPPH assay was used for the determination of the antioxidant capacity. In this regard two concentrrations were tested, namely 100 and 1000 µg/mL. For the evaluation of the cytotoxic effects Alamar blue technique was performed and Scratch assay for the evaluation of the antimigratory potential. Two concentrations (50 and 100 µg/mL) of each extract were tested and incubated for 24 h. Both extracts displayed a high antioxidant activity, especially at the highest dose tested. At both concentrations used, O.f. 2 extract showed a higher antioxidant capacity as compared to O.f. 1 extract. In terms of antitumor activity, the results obtained indicate that both extracts elicited a dose dependent cytotoxic effect on MCF7 breast adenocarcinoma cells viability, Furthermore, the data showed that by increasing the concentration, tumor cell migration was reduced, the most notable effect being recorded in the case of O.f. 2 extract. The ethanolic extracts of O.f. possess a significant antioxidant potential, reduced breast adenocarcinoma cells viability and migration, with the most significant effects being recorded in the case of O.f. 2 extract.

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PHYTOCHEMICAL ANALYSIS AND ASSESSMENT OF ANTIPROLIFERATIVE POTENTIAL AGAINST MCF-7 HUMAN BREAST CANCER CELL LINE OF THE HYDROALCOHOLIC EXTRACTS OBTAINED FROM *PETROSELINUM CRISPUM* (MILL.) FUSS GROWTH IN THE WESTERN PART OF ROMANIA

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Petroselinum crispum (Mill.) Fuss. presents nutraceutical properties being used both in the culinary as well as in the medical field for its carminative, stomachic, antioxidant, spasmolytic, hepatoprotective, diuretic, antiseptic with tropism for the urinary tract, anti-inflammatory, antibacterial and antifungal properties. The most studied part of the plant because of the complex chemical composition are the seeds [1, 2]. The aim of the study was the phytochemical characterization of *P. crispum* (Mill.) Fuss. seeds extract (PC) as well as the evaluation of the anticancer potential against MCF-7 human breast cancer cell line. Results have shown the presence of polyphenolic compounds (µg/100 µg extract): chlorogenic acid (4.572), caffeic acid (2.213), apigenin glucoside (22.371), cinaroside (0.776), luteolin (4.078), apigenin (14.562), and kaempferol (2.662). Spectrophotometric quantification has shown an amount of 172.2 µg / mL extract total flavones and 292.7 µg GAE/mL extract total phenols. PC in the concentration of 60 µg/ml induced a statistically significant inhibition of proliferation of MCF7 human breast cancer cell line compared to control, as revealed by consecrated MTT assay as well as cytotoxic events as revealed by LDH assay. Migratory ability of cancer cells was also diminished fallowed incubation with PC and a G0/G1 cell cycle accumulation could be observed.

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IN VITRO EVALUATION OF CYTOTOXIC AND ANTIPROLIFERATIVE ACTIVITIES OF *VERBASCUM NIGRUM* L. ON A431 SQUAMOUS CARCINOMA CELL LINE

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Non-melanoma skin cancer (squamous and basal cell carcinoma) has the highest incidence rate amongst cancers, due to overexposure to UV radiations as a consequence of the stratospheric ozone layer depletion and climate changes. Current available treatments (surgery, cryotherapy, radiotherapy, chemotherapy and photodynamic therapy) are invasive and cause side effects; recurrence might also occur [1]. Therefore, less invasive therapeutic agents are needed. Plant extracts are able to modulate multiple mechanisms that trigger a disease with fewer side effects. In this respect, *Verbascum* species were reported to possess multiple medicinal properties due to a complex phytochemical composition [2]. In the present study, anticancer properties of *V. nigrum L.* were tested on A431 squamous cancer cell line. The methanol extract of the aerial parts and the methanol fraction, isolated by the fractionation of delipidated methanol extract on C18, significantly reduced A431 cell viability in MTT assay. Further, annexin V – propidium iodide staining showed that the cytotoxic effects of the raw extract and methanol fraction were based on induction of apoptosis. In addition, fluorescence microscopy revealed caspase 3/7 activation as a mechanism of apoptosis induction. No cytotoxic effects were detected on HaCaT keratinocytes. A moderate but selective antiproliferative effect on A431 cells was revealed for the methanol fraction. These findings suggest that *V. nigrum* methanol fraction could be a potential therapeutic agent for squamous cell skin cancer.

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RECENT ADVANCES IN TACKLING ANTIBIOTIC RESISTANCE WITH NATURAL PRODUCTS: COMBINATORIAL AND NANO-BASED STRATEGIES

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Despite advances in anti-infective therapy, antibiotic-resistant bacterial infections remain an important cause of morbidity and mortality worldwide. According to WHO, carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, carbapenem-resistant and extended spectrum beta-lactamase producing Enterobacteriaceae, methicillin-resistant *Staphylococcus aureus*, clarithromycin-resistant *Helicobacter pylori* are among the priority drug-resistant pathogens responsible for life-threatening infections [1]. Identification of novel treatment strategies to reverse bacterial resistance is therefore of great interest. Natural products offer a promising approach to overcome bacterial resistance. One strategy is the combination of natural products with antibiotics. In our investigations, we screened volatile and phenolic extractives/constituents for their ability to restore antibiotic effectiveness against resistant bacterial strains. The antibacterial effects of the combinations were assessed by the checkerboard and time-kill assays. Our studies found ajowan and coriander essential oils and their major constituents, thymol and linalool, respectively, but also prenylated flavonoids (xanthohumol, 8-prenylnaringenin) to significantly enhance antibiotic activity against resistant Gram-positive and Gram-negative bacteria [2, 3]. Combination of two natural products, encapsulation of antibacterial natural products in nanocarriers, phytochemicals-mediated synthesized metal nanoparticles are other alternatives to overcome bacterial resistance. Natural product-based combinations and nanoformulations seem to be good candidates for the development of novel resistance modifying antibacterial agents.

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GREEN BIOSYNTHESIS AND PHYSICO-CHEMICAL CHARACTERIZATION OF MAGNETIC IRON OXIDE NANOPARTICLES (FE_3O_4) USING *ARTEMISIA ABSINTHIUM L.* AQUEOUS EXTRACT

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Aims: The present study was carried out to describe the bioreduction of $FeCl_3 \cdot 6H_2O$ with wormwood aqueous extract and physico-chemical characterization of the magnetic nanoparticles obtained.

Methods: Two aqueous extracts based on *Artemisia absinthium* L. (wormwood) from leaves and stems, using Şahin's slightly modified method [1] were prepared. Magnetic iron oxide nanoparticles (MIONPs) were synthesized using the modified method of Basavegowda [2]. An amount of lyophilized aqueous extract was added over 2 mM $FeCl_3$ solution and then the mixture was stirred for 4 hours at room temperature. A solution of 1 M NaOH was added until the formation of MIONPs was marked by the appearance of a black precipitate.

Results: UV-Vis spectra showed maximum absorbance at 250 nm which confirmed that the Fe_3O_4 nanoparticles were formed. TEM analysis revealed that Fe_3O_4 nanoparticles have spherical shape with size up to 20 nm. FTIR spectra confirmed the presence of biomolecules in the extract responsible for the reduction of Fe_3O_4.

Conclusion: An eco-friendly, low toxicity, facile and green approach synthesis of Fe_3O_4 nanoparticles from aqueous $FeCl_3$ solution using an aqueous extract of *A. absinthium* L. was described.

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ARTEMISIA ABSINTHIUM L. – ANTIOXIDANT ACTIVITY AND CYTOTOXIC EFFECT ON MCF7 AND A375 HUMAN TUMOR CELL LINES

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Aims: The present study is focused to investigate the antioxidant activity of two aqueous extract based on *Artemisia absinthium* L. (wormwood), obtained from the leaves and stems. The cytotoxic effect of both aqueous extracts was also investigated on two human tumor cell lines, namely MCF7 breast adenocarcinoma and A375 melanoma cell.

Methods: The antioxidant potential was evaluated by using DPPH method [1]. The cytotoxic activity of both aqueous extracts was determined by means of Alamar blue assay [2].

Results: The antioxidant activity was concentration-dependent in the case of both aqueous extracts. Concerning the cytotoxic effect, the most significant results were obtained at the highest dose tested, 200 µg/mL, for the aqueous extract obtained from stems, on both MCF7 and A375 tumor cell lines. On A375 melanoma cells, the extract based on wormwood stems decreased cells viability to 83% and for the extract obtained from leaves, the results showed that the viability was around 86%.

Conclusion: The aqueous extract based on the stems of wormwood induced a reduction in cell viability on both tumor cell lines, and proved to have a higher antioxidant potential than the extract based on the leaves.

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PHYTOCHEMICAL INVESTIGATIONS OF *SALVIA TRANSSYLVANICA, SALVIA GLUTINOSA, AND SALVIA OFFICINALIS* FROM ROMANIA AND THEIR BIOACTIVITIES

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In the Romanian traditional medicine sage species are used as remedies for coughs, rheumatism, inflammatory and bacterial diseases, as well as antidiabetic remedies.

In this study, an HPLC method was applied for determination of 22 phenolic compounds in extracts of *S. glutinosa, S. transsylvanica,* and *S. officinalis.* The enzyme inhibitory potential of the extracts was evaluated using microtiter assays, and the antimicrobial potential was tested using the microdiluation assay for eight microorganisms. These extracts were further tested on three different cancer cell lines (A549, HepG2 and MCF-7) at increasing concentrations (1.56-200 µg/mL) for 24 h/48 h.

The chromatographic fingerprint revealed that among investigated compounds, the dominant compounds of *Salvia* species are rutin (1357.9 - 4070.2 µg/g) and catechin (1112.6 - 1911.1 µg/g). Concerning the enzyme inhibitory assays, both *S. officinalis* and *S. transsylvanica* extracts exhibited an important inhibitory potential against alpha-glucosidase (27.01 mmolACAE/g extract, and 25.62 mmolACAE/g extract, respectively). The most sensitive bacteria to the extracts were *Enterobacter cloacae* (MIC = 0.01 mg/mL, MBC = 0.02 mg/mL for *S. officinalis*) and *Bacillus cereus* (MIC = 0.09 mg/mL, MBC = 0.18 mg/mL), while *Penicillium funiculosum* was the most sensitive fungal strain to *S. officinalis* extract (MIC = 0.06 mg/mL, MFC = 0.12 mg/mL). From the three extracts, the *S. officinalis* extract exhibited the most potent cytotoxic effect. Interestingly, when testing on the estrogenic responsive cell line MCF-7, an increase in the viability was observed for intermediary doses which we hypothesize to be related to the estrogen-like compounds present in *Salvia* species.

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GSK-3B-TARGETING FISETIN PROMOTES MELANOGENESIS THROUGH B-CATENIN ACTIVATION

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Fisetin is found in many fruits and plants such as grapes and onions, and exerts anti-inflammatory, antiproliferative, and anticancer activity. However, whether fisetin regulates melanogenesis has been poorly studied. Therefore, we evaluated the effects of fisetin on melanogenesis in B16F10 cell and a zebrafish model. Fisetin slightly suppressed *in vitro* mushroom tyrosinase activity; however, molecular docking data showed that fisetin did not directly bind to tyrosinase, which suggested that the fisetin-mediated inhibition of *in vitro* tyrosinase activity was a non-specific or anti-oxidant effect. Unexpectedly, fisetin significantly increased intracellular and extracellular melanin production in B16F10 cells from 48 h. In addition, fisetin promoted α-MSH-induced intracellular and extracellular melanin content. We also found that the expression of melanogenesis-related genes such as tyrosinase and microphthalmia-associated transcription factor (MITF), were highly expressed at 48 h after fisetin treatment. Pigmentation of zebrafish larvae after fisetin treatment increased for concentrations up to 200 µM and then slightly decreased at 400 µM, with no change in heart rate. Fisetin also enhanced α-MSH-induced pigmentation in zebrafish larvae. Molecular docking results indicated that fisetin binds to glycogen synthase kinase-3β (GSK-3β). Therefore, we evaluated whether fisetin negatively regulated GSK-3β, which subsequently activates β-catenin, resulting in melanogenesis. As expected, fisetin increased the expression of β-catenin, which was subsequently translocated into the nucleus. In the functional assay, FH535, a Wnt/β-catenin inhibitor, significantly inhibited fisetin-mediated melanogenesis in zebrafish larvae.

Our data suggested that fisetin inhibits GSK-3β through its direct binding, which activates β-catenin, resulting in melanogenesis through the revitalization of MITF and tyrosinase.

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ROOTING AS A DECISIVE FACTOR AFFECTING ENDOGENOUS HORMONOME AND BIOSYNTHETIC CAPACITY OF *ARTEMISIA ALBA* TURRA *IN VITRO* MODEL

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In vitro culture model characterized by enhanced or suppressed rooting in essential oil bearing *Artemisia alba* Turra was developed with the aim to better understand interrelations between *in vitro* rooting and biochemical processes occurring in this plant.

Two main morphotypes were developed based on modification of cytokinin (N^6-benzyladenine) and auxin (indole-3-butyric acid) supplementation, either alone or in combination. Comparison was made based on the study of essential oil profile, polyphenolics levels, as well as endogenous hormonome *in vitro.*

It was revealed that normally rooting plants were characterized by elevated monoterpenoid/sesquiterpenoid ratio of their essential oil profile, higher salicylic and abscisic acid contents, bioactive cytokinin concentrations and *trans*/*cis*-zeatin ratio on one hand and by lowered polyphenolics levels and decreased jasmonic acid amounts on the other hand as compared with root suppressed plants.

The plant organism consists of different organs, which are constituted of different tissues, build up by cells. Production of secondary metabolites and phytohormones as well as their accumulation and translocation are dependent on the presence and communication between the highly specialized anatomical structures within the plant organism. Root morphogenesis was shown to play an important role in both biosynthetic features as well as physiology of *in vitro* cultured *A. alba* model, which could be applied in targeted cultivation of plant material with desired quality of this species.

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PHENYLPROPANOIDS FROM *CLINOPODIUM VULGARE* WITH ANTIOXIDANT ACTIVITY

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Wild basil (*Clinopodium vulgare* L.) (Lamiaceae) is a perennial herbaceous plant widespread in Bulgaria. Aerial parts are used in Bulgarian folk medicine for treatment of diabetes, gastric ulcers and cancer. The herbal drug alleviates symptoms associated with mastitis, prostatitis, skin irritation and swelling. The aim of the study was to isolate the main bioactive compounds from *C. vulgare* aerial parts. Ultrasound assisted extraction of aerial parts with 80 % methanol aqueous solution at room temperature allowed good extraction of all compounds of interest. *C. vulgare* extract (CVE) was separated in several fractions by low-bar liquid chromatography on a reverse phase column. The subsequent ultra high-performace liquid chromatography coupled with high resolution mass spectrometry (UHPLC-HRMS) was achieved on LC/HRMS system consisting of an Q Exactive Plus mass spectrometer, equipped with a heated HESI-II source. DPPH, ABTS and FRAP methods were used for antioxidant activity determination. Commercial standard of rosmarinic acid was used as positive control. All investigated CVE fractions demonstrated significant radical scavenging and ferric reducing capacity. Based on the UHPLC-HRMS results, comparison with reference standards and literature data, the major compounds in CVE fractions were identified or tentatively elucidated. Moreover rosmarinic acid, the main compound in CVE was isolated (94% purity) and identified.

The obtained results revealed the studied CVE as potential new source of phenylpropanoids with significant potential in safeguarding against various induced oxidative stress.

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DITERPENOIDS OF THE CALIFORNIA PLANT, *GRINDELIA STRICTA VAR. PLATYPHYLLA*: ISOLATION, STRUCTURE DETERMINATION AND ANTIMICROBIAL ACTIVITY

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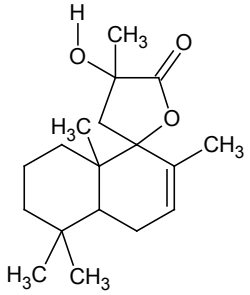
Gumweed (*Grindelia stricta var. platyphylla*, Asteraceae) is a variety of flowering plant, native to a coastal area in central California, USA, and belonging to the genus *Grindelia* which has traditionally been used for the treatment of respiratory ailments, tuberculosis, skin inflammations and many other ailments. The genus *Grindelia* contains over 65 species and over 75 varieties in California alone and is native to arid regions of Mexico and North and South America where it has often been used as a medicinal plant by local native peoples. Here are presented new diterpenoids isolated from this plant and their antimicrobial activity against *Mycobacteria phlei* and *Staphlococus aureus*.



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CORELATION BETWEEN ANTIOXIDANT PROPERTIES AND OPTICAL CHARACTERISTICS OF ALGEA WATER EXTRACTS AT DIFFERENT CONDITIONS

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The physico-chemical properties of water extracts from Spirulina and Chlorella grown in bioreactor at temperature 100 °C and different time of extraction 10 min, 15 min, 20 min and 30 min respectively have been investigated. The data for antioxidant activity of the samples have been determined by using DPPH and ABTS methods. The antioxidant activities decrease at both methods from 5 min to 15 min for extraction and after that increases.

The purpose of this study is to explain existing relations between chemical characteristics and optical parameters, which are determined by method of applied photonic.

The correlation dependences exist between color characteristics and time of extraction for each concentration of the samples. When the time of extraction increasing the lightness for more samples decreases without these with content 6 g *Chlorella* in 100 ml. The samples from *Spirulina* poses chlorophyll a, while these from *Chlorella* – chlorophyll b.

The fluorescence spectra have been obtained for excitaion wavelength 265 nm. There are four main emission maxima which can be related according to literature data with riboflavin, cathehins, chlorogenic and p-coumaric acids and chlorophyll.

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PHYTOTOXIC AND INSECTICIDAL EFFECTS OF *ORIGANUM VULGARE* SUBSP. *HIRTUM* ESSENTIAL OIL AND METHANOLIC EXTRACT

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Plants are an important source of compounds with allelepathic and phytotoxic effects. These mechanisms of protection of plant species from each other are in the base of creation of natural products for pest control. Phytotoxic potential of *Origanum vulgare* have been well documented while *O. vulgare* subsp. *hirtum* (Greek oregano) in particular its essential oil is insufficient studied. The aim of present study was to evaluate *O. vulgare* subsp. hirtum essential oil and methanolic extract as inhibitors of seed germination, seed root elongation and seedling grown of weeds (*Lolium perenne* L. and *Trifolium repens* L.) and potato (*Solanum tuberosum* L.) as well as for insecticidal effects on *Myzus persicae* Sulz.

Assays for seed germination and seed root elongation were conducted in Petri dishes. Growth of seedlings after treatment with essential oils and methanolic extract in the form of spray was evaluated visual. The insecticidal effect was assessed by dip leaf test method (FAO). The chemical composition of essential oil and methanolic extract were determined by GC–MS.

The results showed that the essential oil completely inhibit seed germination of studied weeds at concentration 5 µg/mL. Up to 5 days after treatment on seedling with essential oil solution, 70% of the individuals of *T. repens* died while these of *L. perenne* and potato leaves were very poorly affected. Methanolic extract exhibited low inhibition activity of seed germination, root elongation and seedling grown at concentration 1 mg/mL. The results of insecticidal test showed that essential oil had completely toxic effects on *Myzus persicae* at concentration 5 µg/mL while the methanolic extract caused 44% mortality at concentration 5 mg/mL. The main components of the essential oil were identified as carvacrol, p-cimene, γ-terpinene β-caryophyllene. The received data showed that essential oil of *O. vulgare* subsp. *hirtum* is promising product for pest control.

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METABOLITE PROFILING OF BULGARIAN ENDEMIC *VERBASCUM TZAR-BORISII*

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The Bulgarian endemic *Verbascum tzar-borisii* Stef.-Gat. is one of the rarest and the most endangered plant species in the world. Its populations, numbering less than 1000 individuals, are situated in the northeastern part of the country in two localities in Varna district – "Probitiya kamak" and "Sivri tepe" [1]. With the improvement of chemical analysis techniques, it has been possible to determine the composition of little quantity plant material. This allowed examining the composition of such rare species. The leaf material of 7 individuals of the both populations was collected. Methanolic extracts and their fractions of the studied samples were analyzed by GC/MS. Fatty, phenolic and organic acids, mono-, di- and tri-saccharides - compounds of main metabolite groups were identified. The phenolic fraction was found to be rich in phenolic acids. Eight phenolic acids were identified, of which the *cis* and *trans* forms of hydroxycinnamic acid and *trans*-ferulic acid are the main ones. Hexadecanoic acid and linoleic acid (C18:3) are dominant fatty acids. Flavonoid aglycones - apigenin and luteolin as well as luteolin-7-glycoside were identified by TLC analysis. The high content of phenolic acids also determines the good antioxidant activity that was evaluated by DPPH assay. The relationship between metabolite profiles of studied populations and their inter-population genetic differences [2] is discussed. The present study reported new data about chemical composition and free radical scavenging activity of *V. tzar-borisii*.

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IDENTIFICATION AND QUANTIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN *MELISSA OFFICINALIS* L. EXTRACT

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Lemon balm (*Melissa officinalis*) is a perennial herbaceous plant in the Mint family (Lamiaceae). The leaves of *M. officinalis* are used as herb, in teas, and also as flavouring. The tea of lemon balm, the essential oil, and the extract are used in traditional and alternative medicine, including aromatherapy. In traditional medicine, it is used in the treatment of headache, migraine, toothache, earache, flatulence, indigestion, cramping, nausea, anxiety anemia, vertigo, syncope, asthma, bronchitis, amenorrhea, heart failure, arrhythmias, insomnia, epilepsy, depression, psychosis, hysteria, stomach disorders, ulcers, wounds, rheumatisms and torticollis. Pharmacological reports indicate that *M. officinalis* exhibits a variety of actions including antioxidant and anticholinesterase activities [1]. Several studies have demonstrated the lemon balm's antioxidant activity, obtained through high amounts of flavonoids, rosmarinic acid and phenolic contents [2].

Herein we present a complete characterization of dry extract of *M. officinalis*, obtained after extraction with alcohol, evaporation and subsequent drying with Mini Spray Dryer. We performed a chemical profiling of biologically active compounds in dry extract of *M. officinalis* using high performance thin layer chromatography (HPTLC), UHPLC-ESI-MS (with single quadrupole MS), SFC/HPLC-ESI-MS/MS (with triple quadrupole MS) and 1D and 2D NMR spectroscopy. For quantitative determination of the main components – rosmarinic acid, caffeic acid and rutin, HPTLC and UHPLC-PDA analyses have been done.

Acknowledgements: Support of this work by the Research and Development and Innovation Consortium, Sofia Tech Park (project D-062-2018/20.06.2018) is gratefully acknowledged.

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NEW AURONOLIGNANS FROM *COTINUS COGGYGRIA*

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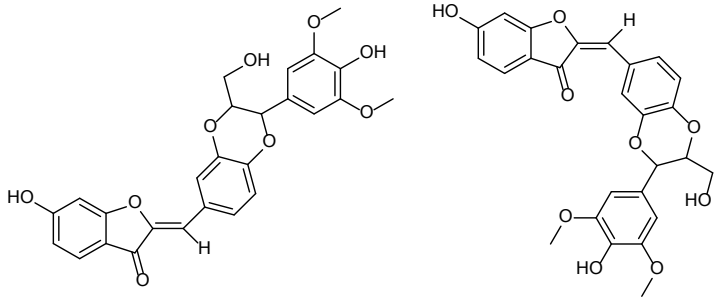
Cotinus coggygria Scop. is a bush or small tree of genus *Cotinus* family Anacardiaceae widely distributed in Europe and Asia. It is a reach source of flavonoid compounds – flavones, flavanones, flavanols, flavanonolos, aurones, chalcones, biflavones, phenolic acids and sterols with significant biological activities.

Aurones are subclass of flavonoid compounds with five-membered C-ring. Well known aurone isolated from *C. coggygria* is sulfuretin containing Z configuration of the double bond. Flavonolignans are secondary metabolites that consist of flavone and lignin moieties. Probably the best known flavonolignans are silybins A and B, isosilybins A and B isolated from *Silybum marianum* with remarkable hepatoprotective activity [1].

In this study, we report isolation and characterisation of two new auronolignans **1** and **2** from *C. coggygria* collected in Deliblatska Peščara in Serbia in 2017. Air dried heartwood was extracted with mixture of methylene chloride and methanol. Extract was fractionated by silica gel column chromatography. Final separation and purification was done using semi-preparative reversed phased HPLC. The structure elucidation was established using 1D (^{1}H and ^{13}C) and 2D NMR (COSY, NOESY, HSQC and HMBC), UV, IR and HRMS.



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IMMOBILIZATION OF CURCUMIN IN HYDROGEL FILMS BASED ON GELLAN/ALBUMIN/PECTIN OBTAINED BY IONIC CROSS-LINKING AND POLYELECTROLYTE COMPLEXATION

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Previous studies have reported that the curcumin has antibacterial and anti-inflammatory activity and in topical applications was successfully used to treat rashes and skin infections [1]. The main drawback in the use of curcumin is determined by its water insolubility and low bioavailability [2].

In this study, β-cyclodextrin inclusion complexes were prepared in order to increase the water solubility of curcumin. Moreover, the gellan/albumin (BSA) films containing the β-cyclodextrin/curcumin inclusion complex were obtained by ionic cross-linking with magnesium acetate at pH 7.8, being subsequently polyelectrolytically complexed with 1% (w/v) pectin solution. BSA was used in order to improve the hydrogel films biocompatibility, the immune response capacity and to increase the systemic level of glutathione [3].

The effect of pH on the cross-linking degree was studied and it was observed that the BSA carboxylic groups from the gellan/BSA solution react at pH 7.8 with Mg^{2+} ions; the amino groups react at pH 3.5 with the pectin carboxylic groups leading to a polyelectrolyte complexed film.

The films obtained were characterized by the swelling degree, SEM, FT-IR, TGA, mechanical tests and the cytotoxicity was evaluated. The curcumin stability under light and pH as well as its antioxidant activity within the films were evaluated and the protective role of the polymer matrix was proved. The release kinetics studies of curcumin from the polysaccharides films were performed in two different pH media (5.5 and 7.4); higher release efficiency was observed at pH 7.4, in accordance with the swelling degree behavior.

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CHEMICAL PROFILES AND PHARMACOLOGICAL PROPERTIES OF TWO *ANTHEMIS* SPECIES: *ANTHEMIS TINCTORIA* VAR. *PALLIDA* AND *A. CRETICA* SUBSP. *TENUILOBA*

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Members of *Anthemis* genus are extensively used in the Turkish folk medicine to treat various ailments. In this present study, the ethyl acetate, methanolic and aqueous extracts of aerial the parts of *Anthemis tinctoria* var. *pallida* (ATP) and *A. cretica* subsp. *tenuiloba* (ACT) growing in Turkey were investigated for their antioxidant and key enzyme inhibitory potentials. Total phenolic and flavonoid contents were determined using colorimetric methods.

The antioxidant capacities of the studied extracts were evaluated using different assays including free radical scavenging, reducing power, phosphomolybdenum, and metal chelating. Additionally, we evaluated the putative protective effects of *Anthemis* extracts on "Cortical Spreading Depression" (CSD) paradigm, on rat cortex specimens treated with an excitotoxicity stimulus. To this regard, we assayed extract capability in blunting CSD-induced cortex 5-HT decrease. All the extracts showed strong antioxidant abilities, with the best activity exerted by MeOH extracts. Enzyme inhibition was tested on AChE, BChE, α-amylase, α-glucosidase, and tyrosinase. Only the EtOAc and MeOH extracts were potent against AChE and BChE. The extracts showed remarkable enzyme inhibitory effects against tyrosinase and α-glucosidase, and modest activity against a-amylase. Finally, in agreement with the evaluation of antioxidant activity, *Anthemis* MeOH extracts revealed the most effective in restoring physiological 5-HT level, in cortex specimens subjected to an excitotoxic stimulus.

The results highlighted on the biological potential of the studied *Anthemis* species and warrant for further studies to explore their potential use in phytomedicines and cosmetics.

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CRUDE FIBER CONTENT ANALYSIS IN *LAVANDULA OFFICINALIS*, *THYMUS VULGARIS*, *MENTHA PIPERITA* AND *SALVIA OFFICINALIS* WASTES

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Knowing the crude fiber content is very important in present, not only because it can be considered as an indicator of feed for ruminants, but also because it could be used in human consumption as a biomaterial support for enrichment with bio-active principles.

The aim of this study is the quantitative analysis of the crude fiber content from four medicinal and aromatic plants waste, *Lavandula officinalis* L., *Thymus vulgaris* L., *Mentha piperita* L. and *Salvia officinalis* L., in an attempt to contribute to the sustainable valorization of plant waste. This work involves a comparison of the methods of determination of the crude fibers, starting from the traditional Weende method to modified fiber extraction methods in order to obtain a better extraction yield. FTIR analysis was performed in order to identify the main functional groups of the components of insoluble carbohydrates as cellulose, hemicellulose and lignin. The statistical validation of the results obtained by the crude fiber extraction methods was performed by analysis of variance (ANOVA).

Therefore, the results of this study indicated that the valorization of plant waste in this way represents an improvement at least in the biomaterial domain.

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CARROT CELL SUSPENSION AND HAIRY ROOT CULTURES FOR THE ESTABILISHMENT OF HIGH-YIELD PRODUCTION SYSTEM OF RECOMBINANT MIRACULIN PROTEIN

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Miraculin is a taste-regulating protein with the ability to interact with human sweet taste receptors and transform sourness into sweet taste, extracted from the fruit 'miracle fruit' of *Synsepalum dulcificum*. Since miracle fruit is difficult to mass-produce due to regional and seasonal limitations, there have been many efforts to express miraculin in various cell systems [1, 2]. Therefore, in order to produce miraculin from hairy roots of carrots, we induced hairy roots by infecting carrot root pieces with *Agorbacterium rhizhogenes*. After 4 weeks of transformation, putative hairy roots induced in 5 of the 22 explants (23%). To confirm the hair roots, the presence of rol gene insertion was confirmed by PCR, and 4 lines out of 5 lines were confirmed to be hairy root. Three lines (HR2, 3, 4) except for HR1, which has a slow growth rate among four hairy root lines, were selected and used for 2 step transformation for miraculin production. The plant expression vector with SWPA promotor was introduced into carrot selected hairy roots and calli via Agrobacterium-mediated transformation methods. The integration of the miraculin gene into the chromosome of the transgenic callus and hairy roots was verified via genomic DNA PCR amplification and miraculin expression in transgenic carrot suspension cells and hairy roots was confirmed via RT-qPCR analysis. In further study, we will investigate the yield of miraculin production through protein extraction and purification in transgenic hairy roots and suspension cultured cells. This study confirmed the optimal system for efficient production of recombinant Miraculin protein.

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ANISOMELIC ACID – A PHYTOCHEMICAL AGENT SPECIFICALLY TARGETING E6/E7 ONCOGENES IN HPV-MEDIATED CANCERS

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Human papillomavirus (HPV) infection is now a well-established cause of different types of cancer including, but not limited to cervical cancer. To date, there is no specific therapy for effective treatment of HPV. The integration of the HPV viral genome into the human genome and the expression of the HPV-encoded E6/E7 viral proteins are critical steps in HPV mediated diseases indicating that E6 and E7 would be valuable targets for therapeutic intervention in HPV treatment. Anisomelic acid (AA), is a natural diterpenoid present in different *Anisomeles* plant species. In our recent studies, we observed that AA acts through a unique mechanism that enables specific targeting of the E6 and E7 viral oncoproteins in HPV positive cancer cells. It's specific targeting of E6 and E7 oncoproteins induce programmed cell death or apoptosis by blocking anti-apoptotic proteins, activating proteins involved in cell cycle arrest, and by inhibiting growth promoting signal transduction pathways. This is the first description of AA as an inhibitor of viral oncoprotein expression and as a chemotherapeutic agent for HPV-induced carcinoma.

Our results reveal the molecular mode of action of AA, which enables specific targeting of the HPV oncoproteins, that can be employed when developing drug molecules both for anti-cancer and for anti-HPV therapy.

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CYTOPROTECTIVE EFFECT OF *SAMBUCUS EBULUS* L. EXTRACT AND ITS LIPID NANOFORMULATIONS AGAINST H_2O_2 INDUCED STRESS

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The aim of this paper was to investigate the cytoprotective effects of *Sambucus ebulus* L. extract and its lipid nanoformulations against H_2O_2 induced stress. *S. ebulus* L. was encapsulated in lipid nanovesicles to improve biodisponibility and achieve maximum pharmacological effect. *S. ebulus* L. (dwarf elder, Adoxaceae), a perennial plant widespread in Europe, is used for its antioxidant, anti-inflammatory, antimicrobial, antiarthritic properties [1-3].

Lipid nanovesicles loaded with *S. ebulus* L. were prepared by film hydration method and characterized in terms of entrapment efficiency, particle size, polydispersity index and stability. *In vitro* release from lipid nanovesicles was assessed using dialysis bags method. Obtained lipid nanoformulation was investigated by DPPH method to observe antioxidant activity. Also, it was evaluated whether a pretreatment of one hour or 24 hours with loaded lipid nanovesicles had an effect on L-929 mouse fibroblasts cells cytotoxicity induced by H_2O_2. Nanoformulations showed good entrapment efficiency, sizes below 200 nm, narrow polydispersity index and good stability over 90 days at 4 °C. *In vitro* polyphenols release study showed a slow release compared to free extract. Encapsulation of *S. ebulus* L. in nanovesicles maintained the antioxidant capacity of extract. In the same manner the pretreatment with lipid nanovesicles loaded with *S. ebulus* L. had a cytoprotective effect against H_2O_2-induced cytotoxicity on L-929 mouse fibroblasts cells. These findings support the use of *S. ebulus* L. and its lipid nanoformulations as cytoprotective phytopharmaceuticals for biomedical applications.

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DESIGN AND EVALUATION OF INNOVATIVE LIPID NANOVESICLES CONTAINING *CENTAUREA CYANUS* L. EXTRACT

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In alternate systems of medicine like Ayurveda and traditional Chinese medicine, *Centaurea cyanus* L. phytomedicine has been used for its antipruritic, antitussive, astringent, diuretic, emmenagogue, mildly purgative and bitter tonic properties, reducing inflammatory process. The aim of this work was the design of lipid nanovesicles based system for *C. cyanus* L. with the purpose to maximize its biodisponibility [1, 2].

C. cyanus L. loaded lipid nanovesicles were prepared by film hydration method. Entrapment efficiency, particle size and polydispersity index were assessed for the characterization of nanovesicles. *In vitro* release from lipid nanovesicles was investigated using dialysis bags. Cytotoxicity effect of empty and loaded lipid nanovesicles was evaluated by MTS method on mouse fibroblasts cells.

The obtained nanoformulations showed good entrapment efficiency, nano-size and narrow polydispersity index. *In vitro* drug release study showed the ability of lipid vesicles to provide slow release of *C. cyanus* L. with reduced burst effect compared to free extract. Both free plant extract and loaded lipid nanovesicles showed a low cytotoxicity effect on mouse fibroblasts cells.
These promising results open up avenues to explore lipid nanovesicles as carriers for delivery of phytopharmaceuticals.

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PHENOLIC COMPOSITION OF FRESH AND DRIED LEAVES OF *PERSICARIA ODORATA*

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Persicaria odorata (Lour.) Soják commonly known as Vietnamese coriander, Vietnamese mint, laksa plant or *rău ram* belongs to the family Polygonaceae. Leaves are used as a pungent additive to dishes of south-eastern Asian cuisine, where it occurs as a native species. Previous research involved composition of essential oil [1, 2], whereas phenolics were studied mostly in terms of total contents in extracts or fresh plant material [3].

The aim of this research was to investigate what are the main phenolics in both dried and fresh leaves: flavonoids, phenolic acids and procyanidins.

Plant material was purchased commercially from six sources, powdered and extracted with methanol-water mixture (1:1, v/v). Extracts were analysed with UHPLC-DAD-MS^n validated analytical method. Identification was based on UV-Vis, mass spectra and comparison of retention times with the available standards.

Comparisons of contents and profile of phenolics were appointed. Main compounds in all samples were methyl gallate, catechin, (epi)catechin gallate, quercetin 3-*O*-*β*-D-glucuronide, feruloylkaempferol, quercetin and kaempferol sulphates. On the other hand, contents of some compounds were significantly different in various samples: trimeric procyanidin (type B), caffeic acid pentoside, quercetin 3-*O*-*β*-D-glucoside (isoquercitrin), quercetin 3-*O*-*β*-D-rhamnoside (quercitrin). Samples varied in terms of total contents' proportions of flavan-3-ol derivatives and flavonols. There were none or minimal differences in phenolic profiles of dry and fresh leaves.

In comparison with other herbs used in traditional European or Chinese medicine, leaves of *P. odorata* do not contain high amounts of phenolics. However, in combination with essential oil it is a valuable food additive.

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QUALITATIVE STUDIES ON RHIZOME OF *PERSICARIA BISTORTA*

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Bistortae rhizoma is the pharmacopoeial plant material acquired from *Persicaria bistorta.* Although it exhibits astringent (*adstringens*) and antidiarrheal (*antidiarrhoicum*) properties, aqueous extracts are used mostly topically on account of high contents of tannins. Research on phytochemical composition of *B. rhizoma* showed contents of gallotannins, procyanidins (catechin oligomers), triterpenoids, coumarins, steroids, fatty acids [1, 2].

The aim of this study was to isolate and identify main compounds from aqueous extract of *B. rhizoma.*

The extraction of rhizome was carried out with water, as the plant material is used in aqueous preparations. Raw extract was subsequently evaporated and fractionated by liquid-liquid extraction with diethyl ether, ethyl acetate and n-butanol. Fractions were separated using column chromatography (using Diaion HP-40, silica gel and Toyopearl HW-40F) and preparative HPLC (column: Kinetex 5u XB-C_{18} 100A, 150 mm × 21,2 mm × 5 μm). The result was the isolation and identification of 30 compounds. Their structures were established based on 1D/2D NMR experiments, MS^n analyses and UV-Vis spectroscopy.

Obtained compounds were classified as derivatives of gallic acid and flavan-3-ol, as well as chlorogenic acid. The main compounds of the extract were chlorogenic acid and procyanidin B3. The latter is reported for the first time in the pharmacopoeial plant material *B. rhizoma*.

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ANTITUMOR ACTIVITY SUPERCRITICAL CARBON DIOXIDE FRACTION OF BLACK PEPPER RICH IN PIPERINE SHOWED CDK2 AND BCL-XL INHIBITORY MECHANISMS PREDICTED *IN SILICO* STUDY

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This study aim to investigate the antitumor activity of a piperine rich fraction obtained through supercritical carbon dioxide extraction (SFE) from black pepper (*Piper nigrum* L. cultivar Bragantina; Piperaceae). *In silico* docking simulations predicted antitumor molecular mechanism. Molecular docking using piperine showed protein-ligand hydrophobic interactions and hydrogen bonds between piperine and residue Ser^5 inside the ATP binding site in CDK2. Besides, piperine interacts with peptide substrate residue Lys^8 inside its binding site in cyclin A molecule. Other predicted interactions showed piperine inside hydrophobic groove of Bcl-xL. In line with this prediction SFE showed cytotoxicity (EC_{50} = 27.8 ± 6.8 μg/ml) inhibiting the proliferation of breast adenocarcinoma cells (MCF-7) correlated to increase of apoptosis. SFE phytocompounds intercalated/bounded with DNA. Balb/c mice-bearing Ehrlich ascites carcinoma cells (EAC) received SFE (10 or 100 mg/kg/day), and the dose of 100 mg/kg/day inhibited EAC growth (60%) increasing mice survival (50%) related to cell cycle arrest (G2/M) and increased apoptosis. SFE increased *in vivo* expression of apoptotic proteins (p53 and Bax) and inhibited *in vivo* expression of cell cycle progression proteins (CDK2, Cyclin A) and anti-apoptotic protein (Bcl-xL) confirming *in silico* predicted inhibitory interactions. In conclusion, SFE piperine-rich fraction showed potential to be used as a cancer complementary therapy.

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IN SILICO AND *IN VITRO* STUDY OF B-DNA INTERACTIONS WITH SS-CARBOLINE ALKALOID HARMINE

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Introduction: Previous studies have shown that β-carboline alkaloids have antitumoral effect and have as one of the main target the DNA. This study aims to predict interactions between DNA and harmine through *in silico* tools, molecular and *in vitro* assays.

Methods: Harmine (Ine) (ZINC18847046) lowest energy 3D conformer was calculated (pH 7.4; Dreiding force field; MarvinSketch ChemAxon), converted (Openbabel), submitted and approved by topology portals (SwisParam, CGENFF). Harmine and structure of B-DNA (PDB 1G3X) without inhibitor were docked (AutoDock tools; PyMOL AutoDock/ Vina Plugin 2.2.0), considering default parameters. *In vitro* cytotoxicity of harmine (0.1 – 1.000 µM; 72h) was evaluated against MCF-7 and NIH 3T3 (10^4 cells/well; MTT assay). Then, was evaluated the interaction between calf thymus DNA (CT-DNA, 150 µM) and harmine (50-250 µM) by spectrophotometry (220-800 nm) and through intercalant propidium iodide fluorescence variations (PI, 300 µM). Finally, effects on nuclear DNA of MCF-7 cells were evaluated by alkaline Comet Assay.

Results/Discussion: Vina predicted lowest binding energy for DNA-Harmine at pose 1 (-7.6 kcal/mol; RMSD 0.000). LigPlot+ showed only hydrophobic interactions between harmine and B-DNA, especially with adenine and thymine nucleotide. Harmine cytotoxicity was dose-time-dependent and selective. CT-DNA with increasing concentrations of harmine revealed both hypochromic and bathochromic effects indicative of harmine-DNA interactions. Indeed, intercalated harmine decreased PI fluorescence. Besides, intercalated harmine probably contributed to nuclear DNA damage and increased cytotoxicity.

Conclusions: Docking predicted DNA-Harmine interactions corroborate with molecular and *in vitro* assays highlighting this ß-carboline alkaloid as a leading compound.

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COMPARISON OF DIFFERENT EXTRACTION SOLVENT MIXTURES FOR CHARACTERIZATION OF PHENOLIC COMPOUNDS IN *ARONIA MELANOCARPA*

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Thirty-two different solvent mixtures containing pure methanol, methanol combined with an acid (acetic, formic, hydrochloric) or with acid and water were tested for their efficiency for extraction of phenolic compounds from aronia (*Aronia melanocarpa*) belonging to three groups of polyphenols: anthocyanins, flavonols and hydroxycinnamic acid derivatives.

Thirteen compounds have been detected and quantified using HPLC/DAD/ESI-MS^n. The yield of each phenolic compound and group was evaluated with regard to the extraction solvent composition. Extraction mixtures containing hydrochloric acid were superior to the ones containing acetic or formic acid for extraction yield of total phenolic compounds, which was especially pronounced for the group of anthocyanins. It is well established that anthocyanins can be found in different chemical forms which depend on the pH of the solution. At pH 1, the flavylium cation is the predominant species and contributes to purple and red colour.

The solvent mixture containing methanol/water/HCl (90 : 8 : 2, v/v/v) gave the best results for the qualitative and quantitative assay of anthocyanins. However, this solvent mixture caused *O*-methylation of 3- and 5-caffeoylquinic acids which were transformed to 3- and 5-feruloylquinic acids respectively, during the extraction. This is a peculiar finding that must be taken into account during sample preparation for analysis of polyphenols. From preliminary trials it was observed that this *O*-methylation is specific for dihydroxycinnamic acid compounds with neighboring hydroxyl groups. These results suggest that more detailed studies should be carried out to elucidate the behavior of various polyphenolic compounds during extraction with solvent mixtures containing methanol/HCl, since it is very often used for analysis of anthocyanins.

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TARAXACUM OFFICINALE EXTRACTS AND DERIVED PRODUCTS AS EFFICIENT PROTECTORS IN UV-DAMAGED SKIN

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Taraxacum officinale (dandelion) is a well-known curative plant, which was used from ancient times in traditional Chinese medicine, with more than 2500 species reported in the present [1]. The plant has an extremely rich chemical composition in biologically active compounds, such as: sesquiterpenoids, triterpenoids, phytosterols, flavonoids, phenolic and organic acids, carbohydrate inulin, vitamins, and volatile compounds. Based on the composition described above, dandelion, has a significant pharmacological potential in terms of antioxidant, anti-inflammatory, hypolipidemic, hypoglycemic, antimicrobial, anticancer activities [1, 2]. In recent years, the plant has captured the attention of researchers that investigates skin diseases (especially of those that lead to skin cancer), due to its beneficial potential for prevention and treatment.

This study was aimed to investigate the influence of different aqueous and hydroalcoholic dandelion (leaves and flower) extracts on healthy human cells – keratinocytes and fibroblasts, both in normal and specific conditions like UV-irradiation. The extracts obtained were characterized by spectrophotometric techniques and were identified and quantified the main bioactive compounds. Cell viability, morphology and other specific effects exerted by the extracts (antioxidant activity, ROS measurement, and immunohistochemistry) were assessed. The concentrations of 10, 100, 250 and 500 µg/mL were added to irradiated cells before and/or immediately after irradiation, being noted a significantly protection.

The main conclusion from the data obtained has highlighted the superior protective activity of hydroalcoholic extracts as compared to aqueous ones on keratinocytes and fibroblasts against UV, at lower concentrations. Future studies aim to evaluate the activity of extracts obtained on tumor cells of skin cancer.

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FIRST STUDY ON PROPOLIS COLLECTED BY THE STINGLESS BEE *LISOTRIGONA CACCIAE* (NURSE)

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Propolis is a beehive product with biological and pharmacological properties. Nowadays, it is widely used as a tincture and as an ingredient in many commercial products. Propolis chemistry depends on the geographical origin, plant species and bee species, and thus various constituents contribute to its bioactivity. At present, the majority of scientific information is on propolis of honey bees (tribe Apini), but in tropical regions the native bee species are stingless bees (tribe Meliponini), which are also key pollinators as well as producers of traditionally used by humans honey and propolis.

In Southeast Asia stingless bees with unique behaviour have been recorded, such as minute lachryphagous species of a rare genus *Lisotrigona* Moure [1]. Till now, there are no data about chemistry and bioactivity of propolis collected by *Lisotrigona cacciae*.

Our analysis of *L. cacciae* (Nurse) proplis from Vietnam resulted in isolation of new for propolis flavanes and xanthones, together with known resorcinols and triterpenes. Additionally, anacardic acids and tocotrienols were identified by GC/MS. Significant antibacterial activity for 70% ethanol extract and isolated compounds was determined. Triple botanical origin of the sample was also defined, consisting of *Dracaena cochinchinense*, *Cratoxylum cochinchinense* and *Mangifera indica*. *D. cochinchinense* is a new for propolis resin source.

The results obtained expanded the knowledge for the chemistry and plant sources of propolis which is of great importance for further standardization purposes.

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ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF EXTRACTS FROM CALLUS CULTURES OF *ASTRAGALUS VESICARIUS L. SSP. CARNIOLICUS* (A.KERN.) CHATER

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Astragalus L. is the largest genus in Fabaceae family. It contains perennial herbs or shrubs, widely distributed in Europe, Asia and North America. *A. vesicarius ssp. carniolicus* is located in Italian Alps and the Balkan region. It has been used in Bulgarian folk medicine as diuretic, to treat high blood pressure and menstrual disorders. It has antioxidant, antitumor and hepatoprotective activities [1].

The aim of the study is to determine the antioxidant and antiproliferative activities of ethylacetate extracts from callus cultures of *A. vesicarius ssp. carniolicus*.

For establishment of callus cultures Murashige and Skoog's medium supplemented with 1 g/l casein, 0.1 mg/l 2.4-D, 2 mg/l kinetin and 0.2 mg/l IAA was used. The ethylacetate extract from callus cultures of *A. vesicarius ssp. carniolicus* was investigated for its antioxidant and antiproliferative activities. The total flavonoids content was 5.56 mg/g DW determined by LC/MC analysis. After enzymatic hydrolysis of ethylacetate extract with β-glucosidase, by means of conventional column chromatography, LC-MS/MS and ^{1}H NMR were identified coumarochromones types aglycones. They have high antioxidant activity evaluated with radical scavenging method using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and determined spectrophotometrically. The coumarochromones are characterised by their high levels of antproliferative activity and they were tested *in vitro* against different cancer cell lines.

Acknowledgements: Financial support from the Bulgarian National Scientific Fund, Contract № DN03/6/17.12.2016, is acknowledged.

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GUT MICROBIOTA BIOTRANSFORMATION OF THE EXTRACTS OF MEDICAL PLANTS USED TRADITIONALLY IN URINARY TRACT INFECTIONS TREATMENT

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Urinary tract infections (UTIs) are one of the most prevalent bacterial infections, affecting 150 million people each year worldwide. While treating UTI, novel medicine struggles with the issues of multi-drug resistance of uropathogens and UTI recurrence. Searching for a therapy alternative to antibiotics may led to reconsideration of testing and usage of traditional UTI treatment methods, based on plant-origin medical remedies [1, 2].

Human intestinal microbiome influences nutrition, immunological functions and bioavailability of xenobiotics. It can also perform biotransformation of compounds before their intestinal absorption. Describing gut microbiota influence on the form of constituents of natural products used in the UTI treatment is important task in the process of investigating mechanisms of their activity [3].

Presented study focuses on phytochemical characterization of water infusions of medicinal plants used traditionally in treatment of UTIs (i.a. *Filipendula ulmaria*, *Solidago virguarea*, *Polygonum aviculare*) and investigating the influence of gut metabolism on the constituents of the extracts. Gut-microbiota biotransformation of the extracts was performed by incubating the extracts with faecal samples. Different times of incubation (from 2 to 24 h) were tested to describe the process. UHPLC-DAD-MS^n was used for the phytochemical screening and analysis of the post-incubation media. Various metabolites, that could be responsible for therapeutic activity, were detected (i.a. aglycons, urolithins).

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EPIGENETIC CHANGES DRIVEN BY SEA BUCKTHORN DERIVED UNSATURATED FATTY ACIDS IN NORMAL AND TUMOR CELL LINES

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Recently, the impact of natural compounds on human chronic diseases, including cancer, started to be studied intensively in order to understand the molecular basis of their benefic properties. Cancer initiation and development is driven by genetic mutations and epigenetic dysregulations. DNA methylation is the major epigenetic regulator of gene expression and its alteration might occur in early stages of tumorigenesis. Emerging evidence suggests that natural compounds, including unsaturated fatty acids, have the ability to modulate the epigenetic events. Epigenetic alterations involving DNA methylation and histone modifications are currently targeted by anticancer therapies, due to their reversibility potential. Sea-buckthorn oil is used in traditional medicine for centuries and has demonstrated antioxidant and anti-cancer properties. Here we present the impact of sea-buckthorn derived unsaturated fatty acids on DNA methylation mechanism of normal versus tumor cells. Cytotoxicity profile of purified unsaturated fatty acids on cells was assessed by end-point assays (MTS, LDH). DNA methylation level and DNA methyltransferases (DNMTs) activity was quantified by ELISA. The pattern of global DNA methylation and DNMTs expression levels were measured by immunofluorescence and qRT-PCR was used for DNMTs mRNA quantification. Unsaturated fatty acids treatment on tumor cells decreased DNA methylation level correlated with decreased DNMTs expression and enzymatic activity. No significant modifications were observed on normal cells. The epigenetic modulator capacity of unsaturated fatty acids on other epigenetic modifications such as histone post translational changes and non-coding RNA expression will be further investigated.

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THE USE OF ARTIFICIAL LIGHT TO REGULATE THE BIOSYNTHETIC ACTIVITY OF MEDICINAL MUSHROOMS

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Mushrooms, one of the most ancient organisms of our planet, have a photoregulating system of mycochrome. Are well-known mushrooms such responses on low-intensity radiation, such as regulation of speed and growth vector, control of the implementation of the genetic program of individual development? The wavelength of light affects a number of metabolic processes.

The research of influence of light with different wavelengths on the biosynthesis of polysaccharides (*Ganoderma lucidum*, *Lentinus edodes* and *Hericium erinaceus*), melanin's (*Inonotus obliquus*) and carotene pigments (*Laetiporus sulphureus*) was carried out. Irradiation of *G. lucidum*, *L. edodes* and *H. erinaceus* sowing mycelium with blue and red light increased the polysaccharides accumulation by 40-64%. Irradiation significantly affects the carbohydrate composition of *G. lucidum* exopolysaccharides. The maximum of melanin's accumulation by *I. obliquus* was observed under the influence of blue light – 10.5 g/l (in control – 6.05 g/l), and the maximum of biomass increase and carotene pigments accumulation by *L. sulphureus* – under red light irradiation. The influence of low-intensity laser light with a wavelength of 633 nm (He-Ne laser) on antibiotic activity of *P. ostreatus* under submerged cultivation was investigated. Antibiotic activity of extracts from mycelium and cultural liquid against *Micrococcus luteus*, *Staphyloccocus aureus,* and *Bacillus mycoides* increased by 50-100%.

The obtained results prove the prospects of the use of light to increase biological activity of investigated mushrooms.

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PHOTOREGULATION OF SOME MEDICINAL MUSHROOMS ANTIMICROBIAL ACTIVITY

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Research on the factors that regulate antimicrobial activity is limited the selection of nutrient media and sources of nitrogen and carbon. Despite the large number of studies in this area, the search for new environmentally friendly growth regulators and the biological activity of fungi remains relevant.

We first studied the effect of low-intensity light on the antimicrobial activity of mushrooms and the possibility of using it to intensify the technological stages of mushrooms cultivation and increasing the yield of the final product. We found that low-intensity light with an energy of 230 mJ/cm^2, obtained from laser and LED sources, has a significant impact on the antimicrobial activity of *Flamulina velutipes*, *Pleurotus ostreatus*, *Ganoderma lucidum* and *G. applanatum* during submerged cultivation. Short-term exposure of seed spawn in red and blue wavelengths helped to reduce the cultivation period before the antimicrobial activity. Coherent (laser) light had a large stimulating effect compared to low-coherence (LED), and exposure to them in given modes increased the inhibitory activity of the culture fluid in relation to different test cultures for *F. velutipes* by 60-150%, *P. ostreatus* - 100-238%, *G. applanatum* - 30-87% and *G. lucidum* - 30-70%.

These results demonstrate the promise of using low-intensity light as an environmentally friendly stimulator of antimicrobial activity in biotechnology for the submerged cultivation of mushrooms.

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ANTI-DIABETIC ACTIVITY OF NOVEL THEOPHYLLINE DERIVATIVES ON HIGH FAT DIET AND LOW DOSE STZ INDUCED DIABETES IN RATS

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The intake of diet rich in fat and sucrose leads to metabolic complications such as obesity, insulin resistance, diabetes and renal disorders. Natural drugs have too many unknown facts about them while a synthetic drug is heavily tested to make sure it is the best product for human consumption. Hence the present study was aimed to explore the anti-diabetic activity of novel theophylline derivatives. *In vitro* α-amylase inhibitory activity and *In vivo* anti-diabetic activity was performed by HFD and STZ in rats. Control group received citrate buffer, treatment groups received compounds 16 (but-3-yn-lyl(1-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7-purin-7-yl) acetate and 17 (but-3-yl (1,3-dimethyl-2,6-dioxo1,2,3,6-tetrahydro-7-purin-7yl) acetate (10 mg/kg, p.o.), while that of standard group received pioglitazone (10 mg/kg, p.o.) at the end of the study blood samples were collected plasma glucose levels and lipid profiles were assessed and pancreas and liver were collected for histopathologically assessed. Test compounds i.e., Compound-16 and Compound-17 when compared with standard Compound-16 showed significant decrease in Plasma glucose ($P<0.001$), Triglycerides ($P<0.05$), Total cholesterol ($P<0.05$), LDL ($P<0.01$) levels and increase in HDL ($P<0.05$) levels. Compound-17 showed decrease in Plasma glucose and Total cholesterol ($P<0.001$), Triglycerides and LDL ($P<0.05$) levels and increase in HDL ($P<0.001$) levels. In histological examinations of the liver and pancreas we observed a significant improvement after treatment. The compounds 16 and 17 has significant α amylase inhibition with IC_{50} values ≤ 50 µm when compared to standard positive control acarbose. Structure and biological activity relationship of the compounds showed the electron donating group enhanced the activity.

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INHIBITION OF HUMAN CYP3A4 ACTIVITY BY METHYLXANTHINE FRACTIONS ISOLATED FROM *PU-ERH* AND *BANCHA* TEA LEAVES

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Methylxanthines, natural products from plants, are part of the most consumed beverages worldwide, such as coffee, tea, and cacao. They are mainly represented by caffeine, theophylline and theobromine and have a variety of pharmacological effects [1, 2]. Methylxanthines are metabolized in the liver by the CYP1A2 isozyme, but other enzymes take part too. The aim of the present study is to evaluate the potential of methylxanthine fractions, isolated from *Pu-erh* and *Bancha* tea leaves, to affect CYP3A4 isoenzyme and to assess the risk of potential drug interactions. Methylxanthine fractions were extracted using an approved standard method and the yield from 50 g dried *Pu-erh* and *Bancha* tea leaves were 0.521 g (or 1.04%) and 0.475 g (or 0.95%) respectively. The activity of human CYP3A4 was analyzed using Vivid P450 screening kit. Methylxanthine fractions have shown concentration-dependent inhibition of CYP3A4. After analyzing the results, we calculated IC_{50} values for each fraction with the 95% confidence interval (CI). The IC_{50} values for the fraction isolated from *Pu-erh* were 1.283 mg/ml (CI 0.9336 to 1.762 mg/ml) and for the fraction isolated from *Bancha* – 1.351 mg/ml (CI 1.119-1.631 mg/ml). The values for the both fractions were close, therefore can be assumed, that they approximate as qualitative and quantitative composition and activity. In conclusion, methylxanthine fractions potently suppress human CYP3A4 activity and can result in an increased risk for the occurrence of drug interactions.

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MULBERRY SEEDS OIL AS A NEW SOURCE OF PHYTOCHEMICALS AGENTS

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Mulberry (*Morus nigra* L.) to being an ancient Chinese plant, but is widespread on the Balkan Peninsula and belonging to the Moraceae family. All parts of the plant have nutritionally valuable components. For research plant material was collected on the territory of the Serbia, from dried seeds essential oil was isolated using the Clevenger method. The isolated oil was analyzed on the GC-FID chromatographic method. Biological activity of isolated oil was investigated. Antioxidant potential was analyzed using *in vitro* antioxidant assays: ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) and CUPRAC (cupric reducing antioxidant capacity). Enzyme inhibitory effects were tested against cholinesterases, glucosidase and amylase. The analysis found that the dominant fatty acids were linoleic and palmitic [81.13 and 8.45% (m/m), respectively]. The results of the biological activity showed exceptional antioxidant and enzyme inhibitory activity. Of the applied antioxidative assays, the highest efficiency was shown by ABTS, while strong inhibitory activity was expressed in enzymes responsible for the metabolism of carbohydrates (α-amylase and α-glucosidase). The comprehensive utilization of mulberry, rich in functional components, particularly those that are less widely used in culinary and medical applications, is still rare. Processing of this plant remains an interesting and useful task, particularly in discovering new sources for functional foods and nutraceuticals.

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ELEMENTS CONTENT AND ANTIOXIDANT ACTIVITY OF *SEMPERVIVUM TECTORUM* L. FROM BULGARIA

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Sempervivum tectorum L. is an evergreen plant belonging to a large family of the Crassulaceae with crassulacean acid metabolism. Fresh juice from squeezed leaves of *S. tectorum* is used as a folk medicine almost exclusively for external purposes. In recent years, studies have focused on the concentrations of the potential bioactive compounds in *S. tectorum* as well as their antioxidant activity, detoxification properties, and other important for human health plant properties. The objective of this study was to investigate the level of elements Ca, K, Na, Mg, Mn, Fe, Zn, Cu, Co, Al, Cr, Ni and other as a total content in plant samples of *S. tectorum* obtained from different regions in the country. Additionally the bioavailable fraction of essential elements Ca, Mg, Fe, Mn, Zn, analyzed after extraction with hydrochloric acid, which mimics processes of food digestion in the stomach, was determined as percentage of total content. Results showed high bioavailability of important for human health elements like Ca, Mg, Fe and Zn. They demonstrated high concentrations of K, Mg, Ca, Zn and Cu which could be accepted as additional explanation of antiinflammatory action of this juice. The antioxidant activity and some essential elements are determined in different of ethanol extracts - 10%, 50% and 95% (v/v), highest in 50% ethanol extract.

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COMPARATIVE PHYSICICHEMICAL ANALYSIS OF ESSENTIAL OILS DERIVED FROM *NIGELLA SATIVA* AND *CORIANDRUM SATIVUM* L

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The black cumin (*Nigella sativa*) and coriander (*Coriandrum sativum* L.) are well-known representatives from Ranunculaceae and Apiaceae families respectively. The essential oil and seeds from black cumin have been known for their physiological activity such as immunomodulating, antimicrobial and antioxidant properties and its effective use in treating respiratory conditions. The oil from coriander seeds has been known to alleviate rheumatoid, joint and gastro-intestinal conditions.

The aim of the present study is to evaluate the physicochemical properties of essential oils derived from black cumin and coriander. The UV and fluorescent spectra of have been measured. The fatty acid profile for both oils has been obtained, indicating the predominant constituent of the oil to be linoleic acid (C 18:2) in 58.2% and 42.29% respectively. The coriander oil has a lower (8.2 h) compared to that of the black cumin oil (12 h). Differential scanning calorimetry (DSC) was used to masure the enthalpy changes and phase transitions in both oils samples.

The concentrations of some elements (essential and toxic) in the oils were determined after acidic decomposition by using inductively coupled plasma mass spectrometry (ICP-MS). Infrared spectroscopic experiments (ATR and transmittance) were used to study the fatty acid profile of the analysed oil mixture. The acid value was determined by measuring the infrared absorbance of the oil in diluted CCl_4 solution.

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METABOLITE PROFILING OF THE MICROALGAE *PHAEODACTYLUM TRICORNUTUM*

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There is an increasing interest in the pharmaceutical and nutraceutical industry to explore resources from marine organisms, for instance microalgae. These organisms contain a considerable amount of high quality oils (omega-3 and omega-6 fatty acids) and pigments, which are used in pharmaceutical and nutraceutical application. The aim of our work is to isolate secondary metabolites from the microalgae *Phaeodactylum tricornutum*. Structural elucidation is performed by comprehensive one- and two-dimensional NMR spectroscopic analysis as well as HR-ESIMS measurements. Fucoxanthin, was the first compound isolated. It shows some activity against *Bacillus subtilis* and *Aliivibrio fischeri* as well as activity as antioxidant.

By means of GC-MS measurements and NIST database supported analysis, fatty acids isolated from the algae were determined as arachidonic acid, docosahexanoic acid, methyl-6,9,12,15,18-heneicosapentaenoate, octacosanol, and α-tocopherol.

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PHYTOCHEMICAL ANALYSIS AND SUPPLEMENTATION OF *RAPHANUS SATIVUS* VAR. *SATIVUS* ETHANOL EXTRACT IN THE MITIGATION OF CARBON TETRACHLORIDE INDUCED LIVER TOXICITY IN SPRAGUE DAWLEY RATS

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The medicinal vegetable *R. sativus* contains several biologically active compounds that can be used for the treatment of human ailments. The present study is attempted to determine total phenol and flavonoid contents of *Raphanus sativus* var. *sativus* ethanolic extract spectrophotometrically and to investigate the effect of its fresh rhizome water extract on carbon tetrachloride (CCl_4) induced liver toxicity in Sprague Dawley rats with its possible mechanisms. Rats were induced with hepatic toxicity using carbon tetrachloride and after 4 weeks of treatment with *Raphanus sativus* ethanol extract, liver samples were collected for detection of antioxidant and anti-inflammatory biomarkers spectrophotometrically. On the other hands, the blood was collected and its serum was used for assessing lipid profile and biochemical analysis. Meanwhile, stained liver tissues with hematoxylin-eosin were used for observing histopathological changes. We found that the intragastric administration of *R. sativus* (50 and 100 mg/kg) ethanol extract is significantly reduced and overcome hepatotoxicity induced by CCl_4 effectively through free radicals scavenging and boosting the antioxidant capacity of the liver. Finally, we concluded that the hepatoprotective effect of the rhizome could be attributed to its high phenolic and flavonoid contents.

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NATURALLY DERIVED COMPOUNDS TO ELIMINATE HUMAN PAPILLOMA VIRUS (HPV)-INDUCED CARCINOMAS

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Human papillomavirus (HPV) infection is one of the major health issues of our time and is responsible for several types of cancers and other dangerous diseases. Based on a report from the Centers for Disease Control and Prevention (CDC), approximately 110 million people in the USA alone are already infected with HPV, and about 20 million new cases of HPV infection is reported each year (www.cdc.gov). Although, it is known since around 1983 that HPV cause cancer and papillomas, no specific therapeutic treatments against HPV exists. We have already successfully shown a natural diterpenoid Anisomelic acid (AA) down-regulates E6 and E7 oncoproteins which are the root cause of HPV. During the optimization of AA synthesis, we have identified few small molecules, which preferentially target the HPV E6 and E7 proteins similar to the AA mode of action, but are more efficient than AA in inducing apoptosis in cervical cancer cells. Furthermore, these molecules also showed specificity in killing HPV positive cells with different genotypes compared with human primary skin fibroblast cells. Indeed, cancer xenograft models in nude mice demonstrated a proof of principle, where a decrease in tumor size was observed in HPV-driven tumors treated with our compounds. These fundamental discoveries served as the basis for a successful awarded grant from Novo Nordisk and TEKES-Business Finland Investment Funds to further develop this compound toward a lead molecule, a key milestone in the drug discovery process.

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PROTECTIVE EFFECTS INDUCED BY ALCOHOLIC *PHLOMIS FRUTICOSA* AND *PHLOMIS HERBA-VENTI* EXTRACTS IN ISOLATED RAT COLON: FOCUS ON ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITY *IN VITRO*

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Phlomis fruticosa L. and *P. herba-venti* are species belonging to the Lamiaceae family, which have been traditionally used to prepare tonic and digestive drinks. Multiple studies also demonstrated the inhibitory effects of *P. fruticosa* extracts and essential oil against oxidative/pro-inflammatory pathways and bacterial strains deeply involved in ulcerative colitis. Considering these findings, in the present study we evaluated the effects of alcoholic *P. fruticosa* and *P. herba-venti* leaf extracts in isolated rat colon challenged with *E. coli* lipopolysaccharide (LPS). The results were compared with sulfasalazine used as reference drug. Additionally, the extracts have been tested in order to evaluate the possible inhibitory role on specific bacterial strains and fungi involved in ulcerative colitis. We found that alcoholic *P. fruticosa* and *P. herba-venti* extracts were able to blunt LPS-induced nitrite, MDA, 5-HT and LDH level, in isolated rat colon. Additionally, microbiological studies revealed that both extracts inhibited the growth of multiple bacterial and fungi strains, such as *P. aeruginosa*, *E. coli*, *S. aureus*, *C. albicans* and *C. tropicalis*.

Taken together, our findings show protective effects exerted by alcoholic *P. fruticosa* and *P. herba-venti* extracts on isolated colon, thus supporting further studies in order to confirm efficacy and safety in ulcerative colitis.

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HEAT STRESS CHANGES *MENTHA PIPERITA* ESSENTIAL OIL COMPOSITION AND ITS ANTIBACTERIAL ACTIVITY

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Heat stress compromises the yield of medicinal plants and reduces biomass and/or metabolite production. *Mentha piperita* L. (2n = 72) is a hexaploid medical plant hybrid of *M. spicata* L. (2n = 48 or 36) and *M. aquatica* L. (2n = 96) and is a perennial plant, belonging to Lamiaceae family, originating from Europe, wide spread and planted in all over the world in many different climates. *M. piperita* is one of the most important medicinal plants due to high consumption in the world and for the size of the area cultivated for essential oil production. Many medical usages are reported for *M. piperita* to treat intestinal colic, spasms of the bile duct, dyspepsia, biliary disorders, gastritis, flatulence, and enteritis. This plant has also high antioxidant, antitumor, antiallergenic, antiviral, antibacterial, fungicidal and antimicrobial activity and its chemical components include: fatty acid, mineral content, vitamins, carotenoids, chlorophylls, polyphenol and volatile oil. *M. piperita*'s volatile oil generally includes: menthol, menthone, isomenthone, 1,8-cineole (eucalyptol), menthyl acetate, menthofuran, limonene, β-myrcene, β-caryophyllene, pulegone and carvone.

Here we report the chemical composition of the essential oils from *M. piperita* samples, which underwent different heat stresses in growth chambers. The antibacterial activity of the essential oils was also evaluated; microscopic observations (fluorescence and electron transmission) were used to assess the effect of the oils on bacterial growth. The results shed light on the mint essential oils' composition and biological activity in relation to heat stress.

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IN VITRO MULTIPLICATION OF RARE *VERONICA CAUCASICA* M. BIEB

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Veronica caucasica has limited distribution and is found only in the Northern Caucasus. The plants from genus *Veronica* have long history of use in ethnomedicine as diuretics, wound-healing remedies and for treatment of cancer, inflammatory and respiratory diseases. In the present work we aimed to develop a protocol for *in vitro* multiplication of *V. caucasica* and its subsequent *ex situ* conservation. *In vitro* shoot culture of *V. caucasica* was induced from ripe dry seeds and micropropagated on basal MS medium with 30 g/L sucrose and 7 g/L agar. To stimulate the growth index, the effect of different concentrations of cytokinin BA (6-benzylaminopurine) on the *in vitro* multiplication of plants was examined. All tested concentrations of BA (0.1; 0.5; 1.0 mg/L) stimulated shoot development but more effective was MS medium supplemented with 0.5 mg/L BA and approximately 96% of explants showed shoot proliferation and produced 12.80 ± 0.2 shoots per explant. *Ex vitro* adaptation was accomplished in growth camera with 92% survival. No accumulation of ROS by staining with 3-3´-diaminobenzidine and 2´,7´-dichlorofluorescein diacetate and very low amount of stress-marker metabolite malondialdehyde were detected in *in vitro* propagated and *ex vitro* adapted plants. The successful initiation of *in vitro* and *ex vitro* cultures is an alternative biotechnological approch for preservation of *V. caucasica* and would allow further analysis of metabolite profile and selection of lines with high production of valuable secondary metabolites.

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A ROLE OF EXTRACTS FROM *MILLEFOLII HERBA*, *SERPYLLI HERBA* AND *ARCTII LAPPAE RADIX* IN CYTOKINES SECRETION BY HUMAN NEUTROPHILS

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Upon skin injury, rapid closure of the wound is critical to restore the barrier function. Neutrophils constitute the first line of defence of the innate immune system by phagocytosing, killing microbes. A wide-range of plant materials are traditionally recognized as medicines in the treatment of skin disorders. Among them *Millefolii herba*, *Serpylli herba* and *Arctii lappae radix* are mentioned. The aim of the study was an examination of the extracts activity on inflammatory response of human neutrophils as well as a determination of their phytochemical composition. Aqueous and 70% ethanolic extracts were prepared from 3 plant materials. The effect of the extracts on the secretion of pro-inflammatory cytokines, such as IL-8 and TNF-α on lipopolisacharide (LPS)-induced human neutrophils using ELISA assays was evaluated. Moreover, the changes of luminol chemiluminescence to assess a generation/scavenging of ROS were measured. Cytotoxicity of the extracts on human neutrophils stained with propidium iodide was also checked. The phytochemical analysis of the extracts was performed using HPLC-DAD-MS^n method. The extracts (25-100 µg/mL) showed the modulation of the IL-8 and TNF-α production. All extracts induced the overproduction of both cytokines. In the higher concentration the more relevant production of IL-8 and TNF-α was noted. The aqueous extract of *M. herba* at concentration of 100 µg/mL stimulated secretion of IL-8 (251.5 ± 26.3%) and TNF-α (277.8 ± 48.2%) in comparison to LPS(+) control (100%). In conclusion, the tested extracts might be engaged in wound healing through overproduction of cytokines in neutrophils.

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GC AND GC-MS ANALYSIS OF VOLATILE COMPOUNDS FROM *BALLOTA NIGRA* SUBSP. *UNCINATA* COLLECTED IN SICILY (SOUTHERN ITALY)

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The genus *Ballota* belongs to the Lamiaceae family and comprises about 30 species widespread all over the world. *Ballota nigra* is commonly distributed in Western, Eastern and Central Europe, where is used to treat inflammation, as an antiseptic for wounds, and against gastrointestinal disorders [1]. Due to the importance of the genus and in the frameshift of our research work on volatiles from *Ballota* species [2, 3], in this paper we report the composition of the essential oil obtained from aerial parts of *B. nigra* L. subsp. *uncinata* (Fiori et Beg.) Patzakis, a perennial herb bearing simple hairs whose anti-listerial activity against *L. monocytogenes* has been demonstrated [4]. For our study, aerial parts of the plant were collected on Lipari (Sicily, Southern Italy), in July 2016. The essential oils, extracted by hydrodistillation according to the *European Pharmacopoeia*, were analysed by GC and CG/MS: peak identification was accomplished by comparison of their mass spectra with NIST 08 and Wiley 275 libraries, as well as by comparison of their retention indices with literature values. Results showed that the major components of *B. nigra uncinata* were (*E*)-phytol (20.0%), α-pinene (9.0%) and hexahydrofarnesyl acetone (5.7%). On the whole, in the oil the sesquiterpene hydrocarbons (30.7%) and diterpenes (21.5%) were the most representative fractions. This is the first report on the chemical composition of the essential oil of *B. nigra* L. subsp. *uncinata* collected in Sicily.

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NEUROTROPHIC ISOINDOLINONES FROM THE FRUITING BODIES OF *HERICERIUM ERINACEUS*

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Hericium erinaceus is an edible and medicinal mushroom which has been traditionally used to improve memory and enervation and for the treatment of gastric ulcer [1]. In this study, neurotrophic constituents were identified to support the traditional usage of *H. erinaceus*. The components of *H. erinaceus* were purified using various column chromatography techniques. The structure of the separated compounds was determined based on spectroscopic data analysis, i.e., 1D and 2D NMR analysis. The neurotrophic activity of the isolated compounds was evaluated by measuring the secretion of neurotrophic factors such as NGF and BDNF and effect on neurite outgrowth in C6 glioma and N2a cells, respectively. Four compounds, hericerinol A (1), hericerin (2), *N*-de-phenylethylisohericerin (3) and corallocin A (4) were isolated from *H. erinaceus* [2]. Among them, hericerinol A (1) was newly reported in nature. Further investigation of the neurotrophic effect on isolated compounds demonstrated that hericerinol (1) strongly increased the NGF production in C6 astrocytes followed by corallocin A (4) and hericerin (2). Increased NGF production by these compounds promoted the neurite outgrowth in N2 neuronal cells. Western blot analysis also showed the increased protein expression of NGF and BDNF in N2a cells. Our present study characterized the neurotrophic constituents of *H. erinaceus*, which support the traditional use of memory improvement.

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EFFECTS OF THE MESEMBRENONE RICH ALCALOID FRACTION FROM *NARCISSUS CV. "HAWERA"* ON THE ANXIETY, DEPRESSION-LIKE BEHAVIOR AND DIABETES MELLITUS IN RATS

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Mesembrine type alkaloids containing plants have a long history of traditional use, but recently it is emphasis on their use as supplements in maintaining a sense of well-being, and for the treatment of anxiety, stress and major depression disorder. Diabetes mellitus is characterized along with metabolic disturbance also with elevated anxiety and depressive-like behavior. The aim of present study was to study the effects of enrich mesembrine fraction (MZM) from *Narcissus cv. "Hawera"* on the anxiety and depressive-like behavior in healthy female Wistar rats and in female Wistar rats with an experimental model of diabetes mellitus type 1 (T1DM).

Using Gas chromatography–mass spectrometry (GC-MS) were established that mesembrenone represented 64.1% of the total alkaloid fraction extracted from the leaves of *Narcissus cv. "Hawera"*. T1DM caused a significant decrease in overall motor activity, increased depressive-like behavior without altering the anxiety behavior in Open field, Forced swimming tests and Elevated plus maze, respectively. Chronic treatment with an effective dose of MZM significantly suppressed depressive-like behavior and reduced anxiety behavior in healthy rats without altering overall motor activity. MZM treatment decreased weight gain, water intake and urine output but did not influence significantly the behavioral parameters in rats with T1DM.

Present results suggested that MZM fraction effectively modulates anxiety and depressive-like behavior, takes a part in the mechanism of regulation of water balance, however it is not included in the diabetes-induced behavioral alterations.

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THE SUCCESSFUL BREEDING WORK OF MEDICINAL PLANTS IN SLOVAKIA

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The large-scale cultivation of medicinal, aromatic and spice plants in Slovakia belongs to the special agricultural production. It is an only way of supply the contracted volume and quality of these crops. From the current state of medicinal, aromatic and spice plants cultivation in Slovakia, it shows a partial increase of growing areas on arable land [ha], total production [kg] and yield per hectare [kg/ha]. Presented data were taken directly from the producers from the period of 2010 to 2017. There is shown, that milk thistle [*Silybum marianum* (L.) Gaertn], plantain (*Plantago lanceolata* L.), German chamomile (*Matricaria recutita* L.), mint (*Mentha ×piperita* L.), and lavender (*Lavandula angustifolia* L.) were the most cultivated medicinal plants from the acreage of arable land viewpoint. The special crop production at the

Slovakia is one of the European countries in which particular attention has been devoted to research of medicinal, aromatic and spice plants in all its aspects, including the breeding and selection. Based on the study of pharmacodynamics properties of several medicinal crops, the chamomile variety "Lianka" and the peppermint variety "Kristinka" were bred at the University of Presov, Slovakia, between the years 2008 – 2013. Currently, both varieties have the certificates by the Community Plant Variety Office in Angers, France. The chamomile variety is characterized by its high percentage of sequiterpenes (/-/-α-Bisabolol [52 – 55%], Chamazulene [18 – 19%], the low contents of /-/-α Bisabololoxides A and B [<3%] and essential oil content is from 0.65 to 0.85%). The peppermint variety has very high content the Menthol [70 – 75% of herbs and 80 – 85% of leaves] of essential oil [2.6%] into the dry raw material. There is a strong premise that the cultivation of these two varieties of medicinal plants throughout the European Union will be expanded in the coming years.

A long-term goal in the production of medicinal, aromatic and spice plants is the targeted expansion of growing areas with the simultaneous increase in the overall harvest and quality of the raw material suitable for the pharmaceutical, cosmetic and food industries.

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INFLUENCE OF ABIOTIC STRESS ON SPECIALIZED METABILITES LEVEL IN *BRASSICA OLERACEA* VAR. *ACEPHALA*

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Kale (*Brassica oleracea* var. *acephala*) is a cruciferous vegetable, characterized by leaves along the stem, which, in recent years, have gained a great popularity as a "superfood". This is trigged by the fact that scientific evidences support the idea that cruciferous vegetables included in human diet can positively affect health and well-being. Mainly, health benefits of cruciferous vegetables are associated with the presence of various phytonutrients from the glucosinolate, polyphenol and carotenoid group. Kale is also known as a crop which posses good tolerance for the extreme environmental condition such as drought and very high or low temperature. In the present study we aimed to evaluate how abiotic stressors, low temperature and drought, influence the level of the main specialized metabolites in kale. The level of analysed metabolites was influenced by development stage but also by abiotic stresses. In a stress response may be included mainly compounds from phenolc acids group, expecially synapinic acid, and glucosinolates.

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ESSENTIAL OIL YIELD AND COMPOSITION OF *SATUREJA PILOSA* VAR. *PILOSA* VELEN. (LAMIACEAE) FROM BULGARIA

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The genus *Satureja* L. (Lamiaceae) comprises five species in the Bulgarian flora. The plants are known for their biologically and pharmacologically active essential oils (EO). *Satureja pilosa var. pilosa* Velen (Lamiaceae) is a Balkan endemic plant, found on rocky outcrops on limestone base in Stara Planina (the Balkan Mountains), the Rhodope Mountains, and the Thracian Lowland. The objective of this study was to assess the variability of EO content and composition of *S. pilosa var. pilosa* populations in the Bulgarian flora. Overall, the EO content in dried aboveground biomass was around 2.3%. More than 20 EO constituents were identified, belonging to the groups of monoterpenes, sesquiterpenes, and palmitic and stearic acids. The monoterpene hydrocarbons and oxygenated monoterpenes were the predominant groups of compounds representing around 52% of the total oil, with thymol, p-cymene, and carvacrol being the major constituents of this group. Overall, thymol (around 36%) and p-cymene (around 26%) were the major oil constituents. The second largest group was the one of palmitic acid. The third largest chemical group comprised sesquiterpenes (sesquiterpene hydrocarbons and oxygenated sesquiterpenes). Overall, there was significant variation with respect to the EO content and composition between different populations. The results from this study can be utilized by researchers for the development of new cultivars of *S. pilosa,* and by the industry utilizing Satureja oil as flavor and fragrance component.

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PYRROLIZIDINE ALKALOIDS FROM *ALKANNA* SPECIES IN THE BULGARIAN FLORA

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The genus *Alkanna* (Boraginaceae) comprises about 50 species and shows greatest diversity in the southern part of the Balkan Peninsula and South Anatolia. Most species of the genus contain pyrrolizidine alkaloids and possess hepatotoxic, mutagenic, and cancerogenic activities. There are six *Alkanna* species in Bulgaria, five of which are either Balkan or Bulgarian endemic species. *Alkanna primuliflora* Griseb. and *A. stribrnyi* Velen., A. *graeca* Boiss. & Spruner are Balkan endemics. *Alkanna stojanovii* Kozuharov and *A. jordanovii* Kozuharov are the two Bulgarian endemics. The objective of this study was phytochemical investigation of the alkaloid composition of the Balkan endemic species *A. primuliflora Griseb. and A. stribrnyi Velen., A. graeca Boiss. & Spruner.* Eight pyrrolizidine alkaloids were identified by GC-MS. The main alkaloid in all investigated plants was 7-angeloylretronecine. *A. primuliflora и A. graeca* showed similar chemical composition that comprised 9-angeloylretronecine, 7-tigloylretronecine, 9-tigloylretronecine, triangularine, triangularicine, dihydroxytriangularine, dihydroxytrian-gularicine, whereas in *A. stribrnyi* 9-tigloylretronecine, triangularicine and dihydroxytrian-gularicine were not found. We continue with the research on the phytochemistry, genetics, and the embryology of these species.

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PLANT SOURCES OF MAURITIANIN AND EVALUATION OF ITS PROTECTIVE POTENTIAL

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Mauritianin is a flavonol glycoside, previously isolated from *A. monspessulanus* subsp. *monspessulanus* (Fabaceae). The cytoprotective effect of the compound was evaluated in a model of tert-butylhydroperoxide oxidative stress on rat hepatocytes [1]. Due to its rare occurrence in plants, there are only few studies of its pharmacological action. Up to date there is no information of its *in vivo* activity.

The aim of the present study was to investigate the chromatographic possibilities to isolate mauritianin from this species in sufficient quantity to conduct *in vivo* pharmacological evaluation of its antioxidant and hepatoprotective potential.

Administered alone, mauritianin proved non-toxic for the liver. It preserved the levels of malonedialdehyde (MDA) and reduced glutathione (GSH), and decreased the activity of the enzyme ethylmorphine N-demethylase (EMND) – a marker to evaluate the activity of the CYP3A isoform [2]. In a model of CCl4-induced hepatotoxicity, mauritianin displayed hepatoprotective and antioxidant effects, comparable to silymarin. The flavonoid decreased the level of MDA and preserved the level of GSH as well as the activity of EMND.

These findings will serve as a perspective for further investigations of mauritianin as possible hepatoprotector and antioxidant.

Acknowledgements: Financial support from Council of Medicinal Science at Medical University of Sofia, project № 8234/20.11.2018 is gratefully acknowledged.

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PHYTOCHEMICAL INVESTIGATION AND CYTOTOXICITY TESTING OF *POLEMONIUM CAERULEUM* EXTRACTS

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Polemonium caeruleum L. is a Chinese medicinal plant used in the treatment of tuberculosis, whooping cough, fever, infertility, epilepsy, endometritis and insomnia. It was also used as sedative and anaesthetic agent. In this work the phytochemical investigation using LC-ESI-QTOF-MS and GC-MS techniques was done for chemical characterization of methanol extracts of underground and aerial plant parts. In the course of our studies on medicinal plants, we evaluated the extracts of *P. caeruleum* for their cytotoxicity towards mammalian cells – HEK293 and Vero cell lines, both originating from kidney and also against pharyngeal squamous cell carcinoma, FaDu cell line. LC-ESI-QTOF-MS analysis confirmed the presence of triterpene saponins – oleanane derivatives (polemoniumsaponins, glycosides of theasapogenol derivatives and β-amyrin among others), as well as flavonoid glycosides with most predominant acacetin derivatives. Saponins were more abundant and varied in the extract from underground parts of the plant, while flavonoids dominated in the extract from aerial parts. GC-MS performed after silanization enabled the identification of carbohydrates, fatty acid esters, amino acids and carboxylic acids. Carbohydrates were the major group of compounds in both extracts, mainly represented by α- and β-glucopyranose, and β-fructofuranose. Among esters of fatty acids, palmitic acid, linoleic acid and oleic acid methyl esters were distinguished. Carboxylic acids dominated in the underground part of *P. caeuleum* and were represented by malic, fumaric, 2,3,4-trihydroxybutyric, glycolic and lactic acid. The cytotoxicity of the extract from underground parts of *P. caeruleum* was in a range 44.5–63.5 µg/ml, while cytotoxicity of the extract from aerial parts ranged from 93.8 to 178.0 µg/ml.

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SIRTUIN ACTIVATION'S EFFECTS ON MURINE INFLUENZA A/H3N2-VIRAL INFECTION

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Sirtuins (Sirt) are enzymes which regulate the transcription of various genes involved in pro- or anti-inflammatory responses. They can alter intracellular pathways, transcription factor transport and chromatin remodelling, thereby modulating the host-cell defence and pathogen replication. Recently, the inhibitory effect of Sirt activators on viral replication *in vitro* has been reported [1] but evidences on their anti-infectious effects *in vivo* remain scarce.

The aim is to investigate how pharmacological Sirt activation can affect influenza infection *in vivo*. The SRT 2183 activator was administered s.c at a dose of 3, 10 and 30 mg/kg/day to ICR mice for 5 consecutive days. We monitored the survival and evaluated changes in lung viral titers, macroscopic and histological pulmonary parameters, and the phenotype of inflammatory cells in lung exudates on day 5 p.i. The antiviral response was compared to a group of oseltamivir-treated mice (10 mg/kg/day). The effect of SRT 2183 was studied in moderate ($1LD_{50}$) and severe infection ($1LD_{90}$).

The SRT 2183 failed to exert a protective effect on the survival of influenza-infected mice with mortality rates comparable to vehicle group in moderate and severe infections. The compound did not influence weight loss and pathological score indicative for pulmonary oedema. However, lung titers of SRT 2183-treated and challenged mice were with up to 1.33 Lg $CCID_{50}$ lower than in the PBS control. Lung exudate of drug-treated and infected mice was enriched with neutrophils but lacked the essential Ly6C+ population of monocytes as compared to the infected group.

We concluded that the SRT 2183 activator worsens the disease outcome most likely by interference with tissue specific inflammatory response mechanisms in infected lung.

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PROTECTIVE EFFECTS OF *NIGELLA SATIVA* OIL ON DEXTRAN SULPHATE SODIUM (DSS) INDUCED COLITIS IN MICE

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There is increasing evidence that the oxidative stress and inflammation play a critical role in the development and perpetuation of ulcerative colitis (UC) and Crohn's disease (CD). The treatment with conventional drugs has many limitations and adverse effects, not all patients respond effectively to the newer therapeutic options. Therefore, there is an increased need to discover effective and safe treatments for these types of inflammatory bowel disease (IBD). *Nigella sativa* (NS; Ranunculaceae) has many beneficial pharmacological properties such as anti-oxidant, anti-bacterial, anti-inflammatory, immunomodulatory etc. This study aimed to comparatively investigate the protective effects of NS oil (NSO), administered orally (2.5 ml/kg/day) and curcumin (42 mg/kg/day) for 7 days on DSS-induced mouse colitis model. Colitis is manifested by increased level of inflammatory markers as C reactive protein (CRP), nitric oxide (NO), myeloperoxidase (MPO) activity, and disturbed oxidative stress markers as reduced glutathione (GSH) and malondialdehyde (MDA). In addition, chemical composition of NSO was analyzed by GC-HRMS. NSO markedly attenuated colonic inflammation by inhibiting MPO activity and by decreasing the augmented levels of NO and CRP. NSO improved colon anti-oxidant defense machinery by decreasing the quantity of malondialdehyde (MDA), a marker of lipid peroxidation and increasing the level of GSH. NSO ameliorated colon injury and inflammatory signs as visualized by histopathological examination. Results revealed that NSO produced a comparable therapeutic effect as the positive control curcumin.

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PRELIMINARY ANALYSIS OF THE CHEMICAL COMPOSITION AND ANTIOXIDANT AS WELL ANTI-INFLAMMATORY ACTIVITY OF EXTRACTS FROM ELDERBERRY LEAVES

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Black elder (*Sambucus nigra* L.) is a shrub from the Adoxaceae family wildspread throughout Poland. Elderberry flowers and fruits are common plant materials used internally to treat colds and to suport immune system [1]. Much less popular, elderberry leaves are mainly used externally to treat wounds, burns, inflammation of the skin and haemorrhoids in folk medicine [2].

Aqueous and 70% (v/v) ethanolic extracts from elderberry leaves were prepared by maceration at room temperature and under reflux. Antioxidant and anti-inflammatory activity was tested in cell-free systems. The ability of the studied extracts to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), hydrogen peroxide (H_2O_2), nitric oxide (NO) and superoxide anion radical ($O_2^{\cdot-}$) was tested with spectrophotometry, luminescence and fluorescence. In addition, the effect of extracts on the activity of xanthine oxidase and lipoxygenase was examined. The content of phenolic compounds was determined by the Folin-Ciocalteu method, and the analysis of the chemical composition was carried out by HPLC-DAD-MS^n.

The antioxidant properties of the tested extracts were confirmed. Statistically significant differences were observed between aqueous and ethanolic extracts. The extracts have a strong NO scavenging ability (SC_{50} from 1 to 8 µg/mL), while SC_{50} for DPPH, H_2O_2 and $O_2^{\cdot-}$ ranges from 50 to 120 µg/mL. The phytochemical analysis revealed the presence of phenolic acid derivatives and their conjugates as well as flavonoids, kempferol and quercetin derivatives wich are likely to be responsible for extracts antioxidant activity.

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NEW TRITERPENOID ACIDS FROM FUNGUS *FOMITOPSIS BETULINA*

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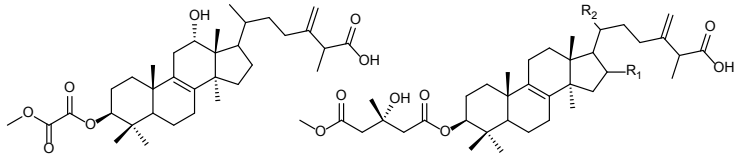
One of the most important groups of secondary metabolites in fungi is triterpenoid acids, the compounds with relevant biological and pharmaceutical properties. In the previous studies SAR was investigated and their cytotoxic effects were reported [1].

As a part of our research, we present isolation and chemical structure elucidation of three new triterpenoid acids from fungus *Fomitopsis betulina* collected in village Oglađenovac, near Valjevo. The crude extract was obtained from the air dried and powdered basidiocarp using the mixture of methylene chloride and methanol, while the ethyl acetate was used for the re-extraction. The final extract was fractioned by column silica gel chromatography and nine triterpenoid acids were isolated by semi-preparative HPLC. Four of these compounds were previously reported from the same species: polyporenic acid A, polyporenic acid C, piptolinic acid B and betulin and two were reported from *Poria cocos* - poricocic acid H and pachymic acid. The compounds 12*α*-hydroxy-3*β*-[(2′-methoxy-2′-(oxo)acetil)oxy]-24-methylenelanosta-8-en-26-oic acid (**1**), 3*β*-(3′-hydroxy-4′-methoxycarbonyl-3′-methylbutyryloxy)-21-hydroxy-24-methylene-lanosta-8,24-(31)-dien-26-oic acid (**2**) and 3*β*-(3′-hydroxy-4′-methoxycarbonyl-3′-methylbutyry-loxy)-16-oxo-24-methylenelanosta-8,24-(31)-dien-26-oic acid (**3**) are the new triterpenoid acids we have isolated from *F. betulina*. The structure elucidation was established using 1D (^{1}H and ^{13}C) and 2D NMR (COSY, NOESY, TOCSY, HSQC i HMBC), UV, IR and HRMS.



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INFLUENCE OF DRYING METHODS ON BIOACTIVE COMPOUNDS FROM *AMARANTHUS CAUDATUS* WASTE

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Plants are an inexhaustible source of bioactive compounds that play an essential role in human health. Therefore, researchers studies are increasingly focusing on improving the ways of separation and purification of bioactive compounds from plants.

The aim of this research paper is to investigate the effects of drying methods on bioactive compounds such as phenols, flavonoids, betalains, etc. from *Amaranthus caudatus* L. aerial parts waste. In this investigation, various drying methods including freeze-drying, oven drying, microwave drying and convective air-drying were applied. In order to obtain a favorable extraction yield, the solvent extraction method by application of the ultrasounds effect on the dried vegetal material was chosen. In order to identify the content of total phenols, flavonoids or betalains, modern chromatographic techniques have been used. The results obtained following the study showed a favorable statistical significance confirmed by factorial analysis of variance (ANOVA). Moreover, depending on the target bioactive compound, one of the investigated drying methods can be chosen, being well known that drying is the most common method of plant preservation and therefore also applied in the case of waste from *A. caudatus* aerial parts.

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EFFECTS OF *NELUMBO NUCIFERA* PETAL EXTRACTS ON BACTERIAL KILLING CAPACITY AND RESPIRATORY BURST OF HUMAN MONOCYTES

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Phagocytosis and respiratory burst are key mechanisms of neutrophil and monocyte in the body defense against infection [1]. Enhanced phagocytic function of murine neutrophil by rhizome and seed extracts of *Nelumbo nucifera* has been demonstrated [2]. This research investigates immunomodulatory activity of ethyl acetate (EA) and ethyl alcohol (ET) *N. nucifera* petal extracts on bacterial killing capacity and respiratory burst induction. Extraction of the petal was succeeded by maceration. HPLC-DAD analysis revealed that phytochemical profile of EA lotus petal extract closely resembled that of ET extract. Effect of the EA and ET petal extracts on phagocytic activity was assessed by *ex vivo* bacterial phagocytosis assay in human peripheral blood mononuclear cells (PBMCs) then the number of viable intracellular bacteria was measured by standard plate count method. The results showed that pretreatment of PBMCs with the extracts significantly increased intracellular killing of the engulfed *Staphylococcus aureus* and *Escherichia coli*. In addition, both extracts markedly increase the reactive oxygen species production in monocytes after analyzed by flow cytometry. Taken together, this research demonstrates immunomodulatory activities of *N. nucifera* petal extracts on enhancing bacterial killing capacity of human monocytes by induction of respiratory burst production.

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NEWER OPTIMIZATIONS PROTOCOLS FOR OVERPRODUCTION OF SECONDARY METABOLITES FROM PLANT CELL/HAIRY ROOT CULTURES

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Plants have an amazing ability to produce several important secondary metabolites for society. However the major drawbacks in the commercialization of natural plant based metabolites production are their seasonal availability and low content of bioactive compound. Besides, the highest content of the plant metabolites is usually in the roots. Therefore the plants are uprooted and recovery of the strategic metabolite in root is done by solvent extraction. This leads to extinction of rare plants primarily due to their overexploitation for production of secondary metabolites. Several attempts have been made in our laboratories to do *in vitro* cultivation of the specialized plant cells/hairy roots in the bioreactors wherein innovative bioreactor designs and use engineering optimizations have not only eliminated the dependence on natural plants but also helped to significantly increase the concentration, yield and productivity of bioactive compounds to facilitate commercialization of plant based metabolites for the growing needs of society.

The above methodology(ies) and some newer bioreactor designs will be presented for the mass production of key secondary metabolites using plant cell/hairy root cultivations in the bioreactor.

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SUSTAINABLE PRODUCTION OF CAMPTOTHECIN FROM AN *ALTERNARIA* SPECIES ISOLATED FROM *NOTHAPODYTES NIMMONIANA*

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Camptothecin, the third most in-demand alkaloid, is commercially extracted in India from *Nothapodytes nimmoniana*. However, due to extensive uprooting from the wild, its population is now endangered in the country. Our research aims at establishing a microbial fermentation based production platform as a sustainable and alternative source of camptothecin. In this regard, bioprospecting of potential microbial strains was carried out from the plant parts of *N. nimmoniana*. Leaf, petiole, stem and bark explants of *N. nimmoniana* were surface sterilized and incubated at 28 °C for 7 days on potato dextrose agar medium. A total of 132 strains were isolated, 94 among them were found to be producing camptothecin in axenic culture based on HPLC (quantitative) and LC-MS/MS (qualitative) analysis. Six different fungal isolates were further selected, based on their high camptothecin yield (g/g biomass), for ITS sequence based characterization. ITS1 and ITS4 primers were used to amplify the conserved regions of 18S, 5.8S and 28S rRNA genes to identify the fungal species. Two fungal strains, *Alternaria alstroemeriae a*nd *Alternaria burnsii* which demonstrated highest camptothecin yield up to 426.7 ± 47.4 µg/g and 403.3 ± 58.7 µg/g, respectively, were investigated for sustainable production of camptothecin with subculture cycles under axenic state. Interestingly, the second highest yielding fungal strain isolated in this study, *A. burnsii,* could sustain the camptothecin production (up to ~200 µg/g in suspension culture) even after 10th subculture cycle of its stock culture maintained as fresh slants, for its use as inoculum for the suspension culture. An optimized bioprocess based on the submerged fermentation of *A. burnsii* will be developed further to achieve maximum productivity of camptothecin.

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FORMULATION OF MAGNOLOL IN ANTI-ACNE TOPICAL GEL: PHARMACO-TECHNICAL CHARACTERIZATION AND RHEOLOGICAL EVALUATION

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The current treatment of acne is complex and includes topical and / or systemic therapy with drugs in the class of anti-seborrheics, antibiotics, anti-inflammatory and keratolytic agents [1, 2]. The increasing resistance to antibiotic therapy is a major problem faced by doctors treating all forms of acne. The number of patients identified with antibiotic-resistant *Propionibacterium* species and *S. aureus* strains has increased greatly in the past few decades [3]. Given this, the need to formulate new anti-acne pharmaceutical preparations becomes a priority.

The aim of this study was to formulate and pharmaceutically characterize *in vitro* and *ex vivo* and to examine the rheological properties of Carbopol-based hydrogels using erythromycin as an active ingredient and the innovative combination of erythromycin and magnolol in cutanous therapy for acne. The results showed large variations in the release profile of erythromycin in gels under investigation, both *in vitro* and *ex vivo*. Magnolol showed an increase in the release rate in *ex vivo* conditions, but the permeation coefficient is constant in the two methods. In gels under investigation, erythromycin and magnolol release kinetics is achieved by diffusion, as a result of fitting to the Korsmeyer-Peppas model.

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VARIATION OF BIOCHEMICAL COMPOSITION OF ORGANIC RASPBERRY *(RUBUS IDEAUS L.)* DURING STORAGE UNDER FROZEN STATE

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Due to their taste, appearance and biochemical composition, many studies have shown that raspberries (*Rubus ideaus* L.) are used in various forms such as fresh, frozen or processed products (concentrates, jam, juice, nectar, syrup, dairy products). Also, organic raspberry contain an important amount of vitamins, anthocyanins and mineral elements and present an important role in pharmaceutical industry because it can be used as ingredient in food supplements formulation, as natural flavor for children's syrups and not only. However, the limited production period and short postharvest storage (because of rapid spoilage, nutritional and moisture losses of the raspberry fruits) remain the most important problems for both food and pharmaceutical industries. Therefore, the purpose of this work is to determine the biochemical composition variation after 5 months of frozen storage at -20 °C respectively at -70 °C after pre-freezing of the organic raspberry under nitrogen stream. Ascorbic acid identification and cuantification through HPLC, total anthocyanin content, mineral elements through ICP-MS and qualitative indicators like dry matter (DM%), total soluble solids (TSS) and total titrable acidity (TTA) were analysed. Results obtained after 5 months of frozen storage were compared with initial moment and it was observed that total anthocyanin content registered small decreases (18%) for raspberries stored at -20 °C in comparation with those stored at -70 °C (36%). Regarding the differences between above results, this work suggest that frozen temperatures of -20 °C preserve better the biochemical composition of organic raspberries, but further studies and trials are required.

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NMR AND LC-MS METABOLOMIC ANALYSIS OF UNRELATED PLANTS WITH ANTI-HSV AND ANTI-HIV ACTIVITY

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Viral diseases are compromising health and quality of living worldwide and improved anti-viral treatments are continually needed. HIV affect 36.7 million people worldwide with 2.1 million people newly infected with HIV in 2015, mostly affecting sub-Saharan Africa [1]. Development of affordable and accessible medicines for treating viral infections is an important objective, especially in developing countries where the majority of the population relies on traditional medicines for primary health care. The anti-viral activity of unrelated plants from the southern African region was investigated using NMR and LC-MS metabolomic analysis. Plants with previously published anti-viral activity were collected from 25 families, comprising 32 genera and 43 species. The plant material was air dried and extracted by a direct extraction method using deuterated water and methanol and then analysed using a 600 MHz NMR and LC-MS. PCA and OPLS-DA analysis were conducted to determine the chemical variation between active and non-active samples, but also between plants with activity against HSV and HIV viruses. Compounds were annotated using published data, as well as databases (Chenomx and the Human metabolome database) and identified with NMR and LC-MS analysis. Chlorogenic type compounds were identified as the major driver for clustering of the samples with HSV activity. The anti-HIV samples were divided into samples active against different targets, revealing that hydroxyphenyl acetate derivatives are common in plants with anti-HIV RT activity. This study therefore provides an opportunity to identify lead compounds without comprehensive screening programmes, reducing costs and time.

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EXPLORING DRUG DELIVERY SYSTEMS LOADED WITH PLANT EXTRACTS RELEVANT FOR MANAGEMENT OF CNS DISEASES

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Human brain is the most complex living structure continuously generating more than a hundred thousand electrochemical signals every second. With the aging of the world population, mental illnesses like epilepsy, Alzheimer and other neurodegenerative diseases are becoming the most serious chronic conditions affecting millions of people [1].

The therapeutic potential of plants has been used for decades for treatment of diseases affecting the central nervous system (CNS). However, low water solubility, poor absorption and extensive and rapid metabolism are main problems which lead to the need of developing drug delivery systems [2].

The aim of this study is to investigate possible activities of the several extracts of *Cannabis sativa*, *Salvia officinalis*, *Melissa officinalis*, *Rosmarinus officinalis*, *Teucrium polium*, *Teucrium chamaedrys* and *Sideritis raeseri* in order to point out the role of these plants as potential sources for the development of therapeutic agents for epilepsy, Alzheimer and other neurodegenerative diseases. When it comes to phytochemicals deliberate for the CNS, main transitory issue is the blood brain barrier, thus development of different controlled drug delivery systems (DDS) was foreseen.

For this purpose, dry extracts of the listed plants were produced and several drug delivery systems have been formulated and characterized in terms of physico-chemical and biopharmaceutical properties for parenteral administration. Therefore, all aspects of research have been conducted: extraction, chemical characterization, bioactivity testing, DDS formulation and evaluation.

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COMPARISON BETWEEN MACEDONIAN AND BULGARIAN PROPOLIS: CHEMICAL COMPOSTION AND PLANT ORIGIN

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Macedonia and Bulgaria have a long history of production and use of bee products, dating back to the 1 Millenium BC. Propolis is one of the most popular bee products, used in prophylactics and treatments of numerous diseases. Propolis is collected by bees from tree excretions and possesses antibacterial, antifungal and antiviral properties and many other beneficial biological activities. It is used as a home-made remedy in Balkan countries, but also in over-the-counter preparations, food additives, as preservative in foods and cosmetics, etc. The chemical composition of propolis is determined by the source plant and varies significantly in different geographic regions. Detailed comparative chemical study of propolis from Bulgaria and Macedonia was performed using GC-MS of silylated ethanol extracts. Over 40 individual compounds were identified in the studied samples. The major constituents of propolis from both countries were flavonoid aglycones (pinocembrin, chrysin, galangin, etc.), phenolic acids (caffeic, coumaric, ferulic) and their esters (pentenyl caffeates, CAPE, etc.): compounds with pronounced antimicrobial and antioxidant activity. There was no significant difference between Macedonian and Bulgarian samples, which was demonstrated by PCA and cluster analysis. The chemical profiles clearly indicated the poplar origin of all samples.

The knowledge about the chemical composition, active principles and botanical origin propolis is a good prerequisite for its chemical standardization.

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REDUCTION OF GASTROINTESTINAL TOXICITY OF CAPECITABINE VIA CES2 INHIBITORS OF NATURAL ORIGIN

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Introduction: According to GLOBOCAN data, there were estimated 18.1 million new cancer cases in 2018. This is the main reason why anticancer drugs usage worldwide is gradually increasing. 5-Fluorouracil (5-FU) and its prodrug forms are one of the most commonly used medicines for the treatment of various types of cancer, especially Capecitabine (CAP).

CAP itself is non-cytotoxic, but it is designed for selective delivery of the cytotoxic agent 5-FU into the tumor cells. Although the prodrug possesses a better safety profile, it still leads to life-threatening side effects (diarrhea, mucositis, abdominal pain, neutropenia etc.).

Aim: The aim of the present study was to investigate the possibility of reduction of the gastrointestinal (GI) toxicity of CAP via substances of natural origin.

Materials and methods: It was made a review of literary sources that are available in electronic databases such as Science Direct, PubMed etc.

Results: The presence of the entire set of CAP-activating enzymes in GI tract leads to formation of 5-FU before the absorption phase. There, the cytotoxic agent damages the healthy cells, which is associated with gastrointestinal toxicity. In order to reduce the early formation of 5-FU in GI it is necessary to inhibit prodrug activation. The first enzyme of the CAP-activation cascade has several isoforms in the human body. The intestinal Carboxylesterase-2 (CES2), is that which is related to GI formation of 5-FU and there are some inhibitors of natural origin (bisbenzene sulfonamides, benzils, isatins), that can inhibit it.

Conclusion: The inclusion of selective CES2 inhibitor of natural origin in the CAP treatment may increase its bioavailability and reduce the observed GIT toxicity.

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TOTAL POLYPHENOLIC COMPOUNDS CONTENT IN COOKIES FORMULATED WITH BUCKWHEAT FLOUR AND ARONIA FRUITS

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The effect of aronia fruits (AF) addition on the total polyphenolic compounds (TPC) in cookies enriched with different content of buckwheat whole-grain flour (BHF) was investigated. To formulate the cookies, the basic recipe was modified by substituting wheat flour (10, 20 and 30%) with BHF and by the addition of AF (10, 20 and 30%) according to central-composite design. Response surface methodology (RSM) was used to optimize the content of potentially functional ingredients (BHF, AF) in order to maximize the content of TPC in final products.TPC was determined by a method with Folin–Ciocalteu reagent [1].

It was found that TPC content, depending on the cookie recipe, ranges from 2.29 to 4.76 mg of gallic acid (GA)/g of cookie. After applying RSM, statistically significant quadratic model was developed. Analysis of variance implies that the AF content is the most significant factor, while BHF enrichment did not significantly improve the TPC of cookies. The cookie formulation proposed after optimization step predicted that cookies containing 30% of AF and 16.9% of BHF represent the best recipe to manufacture final products with maximum content of TPC (4.76 mg GA/g of cookie). This value was close to the TPC experimentally obtained (4.51 mg GA/g of cookie) and almost two times higher than the TPC of control sample without BHF and AF addition (2.33 mg GA/g of cookie).

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HERBAL MEDICINES: THE ULTIMATE THERAPY TO STIMULATE BENEFICIAL GUT MICROBIOTA

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The human digestive tract is cottage to trillions of live microorganisms, which regulate health and illness. It is important to understand the biological mechanisms in the development of clinical applications. The microbiota is essential for preventing infections caused by intestinal pathogens, body growth, and improving immune system. For instance, the intestinal microbiota of patients with *Clostridium difficile* infection, *Helicobacter pylori*, and human immunodeficiency virus are significantly altered. The equilibrium in the composition of the gut microbiome and the presence/absence of key species able to effect specific responses, is essential for the improved health condition of the host. It is now understood that herbal medicines plays a significant role in shaping the gut microbiome to get maximum health benefits. Extensively studied herbal medicines such as Triphala, slippery elm, licorice and other medicinal plants and their formulations are commonly used for gastrointestinal health and disease prevention in traditional medicine. Upon consumption, the active compounds in herbal medicines come in contact with the intestinal microflora and are transformed before being absorbed from the gastrointestinal tract. In other case, gut bacterial metabolism is linked with enhancement of bioactivity/toxicity diminishment of the metabolites in comparison with its parent compound. For example, polyphenols from Triphala and other medicinal plants promote the growth of probiotic bacteria including *Bifidobacteria* and *Lactobacillus* species while inhibiting the growth of undesirable gut residents such as *Escherichia coli*, *Salmonella typhimurium* etc.

Therefore, this study focuses on the role of herbal medicines in promoting growth of beneficial microbes in the digestive tract.

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IN VITRO ANTIOXIDANT ACTIVITY AND GENOTOXICITY EVALUATION OF HIGH-PRESSURE EXTRACTS OF *DASIPHORA FRUTICOSA* LEAVES

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Dasiphora fruticosa is a vigorous, deciduous shrub that is native to subarctic regions of the northern hemisphere. This member of the *Rosaceae* family is commonly used as an ornamental plant whereas in traditional medicine is consumed as a herbal tea for strengthening the stomach and the spleen, boosting metabolism, regulating menstruation and to combat fatigue [1]. Extracts of this plant have shown hypoglycaemic, antioxidant, anti-inflammatory, antitumor and anti-ulcerogenic properties [2]. Although, these properties are linked to various chemical ingredients, mainly found in the leaves, data on optimal extraction conditions as well as the diversity of phytochemistry and pharmacology on this genus are rather scarce [3]. Towards this end, a multi-step biorefining protocol based on high pressure techniques was developed. Lipophilic fraction was obtained by means of supercritical fluid extraction and the fatty acid profile was assessed by gas chromatography. Moreover, the oxidative stability of edible oils enriched with non-polar extract was determined by Oxipress. In a next step, response surface methodology was utilized in order to obtain acetone, ethanol and water extracts with high antioxidant activity. Preliminary phytochemical characterization of obtained extracts was performed by ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-Q-TOF-MS). In addition, the total phenolic content and in vitro antioxidant capacity were evaluated by the Folin-Ciocalteu and $DPPH^{\bullet}$, $ABTS^{\bullet+}$ assays respectively. Finally, extract genotoxicity was evaluated by means of alkaline single-cell gel electrophoresis (comet) and cytokinesis-block micronucleus assays in human lymphocytes in vitro, while their inhibitory activity against physiologically important enzymes were measured spectrophotometrically.

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ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF *CURCUMA LONGA* EXTRACTS AND MACERATES

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Turmeric (*Curcuma longa* L.) rhizome is a raw plant material widely used in traditional medicine. It contains characteristic compounds belonging to curcuminoids, i.e. curcumin and its derivatives. Although clinical trials are still ongoing and their results are sometimes ambiguous or contradictory, the extracts from turmeric are widely ascribed to exhibit high therapeutic potential, showing anti-inflammatory and anti-cancer activity. In the present study, various extracts obtained from powdered spices and fresh turmeric rhizomes were tested for their antioxidant and antimicrobial properties.

Acetone extracts (oleoresins) were prepared from culinary food additive and fresh turmeric rhizomes: dried and powdered either directly, or after traditional processing by steaming. Dried and powdered turmeric rhizome was also used for maceration in linseed oil. The obtained extracts were evaluated for their radical scavenging properties in DPPH assay. Oleoresin of dried non-steamed rhizomes was the most active (EC_{50} = 23 µg/ml). The antioxidant potentials of oleoresin from rhizomes processed by steaming and extract from food additive were a little lower (with values of EC_{50} equalling 36 and 35 µg/ml, respectively), whereas the lowest activity was exerted by linseed oil extract (EC_{50} = 967 µg/ml). Antimicrobial properties were tested against several Gram-negative strains: *Escherichia coli*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Salmonella enterica* sv. typhimurium, Gram-positive strains: *Bacillus cereus*, *Staphylococcus aureus*, and fungus *Candida albicans*. The tested microorganism showed various susceptibility to applied extracts. The highest bactericidal activity was shown for *B cereus*, *S. aureus* and *P. aeruginosa*, moreover, some differences between the effect of steaming and non-steaming turmeric extracts were observed.

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RAPID HIGH-YIELD TRANSIENT EXPRESSION OF SWINE HEPATITIS E ORF2 CAPSID PROTEINS IN *NICOTIANA BENTHAMIANA* PLANTS AND PRODUCTION OF CHIMERIC HEPATITIS E VIRUS-LIKE PARTICLES BEARING M2E INFLUENZA EPITOPE

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Hepatitis E virus (HEV) is a causative agent of acute hepatitis, mainly transmitted by fecal-oral route or zoonotic. Open reading frame (ORF) 2 encodes the viral capsid protein, which is essential for virion assembly, host interaction and inducing neutralizing antibodies. In this study, we investigated if the full-length HEV ORF2 capsid protein and N and C-end modified capsid proteins can assemble into highly immunogenic virus-like particles (VLPs), during transient expression in *N. benthamiana* plants, and can they act as a carrier of foreign immunogenic epitope such as the highly conserved M2e peptide from Influenza virus. Plant codon-optimised HEV ORF2 capsid genes was constructed in which the nucleotides coding the N-terminal, the C-terminal or both part of the protein were deleted. The M2e peptide was inserted into P2 loop after the residue Gly556 of HEV ORF2 protein by gene fusion and designed three different chimeric sequences. Plants produced specifically all modified HEV capsid proteins and chimeras. Only the capsid protein consisting of aa residues 110 to 610 (HEV110- 610) and chimeric M2HEV110-610 spontaneously assembled in higher order structures. In this study, we report the expression and self-assembly of HEV VLPs and chimeric HEV VLPs bearing the M2e influenza epitope in plants, representing also the first record of a successful plant-based transit expression of chimeric HEV VLPs carrying an epitope of a heterologous virus in plants.

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EFFECT OF COPTISINE ON LEARNING AND MEMORY IN MICE WITH EXPERIMENTAL DEMENTIA

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Coptisine (Cop) is the main constituent of variety plant extracts and mixtures used for the treatment of Alzheimer's disease (AD) or age related CNS disorders in Chinese medicine. However, little is known regarding its own effects in neurodegenerative process. Aim of this study was to investigate potential protective effects of Cop in animals with experimental dementia of AD type. Male IRC mice were divided into: i) Control group (Saline, 0.1 ml/10 g, i.p.); ii) Group treated with a single dose of Cop (3.5 mg/kg, i.p.); iii) Mice with dementia (Scopolamine 1 mg/kg, 9 days, i.p.), treated with Cop (3.5 mg/kg, 9 days, i.p.). Effect of Cop on learning and memory (Step-through test) of healthy animals was tested on 1st, 24th and 48th hour as well as on 7th and 14th day after Scopolamine treatment. One hour after behavioral tests AChE activity and changes in oxidative status (lipid peroxidation, glutathione level and antioxidant enzymes) were estimated in brain homogenates. In healthy animals Cop treatment unaltered both memory and oxidative status, and inhibited AChE (38% *vs* control). In mice with Scopolamine-induced dementia Cop treatment led to recovery of memory, inhibited both AChE and oxidative stress (lipid peroxidase, superoxide dismutase and glutathione peroxidase). In conclusion Cop demonstrates significant antioxidant activity, improves memory and inhibits activity of the enzyme acetylcholine esterase in AD type dementia.

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BENEFICIAL EFFECT OF SNAIL (*HELIX ASPERSA*) EXTRACT ON RATS WITH EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

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Crude mucus was collected from snail *Helix aspersa* and fresh extract was purified by different methods. Molluscan snail extract (SE) is a rich source of biologically active substances with antibacterial, antiviral, immunostimulating and anti-tumor activity.The aim of this work was to check potential protective and therapeutic effect of SE on Parkinson's disease (PD). Experimental rat PD model was induced as previously described [1]. Animals were divided into following groups: sham-operated (SO), striatal 6-OHDA-lesioned control group and 6-OHDA-lesioned rats treated for 13 days with fresh water snail extract orally (6 days before and 7 days after striatal lesion). On the 1st, 2nd and 3rd week post lesion animals were subjected to behavioral tests. After that brains were removed and studied for changes in oxidative status indexes: lipid peroxidation (LPO), glutathione level and antioxidant enzymes. In PD group apomorphine-induced rotations, number of falls and memory impairment were increased as compared to SO group. SE treatment reduced significantly apomorphine-induced rotations (by 46%), number of falls (by 90%) and compensate memory deficit (by 23%) as compared to 6-OHDA control group. These changes were accompanied by significant decrease in brain LPO and recovery of the increased by 6-OHDA-treatment superoxide dismutase activity. In conclusion snail extract had a beneficial effect on experimental rat model of PD confirmed by both behaviorally and biochemically.

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ANTIOXIDANT AND ANTITUMOR ACTIVITIES OF BROWN SEAWEED *CYSTOSEIRA BARBATA* (STACKHOUSE) C. AGARDH FROM ROMANIAN BLACK SEA COAST

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The aim of the present study was to assess the *in vitro* antioxidant and antitumor activities of *Cystoseira barbata*, Sargassaceae, a brown alga inhabiting the costal line of Romanian Black Sea. Four different solvents (70% acetone, ethanol, methanol and water) were used for the extraction of the algal material. The antioxidant activity of *C. barbata* extracts was assessed by various assays: DPPH and ABTS radicals scavenging assays, reducing power and 15-lipoxygenase inhibition assays. Cytotoxic activity of the extracts was determined by MTT viability assay using three adenocarcinoma cell lines (mammary MCF7, alveolar A549 and colorectal HT29). The effect of the extracts in cell cycle distribution was evaluated using flow cytometry (FACS) [2, 3]. The 70% acetone extract exhibited the highest antioxidant activities in all antioxidant assays, with IC_{50} values comparable or higher than those of known antioxidants. The antitumor tests showed that 70% acetone extract had the most potent antiproliferative effects. Thus, at 1 mg/mL the extract reduced MCF-7 cell viability to 13.88 $\pm$ 0.35% and HT29 cell viability to 46.63 $\pm$ 10.08%. FACS analysis revealed that the 70% acetone extract induced a significant increase in the subG1 fraction in MCF-7 and A549 cells, indicative of the induction of apoptosis. From these findings, the 70% acetone extract was investigated by

HPLC-DAD-ESI-MS/MS [3]; phlorotannins with molecular weights ranging from 374 to 1466 Da were tentatively identified on the basis of their characteristic MS/MS fragmentation pattern. In conclusion, *C. barbata* represents an important source of bioactive compounds, with potential use in food and pharmaceutical industries as preservatives and nutraceuticals.

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VACCINIUM VITIS-IDAEA L., PICKED IN BULGARIA SHOW *IN VITRO* ANTITUMOR ACTIVITY

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Antitumor therapy aims to be established active ingredients that selectively suppress the proliferation of tumor cells. In this relation, the antitumor activity of natural products with proven phytochemical properties and pharmacological significance are actively investigated [1, 2]. A promising candidate is Bulgarian cranberries from high mountain plant populations, which are rich in various bioactive ingredients including phenolics and anthocyanins. Nowadays they belong to a group of functional foods and many studies have demonstrated their beneficial effects on different functions in the human body [3].

Aim: The present study aims to evaluate *in vitro*, antitumor potential of total methanol extracts and purified fractions (B- nonanthocyanin / C- anthocyanins) of *Vaccinium vitis-idaea* L., picked in Bulgaria on human cervical (HeLa) and breast (MCF7) cancer cell lines.

Materials and methods: A total of four methanol extracts and respective number purified fractions (B-nonanthocyanin/C-anthocyanins) of cranberry picked in Bulgaria were used. Antitumor effect was established by Trypan Blue method and MTT cell viability assay.

Results: The results from MTT analyses showed that B- nonanthocyanin fractions of Bulgarian cranberry have well expressed inhibitory effect on survival of tested tumor cells. The observed effect dependent of the dose administered and were stronger in relation with the high-mountain populations and HeLa cell line.

Conclusions: Evaluation of antitumor activities of Bulgarian cranberries

using modern molecular methods, could contribute to establish the natural substances useful for human health.

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SYNTHETIC APPROACHES TO CAFFEIC ACID PHENETHYL ESTER (CAPE): LITERATURE REVIEW AND EXPERIMENTAL RESULTS

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Caffeic acid phenethyl ester (CAPE) is a simple natural compound, described for the first time in 1988, when it has been isolated from propolis as the carcinostatic active principle with preferential cytotoxicity on tumour cells. Later it turned out to possess many other potent pharmacological activities: antibacterial, antiviral, anti-inflammatory, cytotoxic, neuroprotective, hepatoprotective, etc. Isolation and purification of CAPE from natural sources is expensive and time consuming, but because of its remarkable pharmacological potential and the simplicity of its molecular structure, the ways to obtain CAPE synthetically have been receiving significant attention [1]. In general, they could be classified in three groups: methods using caffeic acid as starting material, methods starting with 3,4-dihydroxybenzaldehyde and enzyme catalyzed esterification.

A problem exists with CAPE low solubility in water. A possible solution is immobilization in block copolymer micelles in order to impart good solubility in biological medium and enhanced bioavailability [2]. In search of appropriate carriers we were also interested in a convenient synthesis of CAPE. We tested the following procedures: direct esterification with acid catalysis; esterification *via* Mitsunobu reaction; heterogeneous Wittig reaction under sonochemical conditions, and „one pot" reaction in the presence of Meldrum's acid; and compared the yields, reaction times, and *E/Z* isomers ratio (NMR) of the obtained product. With respect to yield, the Wittig reaction was superior with over 70%. Concerning *E/Z* ratio however, the "one pot" synthesis was preferable because it gave 100% *E* isomer which is the target bioactive molecule.

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GREEN EXTRACTION OF BIOACTIVE CONSTITUENTS FROM PROPOLIS OF POPLAR ORIGIN

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Recently, public and scientific attention has been attracted to the protection of the environment from chemical pollutants. The idea of "green chemistry" was developed as a response to this public interest, focused on designing of products and processes that minimize the use and generation of hazardous substances. A significant topic in this respect is the development of "green" solvents.

Newly, an innovative type of "green" solvents, deep eutectic solvents (DESs), was developed. DESs consist of solid compounds which produce eutectic mixture with a melting point much lower than the one of the individual components and are liquid at ambient temperature. In a particular combination and ratio natural compounds (primary metabolites) may also be converted into liquids to form so called natural deep eutectic solvents (NADESs), which are present in nature [1].

The present study aims to apply for the first time a new approach for extraction of bioactive compounds from poplar propolis based on the use of NADESs as an alternative to 70% ethanol. We have tested a number of NADESs and compared the efficiency of the extraction. The most efficient NADESs were mixtures of choline chloride-propanediol, and lactic acid–propanediol which gave yields comparable to 70% ethanol. These extracts (without isolation of the extracted substances) were tested for their antimicrobial potential and some of them demonstrated good antibacterial activity against *Staphylococcus aureus* and *Listeria monocytogenes*. In addition they have been probed for cytotoxic and genotoxic activities on human cancer cell lines. Results are promising and demonstrate high biological activities of the tested NADES extracts on human cells with the potential to be used in the future in the design and development of anti-cancer and anti-ageing strategies.

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A CONCISED EVALUATION SYSTEM USED FOR RAPID SEARCH OF ANTI-EBOLA PLANT LEADS BASED ON PLANT DIVERSITY IN LINGNAN REGION

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Ebola virus is the causative agent of Ebola virus disease (EVD), a disease associated with hemorrhagic fever and high case fatality rate. There are currently no approval drugs specific for patients suffered from EVD. The study of anti-Ebola agents is largely hampered by the requirement of biosafety level 4 (BSL-4) containments. We have recently applied a cell-based assay utilizing pseudotyped viral particles, which can be easily operated in a BSL-2 containments, and therefore significantly accelerate the search for the inhibitors of highly pathogenic viruses such as Ebola and avian influenza H5N1 viruses [1]. The assay can be achieved by producing replication-incomplete pseudotyped virus particles with expression of a specific viral glycoprotein on surface. Plants have been extensively used for prevention and treatment of infectious diseases in human history. Many bioactive components have been identified from plants. Lingnan is a subtropical region in southern China with high diversity of plant species. In our study, we have evaluated over 500 extracts of the medicinal plants collected in Lingnan region against the infection of Ebola pseudotyped particles, leading to identification of a number of anti-Ebola plant leads, which can be further investigated to discover active compounds through bioassay-guided separation.

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PP 203

FLEXICAULIN A, A NATURAL *ENT*-KAURANE DITERPENOID, ATTENUATES THE PROLIFERATION OF COLORECTAL CARCINOMA CELLS THROUGH THE INDUCTION OF CELL CYCLE ARREST

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Natural products, particularly medicinal plants, are an important source of therapeutic agents and drug leads because they contain astounding amounts of small molecules that possess diversifying chemical entities with potential to be used in the treatment of human disorders. For instance, *Isodon* (formerly *Rabdosia*), a genus of the Lamiaceae (formerly Labiatae) family comprising 150 species, has been reported as a rich source of natural diterpenes for combating inflammations and malignancies.

In the present study, we evaluated the anti-tumor property of flexicaulin A (FA), an *Isodon* diterpenoid possessing an *ent*-kaurane structure, by means of cell viability assay, flow cytometric assessment, quantitative polymerase chain reaction array, Western blot analysis and different staining experiments. Further, the *in vivo* anti-tumor efficacy of FA was validated in a xenograft mouse model of human colorectal carcinoma.

According to our experimental results, we conclude that FA is a potent antitumor molecule since it significantly attenuated the proliferation of human colorectal carcinoma cells *in vitro* and reduced the growth of xenograft tumors *in vivo*. In regard to its molecular mechanism, FA notably elevated the expression level of p21 and induced cell cycle arrest in the human colorectal carcinoma cells. While executing a non-apoptotic mechanism, we believe FA can be served as a potential chemotherapeutic alternative complementary to the conventional apoptosis-inducing agent in overcoming the rapid development drug resistance in the management of colorectal malignancy.

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USE OF EPHEMERANTHOQUINONE, A NATURALLY OCCURRING PHENANTHRAQUINONE, IN THE TREATMENT OF PANCREATIC FIBROSIS IN CHRONIC PANCREATITIS

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Chronic pancreatitis is a long-standing inflammation of the pancreas concomitant with progressive fibrotic replacement of parenchyma and irreversible scarring, which causes patients a set of debilitating symptoms including serious abdominal pain, nausea, diarrhea and weight loss. Owing to parenchymal fibrosis, patients are prone to develop exocrine pancreatic insufficiency, type 3c diabetes and even pancreatic adenocarcinoma. Though the pathogenesis of chronic pancreatitis is not clear, recent studies demonstrated that the activation of pancreatic stellate cells (PSCs) is prerequisite to the progressive parenchymal fibrosis, which manifestly correlates with the permanent loss of pancreatic functional capacities.

Lately, natural or herbal products have been increasingly recommended by various nutritional orthodoxies for human pathological conditions. As we know, many *Dendrobium* (Orchidaceae) plants have been serving as tonics and anti-inflammatory remedies in the traditional Chinese medicinal practice. In the current study, we isolated ephemeranthoquinone (EPH), which is characterized by polycyclic aromatic hydrocarbons arranged into three benzene rings as its core structure, from the whole plant *D. hongdie*. By means of several biochemical and physiological examinations, we found that EPH effectively attenuated the activation of PSCs and their production of extracellular matrices upon the challenge of different pro-inflammatory/pro-fibrotic stimuli. Importantly, EPH also suppressed the population of M2 macrophages, which play a crucial role in the dampening of inflammation and fibrogenesis. Therefore, we suggest that the natural phenanthraquinone EPH is potentially a remedial agent for the management of pancreatic fibrosis in chronic pancreatitis for which no effective therapeutic measure is currently available.

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TEMPORAL VARIATIONON CHEMICAL COMPOSITION, ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF THE ESSENTIAL OILS OF *THYMUS SIBTHORPII* BENTHAM (LAMIACEAE) GROWING WILD IN KILKIS (NORTHERN GREECE)

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This study is aimed at assessing the essential oil composition**,** anti-inflammatory and antioxidant activities of the essential oil of *Thymus sibthorpii* collected from the same area of Central Macedonia, northern Greece (Neo Gynaikokastro, Kilkis during the main flowering period in May 2014, as well as at four consecutive months of 2016 (March-June).The chemical composition of the essential oils was studied by GC-MS. Furthermore, the oils were evaluated *in vitro* for their: (i) antioxidant activity, using the DPPH interaction and inhibition of linoleic acid lipid peroxidation induced by the dihydrochloric acid of 2,2-azobis-2-amidinepropane (AAPH), and (ii) anti-inflammatory activity, using the soybean lipoxygenase assay. In total, 43 compounds were identified. The oils of flowering samples were mainly composed of phenolic compounds, and belonged to the linalool chemotype, while those of pre-flowering stage to the thymol chemotype. Their antioxidant and anti-inflammatory potential scored average in comparison to reference compounds and were relatively higher in pre-flowering stages or at the end of flowering. The results reported here may serve the complex chemotaxonomy of taxa of the genus *Thymus* and the investigation of chemotypes of *T. sibthorpii* in temporal and/or geographical scales. The essential oils of *T. sibthorpii* due to their biological activities could be used in many cases as natural preservatives, food additives, functional food ingredients, nutraceuticals, pharmaceuticals and cosmeteuticals.

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SECONDARY METABOLITES FROM THE AERIAL PARTS OF *SIDERITIS SIPYLEA* BOISS. (LAMIACEAE) GROWING WILD IN GREECE

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S. sipylea represents one of about 140 species of the genus *Sideritis* L., which belongs to family Lamiaceae. Plants of the genus *Sideritis* L. occurring mainly in the Mediterranean and are commonly known in Greece as 'mountain tea'. Infusions of these plants are widely used in traditional medicine in Greece and Europe due to their antiinflammatory, antirheumatic, antiulcer, digestive and antimicrobial properties which are attributed to their phenolic and terpenoid content [1]. In present study, the methanolic extract from aerial part of *S. sipylea* Boiss. (Lamiaceae) were examined for its content in non-volatile secondary metabolites. So far, seven secondary metabolites have been isolated using different chromatographic techniques:an iridoid: ajugoside (1), three flavonoids: salvigenin (2), apigenin-7-O-glucoside (3), isoscutellarein 7-O-[6‴-O-acetyl]-allosyl(1→2)glycoside (4), 4′-O-methylisoscutellarein 7-O-[6‴-O-acetyl]-allosyl-(1→2)glycoside (5) and two phenylethanoid glucosides: acteoside (6) and martinoside (7). They were identified using spectroscopic methods MS and NMR (^{1}H, ^{13}C, gDQCOSY, NOESY, gHSQCAD and gHMBCAD). In addition, the bioactivity prediction of the isolated compounds was estimated by using *in silico* methods.

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ANTIOXIDANT, CYTOTOXIC AND GENOTOXIC POTENTIAL OF BULGARIAN *ROSA ALBA* L. HYDROSOL

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Rose water is among valuable raw materials of food, perfume, cosmetic, and pharmaceutical industries.

The objective of this study was to evaluate chromatographic profile, polyphenols content, antioxidant activities, and cytotoxic and clastogenic effects of hydrosol of Bulgarian *Rosa alba* L.

It was investigated chromatographic profile of hydrosol, total polyphenols content (TPC); antioxidant activity in systems generating hydroxyl and superoxide radicals, genotoxicity – induction of chromosome aberration and micromuclei in two types of test-systems: *Hordeum vulgare* and human lymphocytes *in vitro*. Rose hydrosol contained geraniol 42%, citronellol 28%, phenylethyl alcohol 5%, linalool 3%, and TPC 72 µg gallic acid/mL. It demonstrates good ability to scavenge hydroxile and superoxide radicals.

The data showed no cytotoxic effect in barley and low cytotoxic effect in human lymphocytes compared to the control. Similar were results concerning the clastogenic effects (chromatid aberrations and micronuclei). Chromosome aberrations observed within the first mitosis included predominantly isochromatid breaks and a small number of chromatid breaks and translocations. In barley a concentration-dependent formation of aberration hot spots at a low level was observed.

In conclusions, rose hydrosol: (i) is rich in oxygenated monoterpenes; (ii) has low cytotoxic and (iii) clastogenic/genotoxic effects in *H. vulgare* root tip meristem cells and in human lymphocytes in vitro. Hydrosol from Bulgarian white rose could be used in preventing free radical-related diseases without damaging normal and healthy cells.

PP 208

BIOSUSTAINABLE APPLICATIONS OF PLANT BASED NATURAL DEEP EUTECTIC SOLVENTS

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Green chemistry emphasizes on the role of tailoring novel green and natural solvents as alternatives to the traditional organic solvents and ionic liquids. A new generation of solvents inspired from nature has emerged in the last decade referred to as Natural Deep Eutectic Solvents (NADES) from plant bioresources/products. NADES are mixtures of natural constituents like sugars, polyalcohols, sugar alcohols, amino acids and organic acids. Curiosity related to NADES has amplified over the last 8 years, clearly evident by the exponential increase in the number of publications. NADES are considered to be highly biocompatible as they can be biosynthesized as well as metabolized by essentially all the living organisms. These solvents pose several noteworthy virtues such as easy synthesis, tunable physico-chemical properties, low toxicity, high biodegradability, solute sustainability and stabilization and low melting point. Research on feasibility of NADES applications in diverse areas is gaining momentum especially related to: media for chemical and enzymatic reactions, yielding essential oils, anti-inflammatory and antimicrobial applications, extraction of bioactive composites, as chromatographic media, preservatives for labile compounds and for drug synthesis. To gather better knowledge related to the significance of NADES in the biological systems and its application in green and sustainable chemistry, this review gives a complete snapshot about their properties, biodegradability and toxicity. Information related to applications of NADES in biomedical, therapeutic and pharma-biotechnology fields are also a highlight of the current draft. The comprehensive research review also briefs about the future perspectives and recent progress in developing novel applications of NADES.

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MORINGA OLEIFERA LAM. LEAVES EXTRACT INHIBITS INFLAMMATION OF HUMAN MACROPHAGES INDUCED BY LIPOPOLYSACCHARIDE

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Moringa oleifera Lam. has been known as anti-oxidation and anti-inflammation activity and can use as anti-inflammatory substance. However, this work was found the effect of *M. oleifera* leaves extract on human macrophages function. Since macrophages cytokines play an important role in inflammation which causes tissue damage, we investigated the effect of Moringa on lipopolysaccharide induced cytokine production by human macrophages. An ethyl acetate fraction of Moringa was prepared from fresh leaves extract and shown high level of phenolic and anti-oxidant activities. Human monocyte derived macrophages treated with the extract showed decrease production of pro-inflammatory cytokines level including IL-6, TNF-α. Furthermore, the Moringa extract inhibit the expression of Cyclooxygenase production, NF-κB nuclear translocation and IκBα phosphorylation in response to LPS. This work shows that activity of Moringa extract potently inhibits the ability of lipopolysaccharide to induce pro-inflammatory marker.

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EFFECTS OF CHLOROGENIC ACID ON INDICES OF LIPID METABOLISM IN OVARIECTOMIZED RATS

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Chlorogenic acid (CGA) is abundant in human diet. It has been demonstrated to perform crucial roles in lipid and glucose metabolism [1] and lower the increased plasma total cholesterol and low-density lipoprotein induced by a hypercholesterolemic diet [2].

This study aimed to investigate the effects of CGA on indices of lipid metabolism in ovariectomized rats.

Female Wistar rats were divided into 3 groups: SO (sham-operated), OV (ovariectomized) and OV+CGA. The oral treatment started 15 days after the operation and lasted 10 weeks. SO and OV groups received distilled water. OV+CGA group was treated with CGA (20 mg/kg). At termination of the experiment, fat deposits (total, retroperitoneal and mesenterial) were measured and blood cholesterol and triglycerides were determined.

The total and retroperitoneal fat deposits of OV group were significantly higher ($p<0.01$) than those of SO group while the mesenterial fat was also increased but not significantly. This accounted for significantly higher ratios of total fat/animal weight and retroperitoneal fat/animal weight of OV rats compared to SO rats. Blood cholesterol level of OV group was significantly higher ($p<0.05$) than that of SO group while blood teiglyceride level was not significantly different between the two groups. CGA did not significantly affect the fat accumulation and blood lipids concentration in OV rats. All determined indices of lipid metabolism for OV+CGA group were not significantly different from those of OV group.

In conclusion, CGA did not counteract the disturbances in lipid metabolism caused by estrogen deficiency in ovariectomized rats.

Acknowledgements: This research was funded by Science Fund – MU-Varna, 2014.

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PHYTOCHEMICAL ANALYSES OF THREE ENDEMIC BORAGINACEAE PLANTS FROM TURKEY: *PHYLLOCARA AUCHERI*, *SYMPHYTUM ANATOLICUM*, *CYNOGLOTTIS BARRELIERI*. BIOLOGICAL PROPERTIES

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In the framework of our research on Boraginaceae plants [1-3], we report in this study the phytochemical investigation on three not previously studied endemic plants from Turkey: *Phyllocara aucheri, Symphytum anatolicum* and *Cynoglottis barrelieri*. The methanolic extracts were subjected to qualitative LC-MS analysis, in which eighteen secondary metabolites have been identified. Some among the most abundant metabolites, were also isolated (rutin, isoquercitrin, together with caffeic acid derivatives –rosmarinic and chlorogenic acid etc), and all structurally determined by modern spectral means. Moreover, pyrrolizidine alkaloids were identified by LC-QTOF-MS analyses, according BfR method, in the form of bases and their corresponding N-oxides like europine, acetyl uplandicine N-oxide and intermedine N-oxide. All extracts have been assayed for their antioxidant and *in vitro* enzyme inhibitory properties. Their antioxidant capacity was evaluated using free radical scavenging, reducing power, phosphomolybdenum and ferrous ion chelating assays, while they also evaluated against cholinesterases, α-amylase and α-glucosidase. *C. barrelieri* exhibited the strongest antioxidant activity due probably to its high total phenolic and flavonoid content and it is followed by *S. anatolicum*, *P. aucheri*, while it is noteworthy that *S. anatolicum* showed the highest inhibitory capacity against AChe and BChe. The phytochemical profiles as well as bioactivities results of all three herbal materials suggest potential candidates for further phytotherapeutic applications.

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ALCHEMILLA MONTICOLA OPIZ AFFECTS ADIPOCYTES DIFFERENTIATION IN SGBS CELLS

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Obesity is an epidemic with accelerating expansion worldwide. Human Simpson-Golabi-Behmel syndrome (SGBS) cell line is an established *in vitro* model of adipocytes differentiation and metabolism [1]. The potential of plants to control weight gain is exploited from centuries [2]. The present study aimed to elucidate whether extract of *Alchemilla monticola* Opiz (ALM) could modulate the processes of differentiation and lipogenesis in SGBS cells. Near confluent SGBS pre-adipocytes were initiated to differentiation and treated with ALM extract in doses of 25, 50 and 100 µg/mL. Lipid accumulation was assessed with the Oil Red O assay in mature adipocytes and protein samples were extracted for Western blotting. Additionally, the phytochemical profile of ALM extract was analyzed by NMR-based metabolomics. The application of 25 µg/mL ALM extract decreased the differentiation rates and the lipid content in SGBS adipocytes compared to the control samples. Our data suggest that ALM extract worth further exploration as a potential source of bioactive metabolites that interfere with lipogenesis in differentiating adipocytes.

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GENOTOXIC AND ENZYME INHIBITORY PROPERTIES OF *BETONICA OFFICINALIS, GRATIOLA OFFICINALIS, VINCETOXICUM LUTEUM* AND *VINCETOXICUM HIRUNDINARIA* EXTRACTS

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Our previous studies demonstrated significant antioxidant potential of woody betony [1], hedge hyssop [2] and swallow-wort [3] extracts, while screening their phytochemical composition revealed the presence of various phytochemicals, mainly polyphenolic compounds. However, scientific knowledge on toxicity and bioactivities of these herbs is scarce. To fill this gap the aim of our study was to evaluate genotoxic and enzyme inhibitory potential of *Betonica officinalis* L. (Lamiaceae), *Gratiola officinalis* L. (Plantaginaceae), *Vincetoxicum luteum* and L. *Vincetoxicum hirundinaria* L. (Apocynaceae) extracts. The Ames *Salmonella*/microsome test, alkaline single-cell gel electrophoresis (comet) assay and cytokinesis-block micronucleus assay in human lymphocytes were used for evaluating genotoxicity, whereas physiologically important enzymes such as α-amylase, β-glucosidase, acetylcholinesterase and angiotensin converting enzyme were used for determining enzyme inhibitory activity.

The extracts isolated from the all tested herbs at the doses of 50-250 μg/mL were not mutagenic in *S. typhimurium* strains TA98 and TA100 with and without metabolic activation. The extracts of *B. officinalis* and *G. officinalis,* were also not cytotoxic or genotoxic in the micronucleus test, while *V. luteum* and *V. hirundinaria* were cytotoxic at all investigeted extract concentrations. In addition, all investigated medicinal plant extracts induced primary DNA damage evaluated by the comet assay. The determined variation in response was due to the plant extract tested and donor susceptibility.

The extracts inhibited selected enzymes, while their activity was dependent on plant species, applied solvent and extract concentration. In general, the results provide important information for further studies leading to the development of functional ingredients for various applications.

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REPRODUCTIVE SYSTEMS OF TWO ENDEMIC *HIERACIUM* (ASTERACEAE) SPECIES IN THE BULGARIAN FLORA

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Hieracium (Asteraceae) is one of the most taxonomically intricate vascular plant genera due to its specific reproductive system which involve normal sexual reproduction, hybridization, polyploidy and apomixis. It is one of the largest genera in the Bulgarian flora with numerous endemics. The aim of the present research was to study the reproductive systems of two recently described endemic species – *H. kittaniae* and *H. petrovae*, and to discuss the consequences of the reproduction modes for the taxonomic treatment and conservation of the taxa. The *H. kittaniae* is an endangered species, local endemic to a small area in the Central Rhodope Mts, whereas *H. petrovae* has a larger distribution area in the Central Rhodope Mts, although it may possibly occur in other mountains in South Bulgaria and Northern Greece. Plant material was collected from the Trigrad and Buynovo Gorges, Rhodopi Mts. Classical embryological methods, castration experiments and chromosome counting were used to achieve the results. Both species are diploid relicts. The results suggest that they are sexually reproducing which is typical for the diploids in the genus. Sporadically, in individual ovules of *H. kittaniae* integumentary embryony (sporophytic apomixis) was established. The chemical composition of these endemics has not been studied yet and both species have potential value as a source of valuable chemical compounds.

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COMPARISON OF CHEMICAL COMPOSITION OF WATER-SOLUBLE CONSTITUENTS OF LAVANDINS AND *LAVANDULA ANGUSTIFOLIA* OF UKRAINE

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"Rabat" and "Inii" are lavandin varieties of interspecies hybrid of *Lavandula angustifolia* (Lamiaceae) [1]. Our investigation supported antimicrobial properties of Rabat and Inii essential oils [2]. But other groups of chemical compounds of new lavandins are little known up to now. The aim of this study was to investigate contents of main water-soluble groups of compounds in lavandins Rabat and Inii with comparison to *L. angustifolia*.

The plant material of lavandins was obtained from the experimental lots of farm "Novokakhovsky" of the Rice Institute, Kherson region in July 2017-2018, *L. angustifolia* – from botanical garden of Lviv. Flowering tops of the plants were harvested manually, at the maximum flowering stage. Water extractions of these plants as well as assess tannins, organic acids, hydrocynnamic acids content in the tested extracts have been made by spectrophotometric methods of Pharmacopoeia of Ukraine.

The presence of tannins amounts to 4.26 mg/g DW, 4.72 mg/g DW and 7.88 mg/g DW in Lavandins Rabat and Inii, and *L. angustifolia*, respectively. The yield of organic acids is about 1.68%/DW in both lavandins and 3.35%/DW in *L. angustifolia*. Within investigated material *L. angustifolia* had 2.1-4.3 fold higher concentration of hydrocynnamic acids (3.59 - 8.40 expressed as % of caffeic acid or chlorogenic acid) than both lavandins.

Modern medicine should pay attention to the plant secondary water-soluble metabolites of lavandins Inii and Rabat.

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THE EFFECT OF VARIATION OF HARVEST SEASON ON WATER SOLUBLE BAS IN SHOOTS OF *VACCINIUM CORYMBOSUM* L

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It has been known, that the fruits of *Vaccinium corymbosum* L. (Ericaceae), several varieties of which introduced in Ukraine, posses a wide range of therapeutic properties. Information on biologically active substances in the vegetative parts of *V. sorymbosum* in the scientific literature occurs episodically. The purpose of our study was to determine the presence and content of total water soluble BAS and extractives in *V. corymbosum* L. shoots harvested during 2017-2018.

Shoots of *V. corymbosum* Elliot cultivar, grown in Lviv region of Ukraine, were collected in season of 2017-2018. The determination of total content of phenolic acids, organic, hydroxycinnamic, ascorbic acids, tannins and extractives in the shoots were performed spectrophotometrically and according to the Ukrainian Pharmacopoea [1].

It was observed that the most of organic acids is accumulated during fruiting period (in August): 8.28 ± 0.85%/ DW, hydroxycinnamic acids in flowering dates (in May): 4.96 ± 0.66%/DW expressed as % of caffeic acid or 7.11 ± 0.56%/DW expressed as chlorogenic acid, respectively. The profile of ascorbic acid was quantitatively similar during May-October (182.8-217.7 ± 4.5 mg/g DW) and by 20% higher in winter. The total content of tannins is maximal in the end of September; 35% lower in December and August, and 2.6 times lower in May.

The results indicate that the shoots of Elliot cultivars of *V. corymbosum* have different water-soluble BAS contents depends of season; so as they have different antioxidant capacities, their content should be taken into account when used for treatment.

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UHPLC-HRMS PROFILINGS OF SELECTED ASTERACEAE SPECIES GROWN ON VITOSHA MT. (BULGARIA)

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In the present study, selected Asteraceae species grown in Vitosha mt. (Bulgaria) were investigated by ultra-high-performance liquid chromatography – Orbitrap high resolution mass spectrometry (UHPLC-HRMS). The dried and pulverized flower heads from *Senecio nemorensis* L., *Tanacetum macrophyllum* (Waldst. & Kit) Schultz Bip. and *Telekia speciosa* (Schreb.) Baumg were subjected to ultrasound-assisted extraction in 80% MeOH. The obtained samples were then put to UHPLC-HRMS targeted analysis for acylquinic acids, flavonoids and pyrrolizidine alkaloids (PAs). PAs are secondary metabolites that are hazardous to humans and animals because of their hepatotoxicity. The tentative LC-MS identification was achieved based on high-accurate mass determination and analysis of the fragmentation behavior. Chlorogenic, feruloylquinic, dicaffeoylquinic acids, as well as flavone and flavonol glycosides were the dominant compounds in the studied extracts. Moreover, *Senecio nemorensis* showed PAs in high abundance including junceine, trichodesmine, retrosine and senecionine and their corresponding *N*-oxides (PANOs).

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EVALUATION THE *IN VITRO* EFFECTS OF SAPONINS' MIXTURE, ISOLATED FROM *ASTRAGALUS GLYCYPHYLLOIDES,* IN A MODEL OF NON-ENZYME AND ENZYME-INDUCED LIPID PEROXIDATION ON ISOLATED RAT LIVER MICROSOMES

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In this study, we investigate the effects of saponins' mixture, isolated from *Astragalus glycyphylloides*, in conditions of non-enzyme and enzyme-induced lipid peroxidation on isolated rat liver microsomes.

The microsomes were obtained by multiple, differential centrifugation, and were incubated with three concentrations of the saponins' mixture: 100 µg/ml; 10 µg/ml and 1 µg/ml. As a marker of lipid peroxidation we measure the production of malondialdehyde (MDA).

Administered alone, the saponins' mixture (at concentrations 100 µg/ml; 10 µg/ml and 1 µg/ml) didn't reveal statistically significant, pro-oxidant effects, compared to the control (non-treated microsomes).

In both models of toxicity: non-enzyme ($FeSO_4$/Ascorbinic acid) and enzyme (carbon tetrachloride/NADPH)-induced lipid peroxidation, saponins' mixture revealed statistically significant anti-oxidant effects (decreased the MDA production), compared to the toxic agents.

The effects were most prominent at concentration 100 µg/ml.

The mixture had more expressive anti-oxidant effect in a model of non-enzyme lipid peroxidation, compared to enzyme lipid peroxidation.

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IMPACT OF GLUCOSE ON THE STABILITY, ABSORPTION AND METABOLISM OF CATECHINS INCUBATED WITH CACO-2 CELLS

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Blood glucose level of type 2 diabetics can be adjusted by dietary polyphenols. On the contrary, the absorption and metabolism of polyphenols may also be affected by glucose level [1]. In this study, an *in vitro* model was established to investigate the impact of glucose on the stability, absorption and metabolism of catechins incubated with caco-2 cells. Cells were incubated either with catechins in the presence and absence of glucose according to our previous methods [2]. The (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) were added into the medium in a concentration of 10^{-4} M. The concentrations of glucose in culture medium are from 1500 to 7500 mg/L. Samples in cell and supernatant were collected at distinct time points, then the content of catechins and the metabolites were analyzed by UPLC-QTOF-MS/MS and UPLC-TQD-MS/MS [3].

ECG and EGCG with gallate on C ring show obvious instability than EC and EGC. Catechins incubated in high glucose level are much unstable than incubated in low glucose. As for absorptivity, catechins incubated in high glucose medium are slightly higher than in low glucose medium. Phase II metabolites with methyl, dimethyl and glucuronide were identified in caco-2 cells in 2 hours incubation. Methylated epicatechin, as the main metabolites of EC in supernatant, was identified after 2 hours incubation.

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A NOVEL SYNTHASIS OF LIPOPHILIC EGCG PALMITATE AND ITS UTILIZATIONS

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A green and novel approach to synthesize a lipophilic EGCG palmitate was submitted by chemical-catalyzed acylation of EGCG with palmitoyl chloride, and thus the significant elevated bioavailability of EGGC is achieved. Various conditions affecting the acylation reaction process have been studied, in order to optimize the acylation procedure. Successive separation on silica gel eluting gave compound 1 (51 % yield). The structure of the compound 1 was confirmed by HPLC-MS and NMR and was identified as 4′-*O*-palmitoyl EGCG (PEGCG). In addition, the pH, long term and thermal stability of the EGCG palmitates mixture was significantly higher than EGCG. Moreover, PEGCG showed better inhibition toward α-amylase and α-glucosidase than EGCG, with IC_{50} values of 1.64 and 0.22 μM, respectively. These observations suggest that the lipophilic PEGCG may act as an antidiabetic agent.

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ANTIFUNGAL ACTIVITY OF APIACEAE TERPENOIDS - NATURAL PRODUCTS WITH PUTATIVE USE IN ONYCHOMYCOSIS

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Onychomycosis are fungal infections of the finger or toenails, mainly caused by fungi of genera *Trichophyton*, *Microsporum* and *Epidermophyton*, and some *Candida* species. The most prevalent isolated agent is *Trichophyton rubrum*, which is found in 60% of these kind of infections [1]. Onychomycosis are often intractable, and relapses occur frequently after cessation of the antifungal therapy due to poor adherence to long-term treatment regimens and to development of antifungal resistance mechanisms [2]. Therefore, development of novel antifungal drugs targeting structures that are unique to fungal pathogens is a challenge. A literature survey showed that essential oils isolated from Apiaceae species (such as *Coriandrum sativum*, *Carum carvi*, *Pimpinella anisum*, *Trachyspermum ammi*) are effective against etiological agents of onychomycosis such as *T. rubrum*, *T. mentagrophytes*, and *Candida* species. Apiaceae essential oils are complex mixtures of small volatile molecules (*e.g.* limonene, thymol, linalool, anethole, carvone) that could provide improved penetration and reach the fungal elements inside the nail plate. In addition, their multicomponent nature confers a broad antifungal spectrum of activity, through interaction with biological membranes and consequently alteration of cell selective permeability, and interference in radical and enzymatic reaction of fungi cells [3]. In conclusion, Apiaceae essential oils may become the source of new therapeutic molecules and also their incorporation into topical formulation represents an interesting, safe, and effective alternative for the treatment of onychomycosis.

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EXTRACTION AND QUALITY CONTROL OF POLYSACCHARIDES IN *LYCIUM BARBARUM* L

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Analytical method of polysaccharides and their hydrolyzed products in *Lycium barbarum* L. was performed by high performance liquid chromatography (HPLC). The separation method of six monosaccharides was established, and it was applied to analyze the composition characteristics of polysaccharides in *L. barbarum* L. extacted from 10 different growing areas. Polysaccharides of *L. barbarum* L. basically consisted of six monosaccharides, named mannose, rhamnose, galactose acid, glucose, galactose and arabinose with an average molar ratio of 1.00 : 1.38 : 0.63 : 92.37 : 1.18 : 3.55. The analytical method was applied to identify the polysaccharides in *L. barbarum* L. bought from 8 different companies. The results showed that the content of polysaccharides and the relative molecular ratio of glucose in two of the samples were higher than average values, they were more likely to be adulterated with other polysaccharides. With its simple, sensitive and repeatable merits, this method was suitable to analyze the composition characteristics of polysaccharides in *L. barbarum* L.

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THREE NEW NAPHTHALENES FROM THE ROOTS OF *HIBISCUS SYRIACUS*

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Hibiscus is a genus of flowering plants belonging to Malvaceae family. The genus includes both annual and perennial herbaceous plants, as well as woody shrubs and small trees, which is widely grown in tropical and subtropical region including eastern and southern Asia. *H. syriacus* is the national flower of Korea, therefore, widely cultivated in Korea. Recently, anti-proliferative activity of the root bark of this plant against cancer cells has been reported and several triterpenoids were isolated as active constituents [1]. In this study, we investigated the roots of *H. syriacus* for the isolation and characterization of its active constituents. As a result, six naphthalenes (1 – 6) were isolated and characterized as three new naphthalene derivatives, namely mukunglenes A – C (1 – 3), together with three known ones, syriacusin B (4), syriacusin A (5) and parvifloral (6) [2]. The isolated compounds dose-dependently inhibited the proliferation of A549 lung cancer cells. Among isolated compounds (1 – 6), compound 2 showed most potent anti-proliferative activity on A549 cells with IC_{50} value of 0.26 μM. Therefore, the roots of *H. syriacus* and its naphthalenes can be useful for the development of anti-proliferative therapeutics.

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IN VIVO AND *IN VITRO* TOXICOLOGICAL EVALUATION OF A NEWLY SYNTHESIZED COPOLYMER FOR MICELLAR DELIVERY OF CAFFEIC ACID PHENETHYL ESTER

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Copolymer micelles are suitable carriers for problematic hydrophobic drugs (including natural substances) due to their high loading capacity. The aim of the present study was to perform an initial safety assessment of a micellar system based on the newly synthesized C_{12}-PAGE-PG60 copolymer consisting of polyglycidol and poly(allyl glycidyl ether). The micelles were loaded with a hydrophobic caffeic acid phenethyl ester (CAPE), because of its promising cytotoxic and antioxidant activities.

The assessment of the micelles included *in vitro* (evaluation of cytotoxicity on HepG2 and L929 cell lines, lysosomolytic activity on a model of lysosome membranes and hemolytic activity on human erythrocytes) and *in vivo* tests (full blood counts, serum markers of liver and kidney injury, oxidative stress markers in liver homogenate and liver histological evaluation, after acute and 14-days' exposure on *Wistar* rats).

MTT and LDH assays after 24 and 48 h treatments with the empty and CAPE-loaded micelles (0.1 - 32 μg/ml) indicated no cytotoxic effects of the empty micelles and retained cytotoxic activity of CAPE loaded in the micelles. No hemolysis or lysosomolysis were observed *in vitro*. *In vivo* hematological, biochemical and histological assays of rats, treated with the empty or CAPE-loaded micelles (375 and 750 μg/kg) did not reveal pathological changes of any of the parameters assayed after acute or 14-days' treatment.

In conclusion, initial toxicological data characterizes C_{12}-PAGE-PG60 as a non-toxic and promising copolymer for development of micellar drug delivery systems, particularly for a hydrophobic active as caffeic acid phenethyl ester (CAPE).

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MICELLAR CURCUMIN SUBSTANTIALLY INCREASES THE ANTINEOPLASTIC EFFICACY OF ERUFOSINE AGAINST TWIST1 POSITIVE CUTANEOUS T-CELL LYMPHOMA CELLS

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The orphan disease cutaneous T-cell lymphoma (CTCL) primarily affects the skin by clonal accumulation of CD4+CD45RO+ helper/memory neoplastic T-lymphocytes. CTCL progression and metastasis are related to expression of the transcription factor TWIST1. Concomitant bacterial infections due to impaired skin barrier and immune deficiency increase the morbidity and mortality rates. CTCL therapy is often empiric and not based on specific molecular alterations. Therefore, development of new treatment modalities is warranted. In the current work, we showed that the antilymphoma potential of erufosine is decreased by TWIST1 expression, but combinations with micellar curcumin (MCRM) included in methoxy poly(ethylene glycol)-block-poly(ε-caprolactone) copolymer carrier overcome this problem. MCRM was characterized by low zeta-potential, slow release of curcumin and fast cell membrane penetration. The median inhibitory concentrations of erufosine were compared in CTCL cells with or without TWIST1 expression using the MTT-dye test. Erufosine and MCRM combined synergistically at a ratio of 1 : 4. The combination effects were presented as isobolograms using the MAPLE software. The synergistic combination inhibited the expression of

TWIST1 and PKB/Akt as proven by western blot. Noteworthy, NFκB activation was reduced as measured with specific ELISA. Cell death ELISA, caspase-3 activity, glutathione and CFU assays revealed induction of apoptosis, altered glutathione levels and significant inhibition of cell clonogenicity. MCRM potentiated the antistaphylococcal activity of erufosine and prevented biofilm formation as shown by broth microdilution test and crystal violet staining. In conclusion, the fundamental knowledge generated by this study contributes to the development of new nano-technological treatment modalities for CTCL based on rational drug combinations with specific antineoplastic and antibacterial activities.

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BRAF KINASE AND SMOOTHENED HOMOLOG AS POTENTIAL NOVEL TARGETS OF FLAVONOLIGNANS FROM *SILYBUM MARIANUM*

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Silymarin, a standardized mixture of flavonolignans, extracted from the fruits of milk thistle (*Silybum marianum)* is well-known for its hepatoprotective properties. Recently the main active compounds of silymarin, silybins and their 2,3 dehydro-derivatives, have been shown to exert anticancer activities, however the mechanisms of these actions are not clarified yet [1].

In this study the chemical similarity between silybin and 2,3-dehydrosilybin diastereoisomers and the drugs in the DrugBank database [2] (2240 unique structures) was evaluated with ROCS software [3]. TanimotoCombo index (TCI) was applied to score similarity and it was hypothesized that flavonolignans may interact with the same protein targets as chemically similar drugs do. The similarity with the anticancer drugs vismodegib and vemurafenib was scored with TCI ≥ 0.8 and the potential of silybins to interact with their targets, Smoothened homolog and BRAF kinase, respectively, was supported by docking. Further *in vitro* studies of the effects of silybins on the Hedgehog signaling pathway including Smoothened homolog, BRAF V600E kinase activity, and mutated BRAF V600E A375 human melanoma cell line were performed. The preliminary results outline dose-dependent profiles and suggest possible effects of the studied compounds on these targets with

dehydrosilybins showing stronger effects than silybins. Further experiments are ongoing to more precisely quantify the observed effects.

Our results reveal novel anticancer targets for flavonolignans from *S. marianum*.

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A FACILE SYNTHESIS OF METHYLATED ISOPRENOID CYTOKININS

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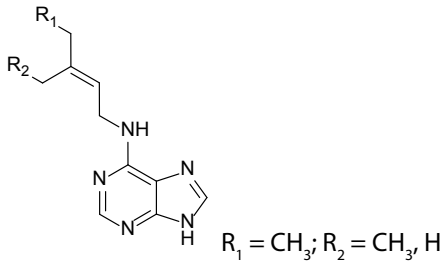
Cytokinins (CK) as group of naturally occuring plant hormones are N^6 substituted derivatives of purine base adenine, responsible for many events in plant growth and development such as cell division, initiation of shoots, apical dominance and leaf senescence [1, 2]. They are according to the substitution in the position N^6 of adenine scaffold divided into two main groups: isoprenoid (N^6- (Δ^2 – isopentenyl)adenine (2-iP), N^6- (Δ^3 – isopentenyl) adenine (3-iP), dihydrozeatin (DHZ), *cis*-zeatin (cZ), *trans*-zeatin (tZ)) and aromatic (kinetin (K), benzylaminopurine (BAP) and hydroxylated derivatives topolins (*o*T, *m*T, *p*T) [3].

Methylated CK analogues (MeCKs), produced by bacteria *Rhodococcus fascians*, are known for their ability to inhibiton of root growth, a hallmark of CK action. Moreover, they are relative stable against CK oxidase/dehydrogenase and therefore they retaine longer in planta in comparison with classical CKs [4]. MeCKs biosynthesis is controlled by S-adenosyl methionin dependent methyltransferases and the best substrate for methylation is N^6- isopentenyl diphosphate [4].

A new facile 3 step synthesis of mono- and dimethylated N^6- (Δ^2 - isopentenyl)adenine, starting from corresponding ketone and diethyl cyanomethyl phosphonate, is described and it is much less complicated than former described method [4]. Final products were purified by prepararive column chromatography and crystallization. Final compounds, as well as intermediates, were analysed by standard physico-chemical metods (ESI-MS, GC-MS, NMR, elemental analysis).



$R_1 = CH_3$; $R_2 = CH_3$, H

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METABOLOMICS PROFILING AND BIO-PHARMACEUTICAL PROPERTIES OF TWO *RUBUS* SPECIES: MULTIDIRECTIONAL APPROACHES

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In the present study, the pharmacological, enzyme inhibitory and antioxidant properties, as well as, the phytochemical profile of the ethyl acetate, methanol, and water extracts of *Rubus sanctus* and *R. ibericus* leaves were determined using *in vitro* bioassays. Wide range of phytochemicals including hydroxybenzoic acids, hydroxycinnamic acids, acylquinic acids, ellagitannins, flavonoids, and triterpenoid saponins were determined using UHPLC-ESI/HRMS technique. The ethyl acetate and methanol extracts of the studied Rubus species effectively inhibited acetyl and butyryl cholinesterase. On the other hand, *R. sanctus* water extract showed low inhibition against a-amylase and prominent inhibitory action against a-glucosidase. Data collected from this study supported the radical scavenging and reducing potential of the studied Rubus species. Pharmacological investigation of the protective effects of the different extracts of *R. sanctus* and *R. ibericus* in experimental model of ulcerative colitis was performed. The extracts were tested on spontaneous migration of human colon cancer cells (HCT116) in wound healing experimental paradigm. Only *R. sanctus* methanol extract inhibited spontaneous HCT116 migration in the wound healing test.

Our results suggested that *R. sanctus* and *R. ibericus* may be a potential candidate as sources of biologically-active compounds for the development of nutraceuticals, pharmaceuticals, and/or cosmetics.

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BENEFICIAL EFFECTS OF DIETARY POLYPHENOLS ON DIABETES MELLITUS

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Diabetes mellitus is the most common disorder of the endocrine system and causes about 1.5 million deaths per year. Polyphenols comprise a large family of naturally occurring secondary metabolites of plant derived foods associated with the health beneficial effects. Polyphenols are generally classified into flavonoids and nonflavonoids. And flavonoids are also classified into further subclasses according to their structural differences (flavanones, flavones, dihydroflavonols, flavonols, flavan-3-ols or flavanols, anthocyanidins, isoflavones and proanthocyanidins). Numerous studies have demonstrated the health benefits of polyphenols as effective supplements for management and prevention of diabetes based on *in vitro* and animal models. Polyphenols affect glycemia through different mechanisms, including the inhibition of glucose absorption in the intestine by sodium-dependent glucose transporter 1 or of its uptake by peripheral tissues. They can inhibit α-amylase and α-glucosidase, stimulate insulin secretion and reduce hepatic glucose output. Polyphenols also enhance insulin-dependent glucose uptake, activate adenosine monophosphate-activated protein kinase, modify the gut microbiome and have anti-inflammatory effects. In addition, dietary polyphenols exhibit protective action against the oxidative stress associated with diabetes. Polyphenols improve glucose metabolism, lipid profile, regulating the hormones and enzymes in human body, further protecting human being from diseases like diabetes, obesity and their complications. In fact, different signaling pathways have been linked to polyphenols. This review brings recent studies which establish the beneficial properties of polyphenols for diabetes mellitus and analyzes the mechanisms involved in these properties.

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NOVEL ARYLNAPHTHALENE LIGNAN GLYCOSIDES FROM *JUSTICIA PROCUMBENS* AND THEIR ANTIVIRAL POTENTIAL

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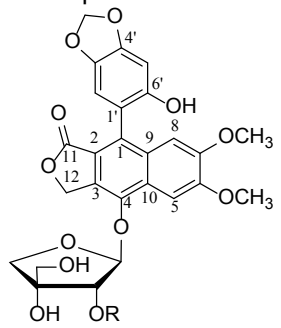
In our previous study, we identified a number of potent anti-HIV aryl naphthalene lignans (ANL) glycosides from *Justicia cf. patentiflora Hemsley* [1, 2]. We subsequently analyzed chemical constituents from other *Justicia* plants and found that *J. procumbens* Linn., was rich in containing ANL compounds. The aerial parts of the plant are used as a popular traditional medicine in China for treatment of fever and inflammation. In the present study, we aim to search for new anti-HIV leads based on the ANL compounds found in this plant. Guided by TLC using chloride ferric as color developing reagent, phytochemical separation of the methanol extract of the stems and barks of this plant led to the isolation of seven new ANL glycosides, together with ten known ones. Their structures were determined on the basis of extensive spectroscopic analyses, and their antiviral potential has been evaluated using a "One-Stone-Two-Birds" system [1]. The ANL compounds (**1**-**2**) demonstrate a unique structural feature by containing a hydroxyl group at C-6' position, which is rare and has not been reported from *Justicia* plants.



1 R = H
2 R = α-L-arabinopyranosyl

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UHPLC-HRMS PROFILINGS OF BULGARIAN AND TURKISH ENDEMIC SPECIES FROM ASTERACEAE

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In the present study Bulgarian and Turkish endemic species belonging to trib Cardueae (Asteraceae) were investigated by ultra high-performance liquid chromatography – Orbitrap high resolution mass spectrometry (UHPLC-HRMS). *Cirsium appedinculatum* Griseb. was collected from Vitosha Mountain (region Zlatni mostove), Bulgaria. *Centaurea patula* DC, *Centaurea urvielli* DC. subsp. *hayekiana* Wagenitz, *Centaurea kotschyi* (Boiss.) Hayek var. *persica* (Boiss.) Wagenitz and *Centaurea drabifolia* Sm. subsp. *detonsa* (Bornm.) Wagenitz were harvested from Central Anatolia Region of Turkey. The sample preparation was performed by ultrasound-assisted extraction with aqueous-methanol. Subsequent UHPLC-HRMS was acquired on LC/HRMS system consisting of a Q Exactive Plus mass spectrometer, equipped with a heated HESI-II source. The UHPLC separations were performed on C18 column with a binary mobile phase consisting of 0.1% formic acid and acetonitrile using a gradient elution mode. A variety of hydroxycinnamic and acylquinic acids, as well as flavonoid aglycons and *O*- and *C*-glycosides were identified or tentatively elucidated for the first time in the investigated accessions. The methylated flavone cirsimaritin was found to be the major compound in *C. appedinculatum* extract, while chlorogenic acid, apigenin-*C*-glycosides, hispidulin and methylated flavone and flavanone glycosides were the dominant components in *Centaurea* species. The obtained UHPLC-HRMS results highlight the studied Asteraceae endemic species as potential new sources of phenolic acids and methylated flavonoids. Further *in vitro* biological investigations are needed for thorough understanding of their activity.

Acknowledgements: The study was supported by Grant D-222/12.12.2018 from the Medical Science Council at the Medical University of Sofia.

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CHEMICAL PROFILE AND PRODUCTIVITY OF INDUSTRIAL HEMP GENOTYPES

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Industrial hemp (*Canabis sativa* L.) is on the most promising old 'new' high-value crops in the world, with a number of potential uses not matched by any other crop. With the current decriminalization of industrial hemp in many countries, there is a need to evaluate the available genotypes for chemical profile and productivity. Therefore, the objective of this study was to evaluate 20 industrial hemp genotypes in a 2-year field experiment. The two primary bioactive constituents in industrial hemp are canabidiol (CBD) and tetrahydrocannabinol (THC), for the health/dietary and the recreational markets, respectively. The canabinoid concentration varied significantly as a function of plant genotype and plant part. The CBD concentration in the herba varied from 0.1 to 2.0%. Overall, the CBD concentration of the flowers was close to the ones in the herba. However, some genotypes had more CBD in the flowers, while others had more in the herba. The highest CBD concentration was found in the glume. The concentration of THC in the herba also varied significantly; from not detected to over 0.08%. Generally, THC concentration was less in the flowers and glume than in the herba, however, some genotypes had higher THC concentration in the glume. Plant genotypes differed in morphological characteristics and fiber yield. Overall, stalk yields varied from around 8,800 (Fedora and Silesia) to >19,000 kg/ha (Fibrinova). Fiber yields ranged from around 2,200 to over 6,000 kg/ha. This study provided comparative information for genotypes with various characteristics for distinct market demands.

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EVALUATION OF EXTRACTION SOLVENTS AND BIOACTIVITIES OF SELECTED CULTIVATED HERBAL SPECIES IN GREECE, PROMOTING SUSTAINABLE AGRICULTURAL DEVELOPMENT

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Medicinal and aromatic plants contain chemical constituents first used by humans as medicines for healing as well as flavoring agents for food and drink. These plant materials continue to play important role in human life, as sources of modern herbal medicines, as food supplements, spices, and in cosmetics' industry [1, 2]. In the framework of the re-evaluation of Greek plants with potential commercial interest, the extracts from selected Greek plants were studied and bioassayed.

Ten cultivated (ordinary, organic cultures) species in Thessaly region, popular and of high economic significance in Greece, were studied: *Pimpinella anisum*, *Rosmarinus officinalis*, *Mentha viridis*, *Origanum dictamnus*, *Origanum vulgare*, *Ocinum basilicum*, *Olea sylvestris*, *Aronia melanocarpa* and two samples of *Lavandula angustifolia.* They extracted with ethanol as well as with "tsipouro" (a Greek pomace brandy, strong distilled spirit containing 40-45% alcohol by volume and is products from pomace – residue of the wine press). It has been shown that "tsipouro" as extraction solvent gave a significantly higher amount of dry extract for all samples. The total phenolic content was estimated by the Folin-Ciocalteu method and the free radicals scavenging activity was determined by DPPH and FRAP assays. *O. vulgare, O. dictamnus, R. officinalis, O. basilicum* and *M. viridis* demonstrated the highest phenolic content (up to 165 mg GAE/g dry extract) and the strongest antioxidant activity (up to 456 μmol Trolox equivalents/g dry extract). The antimicrobial activity of all extracts was tested against six Gram (+) bacteria and three pathogenic fungi, showing a very interesting antibacterial profile.

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PP 234

PIMPINELLA ANISUM ESSENTIAL OIL FROM LESVOS: EFFECT OF HYDRODISTILLATION TIME, COMPARISON OF THE AROMA WITH OTHER SAMPLES OF THE GREEK MARKET

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Ouzo (ούζο) is a dry anise-flavoured famous aperitif widely consumed in Greece and its taste is similar to other anise liquors like raki, pastis and sambuca. Since the essential oil of *Pimpinella anisum* L. (Apiaceae), is used as key flavouring agent of ouzo, we report in this study, to the best of our knowledge for the first time, the effect of extraction technique and duration of hydrodistillation (1, 2 and 3 h) with respect to yield, composition and identification rate of extracted essential oils from cultivar of anise seeds from Lesvos island (Plomari) well-known for ouzo production with PDO.

The essential oils of four samples (Lesvos, Thessaly, Viotia and one Turkish sample) were analyzed by GC-MS and their compositions were compared to the one given in European Pharmacopoeia for medicinal purposes. Thus, trans-anethole, followed by γ-himachalene, estragole and trans pseudoisoeugenyl 2-methylbutyrate were the most important and abundant metabolites.
The effect of hydrodistillation time was very important as the results showed that in 1 h-distillation the oil yield was the highest (80 ml/kg) decreasing to 60 ml/kg and 25 ml/kg as trans-anethole levels were higher after 2 and 3 hours, respectively.

It is noteworthy that Lesvos anise oil showed the best quality according to EurPharm while it had the highest yield and the richest chemical composition (29 identified compounds) among all studied samples, contributing to the excellent quality of ouzo from Plomari.

Furthermore, from the essential oil of this sample, the two major secondary metabolites (trans-anethole and γ-himichalene) were isolated and structurally determined.

Acknowledgments: The authors would like to thank the Pharmacist A. Barbayannis for providing us the fresh anise seeds from Plomari.

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PHYTOCHEMICAL ANALYSES FROM *IN VITRO* CULTURES OF *RINDERA GRAECA* BOISS. & HELDR

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Rindera graeca Boiss. & Heldr. (Boraginaceae) is an endemic plant of South-East Europe and Mediterranean Basin. This species, listed in the WCMC Plants Database as "Rare", grows mainly on stony slopes with shallow soils within the main range 1700-2200 m. To our best knowledge *R. graeca* has never been studied phytochemically before. The chemical analyses of *in vitro* cultures (shoots and transgenic roots), showed the presence of phenolic secondary metabolites as: caffeic acid (CA), rosmarinic acid (RA), lithospermic acid (LA), lithospermic acid B (LAB) together with an unusual phenolic naphthoquinone pigment (rinderol), previously isolated by our scientific team also from *Cynoglossum columnae* Ten. *in vitro* natural roots. All isolated compounds have been determined structurally by modern spectral means ($^{1}H/^{13}C$, ^{1}H-^{1}H and ^{1}H-^{13}C correlation NMR spectroscopy, ESI-MS). *Rindera*'s methanolic extracts from all different *in vitro* cultures were screened for their content of pyrrolizidine alkaloids (PAs) through Mattocks-Molyneux visualization reagent and SPE clean out method as proposed by BfR followed by LC-MS analyses to confirm the potential presence of PAs and N-oxides.

Moreover, the total phenolic by the Folin-Ciocalteu method was determined, while all extracts and isolated metabolites were tested for their antimicrobial activites against a panel of human pathogenic bacteria and fungi showing an interesting profile.

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PHYTOCHEMICAL ANALYSIS OF PHENOLIC COMPOUNDS IN *COTINUS COGGYGRIA* AND *AGRIMONIA EUPATORIA* FROM BULGARIA

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Cotinus coggygria Scop (Anacardiaceae) and *Agrimonia eupatoria* L. (Rosaceae) are two species_widespread in Europe and particularly in Bulgaria [1]. They have been used in folk medicine due to their rich chemical composition [2, 3].

Aim: The aim of this study was to analyse the dynamics of main plant phenolic compounds in one vegetation season and to make a comparison with the same substances acquired from the market.

Materials and methods: Plant materials were collected in one vegetation season. The determination of total polyphenols, tannins, flavonoids and phenolic acids was performed with spectrophotomethric methods.

Results and conclusions: The leaves of *C. coggygria* harvested in the middle of the summer (July) were very high in total polyphenols and tannins, while the difference in dynamics of flavonoids and phenolic acids was very lightly visible in all vegetation periods. Phenolic dynamics in *A. eupatoria* showed a maximum of total phenols, tannins and flavonoids content in June and lack of difference in phenolic acid variation.

A comparison between content of plant phenolics in these herbal substances and those bought at the market showed the need for standardization of herbal drugs before their use.

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COMPARISON OF VOLATILES' COMPOSITION FROM SELECTED *SALVIA* SPECIES, CULTIVATED IN GREECE

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The genus *Salvia* (sage) is one of the largest and most important medicinal and aromatic genera of the Lamiaceae family with about 900 species widespread throughout the world. Extracts and essential oils from these plants are used widely in cosmetic and food industry as aromatic ingredients as well as for their potential therapeutic properties. The chemical composition of non-polar extracts and essential oils from selected, not previously studied, *Salvia* spp. cultivated in Greece, were studied (*S. aurita* L.f., *S. bucharica* Popov, *S. canariensis* L., *S. chionantha* Boiss., *S. desoleana* Atzei et Picci, *S. coccinea* Buc'hoz ex Etl.).

Their aerial parts were collected from J. & A.N. Diomedes Botanic Garden, National and Kapodistrian University of Athens. The fresh leaves were submitted to hydrodistillation and the obtained essential oils together with their cyclohexane extracts have been chemically analysed through GC/MS. Monoterpenenes, sesquiterpenes and diterpenes are the main chemical categories among all identified secondary metabolites, while viridiflorol (sesquiterpene alcohol) appeared as the most abundant compound in *S. canariensis* and diterpenes are the abundant compounds in the other studied species. Moreover, it is noteworthy that sclareol (diterpene alcohol) reaches 50% of the total in *S. desoleana*.

The antimicrobial activities of the extracts of all studied species were also assayed against Gram negative and positive bacterial, as well as against human pathogenic fungi exhibited an interesting biological profile.

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ROSMARINUS OFFICINALIS L. EXTRACTS FROM GREECE AND ITS SECONDARY METABOLITES AS ANTAGONISTS OF THE ARYL HYDROCARBON RECEPTOR

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Aryl hydrocarbon receptor activation is potentially implicated in a series of skin inflammation diseases, like seborrheic dermatitis [1], and cancer development [2] and for this reason it would be very important to identify natural compounds that could inhibit the AhR activation by ligands like dioxin (TCDD) or of microbial origin like FICZ or indirubin.

Clinical data have shown that rosemary can have an alleviating effect on seborrheic dermatitis [3] and this led us to investigate the presence of AhR antagonists.

For this purpose, we first prepared 6 different dry extracts from rosemary leaves (ROS) (with different time or solvent of extraction). The extracts (10 and 100 ppm) were assayed for their activities as antagonists of AhR ligand binding with guinea pig cytosol in the presence of [^{3}H]TCDD. All assayed ROS extracts showed similar dose dependent activities with almost complete inhibition at 100 ppm.

The total methanol extract extract was further assayed in human keratinocytes, towards CYP1A1 mRNA induction using RT-PCR. At 10 μg/ml the extract showed 99% inhibition against TCDD, 50% inhibition against FICZ and 90% inhibition against indirubin.

In a next step we isolated the major metabolites: carnosic acid, carnosol, 7-methoxy epirosmanol, dimethoxyapigenin, and methyl betulinate, and assayed their agonist and antagonist activity in the presence and absence of TCDD with the method of gel retardation assay (GRA). All assayed metabolites exhibited dose dependent antagonist activity. Carnosic acid and carnosol showed 80% inhibition of TCDD activity at 100 μM and 30% inhibition at 10 μM.

Rosemary extract is an interesting natural material for further development of AhR antagonists.

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PP 239

A GREEK CULTIVAR'S ARTICHOKE-HYBRID SEEDS, AS A BIOPRODUCT OF POTENTIAL HIGH NUTRITIONAL VALUE

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Cynara cardunculus L. – artichoke, is a perennial plant of the Mediterranean basin, known since antiquity as food and for its highly appreciated therapeutic properties (leaves) [1]. Furthermore, the plant has been widely used as a bioenergy crop. In this particular study, the seeds of a certain established Greek cultivar of *C. cardunculus* hybrid that has been cultivated in the experimental field and used as biofuel have been investigated. The shifting from conventional crops to artichoke cultivation has brought economical and environmental advantage according to the literature [2]. The main purpose of our study was to evaluate the chemical profile and the potential high nutritional value of its seeds, not previously studied and till now just wasted as a bioproduct. The non-polar cyclohexanic extract was saponificated, esterificated and subjected to GC/MS analyses, where to our results appeared as a rich source in ω-6 and other fatty acids (linoleic, palmitic, oleic and arachidonic acid). Several secondary metabolites have been further isolated and structurally elucidated by NMR, from the methanolic extract (caffeoyl derivatives) and the dichloromethanic one (fatty acids and aryl ester derivatives). Moreover, the methanolic extract displayed the highest content of total phenolic content (142.83 mg GAE/g) followed by dichloromethanic (64.32 mg GAE/g) and aqueous extract (88.87 mg GAE/g). Furthermore, the results of the antioxidant activity (ABTS, DPPH) and enzyme inhibitory (cholinesterases) activity of the methanolic and aqueous extracts are in progress.

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PP 240

CHEMICAL ANALYSIS OF *PRUNUS ARMENIACA* (APRICOT) KERNEL IN COMPARISON WITH BITTER ALMONDS – BIOLOGICAL PROPERTIES

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Apricot is one of the most important fruit species grown in the world, as the third economically most important stone fruit crops [1], with total world production reaching around 4.3 million tones. The fruit is highly appreciated by consumers for their flavor and aroma [2]. Three *Prunus armeniaca* (apricot) seed kernel from the two most important Greek varieties (Bebekou-A1, Diamantopoulou-A2) and one commercial product-B from the market were phytochemically studied to our knowledge for the first time and they were compared with Greek bitter almonds, according to their chemical and aroma profile. Their aroma was studied by Headspace Solid-Phase Microextraction (HSSPME)/GC-MS analysis, showing that benzaldehyde was the abundant aroma compound for all the samples (90-95%). Through GC-MS analysis volatile compounds as fatty acids, aldehydes and alcohols were identified while a characteristic triglyceride of saturated and unsaturated fatty acids (palmitic, stearic, oleic and linoleic acid), which has been isolated identified through spectral means in all three apricot kernels and bitter almonds. It is noteworthy that the apricot kernels A1 and A2 showed chemical similarities with bitter almonds explaining their traditional comparable uses.

Moreover, squalene a hydrocarbon widely used in skin protection products was identified and isolated only from the Greek apricot A1 and A2 kernels.

The total phenolic content was determined by the Folin-Ciocalteu method for all the samples and the antimicrobial activity was assayed against Gram-positive and -negative bacteria and human pathogenic fungi, exhibiting a broad spectrum of antimicrobial activity.

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COMPARISON BETWEEN TRADITIONAL PHYTOTHERAPY AND SCIENTIFICALLY-PROVEN DOCUMENTED PHYTOTHERAPY: *TEPHROSIA*

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Tephrosia ssp. genus contains about 400 species of shrubs indigenous in tropical and sub-tropical regions of Africa, Asia and America. The aim of this study was to highlight the therapeutic potential of the most widely investigated species via the most recent scientific studies and compare their traditional use as medicinal plants with their scientifically-proven documented phytotherapy. They are used by local populations of these regions as insecticides and rat, fish and human poisons, as a healing agent for skin wounds and gonorrhea, as well as a pain reliever effective for headache and toothache. Traditionally, the plant is used in herbal remedies as tonic, laxative and diuretic, useful in the treatment of cough, fever, asthma, allergies, rheumatism, abdominal swelling, biliary febrile attacks, obstructions of the liver, spleen and kidneys, recommended as a blood purifier, and as anthelmintic for children. Pharmacological studies referred that several *Tephrosia* species possess antibacterial, antifungal, insecticidal activities and antidiabetic, antioxidant, anti-inflammatory, analgesic, anxiolytic, antiallergic and estrogenic properties. Some species have found to exhibit cytotoxic capacity and anticancer properties. Phytochemical analysis on many species of *Tephrosia* genus resulted to the isolation of coumarins and several flavonoids (1-3). The traditional use of the species is not totally identical the scientifically proven phytotherapy, but there are similarities like its use as a hepato-protective, nephroprotective, antimalarial and anti-epileptic agent. All this information may be useful for the promotion of the further use of these plants in medicinal products, justifying also their traditional use as beneficial to health.

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PARTICIPATION BY CORRESPONDENCE

PbC 1

IN SILICO AND *IN VIVO* STUDIES OF *ASTRAGALUS GLYCYPHYLLOIDES* SAPONIN(S) WITH RELEVANCE TO METABOLIC SYNDROME MODULATION

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Triterpenoids are well known modulators of metabolic syndrome and one of the suggested modes of action (MoAs) involves PPARγ binding [1, 2]. In this study we aimed to: (i) evaluate *in silico* potential metabolites of the sapogenin of the main saponin present in a purified saponins' mixture (PSM) from *Astragalus glyciphilloides* [3] and their PPARγ binding patterns; (ii) investigate *in vivo* the antidiabetic and hypolipidemic effects of PSM on streptozotocin-induced diabetic spontaneously hypertensive rats (SHRs).

A knowledge-based expert system (Meteor Nexus, Lhasa Limited) was used for *in silico* prediction of sapogenin's metabolites. Their possible PPARγ binding modes were investigated by pharmacophore-based docking (MOE, CCG Inc.). The biological effects of PSM in SHRs with type 2 diabetes were assessed by measuring blood glucose, triglycerides, total cholesterol and markers of oxidative stress, and were compared to those of pioglitazone. Liver histopathology was studied by haematoxylin and eosin staining.
In total, 24 structures of probable/plausible Phase I metabolites and their PPARγ binding patterns as weak partial agonists were predicted. In the *in vivo* experiment, PSM showed beneficial effects on all tested parameters, which were supported by the histological data.

This study stresses how combining the *in silico* approaches allows for a more comprehensive exploration of potential active forms of natural products in terms of their biotransformations and receptor-mediated MoA.

The reported *in vivo* results show the promising pharmacological profile of the PSM with its hypoglycaemic, hypolipidemic and antioxidant effects.

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PbC 2

PREDICTING PPARγ PARTIAL AGONISM OF NATURAL TRITERPENOIDS: DEVELOPMENT OF A PHARMACOPHORE-BASED VIRTUAL SCREENING PROTOCOL

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Naturally-derived triterpenoids are promising scaffolds for metabolic syndrome (MS) modulators' design possibly by nuclear receptor PPARγ targeting [1]. In this study we aimed to: (i) develop and validate a pharmacophore-based virtual screening protocol for PPARγ weak partial agonists and (ii) screen naturally-derived triterpenoids with relevance to MS and PPARγ signalling.

X-ray complexes of PPARγ partial agonists from PDB [2] were analysed and series of their structural analogues were searched in the literature. The NIH PubMed and PubChem systems were used to collect naturally-derived triterpenoid PPARγ agonists/up-regulators with unknown receptor binding mode and maximal relative efficacy. Ligand and structure-based methods were applied to develop a virtual screening protocol for PPARγ partial agonists.

A multi-step VS protocol was developed including: docking in the receptor sub-pocket normally occupied by the PPARγ weak partial agonists, pharmacophore filtering, and re-docking using pharmacophore placement and refinement with induced fit. The protocol was validated over 2500+ PPARγ decoys extracted from the DUD-E database [3], and two in-house virtual libraries consisting of 13 PPARγ partial agonists from PDB and 100+ PPARγ weak partial agonists (X-ray ligands' analogues). Using the VS protocol, it was predicted that triterpenoids known to activate/up-regulate PPARγ receptor exhibit weak partial agonist-like binding patterns.

Our results contribute to the mechanistic explanation of the effects of triterpenoids by a potential PPARγ-mediated mode of action. They can direct further studies of triterpenoid compounds focused on the design of moderate PPARγ activators with relevance to MS-related disorders.

Acknowledgements: This study is supported by National Science Fund of Bulgaria (grant DM 01/1/2016).

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PbC 3

SAMBUCUS NIGRA: CONTRIBUTIONS TO THE PHYTOCHEMICAL INVESTIGATION OF ITS FLOWERS AND HERBAL TEA

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Black elder (*Sambucus nigra* L., Adoxaceae) is a common shrub, widely distributed in Europe as a constituent of forest plant communities. Elder flowers are known for their diuretic, diaphoretic, laxative, and secretolytic effects [1]; more recent studies highlighted their benefits in diabetes [2]. While organic constituents of the flowers have been well studied, the inorganic part of this officinal prime matter is poorly known; the same situation applies for its aqueous extracts. The aim of the present study was to analyze the mineral constituents of elder flowers and to determine their passage onto herbal teas. Forty-eight elements were determined using ICP-AES (Inductively coupled plasma-atomic emission spectroscopy) and ICP-MS (Inductively coupled plasma-mass spectrometry). Furthermore, the total polyphenol content of the aqueous extract was measured employing the Folin-Ciocalteu method, and antioxidant activity was assessed towards DPPH. The dry flowers are particularly rich in K (40.7 g/kg dry weight), Ca (7.4 g/kg), Mg (6.4 g/kg), transferring 63%, 18% and 52% of their K, Ca and Mg content, respectively, onto water upon extraction. Other important microelements of elder flowers are Mn (49 mg/kg), Zn (47 mg/kg), Cu (18.5 mg/kg), and I (1.0 mg/kg) with respective transfer ratios of 47%, 26%, 10%, and 52%. Total phenols were 16.2 mg GAE/g DW, and antioxidant activity represented 61.2 mg ascorbic acid/g. These data show that herbal teas prepared from elder flowers may be useful additives in supplying health-promoting macroelements, while acting in the same time as antioxidants.

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PbC 4

CYTOTOXIC AND ANTIMIGRATORY EFFECTS OF SULFURETIN AND COTINUS COGGYGRIA EXTRACT

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Smoke tree (*Cotinus coggygria* Scop.) is used in the traditional phytotherapy of Southern and Central Europe, Russia and China for the treatment of skin and mucosal lesions. The cicatrizing effect is supported by the presence of tannins, volatile oils and various flavonoids [1]. The aim of the study was to assess the cytotoxic and antimigratory effects of natural products obtained from the wood of the plant. Tests were performed on a diethyl-ether soluble fraction, its main component sulfuretin, and their respective inclusion complexes in hydroxypropyl-beta-cyclodextrin (HPBCD). Cytotoxicity was evalated using Alamar blue, using various cancer cell lines (human melanoma: A375, murine melanoma: B164A5, lung cancer: A549, breast cancer: MDA-MB-231) and normal keratinocytes (HaCat cells). The antimigratory properties were assessed by the scratch-assay, using the above-mentioned cell lines. Sulfuretin and its inclusion complex in HPBCD proved to be non toxic for HaCat cells, but exhibited cytotoxic activity against lung cancer cells in the low micromolar range. The diethyl-ether extract proved to have a good cytotoxic effect against A549 cells (decreasing the cell viability to 30% following the application of 50 mg/mL extract) and a weak activity against B164A5 and MDA-MB-231 cells. The scratch-test supported the nontoxic effect of the natural products from *C. coggygria* wood on keratinocytes, with sulfuretin stimulating the proliferation and migration of cells. For the other cell lines, cellular migration is not inhibited, thus an anti-metastatic effect could not be pointed out for the tested products.

Acknowledgements: The authors acknowledge the financial support from grant UEFISCDI, PN II-CT-789/30.06.2014.

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PbC 5

SOPHORA JAPONICA L. LEAVES AND BRANCHES – ANATOMICAL CHARACTERISTICS AND MICROCHEMICAL INVESTIGATION

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Sophora japonica L. (Fabaceae) flower buds represent a plant product rich in flavonoids, triterpenes and isoflavonoids. It is an important source for rutin extraction, compound with antioxidant, antimicrobial, anti-inflammatory, anticancer properties. Although flower buds are most used, leaves, fruits and branches have also been investigated [1]. Recent pharmacological studies showed that extracts from leaves present antioxidant, antidiabetic and cytotoxic activity [2]. The aim of the present study was to bring a contribution to the assessment of anatomical features of *S. japonica* leaves and branches. To this end, freehand cross sections were made. Toluidine blue and red Congo were used as dyes and polarization filters to assess the presence of calcium oxalate crystals. For microchemical reactions Folin Ciocalteu and iodine-KI reagents were used. The leaflets present a bifacial structure, epidermis with anomocytic stomata and numerous rhomboidal crystals. Uniseriate trichomes are present on both lower and upper epidermis. The rachis presents a narrow collenchyma under the epidermis and a sclerenchymatous tissue. The branch exhibits a typical secondary structure and reducing polyphenols were located mainly in the cortical parenchyma. Numerous starch grains are found in the external parenchymal tissue of rachis and branches as well as in branches pith. The evaluation of the anatomical characteristics is important to ensure proper control of plant material used for the preparation of plant extracts and also to prevent adulteration of medicinal vegetal products. The present study represents a first step for future research regarding *S. japonica* L. plant products.

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PbC 6

ISOLATION OF PHYTOCONSTITUENTS AND SCREENING OF SOME BIOACTIVITIES OF *EUPHORBIA HIRTA* L. AND *OLDENLANDIA CORYMBOSA* L.

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Medicinal plants are abundant in Myanmar. In the present work, two medicinal plants: *Euphorbia hirta* Linn. – Euphorbiaceae (Kywe-kyaung-hmin; KKH) and *Oldenlandia corymbosa* Linn. – Rubiaceae (Sular-na-phar; SNP) plants were chosen for isolation of phytoconstituents and bioactivity studies. By silica gel column chromatographic separation, D-friedoolean -14-en-3-one, taraxerol and b-sitosterol were isolated from pet-ether extract of KKH. Whereas, from ethyl acetate extract, KKH, gallic acid was isolated. In addition, stigmasterol and ursolic acid were respectively isolated from pet-ether and ethyl acetate extracts of SNP. The identities of all isolated compounds were made by modern spectroscopic techniques (UV, FT-IR, 1HNMR, 13CNMR, EI-MS and GC-MS). Acute toxicity of 95% ethanol and watery extracts of both samples were investigated with the dosage of 1 g/kg, 2 g/kg and 4 g/kg in mice body weight in albino mice and no lethality was observed up to fourteen days after administration. Antimicrobial activity of pet-ether, ethyl acetate, 95% ethanol and water extracts successively extracted from both plants and two isolated compounds: b-sitosterol and stigmasterol was investigated against six species of microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albicans* and *Mycobacterium* species by employing agar well diffusion method. According to the results, it can be inferred that since no toxic effect occurred in mice model, both plants may be used as the remedy for the treatment of diseases related to the microorganisms tested.

PbC 7

ULTRASONIC-ASSISTED EXTRACTION OF *ALHAGI PSEUDALHAGI* SECONDARY METABOLITES

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Alhagi pseudalhagi plant is used in natural medicine as a diuretic, laxative, antipyretic, diaphoretic, anti-inflammatory, and anti-microbial agent [1].

The paper presents the results of the study of ultrasonic extraction (USE) of biologically active substances (BAS) from the roots of *A. pseudalhagi*.

Ultrasonic extraction was performed with the use of Bandelin HD 2200 instrument. Activated reaction mass was treated with appropriate solvents and analyzed via GC/MS method. It has been discovered that the main components of the roots were *β*-sitosterol and lupeol. Moreover fatty acids such as oleic and *α*- linoleic acids, as well as alcans: gentriacontan, nonacosan, heptacosan were identified in the roots of *A. pseudalhagi* using GC-MS method.

The optimal condition for ultrasonic assisted extraction is determined by the yield of extractive substances of *A. pseudalhagi* and the content of the main biologically active components of the extract of - *β*-sitosterol and lupeol. Optimal conditions for ultrasonic extraction were power - 30 W, sounding time - 30 minutes, temperature - 46 °C. The yield of extractive substances with the use USE is 0.36%, the content of *β*-sitosterol and lupeol in a hexane extract is 18.46 and 12.27%, respectively.

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PbC 8

ANTIBRUCELLOSIS ACTIVITY OF SOME PLANTS OF FABACEAE FAMILY

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The aim of this study was evaluation of the antibrucellosis activity of the extracts of some Fabaceae family plants (roots of *Goebelia alopecuroides* Bge. and fruits of *Alhagi pseudalhagi*).

For the study of the antimicrobial and antibacterial activity of the obtained phytoextracts against the causative agents of brucellosis in vitro, the reference Brucella 16 M strain was used. When studying the sensitivity of strains to ethanol extraction, the roots of *G. alopecuroides Bge.* in the experiment used the extract with serial dilutions in 90% ethyl alcohol. When the ratio of solvent-extract is 1 : 5; 1 : 2.5; 1 : 0.625; 1 : 0.312; 1 : 0.156; 1 : 0.078 and 1 : 0.039 the *G. alopecuroides* Bge. extract has antimicrobial activity against the reference Brucella 16 M strain (no colony growth). In a ratio of 1 : 0.019 and 1 : 0.009, extruct does not possess antimicrobial and antibacterial activity with respect to the reference Brucella 16 M strain (a growth of colonies is noted). Then, as a hexane extract of the fruits *A. pseudalhagi* in the above-mentioned dilutions with alcohol does not possess anti-brucella activity.

The current findings have clearly demonstrated that *G. alopecuroides* Bge fruits extract has a high antibrucellosis activity.

Acknowledgements: This work was funded by project AP05132934 of the Ministry of Education and Science of the Republic of Kazakhstan.

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PbC 9

AN OVERVIEW ON *THYMUS DACICUS* BORBÁS.: DISTRIBUTION, ANATOMY AND CHEMICAL COMPOSITION OF ESSENTIAL OILS

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Thymus species are known to have medicinal properties due, in particular, to essential oils with numerous antimicrobial effects. The purpose of this paper is to make a synthesis of the accomplishment studies carried out on *T. dacicus*, regarding the distribution, the structure and the composition of the essential oils, known that in this genus exist a high variability of populations (with respect to morphological and the composition of essential oils). *T. dacicus* is a perennial herbaceous plant that grows on rocky pastures and sands in Romania, and NE of Serbia [1]. From histo-anatomical point of view we can mention that: passing to the secondary structure of the stems takes place early do to cambium activity; the endoderm is of Casparian type (specific to Lamiaceae); secretory hairs are always multicellular, being formed by a unicellular base, a unicellular pedicel and a single or multicellular gland [2]. Analyzing the chemical composition of essential oils a number of qualitative and quantitative differences have been observed, depending on phenophase and harvest year [3]. The main chemical components identified are geraniol, linalool, geranyl acetate, γ-cadinol and caryophyllene oxide [3]. The factors determining the composition and quantity of volatile oils are numerous, sometimes is difficult to differentiate between them, because many are interdependent. These factors include seasonal and maturation variations, genetic variations, geographical origin, growth phases and plant parts that are used.

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PbC 10

SALICORNIA EUROPAEA L.: PRELIMINARY ASPECTS ON MORPHOMETRY, ANATOMY AND BIOCHEMISTRY

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Salicornia europaea L., a succulent halophyte (Chenopodiaceae family), is widely distributed in coastal and inland salt marshes [1]. Many studies on halophytes have indicate that these species have a high content of polyunsaturated fatty acids, carotenoids, vitamins, sterols, essential oils, polysaccharides, glycosides, and phenolic compounds [2, 3], being potential candidates in food and pharmaceutical industry. The vegetal material is represented by individuals of *S. europaea* L., collected from 4 different locations in the Danube Delta (Murighiol, Sarinasuf, Plopu, Enisala) in 2017. For each individual specific measurement was made. The structure were analysed through classical techniques of histo–anatomy. The photosynthetic pigments (chlorophyll a, chlorophyll b, total carotenoids) and sugars leaf fraction (monosaccharides, disaccharides and polysaccharides) were analysed by spectrophotometric methods. From a morphological point of view, the tracking of specific characters (height of the plant, height from root to first branching, number of internodes, lengths of internodes, stem branching) highlights the fact that the living environment has a significant influence on the growth and development of plants. All the samples taken in the study highlight, the phenomenon of multiple cambium, at the stem level. During analyzed period, chlorophyll a and chlorophyll b increased from June to August, but the carotenoids content registered almost close values. Disaccharides compounds with an important role in maintaining osmotic pressure registered higher values especially on August followed by the polysaccharides. Sugars leaf fractions were reduced in Plopu where *S. europaea* is formed a monocoenosis on a large surface.

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PbC 11

PLANTS USED IN THE ETHNOMEDICINE OF RURAL AREAS FROM HUNEDOARA COUNTY, ROMANIA

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Ethnopharmacy investigates the classification, preparation, effects and uses of traditional remedies. It can help rediscover the traditional knowledge which may lead to the discovery of new drugs.

The present study was performed in Râu de Mori village, Hunedoara county, located in the Western part of Romania. Interviews were conducted based on a questionnaire. Ethnobotanical indices (Uses Totaled and Subjective Allocation) were calculated. Fifty plants were mentioned, among them, the most important were: *Calendula officinalis*, *Rubus idaeus*, *Betula pendula*, *Armoracia rusticana*, *Carum carvi*. Gastro-intestinal diseases were the most frequently mentioned problems and were treated with 16 different species, followed by respiratory (14 species) and dermatological diseases (9 species). In order to prepare remedies, several formulations were described. Infusions were indicated 59 times, sirups 12 times, cataplasms 9 times. Concerning parts of the plant: *herba*, *folium* and *flores* had the highest scores.

Anti-inflammatory effects *B. pendula* are already known, but an interesting and unusual manner to use it was to sleep with the feet in bags full of freshly collected leaves in order to treat rheumatism. Another method, also used for rheumatism, was to take hot baths with birch leaves for 1-2 hours once a year, at the beginning of summer.

Inquiries related to the traditional knowledge of the area are ongoing, in order to write down ethnomedical information and to inspire new research directions in drug development.

PbC 12

CHENOPODIUM ALBUM: KEY ANATOMICAL ELEMENTS

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Recent studies have shown that the leaves and seeds of *Chenopodium album* are rich in vitamins, minerals, essential aminoacids and it also posesses antiviral, antifungal, anti-inflammatory, antiallergic, antipruritic activities [1]. It is mostly used in veterinary formulations.

The aim of the present study was to investigate the anatomical characteristics which may be relevant for the microscopical quality control of the plant for its future phytoterapetical use. Free-hand cross sections were performed in the root, stem and leaf. They were examined using a light microscope, on the spot or after clarification and dying. The roots have a typical secondary structure with a cork layer, followed by a narrow secondary phloem, cambium and a thick secondary xylem. The stems exhibit an epidermis, an angular collenchyma in the corners of the stem and among them chlorenchyma, followed by a ring of sclerified pericyclic fibres, secondary phloem, cambium, secondary xylem, a ring of vascular bundles and a well developed medullar parenchyma. Leaves display a dorsiventral bifacial structure and an epidermis with anomocytic stomata on the lower surface. Druses of calcium oxalate are found in the stem, leaves and petiole. The pollen grains have a panporate exine. Salt bladders are found on the leaves surface.

This preliminary anatomical study needs further investigations regarding the benefits of *C. album* for future medicinal uses.

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PbC 13

SAMBUCUS NIGRA L.: BIOACTIVE COMPOUNDS WITH MEDICINAL AND NUTRITIONAL POTENTIAL

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Sambucus nigra L. (Caprifoliaceae family) is a shrub species that grows spontaneously in Europe, Asia, North Africa, USA [1], but is also grown in commercial plantations in some countries. The purpose of this paper is to provide a synthesis of knowledge about the bioactive compounds identified in different organs, their biological activities, the environmental significance of some compounds and the ways of using this species.

Studies have shown that the fruit is a potential source of bioactive compounds with medicinal and nutritional potential. Bioactive compounds identified in different organs of this species include: carbohydrates, lipids, sterols, proteins, organic acids, vitamins, polyphenol compounds (anthocyanins, flavonoids, phenolic acids), tannins, minerals, essential oils, etc. The identified bioactive compounds exhibit various pharmacological activities: antioxidant, antibacterial, antiviral, laxative, diuretic, immunomodulatory, analgesic, etc. [2, 3]. Flowers and fruits are used to make syrups, alcoholic beverages, jam, food colorants, pharmaceuticals, cosmetics.

Also, some polyphenolic compounds give color to flowers and fruits, helping to attract animals for pollination and seed propagation and protect plants against viral, bacterial or fungal infections.

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PbC 14

BIOPROSPECTING MEDICINAL FLORA FROM PIETRICICA PEAK, COUNTY BACAU (ROMANIA)

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This paper analyzes the medicinal flora of the Pietricica Peak, Bacau County (Romania). This area is located in the Subcarpathian Region of Moldova (Eastern Carpathian District) and has an area of over 1000 km^2.
The area is crossed by the Tazlau River, which has the largest intake of surface water in the area

In the habitats of the Pietricica Peak there is an high floristic diversity, accounting for more than 900 plant species, representing 24.3% of Romanian vascular Flora. This floral diversity is explained by: the variety of climatic, geographic and ecological factors, the existence of specific local microclimates, the multiple influences (Central European, Mediterranean and Pontic), as well as the traditional practices of vegetal resources valorisation (agriculture and shepherding).

As a result, out of the 900 identified vascular species - 53 species are medicinal, classified in 28 families and 49 genres. Families with the most medicinal species are: Rosaceae with 7 species and Asteraceae with 9 species. For each of the 53 medicinal species are mentioned: organs of plants used, bioactive compounds, indications and pharmacologic activities [1, 2]. Of the medicinal species, we mention, among others, *Sorbus aucuparia* (Rosaceae) and *Hippophae rhamnoides* (Elaeagnaceae) whose fruits have a strong and healthy influence, with a delay in the aging process [3].

The flora of Pietricica Peak and the widespread use of medicinal plants for the treatment of various diseases, constitutes an important local health care resource with significant potential for research and development of phytomedicines.

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PbC 15

EX-SITU BIOLOGICAL PRODUCTIVITY OF THE PROTECTED MEDICINAL SPECIES *ALCHEMILLA MOLLIS* (BUSER) ROTHM. AND *A. ACHTAROWII* PAWL. IN BULGARIA

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The biological productivity of the endangered in Bulgaria medicinal species *Alchemilla mollis* (Buser) Rothm. and *A. achtarowii* Pawl. was investigated in *ex-situ* conditions as a part of a broader study of their potential for cultivation. The studied sample included mature plants of three and four years age grown in common garden conditions. Six morphometric characteristics were analyzed for both species at the phenological stages of mass and late blooming when it is the recommended time for harvest of the aerial parts. These characteristics are flowering stems and basal leaves counts, fresh weight and dry weight. The values of the studied characteristics increase with the age of the plants of both species. At the stage of mass blooming regardless of the larger number of basal leaves the biomass of the flowering stems of all studied plants is about 2 times higher. In all sampled plants at the stage of late blooming the counts, fresh weight and dry weight of the flowering stems decrease with more than 90% than the previous stage. On the contrary the values of these indicators for the basal leaves increase with more that 100%. The yield of the cultivated *A. mollis* (3.39 : 1) and *A. achtarowii* (2.62 : 1) is significantly higher than those of the commercially gathered substance *Alchemillae* herba. Despite the relatively good values for some studied indicators, *A. achtarowii* plants are significantly smaller than those of *A. mollis* and show evidences of water and heat stress.

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HOME GARDENS – GEOGRAPHY OF BIOCULTURE AND QUALITY

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The current study focuses on plant diversity used in production of traditional food from the Balkans. The selected products are part of Ark of Taste e-catalogue of Slow Food and their recognition and promotion is a result of the collaborative network of 8 Balkan countries (including Turkey). All entries involve small-scale farmers and processors engaged in preservation of food biodiversity and traditions through sustainable use of biological resources. From over 250 products (primary and processed), 174 were found to include plant ingredients or to be local varieties/landraces. Most of the latter are cultivated in gardens and/or as small-scale crops being part of the disappearing traditional practices handed down from generation to generation. About half of the products are manufactured by small businesses that offer food at local or regional markets and/or restaurants. Analyzing the threats for food diversity in the Balkans we have detected a high level of similarity that presumes common approaches to safeguarding it. To illustrate the conclusions we show-case Bulgarian traditional products with geographical reference. Challenges and transformations related to plant heritage conservation at home gardens are discussed.

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FOOD, MEDICINAL, AROMATIC AND SPICE PLANTS IN BULGARIAN HOME GARDENS – INTERDISCIPLINARY PERSPECTIVES

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Our project addresses the interrelations and interplays between the plant world and the social world. Its main objective is to study how particular plant species/landraces grown in home gardens affect social relations and cultural patterns and how cultural orientations, local knowledge and experience, social relations and cultural practices affect plant diversity, influence selection, modification or loss of plant genetic resources. The field study is focused on village and small urban settlements situated in four provinces in South and North-West Bulgaria. Ethnobotanical and ethnological perspectives on food, medicinal, aromatic and spice plant diversity in the home gardens, old and modern ways of utilization of the resources, cultural and socio-economic interrelations are documented and discussed. We report examples of cultivation/preservation approaches in home gardens along with preserved and newly developed (bio)cultural (trans)formations in the plant world, incl. an alternative non-agricultural ways for development of plant resources in the rural context. The later is found namely in festivalization of (locally) renowned plants or varieties and related products and practices that provide not only additional source of income but also mobilization of knowledge and experience, the appreciation of local cultural specifics, targeted actions for preservation of the natural and cultural wealth.

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COMPARISON BETWEEN TRADITIONAL PHYTOTHERAPY AND SCIENTIFICALLY-PROVEN DOCUMENTED PHYTOTHERAPY: *OREGANUM VULGARE* AND *O. MAJURANA*

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Oreganum species are some of the most recognized plants of the Mediterranean flora with a wide spectrum of application since they can be used as spices and they can be cultivated. Among these species, *O. vulgare* and *O. majurana* attracted great interest about their pharmacological properties. The aim of this study was to highlight the therapeutic potential of these two species via the most recent scientific studies and compare their traditional use as medicinal plants with their scientifically-proven documented phytotherapy. It is obvious that the traditional use of the species is not totally identical the scientifically proven phytotherapy. There are similarities like its use against diarrhea, but there are also differences concerning their potential use as natural antioxidants or anticancer agents, because of their isolated chemical compounds [1-3]. It is important to emphasize the need to carry out further research studies and phytochemical analysis, so as to isolate more compounds that will justify the pharmacological action that is attributed to extracts or the essential oil of the plants or scientifically prove, or not, the other traditional uses of the plants. All this information may be useful for the promotion of the further use of these plants in food and medicinal products, justifying also their traditional use as beneficial to health.

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IN VIVO WOUND HEALING POTENTIAL OF *PHLOMIS CRINITA* LEAVES' METHANOL EXTRACT

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In recent years there has been a great interest in the antioxidant and antiinflammatory properties of compounds derived from medicinal plants [1]. The present study was designed to explore the *in vitro* antioxidant activity and *in vivo* wound healing potential of methanol extract from *P. crinita* leaves. The antioxidant activity was investigated by three different methods: β-carotene bleaching test (BCB), free radical scavenging test (DPPH) and ferric reducing activity power (FRAP). Studies were performed on excision wound model using albino Wistar rats. The healing potential of the test ointment was assessed by measuring biophysical parameters including wound contraction rate and epithelialization period. Hydroxyproline content was evaluated. Moreover, histological assessments were performed to evaluate fibroblasts proliferation, collagen deposition and neovascularization. *P. crinita* leaf extract expressed as a percentage inhibition of the DPPH free radical of 78.69 ± 2.31%. The test ointment-treated groups healed significantly faster, which was indicated by improved contraction rate (83.24 ± 3.02%) in comparison to control group (73.12 ± 3.51%). Period of epithelialization decreased by 19.33 ± 2.13 days compared to the control treated with Vaseline (25.66 ± 1.26 days). Moreover, biochemical analyses revealed a significant increase in hydroxyproline contents on day 16 (1.06 ± 0.02 mg/100 mg of granulation tissue) of the ointment-treated wounds in comparison to control group (0.89 ± 0.09 mg/100 mg of granulation tissue). Histological findings indicate that *P. crinita* promotes the skin healing process by promoting fibroblasts proliferation, collagen fibers deposition and neovascularization. This study demonstrated that the methanol extract of *P. crinita* leaves promoted the acceleration of the healing process.

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ANTIOXIDANT AND HEPATOPROTECTIVE EFFECT OF *TARAXACUM OFFICINALE* LEAF EXTRACT AGAINST CARBON TETRACHLORIDE-INDUCED LIVER INJURY IN RATS

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Taraxacum officinale commonly, known as dandelion is used for medicinal purposes especialy for the treatment of liver and kidney disorders since it has choleretic, diuretic, antioxidant and anti-inflammatory properties [1]. The aim of this study was to evaluate the antioxidant and hepatoprotective profile of *T. officinale* leaves' methanol extract. Preparation of the polyphenolic leaf extract of *T. officinale* was performed using 80% aqueous methanol. *In vitro* antioxidant profiling by multidimensional assays was carried out. CCl_4-induced hepatotoxic rat model was used. Methanol extract of *T. officinale* was administered at the dose of 250 mg/kg/day by gavage for 12 days and rats were sacrificed to collect the serum samples at the end of the12-days treatment. *T. officinale* leaf extract expressed as a percentage inhibition of the DPPH free radical at 1 mg/mL of 63.57 ± 3.42%. Obtained results revealed that administration of CCl_4 caused a significant increase in plasma ASAT, ALAT, and LDH activities and total bilirubin concentration, compared to the control group. In addition, a significant increase in the level of hepatic MDA content in CCl_4 group than those of the control. However, the treatment of experimental rats with *T. officinale* extract prevented these alterations and maintained the antioxidant status. The histopathological observations supported the biochemical evidences of hepatoprotection.

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IN VITRO AND *IN SILICO* TESTING THE FLAVONOID STABILITY

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Mas-selective detection of flavonoids requires analyte concentrations below 1 mg/L. Being polyphenolics, the flavonoids contain hydroxyl groups that make them prone to tautomerization and dimerization in polar media [1].

The **aim** of the current study was to test the flavonoid stability with the time of storage and their tendency for tautomerization and dimerization in polar solutions.

Methods: Standard solutions (0.05, 0.4, 3.0 mg/L in 70% methanol) of epicatechin, epigalocatechin gallate, quercetin, resveratrol, myricetin, kaempferol, and rutin were analyzed chromatographically. The concentration of each standard was measured threefold immediately after the preparation (t_0), at the 2^{-nd} (t_1), and at the 6^{-th} day (t_2) of storage at -21 °C. The structures of the tautomeric forms of the flavonoids were optimized in ground state at B3LYP/6-31+G(d) level. UV-Vis spectra were simulated using TDDFT/B3LYP/6-31+G(d) calculations.

Main results: The solutions of epicatechin, epigalocatechin gallate, rutin, and quercetin were stable for all tested concentrations at t_0. A decrease from the lowest target value (0.05 mg/L) for resveratrol (22%), myricetin (47%) and increase for kaempferol (42%) was found at t_0. For all tested concentrations the levels of resveratrol, rutin, quercetin, and kaempferol were elevated, more than 20% at t_2. Quantum chemical calculations revealed that some of the flavonoids (miricetin, kaempferol, quercetin, and rutin) may theoretically exist in different tautomeric forms while resveratrol is prone to dimerization.

Conclusion: Based on the quantum chemical calculations these changes in flavonoid concentration with the time of storage may be due to shift of the tautomeric equilibrium (kaempferol, quercetin, myricetin, rutin) and tendency for dimerization (resveratrol).

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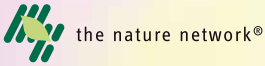

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