PHYTOCHEMICAL SOCIETY OF EUROPE MEETING

PHYTOCHEMICALS IN MEDICINE AND PHARMACOGNOSY

PIATRA-NEAMT, ROMANIA

27 – 30 APRIL 2014

BOOK OF ABSTRACTS

Trends in Natural Products Research

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PHYTOCHEMICALS IN MEDICINE AND PHARMACOGNOSY
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Book of Abstracts

2014 Phytochemical Society of Europe conference Phytochemicals in Medicine and Pharmacognosy

http://ccb-stejarul.ro/PSE2014.html

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Sunday, April 27 2014

10:00 – 16:00	**Registration**
16:00 – 16:30	**Opening ceremony** at Millenium Hall (Central Plaza Hotel)
16:30 – 17:10	**Plenary Lecture PL1** **Simon Gibbons** Phytochemicals as templates for "Legal Highs" and Antibacterials
17:10 – 17:30	**Coffee break** **Chair: Pinarosa Avato, Simon Gibbons**
17:30 – 18:10	**Plenary Lecture PL2** **Satyajit D. Sarker, Lutfun Nahar** Guided or not guided – that is the question
18:10 – 18:50	**Plenary Lecture PL3** **Evelyn Wolfram, Peter Samuel, Beat Meier** Phytochemical analysis using HPTLC and modern LC for application in quality control and medicinal plants research – trends and tools
20:00	**Welcome Dinner** at Avangard Hall (Central Plaza Hotel)

Monday, April 28 2014

Chair: Evelyn Wolfram, Satyajit D. Sarker

9:00 – 9:40	**Plenary Lecture PL4** **Nuria Chinchilla, Rosa M. Varela, Ascensión Torres, José M. G. Molinillo, Francisco A. Macías** How to get the right bioactive cocktail ?
9:40 – 10:00	**Short Lecture SL1** **P. Avato, M. P. Argentieri, P. Leonetti, L. Villanova, T. D'Addabbo** Nematicidal activity of artemisinin
10:00 – 10 :20	**Short Lecture SL2** **Francesco Epifano, Serena Fiorito, Salvatore Genovese** 4'-Geranyloxyferulic acid: an overview of its potentialities as an anti-cancer and anti-inflammatory agent
10:20 – 10:40	**Short Lecture SL3** **Jianbo Xiao, Petra Högger** Structure-uptake relationship of polyphenols by Caco-2 cells
10:40 – 11:00	**Short Lecture SL4** **Tatiana Onisei, Radu Stoianov, Manuela Rascol** Plants and biological active substances responsible for the physiological effects of the main categories of herbal food supplements from the Romanian market
11:00 – 11:20	**Coffee break** **Chair: Monica Hancianu, Christoph Carlen**
11:20 – 11:40	**Short Lecture SL5** **Agnieszka Mroczek, Ireneusz Kapusta, Bogdan Janda, Wirginia Janiszowska** Qualitative and quantitative analysis of saponin content in *Beta vulgaris* varieties

11:40 – 12:00	**Short Lecture SL6** **Guoyin Kai, Qiang Huang, Xiaorong Wang, Yanjie Zhang, Jianbo Xiao, Lijie Cui** Biosynthesis and regulation of tropane alkaloids biosynthesis in *Anisodus acutangulus*
12:00 – 12:20	**Short Lecture SL7** **Simona Carmen Litescu, Sandra A. V. Eremia, Gabriel-Lucian Radu** Alternative analytical tools (sensors/biosensors) in assessment of phytochemical active principles
12:20 – 12:40	**Short Lecture SL8** **Cristina Pavel** Clinical cases of recurrent respiratory infections at children treated with phytotherapy
12.45 – 13:20	**ANNUAL GENERAL MEETING (AGM) of PSE Members** at Baroc Hall
12:40 – 13:00	**Short Lecture SL9** **Camelia P. Stefanache, Samuel Peter, Beat Meier, Doina Danila, Catalin Tanase, Evelyn Wolfram** Phytochemical composition of *Arnicae flos* from wild populations in the northern area of the Romanian Eastern Carpathians
13:00 – 13:20	**Short Lecture SL10** **Camil-Eugen Vari, Amelia Tero-Vescan, Silvia Imre, Bianca-Eugenia Ösz** Controversy regarding the use of weight loss supplements with phytotherapeutic extracts for sportsmen
13:20 – 15:00	**Lunch** at Avangard Hall (Central Plaza Hotel) **Chair: Francisco A. Macías, Franz Bucar**
15:00 – 15:40	**Plenary Lecture PL5** **Christoph Carlen, José F. Vouillamoz, Xavier Simonnet** The importance of the genotype and the harvest stage on phytochemicals in medicinal plants and consequences for quality control
15:40 – 16:20	**Plenary Lecture PL6** **Miroslav Strnad, O. Novák, Danuše Tarkovská, Veronika Turečková, J.Grúz, J.Rolčík, A.Pěnčík, Kristýna Floková, R.Simerský, Karel Doležal** New mass spectrometry technologies for metabolite profiling
16:20 – 16:40	**Short Lecture SL11** **Tatiana Chiru, Fabiana Antognoni, Ferruccio Poli, Anatolie Nistreanu, Anna Benea** The antioxidant and anti-inflammatory activity of *Centaurea cyanus* L. extracts
16:40 – 17:00	**Short Lecture SL12** **Zhi-Zhong Guan, Xiao-Lan Qi, Xiao-Yan Hao** Protective effects of *Herba Cistanches* against neurotoxicity induced by β-amyloid peptide in SH-SY5Y cells
17:00 – 17:20	**Coffee break**
17:20 – 17:40	**Short Lecture SL13** **Anca Miron, Elena Cretu, Adriana Trifan, Maarit Karonen, Juha-Pekka Salminen, Cosmin Teodor Mihai, Pincu Rotinberg, Ana Clara Aprotosoaie** Phenolic profile and *in vitro* screening of Cyprus cedar bark for antioxidant and antitumor activities

17:40 – 18:00	**Short Lecture SL14** **Amir Reza Jassbi, Omidreza Firuzi, Ramin Miri, Ian T. Baldwin** Biologically active natural products from Iranian terrestrial and marine organisms
18:00 – 18:30	**WORKSHOP** (at Baroc Hall) ***Agilrom Scientific* – Alin Mogos** New equipment and methods developed by Agilent Technologies with application in Medicine and Pharmacognosy
18:30 – 19:30	**Poster Session** at Atrium Hall (Central Plaza Hotel) **Chair: Simona Litescu, Simon Gibbons, Franz Bucar**
20:00	**Dinner**

Tuesday, April 29 2014
Excursion
20:00 – Gala Dinner

Wednesday, April 30 2014
Chair: Anca Miron, Amir Reza Jassbi

9:00 – 9:20	**Short Lecture SL15** **Anna Benea, Maria Gonceariuc, Ion Dragalin, Anatolie Nistreanu, Tatiana Chiru** Study of volatile oil from the aerial parts of *Hypericum perforatum* L. by GC-MS
9:20 – 9:40	**Short Lecture SL16** **Petru Harghel, Rotaru Cristina, Sirbu Tatiana, Gheorghe Duca, Nicon Ungur, Veaceslav Kulciţki** Valorization of *Salvia sclarea* wastes. Efficient synthesis of sclareoloxide by sclareol ozonolysis in aqueous solvent system
9:40 – 10:00	**Short Lecture SL17** **Florin Catana Bota** The synergistic action of medicinal herbs extracts. Case study - INTERFERONAT
10:00 – 10:20	**Short Lecture SL18** **R. Albulescu, C. Tanase, A. Motaal, S. Shaker, I. Hassan, S. El-Bahrawy, M. Neagu, A. Grigore, G. Neagu, V. Vulturescu, G. Vassapollo, R. Bauer** Screening for natural anti-diabetic drugs in *Balanites aegyptiaca*
10:20 – 10:40	**Short Lecture SL19** **Veronica Gradinariu, Oana Cioanca, Lucian Hritcu, Elvira Gille, Adriana Trifan, Monica Hancianu** Comparative efficacy of *Ocimum sanctum* and *Ocimum basilicum* volatile oils against amyloid beta (1-42)-induced anxiety and depression in laboratory rats.
10:40 – 11:00	**Short Lecture SL20** **Elvira Gille, Cap Susana, Radu Necula, Georgiana Luminita Gavril, Rogobete Agnes, Valentin Aurica Grigoras, Ciobanu Emilia, Florin Catana Bota** Food supplements used as adjuvants in the therapy of lung diseases – clinical study – The Bisericani Pneumophthisiology Hospital
11:00 – 11:30	**Coffee break**
11:30	**Awards and Grants** **Closing ceremony**

Dear Participants,

It is a great pleasure and an honour to welcome you to the Phytochemicals in Medicine and Pharmacognosy meeting held in Piatra-Neamt, Romania. In this picturesque town, surrounded by mountains, with a rich history and vibrant culture, you will have the possibility to exchange scientific knowledge and put the basis for further collaborations.
In addition to the scientific program, we propose a one day trip to unique painted medieval monasteries in Northern Moldavia.
Both the Phytochemical Society of Europe and Stejarul Biological Research Center/National Institute of Research and Development for Biological Sciences Bucharest celebrate 57 years of activity. We invite you to celebrate together both anniversaries.
We are very grateful to all our sponsors for financial support and to all people involved in organisation of this conference. We deeply hope that this conference will meet all your expectations.

Welcome to Piatra Neamt and we wish you a pleasant stay!

Organizing committee

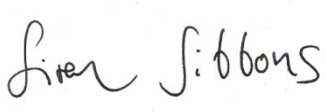
Dr. Elvira Gille, *Chairman*, Regional Representative for Eastern Europe of the Phytochemical Society of Europe

Professor Dr. Pharm. Anca Miron, *Co-chairman*

Dear Colleagues,

On behalf of the Phytochemical Society of Europe, it is my pleasure to welcome you to our conference "*Phytochemicals in Medicine and Pharmacognosy*"in Piatra Neamt, Romania, 27-30 April 2014. This meeting will cover aspects of natural products in medicine, bioactive compounds, their characterisation, ethnopharmacology and nutraceuticals. Dr. Elvira Gille and her team have produced an exciting program of international and national speakers and the Phytochemical Society of Europe is delighted to be part of this meeting.
I wish you a highly enjoyable and interesting conference and look forward to meeting you in Piatra Neamt!

With my best wishes!



Professor Simon Gibbons
President of the Phytochemical Society of Europe

Dear colleagues,

On behalf of the organizing committee, I have the pleasure to welcome you to our conference, entitled *Phytochemicals in Medicine and Pharmacognosy* that takes place at the Piatra Neamț, branch of the National Institute of Research and Development for Biological Sciences. This scientific meeting takes place in a beautiful location in the heart of the Carpathian Mountains, in an area with a great history and a unique conglomeration of Orthodox monasteries.
The aim of this congress is to bring together scientists from the fields of academic research and industry to share the recent developments in plant science and to discuss future prospects.
The study of aromatic and medicinal plants and derived products is included in the area of interest of our institute, especially here. The research of the Piatra Neamț branch is concentrated on this subject.
Herbs have been used to cure people for thousands of years. Mankind, since the beginning of its existence, in the beginning from instinct, intuition and experience, and later through a scientific and rational approach, has used and continues to use medicinal plants in order to cure or alleviate diseases.

In Romania there grow approximately 3600 plant species with medicinal properties, of which about 400 present high officinal values.
The available knowledge on the use of plant preparations in traditional medicine is important, but because the number of traditional healers is decreasing, the dissemination of their valuable knowledge is progressively diminishing. This valuable information constitutes a basis for the investigation of the pharmacological and phytochemical aspects of these natural medications, and thus for the validation of their therapeutic benefits and their possible toxic effects.
A recent survey has revealed that 61% of the 877 drugs introduced world-wide can be traced to or were inspired by natural products. The extensive use of those plants in traditional medicine around the globe has been described in many ethno-pharmacological reports. In recent decades there is a growing research literature on this field, mainly for the validation of ethno-pharmacological usage.

The aim of this conference is to give the participants an overview on plant-derived compounds in respect to human health and an industrial view on biotechnological applications using large-scale production of plant cells. The impact of plant products (phytochemicals) on human health is increasingly recognized. Many of the compounds of current interest have been known and studied for many years, but it is only with the advent of genetic and genomic approaches that their biosynthesis has been understood at a level to permit their engineering in crop plants. Evidence of nutritional value for various classes of natural plant products has been demonstrated. Plants and their products are generally known for their high levels of antioxidants, which may contribute to the positive effects of dietary plant

products for human health. Today, plant biotechnology can make important contributions to food security and nutritional improvement. Also most cosmetic products and their applications are defined by active ingredients and those active ingredients may derive from synthetic sources or from plant sources. The whole cosmetic research and development is desperately seeking new innovative plant ingredients for cosmetic application.

Concluding, I would like to point out that the subject of the conference is always actual; it has been expressed already in the Bible where, in the first chapter, it is said that God gave the multitude of plants for the nourishment and health of people (Genesis 1:29). And this idea is repeated in the last chapter of the Bible which refers to the tree of life for the health of the nations (Revelation 22:2).
My hope is that during this meeting, everybody will have an excellent possibility to meet colleagues, to develop new friendships and to establish fruitful collaborations.

I wish you all a very successful congress and a pleasant stay in Romania!

Dr. Manuela Elisabeta Sidoroff
Director General
National Institute of Research and Development for Biological Sciences

EMANOIL GRIGORESCU, PROFESSOR – 91 years anniversary

Professor dr. Emanoil Grigorescu is an outstanding personality with remarkable scientific achievements in the field of medicinal plants, **one of the foremost exponents of the Romanian School of Pharmacognosy.**

Prof. dr. Emanoil Grigorescu was born on the 10th of May 1923 in Oltenita (Ilfov county), Romania.

He has graduated from the Faculty of Pharmacy, Institute of Medicine and Pharmacy in Bucharest in 1948. In 1964 prof. dr. Emanoil Grigorescu defended his Ph.D. thesis *Contributions to the pharmacognostic and phytochemical study of indigenous Hippophaë rhamnoides L.*, initially supervised by Prof. dr. Elemér Kopp and then by Prof. dr. Teodor Goina.
Between 1948-1953 he was trainer and then assistant in the Department of Biochemistry, Faculty of Pharmacy, Institute of Medicine and Pharmacy in Bucharest.
In October 1953 he started his work as an assistant in the Department of Pharmacognosy and ten years later, in 1963, he became lecturer.
In October 1963, lecturer Emanoil Grigorescu was appointed substitute associate professor in the Department of Pharmacognosy of the Institute of Medicine and Pharmacy in Iasi.
In 1965 he became associate professor and three years later, professor and Ph.D. advisor. He was also involved in administrative activities being vicedean (February-October 1965) and dean of the Faculty of Pharmacy (October 1965-February 1975).
Prof. dr. Emanoil Grigorescu was interested not only in the isolation and chemical characterization of phytochemicals from different plants, but also in the possibility of using phytochemicals in the semi/synthesis of new drugs (several new semisynthetic derivatives with higher bioavailability such as rutin-cysteine, rutin-ε aminocaproic acid, berberine-ε aminocaproic acid and podophyllotoxin-tryptophan complexes).
His innovative research is materialized in more than 200 published works, 12 original drugs in microproduction (Rutin-S, Stomadex, Septazulen, Hipotens, Cerebrodil, Bilogal, Lactoflux), 7 original drugs in industrial production (Variterp, Rutin-S, Perasin, Plavobil, Plavocorona) and 56 patents. He was awarded three times by the Romanian Ministry of Education and Teaching (1963, 1968, 1971). In 1991 he was awarded the Title of Elite Inventor class IV.
Prof. dr. Emanoil Grigorescu supervised the activity of 14 Romanian and 5 foreign Ph.D. students. He has authored and coauthored several books in the field of

medicinal plants, of which the book *Plante medicinale, fitochimie şi fitoterapie* has received the Iuliu Hatieganu Award of the Romanian Academy in 1993.
It is noteworthy that in 1979 Prof. dr. Emanoil Grigorescu became United Nations Organisation (UNO) expert for medicinal plants. As UNO expert he took part in the organisation of three UNIDO (United Nations Industrial Development Organization) international courses partly held in the Department of Pharmacognosy in Iasi. He participated in two UNIDO missions in Burundi (1979) and Rwanda (1984).
Prof. dr Emanoil Grigorescu was president of Section of Pharmacy of the Society of Physicians and Naturalists, Iasi (1968-1975), Commission of Medicinal Plants in the Association of Scientists Iasi (since 1990) and Romanian Society of Phytotherapy (since 1993).
He was member (1976-1990) and then vicepresident (since 1990) of the Commission of Medicinal Plants of the Romanian Academy and member in several professional and scientific commissions (Medicinal Plants Commission for Elaboration of the Romanian Pharmacopoeia, Drug and Pharmacovigilance Commission, Research Commission for the Valorization of Medicinal and Aromatic Plants, Medicinal Plants Commission of the International Federation of Pharmacy).
In recognition of his outstanding merits, in 1991 Prof. dr. Emanoil Grigorescu became member of the Academy of Medical Sciences.

SIMON GIBBONS, Professor

Professor of Medicinal Phytochemistry and Head of Department
Department of Pharmaceutical and Biological Chemistry
UCL School of Pharmacy, London

Education
PhD in Phytochemistry (1994), Department of Pharmaceutical Science, University of Strathclyde (SERC funded scholarship) with Professor Peter Waterman and Dr. Alexander Gray.

Professional experience
Professor of Medicinal Phytochemistry and Head of Department, Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, London.

Research interests
Chemistry and antibacterial activity of natural products from plants; isolation, structure elucidation and biological evaluation of these compounds; analysis of 'legal highs' which are legal drugs of abuse related to existing controlled substances which are stimulants, depressants or hallucinogens.

Membership of journal editorial board and professional bodies
Council Member of the Home Office Advisory Council for the Misuse of Drugs (ACMD) and Chairman of the Novel Psychoactive Substances Committee (NPSW).
Council Member of the Medicines and Healthcare products Regulatory Agency (MHRA) Herbal Medicines Advisory Committee (HMAC).
Member of the Governing Council of the College of Medicine and Vice-Chairman of the Scientific Advisory Council of the College of Medicine 2010-present.
Vice-President and President of the Phytochemical Society of Europe (2010-2016).
Member of medical and pharmaceutical societies (Royal Society of Chemistry, Society for General Microbiology, Linnean Society of London, American Society of Pharmacognosy).
Member of the Phytochemical Society of Europe, membership secretary (2002-2008).
Founding Editor-in-Chief of *Phytochemistry Letters,* member of the Editorial Advisory Board of several ISI and scientific publications (*Natural Product Reports, Progress in the Organic Chemistry of Natural Products* ("Zechmeister"), *Planta Medica*, *Phytochemical Analysis, Phytochemistry Reviews*, *Fitoterapia*, *Phytotherapy Research*, *Chinese Journal of Natural Medicines (CJNM), Scientia Pharmaceutica* and *Phytopharmacotherapy)*.

Honours and Awards: Recipient of the Pharmanex Prize for Phytochemistry; of the first Tshwane University of Technology Vice Chancellor's Seminar Award, Pretoria, South Africa; of the 2005 Phytochemical Society of Europe - Pierre Fabre Award for Phytochemistry.

Author of numerous publications in ISI journals (e.g. *Bioorganic Medicinal Chemistry Letters, The Open Microbiology Journal, European Journal of Pharmacology, Journal of Natural Products*, *Current Drug Abuse Reviews, Phytotherapy Research, Natural Product Reports, Molecules).*

Phytochemicals as templates for "Legal Highs" and Antibacterials

GIBBONS S[1]

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Plants are an underexploited source of templates for new antibacterials and resistance-modifying agents [1]. Some excellent examples in this area include disulphide natural products from species of *Allium* and acylphloroglucinol compounds from *Hypericum*, which display strong *in vitro* potency against *Mycobacterium tuberculosis* and MRSA strains respectively [2,3]. Minimum inhibitory concentrations (MIC) values of some phytochemicals are comparable to commercially used antibiotics (MIC< 1mg/L).

Recently there has been an increase in the use of novel psychoactive drugs [4], some of which are based on plant and fungal natural products. These include mephedrone, which is based on cathinone and 5-MeO-diallyltryptamine, a synthetic tryptamine that is similar to bufotenin and psilocin [5,6].

This lecture will give examples of phytochemical antibacterials from our research and our recent chemical and neuro-pharmacological profiling of psychoactive substances that are being marketed in the United Kingdom as so called “Legal Highs”. These materials are often of dubious identity and have in the majority of cases, little or no pharmacology or toxicology data, which can add to the difficulty in assessing their harms.

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[3] Shiu W.K., Malkinson J.P., Rahman M.M. et al.: *Int. J. Antimicrob. Agents*. 2013, 42, 513-518.
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[6] Arunotayanun W., Dalley J.W., Huang X.P. et al.: *Bioorg. Med. Chem. Lett.* 2013, 23, 3411-3415.

SATYAJIT D. SARKER, Professor

Director of School of Pharmacy and Biomolecular Sciences
Faculty of Science
Liverpool John Moore University

Education

BPharm (Hons), 1st class and 1st in order of merit, University of Dhaka, Bangladesh
MPharm, 1st class and 1st in order of merit, University of Dhaka, Bangladesh
PhD in Pharmaceutical Sciences, University of Strathclyde, UK

Professional experience

2013 – present	Professor of Pharmacy and Director of School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John Moore University
2008 – 2013	Professor of Pharmacy, Deputy Head of Department of Pharmacy & Pharmacy Research Group Leader, Department of Pharmacy, University of Wolverhampton
2004 – 2008	Reader in Pharmacy, Course Director and Chair of the MPharm Course Planning Team, Course Director of the BSc (Hons) Pharmacology and MSc in Pharmaceutical Sciences courses, Department of Pharmacy and Pharmaceutical Sciences, University of Ulster

Research interests

Drug discovery, design and delivery: pharmaceutical, medicinal and natural products chemistry; biosynthesis of pharmaceutically and nutritionally important plant secondary metabolites; application of modern hyphenated techniques (LC-MS and LC-NMR) in natural products research; metabolomics of medicinal plants; toxicological and pharmacological evaluation and quality control of herbal medicine; development of bioassays for screening plant extracts and isolated compounds; synthesis of pharmaceutically important compounds; phytochemistry and bioactivity of mangrove plants from the Sundarbans.

Membership of journal editorial board and professional bodies

Since 2010	Editor-in-Chief, *Phytochemical Analysis*
Since 2011	Associate Editor, *TANG Journal*
2008 – 2013	*Honorary Treasurer* of the Phytochemical Society of Europe (PSE)

Guest Editor, Special issue of *Natural Product Communications* in honour of Professor Peter G Waterman (2008), and Special issue of *Advances in Pharmacological Sciences* on 'Anti-inflammatory, antinociceptive and antipyretic activities of medicinal plants and their constituents' (2012)

Member of the Editorial/Editorial Advisory Board of several scientific journals: *Phytochemical Analysis*, *Chromatographia*, *Natural Product Communications*, *The Open Natural Product Journal, International Journal of Synthesis and Characterisation, Systematic Reviews in Pharmacy*, *Pharmacognosy Research, Analytical Chemistry Letters, Free Radicals and Antioxidants, BioImpacts*.

Fellow of the Higher Education Academy (FHEA). Member of medical and pharmaceutical societies

Author of 361 publications in the field of natural products research*;* citations: well over 4800. Author of 4 books and 11 book chapters related to natural products isolation and their medicinal applications.

Guided or not guided – that is the question

Satyajit D. Sarker and Lutfun Nahar

Medicinal Chemistry and Natural Products Research Group, School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, England, United Kingdom

It may not be as dificult as the dilema that Hamlet faced with, 'to be or not to be – that is the question', but almost all natural products reseaerchers, working towards discovery of bioactive compounds, alsways face with the dilema of making a decision on whether they should follow a comprehensive bioassay-guided approcach or not. A bioassay-guided approach utilises at least one suitable bioassay to monitor any particular bioactivity of crude extracts and chromatographic fractions during an isolation process for purifying bioactive compounds. At times, this approach may feel like 'searching for a needle in a haystack',because crude extracts of plants or any other natural source materials generally contain hundreds of compoundsas complex mixtures. It is imperative to say that the choice of an appropriately sensitive bioassay to assess bioactivity is central to any bioassay-guided approach. The process is not as straigthforward as it seems, and there are obviously 'pros and cons' in any bioassay-guided approach. This talk will try to address this dilema by presenting some specific examples taken from the presenter's well over 25 years of research experience in the area of natuarl products.

Keywords: Bioassay-guided isolation, Natural Products, Bioactivity, Bioactive compound

Sarker SD and Nahar L (2012) Natural Products Isolation, 3rd Edition, Humana Press/Springer-Verlag, USA.

EVELYN WOLFRAM-SCHILLING, Dr.

Senior Scientist Phytopharmacy
ZHAW
Institute for Biotechnology
Research group Phytopharmacy, Wädenswil, CH

Birth date 5.03.1972

Education

2000 – 2001	Doctoral degree at the Department of Bioprocess Engineering, TU München
1991 – 1997	Environmental Engineering – TU Berlin, Degree: Diplom-Ingenieur
1994 – 1996	Environmental Engineering - University of Massachusetts, Amherst, USA, Degree: Master of Science

Professional experience

2009 – present	ZHAW, Institute for Biotechnology, Wädenswil, CH Senior Scientist Phytopharmacy
2006 – 2009	Cosmetochem International AG, Steinhausen, CH: Head of Research and Development, Quality Control and Quality Management

Research interests

Development of innovative active ingredients from plant extracts for application in pharmaceutical, food and dietary supplements; phytochemical analytical method development; process engineering and scale-up of extraction and drying processes from bench to production including outsourcing of process steps to toll producers, development of innovative cosmetic ingredients on the basis of plant extracts.

Stipends

1991-1996	Studienstiftung des deutschen Volkes – Promotion of the best 10% High school graduates. Including stipend for US study program.
1994 – 1995	Tuition Waver Stipend from the University of Massachusetts, Amherst
1995 – 1996	Research Assistant Stipend from the University of Massachusetts

Author of chapters and numerous publications in ISI journals (*Journal of Liquid chromatography, Applied Microbiology and Biotechnology, Water Science and Technology*) related to isolation of active principles from plants and their for application in pharmacy, food and dietary supplements.

Phytochemical analysis using HPTLC and modern LC for application in quality control and medicinal plants research - trends and tools

Wolfram Evelyn, Peter Samuel, Meier Beat

Zurich University of Applied Sciences ZHAW, Institute of Biotechnology, Phytopharmacy, Wädenswil, Switzerland

Introduction. Due to the complexity in number and structural diversity of natural substances in plant materials, phytochemical analysis has been a challenge since the times, when pharmacists like F. Sertürner and A. Séguin started to explore the active principles of potent plant derived remedies by chemical isolation at the beginning of the nineteenth century. It was a botanist - Mikhail Semenovich Tswett - who was first at the beginning of the twentiest century in using a solvent and paper as stationary phase to separate multicomponent mixture of plants and to compare "chromatograms", as he called the result of this method (1). Since then, the research and development in phytochemical analysis has been addressed intensively in science and industry and the number of variants of separation and detection methods is vast. The purpose of phytochemical analysis, could be ranging from fundamental research on secondary metabolites in plant material from different culture conditions and manipulations to routine quality control of starting material or end products of herbal remedies in Phytopharmaceutical and Nutrition Industry according to methods in Pharmacopeia monographs.

Aim of the presentation. This lecture presents the current status of instrumentation and methods for phytochemical analysis using HPTLC for qualitative comparison and its hyphenations to MS and bioactivity detection and HPLC as well as UHPLC for quantitative analysis. Focus is laid on the application in Quality control routines from longterm experience in development of analytical methods for the European Pharmacopeia (PhEur) as well as medicinal plants research from wild habitats, horticulture as well as biotechnological cultivation.

Methods. Modern and highly automatized HPTLC combined with MALDI-TOF and enzymatic bioactivity detection. HPLC and UHPLC with DAD and MS Detection.

Results. Instruments and methods have to be chosen in accordance to the aim of the analysis as well the financial possibilities of research groups and companies. Trends and tools are presented from literature and own experience with HPTLC (2) and UHPLC method development (3). Combinations and hyphenations for the more simple and qualitative HPTLC for rapid information on bioactivity and molecular mass of separated substance zones are presented. Moreover, the challenge of meeting the requirements of the broad applicability of LC methods for PhEur monographs and the first experience in using chromatographic modelling software for method optimization is covered.

Conclusion. Phytochemical analysis is and will be an ongoing challenge to be met by scientists, authorities and industry in the field of medicinal plant research and phytotherapy.

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2. Meier B and Spriano D. (2010) *Journal of the AOAC International. 93(5*):1399-1409
3. Rosenthal I, Wolfram E, Peter S, Meier B. (2014). *Journal of Natural Products*. Accepted for Publication. http://pubs.acs.org/doi/abs/10.1021/np400736s.

FRANCISCO A. MACIAS, Professor

Department of Organic Chemistry, Faculty of Science,
University of Cadiz, Spain

Birth date 18.09.1956

Education

1978 – BS in Chemistry, Faculty of Sciences, University of Seville, Spain.
1979 – Ms. in Organic Chemistry, Faculty of Sciences, University of Seville, Spain.
1984 – Ph.D. in Organic Chemistry, Faculty of Sciences, University of Cadiz, Spain.

Professional experience

2000 – present	Full Professor of Organic Chemistry
2007 – 2011	Vice – Rector for Research, Technological Development, and Innovation. University of Cadiz
2002 – present	Director of the Institute of Biomolecules (INBIO–UCA)
2002 – 2007	Dean of the Faculty of Sciences of Cadiz University

Research interests

Allelopathy studies in plants, lichens and microorganisms; plant – plant and plant microorganism interactions in natural and agroecosystems; parasitic plants; biosynthesis and synthesis of bioactive natural products; structure – activity relationship; bioassays in allelopathy studies; physiological effects of allelochemicals; search for natural agrochemical models.

Membership of journal editorial boards and professional bodies

Editorial Board member of the following journals: *Phytochemistry Letters, Natural Product Communications, Pesticide Management Science, Allelopathy Journal, Medicinal Chemistry Research, Phytochemistry Reviews, Phytochemical Analysis*.
Current Scientific evaluator of ANEP (National Agency for Evaluation and Prospective), Spain; International Foundation for Science, Sweden; American Chemistry Council, USA.
Current reviewer of numerous ISI journals (e.g. *Phytochemistry, Phytochemistry Review, Phytochemistry Letters, Phytochemical Analysis, Journal of Chemical Ecology, Planta Medica, Journal of Natural Products, Journal of Organic Chemistry, Journal of Agricultural and Food Chemistry, Medicinal Chemistry Research, Environmental Science and Technology, Plant Pathology; Plant Physiology).*

Editor of 2 books: *Recent Advances on Allelopathy*. Vol I, "A Science for the Future", Cadiz University Press, 1999 and *Recent Advances on Allelopathy*. Vol II, "Allelopathy. Chemistry and Mode of Action of Allelochemcals", CRC Press, 2004.

Author of 36 book chapters and 220 publications in ISI and scientific journals (*Pest Management Science, Chemistry & Biodiversity, Phytochemistry Letters Phytochemistry, Ultrasonics Sonochemistry, Natural Product Communications, Journal of Agricultural and Food Chemistry, European Journal of Organic Chemistry*, *Biochemical Systematics and Ecology, Journal of Natural Products)*.
Invited speaker and invited presentations: over 230.

How to get the right bioactive cocktail?

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In the process of isolating, identifying, characterizing, and determining the bioactivity of biocommunicators, proper extraction techniques are paramount. In ecological studies, the most appropriate methodology involves mimicking natural conditions by using water as a solvent and then re-extracting with organic solvents, or other procedures. This way presents some important drawbacks due to the complex mixtures obtained, the low amount of metabolites isolated, the formation of artefacts, and easy microbial degradation. For that reason, direct extraction with organic solvents has been widely used because this method yields less complex extracts and significantly higher yields, which is particularly useful when allelochemicals are localized on an specific organ, such as trichomes. In these cases, some corrections have been proposed to ensure that the original concentrations of the metabolites in the plant and the ecological role of the isolated compounds can be determined.

Several advanced extraction techniques can be applied to yield bioactive extracts and these include ultrasound-assisted extraction, supercritical fluid extraction and pressurized liquid extraction. These methods allow the recovery of compounds in shorter times and at lower temperatures. This avoids the destruction of active molecules due to high extraction temperatures. Ultrasound extraction is a very common extraction technique for the recovery of active components, mainly due to the mild extraction conditions applied. Cavitation is the ultrasound mechanical effect that enables greater penetration of solvent into the sample. Additionally, In the same way, supercritical fluid extraction technology, with or without the help of co-solvent, has been applied to the extraction of bioactive compounds.

During the design and development of an extraction process it is important to optimize highly significant factors that affect the extraction in order to obtain the most active extract. In this respect, it is necessary to carry out an effective bioassay to assess the activity during the extraction process. Experimental design provides techniques that can be used for both the evaluation of the effects of extraction variables and interactions between them. The bioassay selected in this study was the etiolated wheat coleoptile bioassay, which is both rapid (24 h) and sensitive. Furthermore, this bioassay can be considered as an initial assessment of phytotoxicity in which undifferentiated tissue cells are used.

CHRISTOPH CARLEN, Dr.

Director of Mediplant
Research Centre for Medicinal and Aromatic Plants
Switzerland
Birth date 14.03.1964

Education

1984 – 1989	ETH Zurich, Master in Agronomy, Plant Production Sciences
1990 – 1994	PhD in Plant Physiology at the ETH Zurich, Institute of Plant Sciences
2013	EPFL Lausanne, CAS in Management of Biotech, Medtech & Pharma Ventures

Professional experience

Since 2005	Head of the research divison 'sheltered crops and crops from alpine region, Agroscope, Institute for Plant Production Sciences, Centre de Recherche Conthey Director of Mediplant, Research Centre for Medicinal and Aromatic Plants

Research interests

Novel analytical methods to determine bioactive plant metabolites with rapid and/or non-destructive tools; plant bioprospecting and domestication of selected plant species or ecotypes for applications of high value products, such as cosmetics, food additives, food supplements and pharmaceuticals; development of cultivation procedures in order to allow a production of high-quality raw material in a sustainable manner, exploration of interactions of plants and other natural organisms (e.g. fungi, microorganisms) to increase quality, development of efficient control methods against pests and diseases; applied research in plant science in order to provide results and recommendations for Swiss and international agriculture for a high-quality and sustainable production.

Membership of professional bodies

Since 2009	Chairman of the ISHS (International Society of Horticultural Sciences) Working Group 'Medicinal and Aromatic Plants: Genetic Resources and Breeding'
Since 2011	Chairman of the ISHS Working Group 'Medicinal, Aromatic and Nutraceutical Plants from Mountainous Areas'
Since 2013	Board of PhytoArk SA, technology site specialised in the commercial development and pilot production of natural plant ingredients, particularly from alpine plants
Since 2014	Chairman of the Swiss Society of Agronomy

Recent Publications

Author of book chapters and numerous publications in ISI journals (*Food Chemistry, Acta Horticulturae*) related to Aromatic and Medicinal Plants and Phytochemicals in Plants.

E.g. Carlen, C. (2012). *Breeding and Cultivation of Medicinal Plants*. In: *Herbal Medicines: Development and Validation of Plant-derived Medicines for Human Health*. Hrsg. CRC Press Taylor & Francis, Taylor & Francis, 79-91.

Crespo, P., Bordonaba, J.G., Terry, L.A., Carlen, C. (2010). Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. *Food Chemistry*, (122), 16-24.

Carlen, C., Carron, C.A., Simonnet, X. (2012). Optimising the cultivation of white genepi to achieve stable yields and high quality. *Acta Horticulturae* 955, 211-217.

The importance of the genotype and the harvest stage on phytochemicals in medicinal plants and consequences for quality control

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Plants are the source of many important pharmaceuticals. Especially plants rich in secondary metabolites are of interest. Collection from wild and agricultural production of medicinal plants still remain the most important supply for plant–derived pharmaceuticals and natural products. However, harvesting from wild, especially for species with a high demand, can cause loss of genetic diversity and habitat destruction due to overharvesting. The agricultural cultivation of medicinal plants is an interesting alternative and offers several advantages such as reliable botanical identification, availability of well-defined genotypes adapted to the requirements of the stakeholders, a steadier source of raw material and less plant extract variability due to well-defined harvest stages.

Agronomic research and development play an essential role to improve cultivation of medicinal plants by increasing their quality, profitability and sustainability. In this context, breeding of new genotypes (cultivars) is a key factor allowing to adapt phytochemicals in the plants and to increase the yield potential. Breeding for increased yield of valuable compounds, for elimination of unwanted compounds, for tolerance against abiotic and biotic stresses and for better homogeneity of the cultivars are important issues.

To optimize yield and quality potential of the selected genotypes, research on best cultivation practices is also essential to get information on optimal conditions for seed germination, plant growing (planting, fertilization, irrigation), drying and conservation. A main issue in this context is the definition of the optimal harvest stage. The harvest stage has a high importance on the content and the composition of phytochemicals within the plant. These research results are integrated in the guidelines for GAP (Good Agricultural Practice) for medicinal plants recommending cultivation procedures optimizing the quality of medicinal plants.

The importance of the genotype of a plant species and the harvest stage on phytochemicals in medicinal plants are shown and consequences for quality control are discussed.

New mass spectrometry technologies for metabolite profiling

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The identification and quantification of natural low molecular weight substances in plant tissues are necessary for physiological studies of their metabolism and mode of action. The major problem associated with phytohormone analysis is that the amount of phytohormones present endogenously in plant tissues is very low, usually in the range of fmol to pmol/g fresh weight. We discovered that a combination of different sorbents, reverse phases and ion-exchange phases, is the best tool in the one-step purification, giving a total extraction recovery ranging between 50-80% for all studied phytohormone compounds.
A fast chromatography technique, the ultra-performance liquid chromatography was coupled to triple quadrupole mass spectrometer equipped with an electrospray interface (ESI) and the unique performance of collision cell – ScanWave^{TM}. In MRM mode, the detection limits for most of phytohormones (cytokinins, auxins, abscisic acids, gibberellins, brassinosteroids) as well as phenolic acids and mammalian steroids were close to 1 fmol and achieved linear range was at least five orders of magnitude. Using the technology can allow the quantification of phytohormones and their derivatives (in total around 150 compounds) in very limited amounts of material, less than 50 mg FW. The methods provide substantial improvements in terms of robustness, sensitivity, selectivity, convenience, through-put and cost-effectiveness over previous methods published. The new analytical approach makes possible a new direction in plant hormone metabolite profiling. We believe that UPLC-ESI(+)MS/MS technology can be used for fast and sensitive quantitative analysis showing reproducibility in the plant hormone profiling in different plant tissue extracts.

Tarkowska D, Novák O, Floková K, Tarkowski P, Turečková V, Grúz J, Rolčík J, Strnad M (2014) Quo vadis plant hormone analysis? *Planta, DOI 10.1007/s00425-014-2063-9*

Nematicidal activity of artemisinin

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Artemisia annua L. (Asteraceae) is an old Chinese medicinal herb traditionally used in Asia for curing chills and fever. The chemical profile of the plant has been extensively studied and a large number of sesquiterpenoids, mainly sesquiterpene lactones, unique for the species has been identified [1]. Among them, artemisinin, the unusual endoperoxide sesquiterpene lactone produced in the secreting glands of the aerial parts, has acquired particular interest for its therapeutic application to treat mild and severe forms of malaria. In addition, some studies have indicated that the molecule is a useful antiparasitic agent against human and animal parasites [1].

Continuing our studies on the nematicidal activity of phytochemicals, we have assayed *in vitro* the nematotoxic potential of artemisinin against some phytoparasitic nematodes selected among the most harmful to economic crops: the root-knot nematode *Meloidogyne incognita*; the golden cyst nematode, *Globodera rostochiensis* and the ectoparasite dagger nematode, *Xiphinema index* [2].

Nematodes were exposed to a 50 µg ml^{-1} solution of artemisinin for 2, 4, 8 and 24 h. The sesquiterpene solution was lethal to more than 50% of *G. rostochiensis* juveniles within 24 h, but did not highly affect *M. incognita* juveniles (8% mortality) and *X. index* females (22% mortality). Results suggest a potential of artemisinin as nematicide with a species-specificity against target nematodes.

As a comparison, bioassays with artesunate, a semi-synthetic derivative of artemisinin with a better bioavailability in water, have been also carried out. Obtained results allowed some considerations on the structure-activity relationships [2].

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2. D'Addabbo T, Carbonara T, Argentieri MP, Radicci V, Leonetti P, Villanova L, Avato P. (2013) *EJPP* 137:295-304.

4'-Geranyloxyferulic acid: an overview of its potentialities as an anti-cancer and anti-inflammatory agent

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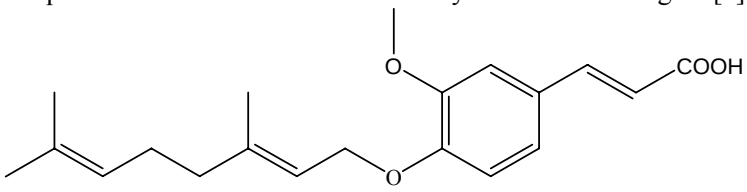
3-(4'-Geranyloxy-3'-methoxyphenyl)-2-*trans* propenoic acid (4'-geranyloxyferulic acid, GOFA) **1** is a secondary metabolite biosynthetically related to ferulic acid in which a geranyl side chain is attached to the phenolic group. It has been extracted for the first time in 1966 from the roots and bark of *Acronychia baueri* Schott (Fam. Rutaceae), an Australian small tree. In the last decade the pharmacological properties of the title compound began to be characterized, revealing its valuable potentialities as an anti-inflammatory and anti-tumor agent [1].



1

During the last years our research group made several efforts to characterize in more details the phytochemistry and pharmacology of compound **1**, as well as to get insights into the mechanism of action underlying the observed effects. In this context the aim of this communication is to report the insofar described biological properties of 4'-geranyloxyferulic acid.

Data collected during the last decade indicates that **1** is an effective dietary feeding colon cancer chemopreventive agent *in vivo*. When administered to animals, in which colon adenoma and adenocarcinoma have been induced by the administration of azoxymethane in the diet, as a large bowel delivered prodrug linked to dipeptides, or as an inclusion complex in cyclodextrins, or coupled to known anti-inflammatory agents like L-NAME, a protective effect on colon cancers growth and development up to 83% in terms of incidence and up to 95% in terms of multiplicity have been recorded. In every cases a large decrease of the concentration of pro-inflammatory mediators of the expression and activity of key enzymes like COX-2 and *i*NOS and parameters related to tumour pathology, like the mitotic index, have been shown.

Investigations on the mechanism of action and interactions of 4'-geranyloxyferulic acid with biological targets revealed that this natural product is able to inhibit geranylgeranyl transferase I, to activate peroxisome proliferator-activated receptor-β/δ (PPARβ/δ), to moderately inhibit glycoprotein P, and finally to inhibit the genomic expression of COX-2.

Recent studies on the natural occurrence of 4'-geranyloxyferulic acid, showed the presence of this oxyprenylated secondary metabolite in widely consumed edible fruits and vegetables like agrumes and their phytopreparations.

[1]. Genovese S, Epifano F. (2012). *Curr Drug Targets* 13: 1083–99.

Structure- uptake relationship of polyphenols by Caco-2 cells

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Natural polyphenols especially flavonoids are the most abundant antioxidants in human diet and have attracted great interests since the 1990s due to growing evidence of their beneficial effect on human health. Biological properties of polyphenols depend on their bioavailability. However, the relationship between the chemical structures of polyphenols and their pharmacokinetics are not well understood. The specific objective of the current research project is to discover the relationship between the molecular characteristics of polyphenols and their absorption, distribution and metabolism.

Caco-2 cells are grown as monolayers in75-cm^2 tissue culture flasks at 37 °C in a humidified atmosphere of 5% CO^2. Medium will be changed every second day. After 14 days cultured, cells were removed from flasks by trypsinization and seeded out in 12-well plates at a density of 3×10^5 cells/cm^2, allowed to adhere, and grown to confluence for one additional day. Fifty-eight polyphenols (flavones, flavonols, isoflavones, flavanones, stilbenoids) (1×10^{-2} mol/L) are dissolved in DMSO or methanol as stock solutions. Stock polyphenols were freshly diluted with DMEM to a concentration of 1×10^{-4} mol/L and stored at -20 ºC and the final concentration of individual polyphenol in the culture media is 10.0 µM. After 1 h incubation with polyphenols, culture media of Caco-2 cells are stored at -20 ºC for measurement of polyphenols. Control polyphenols are carried out without cells.

Structure-uptake relationship:

1) Hydroxylation of polyphenols improves the uptake;

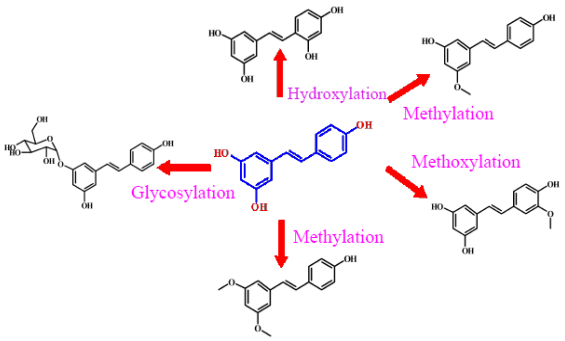
2) Glycosylation of polyphenols decreases the uptake;

3) Methylation and methoxylation of polyphenols weakens the uptake.

4) Presence of an unsaturated 2,3-bond in conjugation with a 4-carbonyl group has been associated with higher uptake.

Molecular property-uptake relationship of polyphenols aglycones on Caco-2 cells:

(1) For the polyphenols aglycones (stilbenoids, flavones, flavonols, isoflavones), there is a relationship between the XLogP3/hydrogen bond donor numbers and their uptake in Caco-2 cells. The uptake of aglycones in Caco-2 cells decreased with increasing partition coefficient and increased with increasing hydrogen bond donor numbers.



(2) For flavanoids aglycones, the uptake in Caco-2 cells increased with increasing partition coefficient and decreased with increasing hydrogen bond donor numbers.

(3) The polyphenols glycosides all showed very lower uptake, so their molecular property-uptake relationship is meaningless.

Plants and biological active substances responsible for the physiological effects of the main categories of herbal food supplements from the Romanian market

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Dietary supplements have registered during the last 10 years a constant increase of the global market. Medicinal herbs constitute one of the most rapidly growing segment of the alternative medicine: 29,000 herbal substances have been registered only in North America [1], while in China there is reported a constant growth of 15% per year of the manufactured herbal products.

The popularity of herbal food supplements (HFS) rises from „all natural" promotion of the products but also from the consumers' trust in health benefits and no side effects. Attractive presentation, spectacular health claims together with the new lifestyle of the people and the easy access to the products by internet and direct sales resulted in an increased consumption of HFS. Scientific evidence demonstrated that certain HFS have genuine health benefits and may be effective in managing some health issues (blood pressure and cholesterol control, mental and physical performances, fatigue relief, bone strength, menopause discomfort, recovering from an illness, etc). Numerous HFS have been used historically in traditional medicine and are still utilized today to relieve a wide range of health issues or to assist in health prevention regimes. Plant species, herbal extracts, biological active compounds and functional ingredients could be found in the huge number of products from the Romanian market (over 18,000 products). Most of them have antioxidant properties, anti-inflammatory or tonic effects, being involved in detoxification, immunostimulation, managing of erectile dysfunction, slimming or supporting the body essential functions. Despite this, there were also reported toxic effects, permanent damage of liver or kidney tissue, stroke and heart attack, cancer and even death [2] due to the herbal products (heavy metal contamination, poisonous plants not listed on the label, substitution of authentically herbs, addition of fillers or pharmaceutical substances).

The most frequent adulterated HFS are those categories for which the market demand is increasing: body building, slimming, sexual enhancement, anti-diabetic and fatigue relief products.

According to World Health Organization [3], adulteration of natural products with pharmaceutical active compounds is a serious safety threat. Due to the widely variation of the standards of manufacturers and distributors with respect to quality, safety and effectiveness of their ingredients and products, the novel European concept "integrated benefit-risk assessment" must be applied to HFS, aiming to identify the health threats, to evaluate the range of consumers' exposure and to define the risks of the unsafe HFS consumption.

1. Astin JA, Marie A, Pelletier KR, Hansen E, Haskell WL, (1998): A review of the incorporation of complementary and alternative medicine by mainstream physicians. Arch Intern Med 1998, 158: 2303-2310

2. Wheatley, M.V, Spink, J. (2013) Comprehensive Reviews in Food Science and Food Safety, vol. 12: 599-613

3. WHO, World Health Organization, 2007. Guidelines for assessing quality of herbal medicine with reference to contaminants and residues. Retrieved November 30, 2012, from http:// apps.who.int/medicinedocs/index/assoc/s14878e/s14878e.pdf.

Qualitative and quantitative analysis of saponin content in *Beta vulgaris* varieties

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[a] Department of Plant Biochemistry, Faculty of Biology, University of Warsaw, Miecznikowa Street 1, 02-960 Warsaw, Poland; [b] Faculty of Biology and Agriculture, University of Rzeszów, Ćwiklińskiej Street 2, 35-601 Rzeszów, Poland; [c] Department of Biochemistry, Institute of Soil Science and Plant Cultivation, State Research Institute, Czartoryskich Street 8, 24-100 Puławy, Poland

Chard and red beet are edible cultivars of *Beta vulgaris* var. *vulgaris*. Red beet is cultivated throughout the world for its roots, which are used as a food and as a source of natural dye. On the contrary, chard is cultivated for its edible leaves. Both *Beta vulgaris* var. *vulgaris* varieties are very popular in USA and Europe due to their high nutritional value. In recent years, they have attracted significant attention because of their possible beneficial effects on human health. These cultivars play a well-established role in pharmacognosy, being widely used in traditional herbal medicine, mainly to stimulation of haematopoietic and immune systems as well as in the protection of kidney, liver and gut from toxic compounds. Pharmacological test demonstrated their anti-diabetic, anti-inflammatory, antioxidant and anticancer activities. There have been numerous reports on betalain and phenolic compounds occurring in the roots of red beet and the leaves of chard, which contribute to antioxidant activities of these plants, but the occurrence of these compounds do not fully explain their other health properties. Nevertheless, there was no available information regarding the triterpenoid content of both plants, when these compounds are known to display various pharmacological activities and they may contribute to the physiological benefits arising from the consumption of red beet or chard.

Thus, the aim of the present study was qualitative and quantitative analysis of saponins in red beet and chard roots and leaves. The analyses were carried out by electrospray ionization tandem mass spectrometry (ESI/MS/MS) and ultra performance reverse-phase liquid chromatography coupled to electrospray ionization mass spectrometry (UPLC-ESI/MS).

The presented report showed that the profiles of saponins in chard and red beet were similar. The roots of both varieties contained 9 saponins consisting of oleanolic acid or hederagenin aglycone and varying numbers of sugars, with the dominant triglycoside derivative of oleanolic acid. Leaves of both varieties contained 9 derivatives of oleanolic acid already identified in roots and 4 additional saponins. Complete quantifications were performed for 9 saponins in roots, and additional 4 saponins in leaves. It was stated that organs of both varieties differ in the total concentration of saponins. Moreover, differences in the relative content of individual saponins were observed.

Being a cultivated plant, *Beta vulgaris* cultivars have been subjected to agrotechnical methods of selective breeding or plant protection, what resulted in morphological and phytochemical differences when one regards the content of betalains. On the other hand the above report shows that the differences in saponin content in chard and red beet are not striking and both varieties seem promising source of bioactive compounds in human diet.

Biosynthesis and regulation of tropane alkaloids biosynthesis in *Anisodus acutangulus*

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Tropane alkaloids (TA) such as hyoscyamine and scopolamine, which existed in some solanaceous plants including *Anisodus*, *Atropa*, *Datura*, *Duboisia*, *Hyoscyamus* and *Scopolia,* are used medicinally as anticholinergic agents with increasing market demand. It is of significance to enhance the production of TA using metabolic engineering to benefit human healthcare. *Anisodus acutangulus* TA-producing endemic herb plant in China with a long-period of application history, so it is significant to improve TA production in this resource plant by biotechnological approaches. Based on the isolation of genes involved in tropane alkaloids biosynthesis pathways (such as *AaPMT1*, *AaPMT2, AaTRI*, *AaTRII* and *AaH6H*) and optimization of hairy root culture system in *A. acutangulus*, the co-expression of AaPMT and AaTRI genes in *A. acutangulus* hairy roots significantly improved the yields of total TA and showed higher antioxidant activity than control because of higher total TA content [1]. Furthermore, the simultaneous introduction of genes encoding the branch-controlling enzyme tropinonereductase I (TRI) and the downstream rate-limiting enzyme hyoscyamine-6b-hydroxylase (H6H) into *A. acutangulus* hairy roots also produced significantly higher levels of TA compared with the control [2]. The above results showed that metabolic engineering of biosynthesis pathway was an effective strategy to improve the production of medicinally active compounds in plants including *A. acutangulus.*

1 . Kai GY*, Yang S, Luo XQ, Zhou WT, Fu XQ, Zhang A, Zhang Y, Xiao JB. (2011) *BMC Biotech.* 2011,11:43

2 . Kai GY, Zhang A, Guo YY, Li L, Cui LJ, Luo XQ, Liu C, Xiao JB. (2012) Mol. BioSystems.8(11):2883-90

Alternative analytical tools (sensors/biosensors) in assessment of phytochemical active principles

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The wide use of phytochemicals like flavonoids, flavonoid derivatives, polyphenols, carotenoids and anthocyans from various plant sources as nutrients or as antioxidants preserving or enhancing the food nutritional quality and foodstuff shelf-life made necessary the development of accurate, versatile and in the same time rapid analytical tools to assess them.
The present work is focusing on presenting the availability and versatility of alternative analytical tools, sensors and biosensors, as analytical tools in quantification of phytochemical compounds (especially phenolics and phenolic derivatives) and in the evaluation of their properties. The available designs of such analytical devices and bio-analytical protocols used will be presented: enzymatic biosensors for phenolic content determination [1,2] and bio-mimetic sensors based on lipids immobilisation for antioxidant efficacy assessment [3,4]. Biosensors applications on real samples (polyphenols assessment from plant and berries extracts), limitations and advantages of their use in phytochemical active principles fast screening will be discussed. The capabilities of biosensors to perform highly sensitive quantitation of phenolic secondary metabolites from plants is supported by obtained limit of detections of 10^{-6} $molL^{-1}$ concentration levels, while the availability to feasible measurement of the total phenolic content is supported by result comparison with LC-DAD-ESI-MS determination which proved that the biosensor systematically reached 90% to 94.3% from the chromatographic assay. The use of electrochemical sensors to assess the antioxidants efficacy benefits from the accurate evaluation of the antioxidant properties with respect to relevant oxidative markers [5] since low-density lipoproteins and phosphatydilcholine are the immobilised substrates. MALDI-ToF analysis results provided the validation of sensors/biosensors responses with respect to polyphenolic derivatives efficiency against lipoperoxidation [4].
Validation issues will be discussed and exemplified on *Salvia* and *Basilicum* extracts, using as model compound rosmarinic acid and its efficacy against *in vitro* induced lipo-peroxidation.

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Clinical cases of recurrent respiratory infections at children treated with phytotherapy

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Respiratory infections are very common in childhood and they are usually treated with antibiotics and other symptomatic medication. Sometimes allopathic drugs are not efficient to heal reccurency of these infections. The paper will present cases of 3 children of 4,6 and 7 years old which were treated successfully only with phytotherapy, after they were prescribed a lot of drugs without efficiency.

First case is a 4 year old boy, P.D., who developed atopic dermatitis after vaccination, since 6 months age. Parents brought him to the medical office for recurrent laringitis and otitis and frequent influenza in the last 2 years before first consultation. He used antibiotics and also homeopathic remedies, without significant result. His treatment in my office included *Nigella sativa* oil capsules, *Ribes nigrum* gemmotherapy extract, *Aloe* leaves juice, propolis tincture, royal jelly. He received a mixture of herbs which solved his respiratory problems: *Verbena officinalis, Melissa officinalis, Carum carvi, Thymus serpyllum, Viola tricolor, Populus nigra* and *Ribes nigrum*. They were effective also on his dermatitis and he did not have any other health problems.

Second case is a 6 year old boy, R. A., who had *influenza* with *fever* and *vomiting*. Parents gave him herb remedies, but they didn't solve his health problems. With a tincture which combined imunostimulant herbs like *Echinacea purpurea* and *Olive* leaves extract, administered together with *Tilia* flowers, coloidal siver, bee pollen and royal jelly, his disease has completely gone.

Finally the third case is a 7 year old girl, C.B., whose parents were desperately seeking a remedy for her cough and respiratory allergy. They tried several treatments with antibiotics, antiallergic syrup (Ketof) and allopathic immunostimulants (Polimod, Ribomunyl), but without results. They tried also homeopathy but it was not effective too. My recipe for her included propolis tincture, *Nigela sativa* oil, royal jelly and a mixture of herbs (*Echinacea, Viola, Thymus serpyllus, Pulmonaria officinalis, Melissa officinalis, Populus nigra, Salvia officinalis, Crateagus monogyna, Hippophae rhamnoides*.) Parents were very content about the great improvement and finally healing that herbs brought to their child.

After these experiences and lot of others, too, we can conclude that phyotherapy is a very good alternative for respiratory disorders, if it is well conducted and there are chosen the best herbs for every situation.

Phytochemical composition of *Arnicae flos* from wild populations in the northern area of the Romanian Eastern Carpathians.

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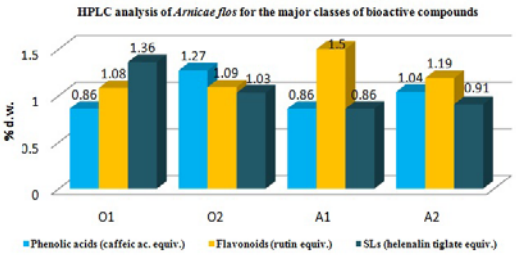
Arnica montana L., *Asteraceae* family, is an important medicinal species, with traditional use, mainly due to the sescviterpene-lactones (SLs), and also to the flavonoids and phenolic acids. In addition to the traditional use of *A. montana* extract - for their anti-inflammatory proprieties, recent studies focused on the applicability of the extract or isolated compounds in the management of various types of cancer. For the antitumoral activity, several mechanisms were suggested. Thus, the species shows great potential for developing new drugs. The interest for the *A. montana* species is still high, but in Romania the species is poorly valorized. Currently, a high ration of the plant material is exported as raw material. Thus, we observed a lack of information regarding the quantitative and qualitative peculiarities of the autochthonous plant material. This reflects also on the low local added value.

The aim of our ongoing study is to assess the quality of the autochthonous plant material, especially for the northern area of the Romanian Eastern Carpathians, in the framework of the current tendencies. Our phytochemical studies can contribute for the preservation of the high yielding varieties with a phytochemical profile adequate for their use in the modern phytotherapy.

Material and Methods. *The plant material* was harvested from 4 wild populations of *A. montana* from the northern area of the Romanian Eastern Carpathians (Suceava County), in June 2012. *The phytochemical assessments* were performed by means of HPTLC and HPLC.

Results and Discussion. *The HPTLC analysis* highlighted the presence of the phenolic acids cynarin, chlorogenic acid, caffeic acid and the flavonoids isoquercitrine, luteolin-7-*O*-glicoside, hyperoside and astragalin in all analysed samples.

The HPLC analysis revealed intra-populational variations, but the content in phenolic acids and flavonoids was comparable with the literature data [2]. The total content in SLs was higher than the minimum content recommended by the European Pharmarmacopoeia (0.4%) and it is comparable with the values cited for the Central-European chemotype (0.40-1.55%) [1].



The current study will be deepened by assessing the plant material harvested in 3 consecutive years, and by performing HPLC-MS analisys for a more thorough characterization of the extracts and extractive fractions which can be used in phytotherapy. Furthermore, the *in vitro* biological activity of the extractive fractions will be tested (enzymatic and cell culture tests).

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Controversy regarding the use of weight loss supplements with phytotherapeutic extracts for sportsmen

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Using weight loss supplements in athletes must meet three essential goals: the conservation of muscle mass, decrease body fat by lipolysis and maintaining a high caloric intake to facilitate training.
Answers of 113 respondents form gyms in Tîrgu Mureş were analysed and the benefit / risk ratio was quantified based on the declared composition on the label of dietary supplements. Considering the recommended dosage, doses actually used and potential risks arising from the misuse of active compounds of vegetal origin (caffeine, yohimbine, hydroxy citric acid) correlations were made when athletes simultaneously used restrictive diets (hypoglycemic / high protein or hypoglycemic / hyperlipidemic).

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The antioxidant and anti-inflammatory activity of *Centaurea cyanus* L. extracts

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The aim of this study was to assess the efficacy of polyphenols and polysaccharides extracts of aerial part of *Centaurea cyanus* L. for their potential antioxidant and anti-inflammatory activity. *In vitro* antioxidant activity of extracts was assayed by DPPH [1], ABTS [2], FRAP [3] and ferrozine [4] tests. *In vivo* anti-inflammatory activity was studied using histamine induced rat paw edema [5].

In all assays, the polyphenols extract of *Cyani herba* showed high values of antioxidant activity (DPPH – IC_{50}=54.14±0.42 μg/ml; ABTS – 0.54±0.02 μM TE/g dried weight; FRAP – 52.43±0.06 μM TE/g dried weight). The results revealed that polysaccharides extract had a significantly ($P<0.05$) higher Iron chelating activity (95.66±1.99%) than polyphenolic extracts (3.88±0.6%). Both extracts demonstrated anti-inflammatory activity. The effect was found to be more pronounced in case of polyphenol extract (Table 1). This bioactivity compared favourably with diclofenac sodium, which was used as positive control, thus showing its usefulness for the treatment of inflammation.

Table 1. Effect of *Centaurea cyanus* L. extracts on histamine induced paw edema in rats[1,2]

Treated groups *50 mg/kg*	Difference in paw edema *g*	Percent inhibition *%*
Natrium chloride 0.9	0.5503±0.03[a]	–
Diclofenac sodium	0.3090±0.05[b]	42.47±6.09[a]
Polyphenol extract	0.2986±0.05[b]	38.36±5.93[a]
Polysaccharedes extract	0.3678±0.07[c]	24.57±3.66[b]

*Values represent the mean ± of three replications

*Different small letters within a column denote significant differences ($P<0.05$)

Based on the present study, it can be concluded that *Centaurea cyanus* L. polyphenol extract has potent antioxidant and anti-inflammatory activity. Polysaccharides extract has a strong capacity on chelating free iron. So, a combined extract is more preferable than that of having one of these activities.

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Protective effects of Herba Cistanches against neurotoxicity induced by β-amyloid peptide in SH-SY5Y cells

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In traditional Chinese medicine, *Herba Cistanches* can encourage kidney yang, enrich blood and loosen the bowel to relieve constipation. The study is designed to investigate the prohibiting effects of extracts from *Herba Cistanche* on the neurotoxicity of β-amyloid peptide (Aβ) that results in the decreased expression of nicotinic acetylcholine receptors (nAChRs) and damages of neurons. The human SH-SY5Y neuroblastoma cells were treated with a certain concentration of extract from *Cistanche deserticola*, and then exposed to $A\beta_{25\text{-}35}$. The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) reduction and lipid peroxidation were measured by spectrophotometry; the α3 and α7 nAChR subunits at the protein and mRNA levels were measured by western blotting and RT-PCR, respectively. The results showed that *Herba Cistanche* can prevent the decreased levels of α7 and α3 nAChRs at protein and mRNA levels, the declined cellular viability and increased lipid peroxidation induced by Aβ, showing a similar protective effect as vitamin E. The results indicated that the anti-neurotoxic effect of *Herba Cistanche* may relate to the up-regulation of the expression of nAChRs stimulated by the drug, which might be important in the therapeutic strategy in Alzheimer's disease.

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Phenolic Profile and *In Vitro* Screening of Cyprus Cedar Bark for Antioxidant and Antitumor Activities

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Cedrus brevifolia (Hook. f.) Henry (Cyprus cedar) is endemic to Cyprus, the area being limited to the mountains of the Paphos forest. A literature survey showed no reports on the chemical composition and possible biological effects of Cyprus cedar. As barks of many conifer species have proved to be a rich source of polyphenols with different biological activities, the aim of the present study was to investigate the phenolic content, antioxidant and antitumor potential of *Cedrus brevifolia* bark. A raw extract and four extractive fractions were obtained from Cyprus cedar bark. The phenolic profile was studied by HPLC-DAD-ESI-MS [1]. The antioxidant activity was investigated using several *in vitro* assays: DPPH, ABTS, superoxide and hydroxyl radicals scavenging assays, nitric oxide scavenging assay, reducing power assay and 15-lipoxygenase inhibition assay [2,3]. The effects on viability, apoptosis and cell cycle of HeLa cervical carcinoma cells were studied by MTT assay and flow cytometry [4]. Ethyl acetate fraction had the highest total phenolic and proanthocyanidin contents (415.00±2.15 mg gallic acid/g extract and 272.1±2.9 mg cyanidin/g extract, respectively); a taxifolin-*O*-hexoside, catechin, epicatechin and procyanidin oligomers (three dimers, two trimers) were identified in this fraction. Ethyl acetate fraction was found to be the most active as scavenger of nitric oxide (EC_{50}=170.3±2.7 μg/mL), DPPH (EC_{50}=13.9±0.3 μg/mL), ABTS (EC_{50}=2.3±0.0 μg/mL) and hydroxyl radicals (EC_{50}=580.20±18.72 μg/mL), reducing agent (EC_{50}=9.13±0.13 μg/mL) but also as inhibitor of 15-lipoxygenase (EC_{50}=34.0±0.9 μg/mL). The highest cytotoxic activity against HeLa cells was attained with diethyl ether fraction (90.58 ± 3.05% cytotoxicity at 100 μg/mL); HPLC-DAD-ESI-MS allowed the identification of taxifolin in diethyl ether fraction. In conclusion, Cyprus cedar bark showed remarkable biological properties and therefore a high potential for use in both food and pharmaceutical industries.

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Biologically active natural products from Iranian terrestrial and marine organisms

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Different plants or organisms either with terrestrial or marine origin produce diverse arrays of natural products. These compounds have important roles in surviving the organisms in their natural habitat. I am presenting a report on bioassay-guided isolation and identification of natural products from different plants using different types of on-line analyses or the classical isolation with chromatography and further identification by various spectroscopy methods including 1 and 2 D NMR.

The chemical constituents of the essential oils of *Mentha mozaffariani* an endemic medicinal plant were analysed by GC-MS in different phenological stages (vegetative, flowering and seed set) and found to be rich in piperitone oxide (32.9±1.2, 35.3±1.2 and 51.5±0.6%), linalool (14.1±0.3, 17.1±0.6 and 8.7±0.16%), 1,8-cineole (11.6±1.4, 9.5±0.3 and 8.3±0.26%) and piperitenone oxide (9.8±0.4, 8.1±0.3 and 1.7+0.05%), during developmental stages respectively. The EOs were evaluated for their cytotoxic potential on three human cancer cell lines namely, HL60, Molt 7, and MCF 7.

The LC-MS analyses of cytotoxic methanol extracts of *Salvia eremophila* and *S. Santolinifolia* [1] resulted in identification of two phenolic abietane diterpenoids, carnosol and carnosic acid and rosmarinic acid as the main anticancer agents in these plants.

Marine red alga from the Persian Gulf, *Dichotomaria obtusata* is searched for its natural products and its anticancer potential against the above cancer cell lines. Three compounds were isolated from a methanol extract of the organism with moderate antitumor activity which were subjected to MS, and ^{1}H and ^{13}C NMR to elucidate their structures.

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Study of Volatile Oil from the Aerial Parts of *Hypericum perforatum* L. by GC-MS

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The aerial parts of the *H.perforatum* L. species were harvested at the stage of full flowering from the spontaneous flora of the Republic of Moldova and the collection of the Medicinal Plant Cultivation Center (MPCC) of State University of Medicine and Pharmacy,,Nicolae Testemitanu" [2].

The volatile oil from *Hyperici herba* (fresh and dry vegetal product) was isolated in the Ginsberg unit through hydrodistillation for 3 hours. The findings were recalculated for absolute dry matter [3]. It was established that the dry *Hyperici herba* product collected from the spontaneous flora contained 0.26% of volatile oil [1]. It was found that the content of the volatile oil in the fresh aerial parts harvested from the spontaneous flora was 0.303%, while that from the MPCC collection made 0.204%.

The chemical composition of the volatile oil was elucidated through gas chromatography-mass spectrometry (GC-MS) [1]. Seventy four components have been isolated from the essential oil of the dry aerial parts, thirty three of them have been identified and quantified. Their content totals 65.9%, and the major components are as follows: caryophyllene (12.155%), β-pinene (8.574%), caryophyllene oxide (12.119%). Ninety seven components have been found in the fresh aerial parts harvested from the spontaneous flora, thirty three of them, that constitute 82.26%, have been identified with the following main components: β-caryophyllene (18.391%), germacrene D (15.546%), β-pinene (12.470%), β-cis-ocimene (7.116 %). The major components of the essential oil isolated from the *Hyperici herba* harvested from the MPCC collection include germacrene D (35.777%), β-caryophyllene (24.751%), biciclogermacrene (5.635%).

The findings demonstrate that the volatile oils studied are distinguished by the content of various vegetal products of *Hyperici herba*, the quality and quantity of the major components.

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Valorization of *Salvia Sclarea* Wastes. Efficient Synthesis of Sclareoloxide by Sclareol Ozonolysis in Aqueous Solvent System

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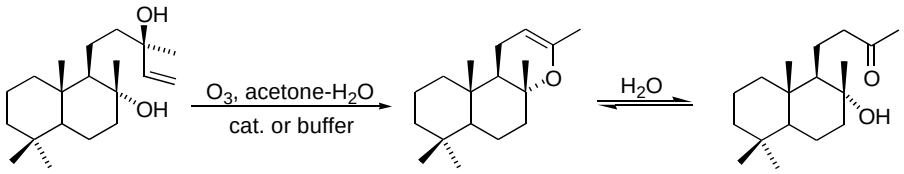
Sclareoloxide is a *bis*-norlabdanic terpenoid broadly used at industrial scale as ingredient of different aroma compositions [1], as well as intermediate in the synthesis of other valuable odorants of terpenic structure [2]. Its olfactive properties are due to a very fine ambergris odor. The large scale production of sclareoloxide is based on a degradation procedure of the closely related sclareol. It is a compound of labdanic structure, readily available from *Salvia Sclarea* plant material and in fact represents a waste product of corresponding essential oil production. Transformation of sclareol into sclareoloxide requires removal of two carbon atoms from the lateral chain, which is best achieved by an oxidative degradation. Different oxidants have been used for this purpose, including potassium permanganate, hypochloric acid salts, ruthenium salts, but most effective proved to be ozone. It converted sclareol to sclareoloxide with a 78% yield on the use of ethylacetate - pyridine solvent mixture [3].



Sclareol **Sclareoloxide**
90% to quantitative yield

We report in the current communication a substantial improvement of the ozonolytic sclareol cleavage which allows to avoid the highly toxic pyridine as co-solvent and ozonide reduction agent. The new procedure includes a single operation of oxidative degradation of sclareol lateral chain on ozonolysis in the presence of a co-oxidant catalyst or under controlled pH conditions in an acetone-water solvent mixture at a temperature close to normal room temperature conditions. This homogeneous solvent system allows application of inorganic catalysts for process efficiency and also elimination of post-ozonolitic reduction agents. Under these circumstances the reducing agent is water [4], which efficiently quenches free radical species generated during ozone treatment to produce hydrogen peroxide as the only by-product. Product isolation includes acetone distillation, extraction of the resulting suspension with a suitable solvent, followed by its evaporation at reduced pressure. Catalyst transformation products can be easily recovered from aqueous phase and further recycled. The crude sclareoloxide has a purity over 97% and can be used without additional purification operations.

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The synergistic action of medicinal herbs extracts
Case study – INTERFERONAT

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Nowadays, in an environment in which the chemical substances from the air we breathe, food or drinks we consume cause health problems, more and more people seek solutions, diets and healthy ways to keep their bodies healthy.
When the liver, the most used and requested organ, becomes toxic many other organs will be affected.
Thesynergistic action.
It represents, by definition, a simultaneous action of more organs or tissues aiming in the same direction.
It is considered in the traditional phytotherapy that the use of complex medicinal herbs extracts has a superior therapeutic value, due the synergistic effect of its compounds. This synergistic effect makes these smaller doses from each active principle have, by summing up, more intense and complex therapeutically effects and also diminish the side effect of these substances. The synergistic effect resulted from a well-chosen medicinal herbs mix is bigger than the one resulted by summing up each plants' effect individually.
The phytotherapic compounds from the medicinal herbs are, as matter a fact, substances synthesized in plants for their own protection and to ensure the species perpetuance. It is this mix of complex substances that helps plants survive in an environment with a high potential attack of pests/abusers. In time, pests/abusers found ways to resist the plants shields and defense systems forcing plants to develop more complex defense systems; this led to the appearance of a big complexity of phytochemical compounds in each vegetal species. If the plants had fallowed the pharmacological model of developing only one single chemical compound against the attaching macro and micro-organisms, most probably the species would have not survived to long.
One basic principle regarding the existence of this synergistic effect in nature can be described by the expression "nature knows best" or, in other words, everything that is naturally "created" is based on nature's native wisdom and intelligence.
There are many clinical and laboratory studies that prove the existence of the synergistic processes betweenthe chemical compounds synthesized by the medicinal herbs.
In the case of INTERFERONAT, the main porpoisewas to harmoniously combine certain plants, with a wide range of chemical compounds, able to determine synergistic action on more levels: hepatic cells regeneration, stimulation of endogenous synthesis of interferon and obtaining an immunomodulation effect, increasing the organism's level of protection with a proficient antioxidant effect.

Screening for natural anti-diabetic drugs in *Balanites aegyptiaca*

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Diabetes mellitus is one of the most frequent diseases all over the world. Both type I and type II diabetes are chronic diseases, with only simptomatic treatments. From their on-set, they progress, and the primary disease is leading to severe complications, among which neuropathy, nepropathy, retinopathy, vasculopathy, to name a few, progress towards organ failures. The use of primary medication, oral antidiabetics or insulin, usually is assisted by administration of other adjuvant terapies, such as plant extracts and/or synthetic drugs to alleviate the complications. *B. aegyptiaca* extracts are used in Egypt and surrounding countries as a food and also medicinal plant [1]. Extracts and fractions from *B. aegyptica* were subjected to *in vitro* studies in order to identify the antidiabetic fractions, to further assess citotoxicity and to estimate the potential of action. The assessment evaluated the modulation of secretory cytokines, feature that is often present in various polyphenolic extracts, since inflammatory cytokines are often elevated in diabetic disease [2]. More, a disease specific mechanism, involved in the onset of diabetic retinopathy and nephrophathy, involving the enzymes aldose-reductase and sorbitol-dehydrogenase, was investigated. A primary *in vitro* cytotoxicity screening was performed using Huvec cells, Jurkat cells and total blood , with MTS assay as end-point. Secretory cytokines were determined in total blood cultures by Luminex-xMAP assay. Further testing was based on inhibition of aldose-reductase [3]; a first assay classiffied the primary extracts, followed by a series of tests on fractions. Finally, the most active primary extract was found to be BA/A (95 % ethanol extract), which further, upon fractionation with second solvent, gave the most active fraction in dichloromethane, followed by ethyl acetate and butanol fractions. For the most active fraction, a maximal inhibitory activity of 60% at 0,01 microM/ml was achieved. Thus, a clear inhibiton of aldose-reductase activity, that efficiently decreases the production of D-sorbitol or glycerol, providing protection against neuropathic disorder and retinopathy was demonstrated.

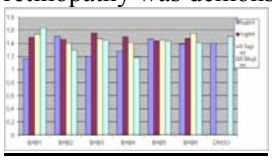

Fig. 1: Citotoxicity of B. aegiptiaca extracts on Huvec cells

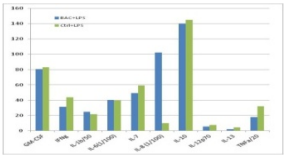

Fig. 2 Influence of B. Aegiptiaca extracts on cytokine secretions in total blood culture

Acknowlegdment: The study was supported by the E.U. FP7 Grant PIRSES-GA-2008-230816.
Keywords: In vitro, anti-diabetic, *B. aegyptiaca.*

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Comparative efficacy of *Ocimum sanctum* and *Ocimum basilicum* volatile oils against amyloid beta (1-42)-induced anxiety and depression in laboratory rats

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Basil isone of themedicinal plants used both for therapeutic and culinary purposes. For the present study the plant material was cultivated in similar conditions, included in bio-cultures from Neamt County, Romania. The volatile oil was isolated by Neo-Clevenger technique and thechemical composition of the essential oils was assessed by GC/MS and GC-FID techniques.
The present study analyzed the possible anxiolytic and antidepressant proprieties of inhaled basil volatile oils extracted from *Ocimum sanctum* (Os) and *Ocimumbasilicum*(Ob) in beta-amyloid (1-42) rat model of Alzheimer's disease.
The anxiolytic- and antidepressant-like effects of inhaled basil volatile oils were studied by means of *in vivo* (elevated plus-maze and forced swimming tests) approaches [1,2]. The beta-amyloid (1-42)-treated rats exhibited the following: decrease of the exploratory activity (number of crossing), the percentage of the time spent and the number of entries in the open arm within elevated plus-maze test and decrease of swimming and immobility times within forced swimming test [3].
The chemical composition varied from one sample to another. The main compounds found in both samples were l-linalool (31% - Ob, 19% Os), camphora, l-beta-elemene, l-alpha-bergamoteneandn-bornyl-acetate, estragole (15.57%, respectively 7.59%), eugenol (2.64%, respectively 1.39%) and 1,8-cineole (3.29% respectively 3.90%). Wecansupposethattheratioandthequantity of theidentified compound willbe determinant for theantidepressantandanxiolyticeffects.
As a result, exposure to basil volatile oils significantly improved these parameters, suggesting anxiolytic- and antidepressant-like effects. Our results suggest that multiple exposures to basil volatile oils can be useful as a mean to counteract anxiety and depression in Alzheimer's disease conditions.

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Food supplements used as adjuvants in the therapy of lung diseases – clinical study The Bisericani Pneumophtisiology Hospital

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Due to the agreement with the Ministry of Health (address Nr. 75/2012), we tested 4 food supplements offered by the producer/processor SC Dacia Plant SRL - Brasov, used as adjuvants in the treatment of some lung affections in the Bisericani Pneumophtisiology Hospital.
The 4 supplements: *Emocalm – tablets, Calmotusin – tablets and syrup, Interferonat – tablets*, were administered - with their consent - to patients suffering of lung tuberculosis, COPD (chronic obstructive pulmonary disease) bronchiectasis, chronic bronchitis. For each patient, we completed files that contain: *information for the patient, the informed consent of the patient, the attestation of the study accept, the inclusion criterion, the laboratory exam, the physical examination, the diagnosis, medical concomitance, the finalizing of the treatment, the final conclusions and the adverse reactions.* The study was achieved over a period of two years and aimed patients of different ages and sex (about 50 patients, out of which 7 patients abandoned before finalizing).
All the supplements were characterized in the "Stejarul" Biological Research Centre laboratory from the qualitative and quantitative phytochemical point of view to identify/dose the antioxidant compounds, of the polyphenolic and flavonoidic type, due to the formula of the product. The phytochemical analysis (spectrophotometrically, TLC, HPLC, GC-MS) of the food supplements highlighted the presence of numerous compounds of the polyphenolic, flavonoidic, terpenic, iridoidic type. The medicinal plants that form the 4 formulas contain biologically active phytocomplexes with antioxidant, antiinflamatory, sedative actions [1,2].
The present study was not a *double-blind* one but one of clinical comparison, and the food supplements had an effect similar to the one produced by the medicine of the same range (anticoughing, mucolytic, hepatoprotecting and anxiolytic). The treatment was generally well tolerated, there were no adverse reactions, the patients were co-operant, the symptomatology has improved. The administration of the food supplements as adjuvants in the treatment of the lung affections was a benefit for the patients.

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Acknowledgements: The work was sustained from the National Project PN 09-360401 financed by UEFISCDI/National Agency for Scientific Research Romania.

Secretory tissues and volatile substances in plants, by **Ioan Burzo, Constantin Toma**

Publishing House of the University"Alexandru Ioan Cuza" from Iasi, 2012, 278 pages

Reviewed by Maria Magdalena Zamfirache, „Al. I. Cuza" University from Iaşi (Romania), Faculty of Biology, Department of Biology

The volume ***Secretory tissues and volatile substances in plants*** **(**ISBN: 978-973-703-762-6**)**, written by Ioan BURZO and Constantin TOMA, represents a high value publication for the theoretical and applied plant biology area of knowledge, of real importance for plant anatomy, physiology and biochemistry specialists in Romania and not only.

Displayed in an estimable number of pages (278), the scientific content of the work is organized in **6** consistent chapters, that advance in a logical sequence information.

The importance of the synthesized substances in plants for their survival in the environment (implications in the pollination process as warning signals against being consumed by herbivores, allelochemical role or antifungal and antibacterial protective role), also as raw material for humans (resources of widely used compounds in food industry, medicine, and of raw material in perfume, dye and preservers industries etc.)– **Chapter I – The importance of the substances in plants**.

Theoretical data referring to the existence and general structure of the secretory structures in cormophytes – **Chapter II – Secretory structures in plants**.

Informations regarding synthesis processes of volatile substances in plants (the location and regulation of the synthesis, the release from the producing cells of the synthesized compounds) – **Chapter III – Synthesis of the volatile substances in plants**.

The main groups of volatile compounds synthesized by cormophytes (phenols, phenolic acids and related substances; phenilpropanoid and benzenoid substances; terpenes; alcohols, aldehydes and non terpenic aliphatic esters; sulphur compounds; aliphatic and aromatic hydrocarbons) – **Chapter IV – The main volatile substances in plants**.

Informations regarding extraction and analysis methods for volatile compounds produced by plants – **Chapter V – Extraction and analysis of volatile substances in plnts**;

Theoretical and practical data regarding plants producing useful substances, obtained by the authors, the merged data form the last chapter of the work – **Chapter VI – The volatile substances in the main plant species and the structures that produce them**, chapter reuniting, in more than 150 pages, representing ½ from the total containment of the work, informations regarding the anatomy of the secretory structures that characterise over 130 plants that have been investigated and also the composition of the volatile oils that they produce.

The title of the work, fully corresponding with the aim, vision and manner of discoursing the chosen subject to be presented by the authors, imposes the informed reader the present work as a monography of real scientific value, ment to bring theoretical edifications, sustained by practical data, regarding the nature, production and use of different classes of plant substances, the present work successfully integrating through the data presented to the reader the informations inserted in the Romanian and foreign traties of profile.

The text of the work is followed by extremely abundant graphycs (diagrams, tables, optical and electronic microscope (SEM) images, chromatograms, mainly original). The latter comprises over 280 reference titles, containing classical works of plant anatomy, physiology and biochemistry that lay over an extremely wide period of time (over a century of research and publishing activity).

In the same prestigious publishing house, in 2013 was published the 2nd edition of volume (revised and enlarged) to which we refer, with 349 pages which includes 196 figures (drawings, photographs), 207 chromatograms and 313 reference titles, in most of the 160 species investigated by the authors is presented and composition of volatiles from flowers.

Capsaicinoids as Efflux Inhibitors in Mycobacteria

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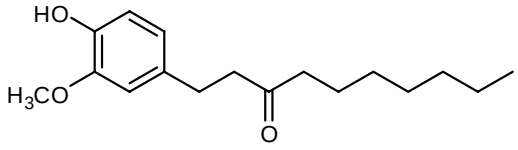
Efflux pumps (EP) are essential proteins in the development of resistances in various diseases such as cancer or bacterial infections. These ubiquitous membrane transport proteins are responsible for the efflux of xenobiotics out of the (bacterial) cell. Thus the concentration of an administered active compoundis decreased below the effective concentration and cells become resistant against this specific drug [1]. In case of tuberculosis (TB) many EP are known to be important for resistances against first-line drugs in TB-treatment. For instance, MmpL7 found in *Mycobacterium tuberculosis* mediates resistance against isoniazid, the most important TB drug at present [2].

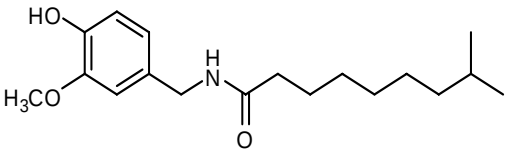
The aim of this study is the isolation and characterisation of efflux pump inhibitors (EPI) out of various plants.EPIare putative tools to overcome the vicious circle of resistance due to their ability to block EP with the consequence of achieving the effective concentration of a co-administered antibiotic [3].

For this purpose we screened several plants for their antimycobacterial and resistance modulatory effect on *Mycobacterium smegmatis*and *M. fortuitum*. Chilies, fruits of *Capsicum spp.* (Solanaceae), were included in this study due to structural similarities of capsaicinoids to hydroxyarylalkanones (e.g. 6-paradol) of Melegueta pepper, *Aframomummelegueta* (Zingiberaceae). In a former study we found that these compounds are putative EPI [4]. Similarly to capsaicinoids, 6-paradol activates the chemosensory ion channel TRPV1 [5], indicating its close structural relationship.

The hexane and dichloromethane extracts of chilies were devoid of antimycobacterial activity (minimal inhibitory concentration of 512 mg/L), however, they revealed notable modulation factors of 8 and 4, respectively, for ethidium bromide, a substrate of many efflux pumps.The results correlated with estimated capsaicinoid content. Experiments with pure capsaicin and dihydrocapsaicin emphasized that the pungent capsaicinoids are theresistance modulating components of these extracts.



6-Paradol



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Biological, Ontogenetic and Phytochemical Research of *Perilla frutescens* (L.) Britton (*Lamiaceae*)

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Perilla frutescens L. (fam. *Lamiaceae)* is an annual species native to East Asia and recently cultivated in many regions of the world for medicinal, culinary and ornamental uses [1, 2]. The plant possesses carminative, anti-allergic, tonic, antitumor, antimicrobial, antibacterial, anti-inflammatory and antioxidant properties [3]. A field experiment was conducted with biennial cultivation of the plant in 2012 and 2013 at the Botanical Garden (I) of ASM (Collections of Medicinal and Ornamental Plants), in order to assess the biological, ontogenetic and phytochemical peculiarities of *P. frutescens* under culture condition specific to R. of Moldova. Plants were propagated by seedlings grown in heated greenhouses. Seeds were sown in special substrate in the last decade of February, previously being moistened. The seeds germinated after 10-15 days, with the germination rate – 80%. In the second decade of May, seedlings were transplanted into open ground under 20x30 cm planting scheme. Four life periods (latent, pregenerative, generative, postgenerative) and 9 stages (plantlets, juvenile, immature, virginal, generative, senile) in the cycle of the development of *P. frutescens* were highlighted. Pregenerative period (seed germination, plantlet and immature stages) takes about 100-120 days. Generative period (budding, full flowering, full seed set) were noted in the second and third decade of July and lasted till September-October.

The plant material for the phytochemical analyses was collected during flowering stage in July and August 2013. The plant material was extracted with methanol and ethanol: water (70:30 v/v) mixture and the extracted were subjected to both spectrophotometry (for total quantitative determinations) and high performance liquid chromatography (HPLC) for qualitative and semiquantitative analysis (Tab.1).

Tab.1. Results of phytochemical analyses for *Perilla frutescens* extracts.

Samples (2013)		Spectrophotometry		HPLC		
		Total phenolic ac.*	Total flavonoids**	*Rosmarinic ac.*	*Caffeic ac.*	*Luteolin*
		mg/g d.w.				
July	MeOH	19.12	5.45	16.25	0.16	0.16
	EtOH	31.33	11.79	21.69	0.28	0.52
August	MeOH	16.84	4.01	16.20	0.15	0.12
	EtOH	27.55	6.59	20.33	0.20	0.29

Legend: * expressed as rosmarinic acid equivalents; ** expressed as luteolin equivalents.

Results of phytochemical analysis show that the optimal harvest period is month July when content in polyphenolic compounds is higher. Rosmarinic acid is the major compound in all the samples. The ethanolic extracts showed higher amount for all envisaged compounds.

The work was supported by the National Agency for Scientific Research Romania (projects PN 09-360401 and 694/24.04.2013) and the Academy of Science of Moldova (04/RoA/2013).

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The screening of anti-inflammatory potential of Allium cepa L extracts by digital infrared thermal imaging (DITI)

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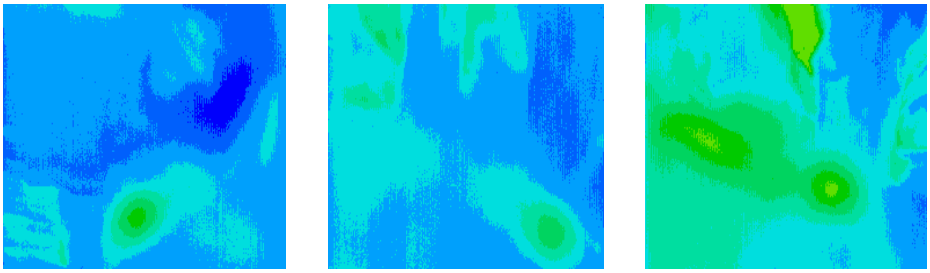
Local inflammatory reaction can be systematically investigated resorting to *in vivo* models (New-Zeeland white rabbits) that mightbe evaluated by digital infrared thermography.
The aim of this work was to assess the local temperature changes due to the inflammatory response after the administration of two extracts obtained from *Allium cepa* L. bulbs, EC1 (hydro-alcoholic extract) and EC2 (aqueous extract). The right eyeball temperature was considered the control, whereas the left eyeball temperature was measured for comparison after the instillation of the investigated extracts. The results are presented below (image 1).



right eyeball *left eyeball with EC1* *left eyeball with EC2*

Image 1. IR termal images registrated after 24 hours from the instillation of 2 drops of *Allium cepa*extracts (left eyeball), 2 drops of terebenthine for control (right eyeball)

The results indicated that the temperature of the left eyeball wasdiminished for EC1 compared to control and slightly higher (with 0.4 ^{0}C) for EC2 extract. Therefore, the investigated onion extracts have anti-inflammatory and antiphlogistic activity, more evident in case of EC1 extract. This is the result of complex mechanisms activity of the different groups of bioactive components that are found in higher concentrations in EC1 compared with EC2.

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Structure activity relationship analysis of natural phenyl propanoic acid & its derivatives for inhibition of inflammatory mediators (TNF-α and NO) in LPS-stimulated J774A.1 macrophages

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Inflammation is root cause for many diseases like cancer, multiple sclerosis, diabetes and many others. Pro-inflammatory cytokines (TNF-α, IL-6, IL-10, PGE-A_2 and IL-1β etc.) and NO are considered as pivotal mediators in inflammatory conditions like rheumatoid arthritis, Crohn's disease etc [1-3]. Thus inhibition of pro-inflammatory cytokines and NO production are important targets for treatment of inflammatory disorders [4]. Various terrestrial plants such as *Desmodium gangeticum*, *Lagenaria siceria*, and so many were reported to contain plant acids like cinnamic acid, caffeic acid, ferulic acid, quinic acid, protocatechuic acid, shikimic acid, gallic acid etc which are used in ayurveda for the treatment of inflammatory conditions [5, 6]. In this study, fifteen plant acids are evaluated for inhibition of TNF-α and NO production in LPS-activated murine macrophage cell lines (RAW264.7 and J774A.1) at various concentrations. In addition, cell viability was also studied by MTT assay on both cell lines (RAW264.7 and J774A.1). Structure activity relationship (SAR) analysis was developed for inhibition of TNF-α and NO by natural phenyl propanoid scaffold. Thus, comparative study of plant acids against inhibition of TNF-α and NO will provide one equal platform to study the potency of plant acids.

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In Vitro Antioxidant Activity of Hydromethanolic Extracts from Romanian *Melissa officinalis* and *Thymus vulgaris* Plants

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Antioxidant activity of plant extracts is of particular interest to medicine, food and cosmetic industry but also in the development of effective radioprotective agents. This study aimed to evaluate the *in vitro* antioxidant activity of hydromethanolic extracts from Romanian *Melissa officinalis* and *Thymus vulgaris* species by six different assays (DPPH, ABTS, superoxide anion and hydroxyl radicals scavenging assays, ferric reducing power assay, ferrous ion chelating ability assay) [1,2,3]. The flavonoid and total phenolic contents (mg/g dried extract) were also determined [1]. The extracts exhibited a concentration-dependent antioxidant activity. According to the EC_{50} values, the extracts were more active as DPPH and ABTS radicals scavengers. Lemon balm and thyme extracts showed important superoxide anion radical scavenging properties. The intensity of other antioxidant activities was moderate (table 1). In comparison to controls, the extracts were more efficient DPPH and superoxide anion radicals scavengers.

Table 1. The antioxidant activity of *Melissa officinalis* and *Thymus vulgaris* extracts

Antioxidant assay	**EC_{50} (µg/mL)**		
	Melissa officinalis	***Thymus vulgaris***	**Control** (gallic acid[a]/catechin[b])
DPPH radical scavenging activity	22.56 ± 0.15	34.76 ± 0.25	25.86 ± 0.05[a]
ABTS radical scavenging activity; *Trolox Equivalent Antioxidant Activity* (TEAC)	7.3 ± 0.1 1.32 ± 0.01	10.2 ± 0.1 0.76 ± 0.02	0.5 ± 0.1[a] 18.61 ± 0.19[a]
Superoxide anion scavenging activity	428.8 ± 1.4	462.8 ± 2.2	1960.6 ± 13.7[b]
Ferric reducing power	10.00 ± 0.01	30.00 ± 0.02	4.00 ± 0.01[b]
Ferrous ion chelating activity	30.83 ± 0.25	34.73 ± 0.15	2.70 ± 0.00[a]

Melissa officinalis extract, containing the highest amounts of total polyphenols (28.53±0.69 mg gallic acid echivalents/g extract) and flavonoids (0.82±0.00 mg catechin echivalents/g extract), was found to be the most potent in all antioxidant assays. The results indicate that *Melissa officinalis* could be a possible candidate for the development of radioprotective agents.

Acknowledgements: The study was supported by University of Medicine and Pharmacy „Grigore T. Popa"-Iasi Internal Research Grant no. 1639/01.02.2013 (INVESTIGATIONS ON THE RADIOPROTECTIVE POTENTIAL OF SOME VEGETAL EXTRACTS).

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Can phytochemicals be a bridge to develop new radioprotective agents?

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Humans are constantly exposed to ionizing radiations from natural and artificial sources. In modern times, the high technological advance has increased radiation exposure. Depending on the exposed and absorbed dose, time during and after exposure, ionizing radiations induce deleterious effects on the mammalian cells. The radiations affect biological systems through a series of molecular events, triggered by an increased reactive oxygen species production (ROS). ROS accumulate in irradiated cells and they interact with biological macromolecules (DNA, RNA, proteins, lipids) causing cell dysfunction, mutations and death [1,2]. Although some synthetic compounds like aminothiols exhibited radioprotective effects, they are toxic at the maximum effective dose, show narrow time windows and limited routes of administration [3]. Various phytochemicals, mainly phenolic derivatives, have been screened for their radioprotective properties. Naturally occurring compounds such as curcumin, resveratrol [4], genistein [5], EGCG [6], silibinin [7] have shown significant radioprotective potential. This review summarizes the current knowledge in radioprotection using plant bioactive compounds with emphasis on their structural characteristics, mechanisms of action and relevant approaches in the development of radioprotective agents. Free radical scavenging abilities, maintaining cellular antioxidant status, modulation of DNA repair or prevention of DNA damages and anti-inflammatory activity are the main mechanisms involved in radioprotection exerted by phytochemicals. Also, they may provide an extended window of protection against low-dose and low-dose-rate irradiation. The limits of existing research as well as the suggestions on further studies including the modulation of long-term radiation effects, are also discussed.

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Phytochemical and bio-ecological aspects of some *Nepeta* species from Republic of Moldova and Romania

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Nepeta species are used as traditional remedy in many countries due to a vast number of ethnobotanical effects: diuretic, antitussive, antispasmodic, anti-asthmatic, expectorant, tonic, febrifuge, and carminative. Current studies [1, 2] confirm also anti-inflammatory, antioxidant, spasmolytic and bronchodilatory properties of these species indicated by traditional medicine.

The aim of this study is to reveal the natural distribution of *Nepeta* (*N. pannonica* and *N. cataria*) species in the flora of Republic of Moldova and their habitat preferences. Phytochemical study conducted on *N. cataria* , *N. pannonica* and *N. transcaucasica* samples aimed to identify the polyphenolic compounds from plant material collected from the native flora and experimental fields. The results of the fieldwork allowed to identify three locations for *Nepeta pannonica* and *Nepeta cataria*: Scientific reservation "Codrii", Lozova village, Landscape reservation "Carbuna", Zloti and Garnetz forest with *Quercus pubescens* near Miresti village, Nisporeni district. Two types of habitats for *Nepeta pannonica* and *N. cataria* were identified and described in details: Ponto-Sarmatic Forest vegetation with *Quercus pubescens* (code number 91HO) and Ponto-Sarmatic deciduous shrubs with code 40CO, according to the Directive 92/43/EEC (Interpretation Manual of EU Habitats, 2003). The aerial part of *N. pannonica* and *N. cataria* were collected from their natural habitats in June 2013 and October 2013 respectively. The plant material of *N. transcaucasica* and *N. cataria* from the experimental fields of the Botanical Garden (Institute) of ASM were collected in June and July 2013 respectively.

For the comparative phytochemical study, the methanolic extracts of *N. cataria, N. pannonica* and *N. transcaucasica herba* were analyzed by means of TLC, HPLC and Spectrophotometry. The phenolic acids and flavonoids were the main classes of bioactive secondary metabolites envisaged in this study. The HPLC analysis highlighted the rosmarinic acid as the main phenolic acid in all analyzed samples (87.49 – 793.45 mg/100 g d.w.) with the highest values in *N. pannonica. N. transcaucasica* extract had the highest average amounts of chlorogenic acid (53.61 mg/100 g d.w.), compared with *N. pannonica* (31.31 mg/ 100 g d.w.) and *N. cataria* (8.89 mg/100 g d.w.). The species *N. cataria* and *N. transcaucasica* showed the highest amounts for the flavonoid apigenol-7-*O*-glicoside (21.50, respectively 18.92 mg/100 g d.w.). The spectrophotometric analysis revealed that *N. pannonica* samples had the highest values both for the total polyphenols - expressed in gallic acid equivalents (966 mg/100 g d.w.) and phenolic acids - expressed in rosmarinic acid equiv. (750 mg/100 g d.w.). *N. cataria* species showed the highest content for flavonoids – expressed in luteolin equiv. (282 mg/100 g d.w.). The species *N. transcaucasica* had a less complex phytochemical profile, but the total content in polyphenols, phenolic acids and flavonoids was comparable with the other studied *Nepeta* species. Further studies are required in order to obtain more information on the phyochemical profile an on the biological activity of the specific phytochemicals.

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Phytochemical composition of the endangered species *Nepeta parviflora* Bieb. from the flora of Republic of Moldova

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The genus *Nepeta* L. (*Lamiaceae* family) includes species that are wildly distributed, several having a special sozological status. It has been shown that many medical properties of *Nepeta* species are the characteristic of its monoterpene derivatives, sesquiterpenes, diterpenes, triterpenes, flavonoids and phenolic compounds, essential oils and some others [1].

In the spontaneous flora of the Republic of Moldova *Nepeta* L. genus is represented by three species (*Nepeta cataria* L., *N. pannonica* L. and *N. parviflora* Bieb.) [2]. The present study refers to *N. parviflora* Bieb., species threatened with extinction in the local flora (regional assessment for the Red Book of Republic of Moldova, 3rd edition is **Vulnerable [VU]**) known from several extant locations.

The aim of our study is to assess the polyphenolic compounds content of this species, for which we found no literature data. The previous phytochemical studies on *N. parviflora* species focused on the essential oil content [3]. This research was initiated in the spring of 2013 and included the field observations in different stages of vegetation. The field studies were preceded by an extensive literature survey regarding this medicinally important species. An ample revision has been made in the Herbarium of the Botanical Garden (I) of ASM. Habitat types are given according to NATURA 2000 **(**Interpretation Manual of EU Habitats, 2003, Directive 92/43/EEC).

Material and Methods. *The plant material* was harvested from three growing locations in the Natural reservation with multifunctional management "Bugeac" in May for germplasm preservation and in June for phytochemical analysis. *The phytochemical assessments* for the *N. parviflora* methanolic extracts were performed by means of TLC and HPLC.

Results and Discussion. *The TLC analysis* revealed the presence of the phenolic acids and for the flavonoids only the presence of luteolin and apigenin-7-*O*-glucoside. *The HPLC analysis* highlighted quantitative intraspecific differences for the samples from different locations in the wild population from "Bugeac" Natural reservation. Rosmarinic acid is the major compound in all the samples.

Tab.1. Results of phytochemical analyses for *N. parviflora* methanolic extracts (June, 2013)

Samples (*herba*)	***Rosmarinic acid***	***Caffeic acid***	***Chlorogenic acid***	***Apigenin-7-O-glucoside***	***Luteolin***
	mg / 100 g d.w.				
NPv1	243.94	2.56	2.71	7.14	8.00
NPv2	219.57	3.71	7.96	30.26	11.26
NPv3	159.92	2.08	1.88	5.16	4.04

The studies will be deepened by comparative phytochemical analysis for the plant material harvested from the wild populations in Romania, *N. parviflora* having the status of rare species within The Vascular Plant Red List of Romania.

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To identify the activity within *Andrographis paniculata* and *Silybum marianum* plant extracts against photo-oxidative damage

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Ultraviolet radiations produce photo-oxidative damage which may lead to mutagenesis and genetic instability if not protected or repaired. The goal of this study is to identify the activity within *Andrographis paniculata* and *Silybum marianum* plant extracts against photo-oxidative damage.

Sequential extraction of both plant materials was performed with different polarity solvents. Quality control was performed with Gas chromatography and High performance liquid chromatography. Primary extracts screening studies were performed with cytotoxicity, antioxidant, proliferation assays and Sircol dye assay. Further bioactivity will be confirmed using indirect immunofluorescence assays using antibodies against markers of DNA damage and repair and finally the single-cell gel electrophoresis assay (Comet assay) to estimate levels of DNA damage.

Acetone, methanol 1 and methanol 2 extracts from both plants do not caused cytotoxicity. High antioxidant activity is observed in methanol 1, methanol 2 extracts from *Andrographis paniculata* and in acetone, methanol 2 extracts from *Silybum marianum*. In addition, good proliferation activity is found in acetone, methanol 1 and methanol 2 extracts of both plants which help to increase the soluble collagen in human dermal fibroblasts against UVA and UVB induce damage.

These preliminary screening results indicate that both plant extracts have protective activity against UVA and UVB induce photo-damage. This finding is promising and future work will be focused on validation with more specific photo-oxidative damage with the aim to isolate and identify the bioactive compound.

Studies on the Permeation of *Curcumin* in Human Skin Using Organotypic In Vitro-Models

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In vitro-penetration studies are usually performed using Franz cell diffusion porcine skin models. Because porcine skin is limited comparable with human skin, we present alternative methods based on human skin cells. Curcumin has been chosen as a test substance because it is a potential candidate for the topical treatment of actinic keratosis and epidermal tumors [1,2].
For the penetration studies we developed organotypic epidermal- and full thickness skin-models. In case of epidermis models keratinocytes were isolated from human surgery material, expanded in cell cultures and seeded on polycarbonate membranes of cell culturing plate inserts. After transferring the cells to the air liquid interface keratinocytes developed a stratified epidermal equivalent showing all characteristic epidermal *strata*. In case of full thickness skin models keratinocytes and fibroblasts were isolated and cultivated in separated cell cultures. Fibroblasts were seeded onto collagen scaffolds and cultivated submersed for 2 weeks followed by seeding of keratinocytes on top of the fibroblast containing scaffolds. After further 1 week the constructs were transferred to the air liquid interface to gain stratified and fully differentiated epidermal equivalents (for details see [3]). After 2 weeks, the penetration of aqueous curcumin solutions and curcumin containing basis ointments was investigated measuring the curcumin derived fluorescence in the culture medium beneath the models.
Curcumin applied in aqueous solution, or in DAC basis ointment penetrated very well into the epidermal model. After 30 min curcumin is detected in the culture medium, maximum concentration was reached after 1 hour. In organotypic full thickness skin equivalents curcumin was detected in the medium 1 hour after application reaching a maximum value after 3 hours. In case of curcumin containing UEA ointment no curcumin could be detected in the medium. Cryosections of the models revealed a strong accumulation of curcumin specific autofluorescence in the *stratum corneum*.
Our results emphasize the suitability of organotypic skin models for penetration studies and show that curcumin is able to penetrate the whole skin compartments depending on the basic principle of the supporting material.

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Phytochemical investigations on *Lamium maculatum* L. species from Romanian Eastern Carpathians

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The enhancement of capitalization for autochthonous medicinal and aromatic plant bioresources, by developing the scientific strategies for preservation, cultivation, processing and phytochemical authentication of ethno-pharmaceutical products, represents a priority also for the mountain sustainable development. Among the *Lamium* species found in Romania, *L. maculatum* and *L. purpureum* are less studied although these species shows a specific pharmacological potential due to the genus they belong to [1]. *L. maculatum*, grows on rich and moist soil, having a wide distribution. The *Lamii herba* is used in folk medicine to improve circulation, treat injuries, fractures, paralysis and hypertension, and also for digestive problems. The phytochemical composition cited in the literature is specific for *Lamioideae* [2, 3, 4].

The results presented in this paper are a part of a wider phytochemical study on the plant material harvested during several seasons of the vegetation period, from different wild populations located at altitudes of 700-800m.

Material and Methods. *Plant material:* The aerial parts of all plants were harvested at full blooming, dried in a heated herb drier at 40°C, ground, weighed and submitted to methanolic extraction. *The phytochemical analysis* was performed by means of TLC, HPLC and Spectrophotometry.

Results and Discussion. *The TLC analysis* showed the presence of phenolic acids and flavonoids in all analyzed samples, the results being confirmed by *the HPLC analysis*. For all envisaged compounds, the sample LM3 had the highest values, fact that can be explained by the pedo-climatic differences in the studied location, mainly due the UV amount available in the habitats.

Results of phytochemical analyses for *Lamium maculatum* extracts.

Samples	Spectrophotometry		HPLC		
	Total phenolic acids*	Total flavonoids**	*Chlorogenic acid*	*Rosmarinic acid*	*Rutoside*
	mg/ 100 g d.w.				
LM1	977.00	423.00	1101.27	283.61	143.52
LM2	1082.00	521.00	1333.90	267.22	157.58
LM3	1564.00	831.00	1718.05	547.16	259.43

Legend: * expressed as caffeic acid equivalents; ** expressed as rutoside equivalents.

The chlorogenic acid is the major compound in the samples. The HPLC analysis also highlighted the presence of rosmarinic acid, although this compound is not characteristic for *Lamioideae* subfamily.

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Phytochemical study of *Pentzia monodiana*: application of accelerated solvent extraction and centrifugal partition chromatography for identification of antiplasmodial and antileshmania bioactive compounds

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The *Pentzia* genus, from *Asteraceae* family, includes about 27 species distributed mainly in South of Africa, three in North Africa and four in Yemen [1]. Traditional uses of *Pentzia* species concerns infectious and digestive troubles [2]. *Pentzia monodiana* is an annual plant, with medium sized leaves, golden yellow and small flowers. Samples have been collected in National Cultural Park of Ahaggar in southern Algeria. Although some of these species have been studied, there is no report of phytochemical study on *Pentzia monodiana* [3]. Main phytochemicals isolated in this genus are sesquiterpene lactones from germacranolides and guaianolides type, but also acetylenes compounds and methoxyflavones. The vegetal material was submitted to a High-Pressure Automatized extraction (Speed Extractor, Buchï), first with ethyl acetate, in order to recover a terpenoid-rich fraction, and then using methanol to complete the extraction. The methanol and ethyl acetate crude extracts were evaluated for their antileshmania activity and also antiplasmodial activity through HEME binding detection test developed by Dr A. Maciuk. In this test, adducts formed between bioactive compounds and plasmodial heme (*m/z*=616) can be detected using mass spectrometry [4]. No adduct was observed with the methanolic extract whereas an intense peak at *m/z*=974 was observed with the ethyl acetate extract. Thus the corresponding heme-binding metabolite should have a *m/z* value of 358 (974-616). Isolation of *Pentzia monodiana* metabolites was performed with both Centrifugal Partition Chromatography, and preparative liquid chromatography. Centrifugal Partition Chromatography conditions have been optimized (system Arizona U : Cyclohexane / Ethyl Acetate / Methanol / Water - 4/1/4/1) to isolate the major metabolite of ethyle acetate extract, identified as the sesquiterpenic lactone **Ketopelenolide B** (2.3%). Structural elucidation of two other compounds **CPC7** (0.8%) and **CPC19** (1.2%) remains to be elucidated. Two others CPC fractions were submitted to preparative HPLC separation and afforded two methoxyflavones, **5-hydroxy-6,7,3',4'-tetramethoxyflavone** (0.034%) and **5-hydroxy-3, 6,7,3', 4'-pentamethoxyflavone** (0.041%). This is the first report of these compounds in *Pentzia* genus. All compounds were characterized by ^{1}H and ^{13}C NMR, 2D-NMR (HMBC and HMQC), EIMS and compared with data in the literature.

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Micromorphological and biochemical studies regarding *Thymus* taxa from Romania flora

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The genus *Thymus* can be considered, due to the large number of taxa, one of the most important genera of the *Lamiaceae* family [4]. A common feature of these taxa is the presence of secretory hairs responsible for production and secretion of essential oils, oils that shows a great significance for producing plants and with clear practical uses.

The purpose of the present paper is to conduct a micromorphological assessment of different secretory hairs that exist on aerial parts of vegetative organs, concomitantly with a qualitative and quantitative analyses of essential oil secreted by some *Thymus* taxa from Romanian flora. Highlighting the possible micromorphological and biochemical changes which could be identified on analyzed biological material, changes induced by environmental factors and by ontogenetic stage of the investigated plants represent, also, another objective of this study. For the identification of morphological types of secretory hairs, cross and superficial sections at the aerial parts of investigations species, were made. The volatile oils were extracted by hydrodistillations, using a Neo Clevenger device and the chemical analysis of these essential oils was performed using a gas chromatograph Agilent Technologies, type 6890N. The separated compounds were identified by means of the Nist spectrum database, and the peak position was confirmed by the Kovats retention index [2].

The micromorphological study of secretory hairs pointed out the fact that they are formed from a unicellular base, implanted between epidermal cells, a one- or bicellular pedicel and a gland with 1, 2 or more secretory cells. These results confirm the already existing data in the literature [1,3,4]. Analysis of the influence of environmental factors and ontogenetic stage of the plants on the micromorphology of secretory hairs, has not recorded significant effects, the observed differences referring to the chemical composition of the volatile oil from these formations. Therefore, the present study shows that geographic zoning and ontogenetic stage of the plant may be important factors that induce qualitative and quantitative changes in the products of secondary metabolism of plants investigated. Correlating the micromorphological data with those of biochemistry, the study aims to identify wild populations of *Thymus* sp. with a high level of their medicinal and aromatic value.

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Chemical composition and *in vitro* activities of *Angelica archangelica* L. and *Angelica sylvestris* L. from Romania

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Several species of the *Angelica* genus are well-known for their medicinal properties [1], with an overall distribution ranging from Eastern Asia and Europe to the American continent [2]. Known bioactive compounds in these species include phenolics, polysaccharides and volatile oils, with activities such as antiinflammatory, antiproliferative, antimicrobian or anti-acetilcholinesterase among many others [1]. In Europe, *Angelica archangelica* is the best-known species, its product being included in official listings of medicinal herb preparations [3], while *Angelica sylvestris* is less often used. The current study aims to characterize side-by-side the two species, found in Romania's wild flora, from a chemical point of view, as well as to asses some *in vitro* activities of extracts from the two species. The research was carried bearing in mind that both species are used in a traditional fashion in alimentation [4], and better knowledge of chemical composition and possible positive effects on human health are necessary. Findings in our study, among other analyses, revealed a calcium content of 0.01 – 5.50 % dry matter (d. m). in the case of *A. sylvestris* and 0.03 – 5.33 % d. m. for *A. archangelica*. Meanwhile, magnesium content ranged from 0.08 to 0.78 % d. m. for *A. sylvestris* and from 0.13 % to 0.62 % d. m. for *A. archangelica*. Total lipid content registered values of up to 8.73 % d. m. for *A. archangelica* and up to 9.30 % d. m. for *A. sylvestris*. Other evaluated parameters were water, ash, phoshorus and nitrogen contents. Total phenolic compounds content determined for roots and aerial parts of the two species revealed quantities as high as 688.39 mg/100 g d. m. for *A. archangelica* and 838.60 mg/100 g d. m. for *A. sylvestris*. HPLC analyses of selected phenolic compounds showed the presence of up to 168.32 mg/100 g d. m. of chlorogenic acid in *A. archangelica* leaves, and of 244.72 mg/100 g d. m. in leaves of *A. sylvestris*. Cumaric and ferrulic acids peaked at 6.17 and 6.08 mg/100 g respectively in *A. archangelica* and at 8.44 and 7.63 mg/100 g respectively in *A. sylvestris*. Other compounds quantitatively determined were caffeic acid, rutoside and luteolin. All the above chemical determinations were carried over several growth stages and plant organs. Bioactivity assays showed antioxidative capacities of up to 92.40 ± 0.13 % inhibition for various concentrations of *A. archangelica* ethanolic extracts and up to 93.14 ± 0.43 % inhibition for same concentrations of *A. sylvestris* extracts in tests using DPPH free radical when compared to untreated controls. Thermal denaturation of albumin was inhibited up to 42.90 ± 0.86 % by *A. sylvestris* extracts and up to 40.90 ± 1.78 % by *A. archangelica* extracts. Results show that analyzed species top other species of the genus in chemical composition [5] or in *in vitro* performances [6], also hinting at a greater interest *A. sylvestris* might receive as a medicinal and alimentary plant.

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Bioautographic Xanthine Oxidase Assay: Combining phytochemical separation and activity assessment as a tool for phytopharmaceutical research on medicinal plants

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Xanthine oxidase (XO), a molybdenum containing flavoprotein, catalyses the oxidation of hypoxanthine and xanthine to uric acid under the formation of superoxide radicals and hydrogen peroxide. An overproduction of these reaction products in the human body is associated with diseases such as hyperuricemia, gout, hypertension, diabetes and different inflammatory diseases. Prospective long-term studies have demonstrated that the intake of Allopurinol as competitive xanthine oxidase inhibitor can cause a number of serious side effects. For this reason the research for specific xanthine oxidase debilitating compounds in Drug Discovery and thus also medicinal plants research is very important.
Bioautography offers a rapid and simple tool for screening of secondary metabolite profiles of medicinal plants by HPTLC combined with screening of potential health beneficial activities. The aim of this work has been to optimize and validate a bioautographic XO Inhibition assay described by Ramallo et al. [1].
To establish a reliable, reproducible and validate bioautographic XO assay, the concentrations of redox dye and substrate, enzyme activity as well as the buffer conditions were optimized using allopurinol as known inhibitory substance. Moreover, the assay procedure has been improved by using a low gelling temperature agarose and adjustment of incubation time and temperatures according to the XO thermal activity characteristics. XO inhibitory effects were visualised as white zones on a purple coloured thin layer chromatogram based on the reaction of superoxide radicals with nitroblue tetrazolium chloride. The visual detection limit of the competitive XO inhibitor allopurinol was 45.4ng. Extracts of *Camellia sinensis* and *Artemisia alba* showed also to contain constituents with XO inhibitory activity, that could be visually detected down to an applied amount of 10µg dry weight (dw) for *C. sinensis* extract and 100µg dw for *A. alba*. Since this assay uses superoxide radicals for the measurement of xanthine oxidase activity superoxide radical scavengers can also generate positive results on the HTPLC plate. However, such compounds can be differentiated from pure XO inhibitors by the direct measurement of uric acid by using a standard XO microtiterplate assay [2].
From the results it can be concluded, that the improved bioautographic Xanthine Oxidase inhibition assay is a rapid and valid research tool for assessment of active secondary metabolites from medicinal plants.

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Progesterone 5β -reductase from horseradish (*Armoracia rusticana* Gaertn., Mey., & Scherb.): cloning, sequence comparison and molecular modelling

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The evolution of bioactive plant natural products is an issue of fundamental and practical interest. Many of these compounds still are important drugs used in therapy, such as paclitaxel, morphine, quinine, scopolamine and digoxin. The occurrence of several so-called secondary metabolites seems to be restricted to certain taxa whereas others are produced by many plant species. 5β-Cardenolides, i.e., a group of cardioactive steroid glycosides, have been detected in many orders of the angiosperms (Kreis and Müller-Uri, 2010) but it is rather the rule than the exception that only one or few genera within a given plant family produce cardenolides. This has been termed an 'erratic' occurrence since the biosynthetic origin of cardenolides in taxonomically distant taxa has yet to be elucidated (Kreis and Müller-Uri, 2010).
Protein extracts prepared from *Armoracia rusticana* leaves exhibited progesterone 5β-reductase activity (P5βR). Using primers deduced from known P5βR genes a full-length cDNA encoding a progesterone 5β-reductase (ArP5βR, EC 1.3.99.6) was isolated from *A. rusticana* leaves. A SphI/SalI ArP5βR PCR fragment was cloned into the pQE30 vector and transformed into *Escherichia coli*. The recombinant His-tagged fusion protein gene was expressed in *E. coli* M15. Progesterone was enantio selectively reduced to 5β-pregnane-3,20-dione. The ArP5βR of *A. rusticana* (JF460011) showed 92 and 91 % sequence identity to steroid 5β-reductases isolated from *Arabidopsis thaliana* and *Erysimum crepidifolium*, respectively. A three-dimensional model of ArP5βR on the basis of the Digitalis lanata P5βR (PDB ID: 2V6G, DlP5βR) highlights the close structural relationship to Vein Patterning 1-encoded enone reductases described previously.
Investigating genes and enzymes involved in cardenolide biosynthesis in different plants may help to understand evolution of the cardenolide trait and shed light on the conservation and/or multiple evolution of genes coding for cardenolide-biosynthetic enzymes. Steroid 5β-reduction is a mandatory step in the biosynthesis of 5β-cardenolides.

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Flavonoids contents and bioactivities of 19 species of ferns from China

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Ferns are well-known traditional Chinese medicinal herbs and there are about 61 families, 223 genera, about 2600 species in China. Moreover, 300 species of ferns are used as medicinal herbs by the minority mainly in Southwestern China. Specially, there are about 1500 species of ferns in Yunnan province of China. However, till now, there is little information on the phytochemical and pharmacological characteristics of most species of ferns. Many subclasses of flavonoids, specifically flavonols, have been identified in ferns. The epidemiological and medical evidence has suggested that the natural flavonoids play an important role in preventing and managing of diseases such as cancer, diabetes, and cardiovascular diseases. Moreover, most interest has been devoted to their antioxidant activities, which are due to their ability to reduce free radical formation and also to scavenge free radicals. However, there is little information on the bioactivities of most species of ferns. Herein, the total flavonoids contents, antioxidant, anticancer and antiacetylcholinesterase activities of flavonoids extracts from 19 species of ferns were investigated.

In current research, the total flavonoids content, antioxidant, anticancer, and anti-acetylcholinesterase activities of extracts from 19 species of ferns from China were investigated in detail. The total flavonoid contents were within the range of 8.60 to 306.4 mg/g (w/w). The antioxidant activities including DPPH free radical, ABTS radical scavenging, and superoxide anion scavenging potential, reducing power and ferric reducing antioxidant potential (FRAP) of flavonoid extracts from these ferns showed a weak relationship with their total flavonoid contents. *E. sinensis*, *D. cylindrica*, *W. magnifica*, *W. japonica*, *S. chusanum*, *D. boryanum*, and *D. erythrosora* show very high total flavonoid contents (> 140 mg/g). The extracts from Group I (*S. chusanum*, *E. sinensis*, *D. boryanum*, *D. cylindrical*, *P. smithii*, *A. yunnanensis*, *P. gralla*, *D. erythrosora*, *W. magnifica*, and *W. japonica*) showed significant antioxidant potential. *D. cylindrical* showed the highest DPPH radical scavenging potentials. The ferns with higher contents of total flavonoids showed more obvious DPPH radical scavenging activities. *S. frondosa* with very low total flavonoids content (13.4 mg/g) showed highest inhibition against A549 cells and it is necessary to further investigate the anti-cancer compounds in *S. frondosa.* The extract from *D. cylindrica* and *S. chusanum* significantly inhibited AChE activity, which illustrated that it is worthy of attention to investigate flavonoids from *D. cylindrica* and *S. chusanum* as AChE inhibitors.

Quantitation of annonacin in Rat plasma: Application to a pharmacokinetic study

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Annonaceous acetogenins [1] are neurotoxic molecules distributed in the Annonaceae family. They have been found in several parts of these plants, including worldwide consumed fruits of the *Annona* genera, like soursop (*A. muricata*) [2]. A link between the consumption of soursop and a high prevalence of atypical parkinsonisms has been established by epidemiological studies [3]. The most abundant acetogenin in soursop is annonacin, a molecule which proved neurotoxic in several models [4]. Hence it has become a main concern to evaluate its pharmacokinetics parameters, especially to get some information about bioavailability.
We developed a quantitation method for annonacin in rat plasma, using UPLC-ESI-TQ, with its analogue annonacinone as internal standard. We used SRM mode with a focus on 112 uma loss, which is characteristic of this class of molecules. We used a gradient water / acetonitrile: 35 / 65 to 15 / 85 in 5 minutes as mobile phase, and an Acquity UPLC BEH C_{18} column. We prepared calibration standards from 0.1 ng/mL to 50 ng/mL in rat plasma spiking 10 µL of annonacin solution of the appropriate concentration, and 10 µL of IS solution at the concentration of 100 ng/mL, in 100 µL of plasma. Sample preparation was performed by two consecutive liquid/liquid extractions with 0,5 mL of ethylacetate, evaporation of the supernatant under stream-flow, reconstitution in 100 µL of acetonitrile. 5 µL were injected in 3 replicates. The calibration curves were linear ($R^2 > 0.990$). The lower limit of quantitation was 0.1 ng/mL, with a S/N ratio > 300, a CV < 20% and a relative error < 20% calculated with the low range of the calibration curve (6 dots). Intraday and interday repeatability were calculated at 5 concentrations with n = 5 replicates, 3 consecutive days, 3 injections a day. They were < 15% for each concentration (0.1; 0.25; 2.5; 10; 100 ng/mL). Extraction recovery was > 80%. We compared matrix effect in different plasma matrices. Stability studies were also performed according to the EMA guidelines on bioanalytical analysis. The method was applied to plasma samples collected after intravenous and oral administration on rats. Annonacin was administered to rats at the concentration of 0.5 mg/kg by intravenous route, and 10 mg/kg by oral route. Blood samples were collected periodically during 48 hours. At these doses, the molecule could be detected in plasma until 48 hours, after administration by both routes. The full method is presented as well as pharmacokinetics results.

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Identification of the environmental neurotoxins Annonaceous acetogenins in an *Annona cherimolia* Mill. alcoholic beverage using HPLC-ESI-LTQ-Orbitrap®

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Epidemiological and toxicological studies suggested Annonaceaeous acetogenins to be environmental neurotoxins responsible for sporadic atypical parkinsonism / dementia in tropical areas [1]. These compounds are present in the tropical genus *Annona* (Annonaceae) [2], known for its fruits-yielding cultivated species such as *A. cherimolia*. This species is widely cultivated in South America, Spain and Portugal and yields acetogenins in its seed, stem and root. Presence of these compounds in the pulp of its fruit and in derived food products is unclear [3]. Using an innovative and sensitive methodology by HPLC-ESI-LTQ-Orbitrap® with post-column infusion of lithium iodide [4], we were able to identify the presence of low-levels of acetogenins in an *Annona cherimolia* Mill. fruit-based commercial alcohol. More than 80 representatives were detected, and among them 31 major acetogenins were identified, and among which several were shown to be neurotoxic *in vitro* or *in vivo* [5]. According to the MS2 spectra of resolved or co-eluted peaks, approximately 17 AAGs (21%) are of A-type (mono-THF), 17 (21%) of B-type (bis-THF), and 7 (9%) belong to other types. Regarding the lactone, 29 AAGs (36%) belong to sub-type 1a (unsaturated γ-lactone) and 48 (60%) to sub-type 1b (unsaturated γ-lactone with OH in position 4). Atypical hydroxylation of the lactone, which distinguishes *A. cherimolia* from other *Annona* spp., was observed. These structural features are in agreement with the compounds isolated from the plant [2]. All together these findings indicate that this species should be considered as a risk factor within the frame of a worldwide problem of food toxicity.

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Fraternine, a new norsecurinine-type alkaloid from *Phyllanthus fraternus* (Phyllanthaceae)

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Phyllanthus fraternus G.B. Webster (Phyllanthaceae) is traditionally used against malaria in Ghana. It was selected on the basis of significant use, including prescription in traditional hospitals. Convergence in the use of the genera in West African medicine is remarkable. From the aerial parts of the plant, norsecurinine-type alkaloids, which are chemotaxonomical markers of the genera, were obtained. Along with norsecurinine, a new hydroxylated analogue was purified. This compound, which was named fraternine, appears as a potential precursor of nirunine, previously identified in *P. niruni* L . Structural elucidation is provided. Biological evaluation of these alkaloids is under way.

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Identification of medicinal plant species through DNA barcoding for a safe and efficient use of phytochemicals

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A large number of molecular techniques have been used to accurately identify medicinal plants based on species-specific variations in the sequences of various chloroplast and nuclear DNA regions. DNA-based authentication of medicinal plants is a work in progress that offers powerful new tools and entry points for measures aimed at quality control and quality assurance in medical plant research as well as the production, clinical use, and forensic examination of herbal medicines. For example, genome based methods can be useful to quickly and efficiently pinpoint adulterated or misidentified raw materials, which can then be discarded without further need for time- and resource-consuming morphological, physical and phytochemical examinations.
Using PCR-based methods, species identification has been achieved using DNA that was isolated from fresh and dried plant parts, plant extracts, processed herbal drugs, as well as finished products such as herbal teas, tablets and capsules [1,2]. DNA barcoding can differentiate between individuals, species and populations and has been proved useful for the characterization of sample homogeneity and detection of adulterants. Rapid advances in "next generation sequencing" (NGS) technology are reducing the cost of sequencing and bringing large-scale sequencing projects into the reach of individual investigators [3].
The paper will review and present the use of some important markers (nrITS1, nrITS2, trnH-psbA, matK, rbcL, rpoC1, and trnL) for the identification of some medicinal and aromatic plant species and comment on the already proven potential of the plant DNA barcoding to be used in the near future as a reliable and standardized method for authentication of medinal plant species for their safe and efficient use as herbal food supplements. Medicinal plant species substitution and adulteration do introduce, beside quality assurance, both safety and efficacy concerns related to the use of phytochemicals.

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Effect of some cytokinins on morphological development and hypericins content in shoot cultures of *Hypericum richeri* ssp. *transsilvanicum* and *H. umbellatum*

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Hypericum is a large genus, which includes about 500 species [1]. Some of them are already considered as high value medicinal plants due to their rich phytochemical profile and the presence of unique compounds such as hypericins, hyperforin and benzophenones/xanthones. However, 75% of *Hypericum* spp. are still unexplored for their pharmacological activities. Hypericins are accumulated in specialized glands "dark glands" present on stems, flowers and leaves. The aim of this work was to study the influence of two cytokinins: 6-benzylaminopurine (BA) and thidiazuron (TDZ) in different concentrations (0.10; 0.25 and 0.50 mg l^{-1}) on the morphology and hypericins (hypericin-Hyp and pseudohypericin-PsHyp) production of previously established shoot cultures [2] of *H. richeri* ssp. *transsilvanicum* (Čelak) Ciocârlan (an endemic taxon for Romania) and *H. umbellatum* A. Kern (a Daco-Balkan element). Results showed that BA-supplemented MS [3] stimulated shoot multiplication and dry weight accumulation in both species. Instead TDZ treatment mainly promoted fresh weight gain, due to an increased water accumulation, which resulted in high occurrence of hyperhydric shoots depending on species. Thus, at 0.50 mg l^{-1} TDZ, over 60% of *H. umbellatum* shoots showed hyperhydricity. Shoots water content was dependent on the cytokinin type and its concentration. Hence, *H. umbellatum* shoots cultured on TDZ (0.50 mg l^{-1}) supplemented MS, showed a higher water content (over 96%) than control shoots (81%) cultured on MS medium without growth regulators. Moreover, in both species, in shoots cultured on MS with TDZ, the basal part of the stems showed hypertrophy, exhibiting a callus-like appearance. The amount of hypericins was determined by high performance liquid chromatography (HPLC). Wild-growing plants contained appreciable amounts of total hypericins: 1.95 ± 0.16 mg g^{-1} dry weight (DW) in *H. richeri* ssp. *transsilvanicum* and 2.47 ± 0.21 mg g^{-1} DW in *H. umbellatum*. PsHyp was found to be the major naphthodianthrone in both species, *in situ* and *in vitro* as well. The average ratio between PsHyp:Hyp was 3.53:1 in the wild growing *H. richeri* ssp. *transsilvanicum* and 4.89:1 for *H. umbellatum*. Significant differences were found between the content of Hyp and PsHyp in *in vitro* regenerated shoots compared to wild plants. Hypericins levels and number of leaf dark glands (DGN) varied with species, cytokinin type and concentration. Thus, in *H. umbellatum*, BA (0.10 mg l^{-1}) significantly stimulated both DGN (from 52.3 to 63.71) as well as total hypericins levels, mainly of PsHyp (from 1.72 to 2.79 mg g^{-1} DW). In *H. richeri* ssp. *transsilvanicum*, BA had no influence on DGN but strongly inhibited hypericins production. TDZ regardless of concentration, negatively influenced DGN and hypericins synthesis in both species. Our results show a strong correlation between tested cytokinins, DGN formation and hypericins production.

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Effects of *Lamium album* and *Lamium purpureum* Extracts Administration on the Thymus, Adrenals and Kidney Function in Anakinetic Stress Conditions

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Aims. The aim of our study was to emphasize the protector potential of both *Lamium album* and *Lamium purpureum* hydroalcoholic extracts on some thymus, adrenal and kidney morphological parameters, in white Wistar rats in anakinetic stress condition.
Materials and methods. Experiments were performed on white female Wistar rats, weighing 165 ± 20 g during 17 days. Animals were divided into 6 groups of 6 animals each, as follows: control group (C); anakinetic stressed group (S); *Lamium album* hydroalcoholic extract treated group (LA), which received 20 mg extract/100 g bw, *á jeun*; *Lamium purpureum* hydroalcoholic extract treated group (LP), which received 20 mg extract/100 g bw, *á jeun*; anakinetic stress + LA treated group (SLA) and anakinetic stress + LP treated group (SLP). The hydroalcoholic extracts of *Lamium album* and *Lamium purpureum* were obtained at the "Stejarul" Biological Research Center, Piatra Neamț. The alcoholic extract (1:1) was obtained with alcohol 70^0.Qualitative analysis of the polyphenolic compounds of the *Lamium album* and *Lamium purpureum* extract was done by thin-layer chromatography (TLC), spectrofotometry and high performance liquid chromatography (HPLC). In the 18^{th} day, animals were killed by decapitation after a pre-anesthesia with ether. Fragments of organs were removed and fixed in Bouin liquid fixative and prepared for histology. Staining was made by haematoxylin-eosin method for histological structure of thymus, adrenals and kidney [1].
Results. At the kidneys level *L. album* extract showed a stronger diuretic effect than that of the *L. purpureum* extract, who presented a lower diuretic potential. In SLA group there has been a stronger diuresis probably due to a concerted action of two factors: stress and the extract of *L. album*. In the SLP group there has been observed a protective effect of the *L. purpureum* extract against the adverse effect of stress, as well as a lower diuretic potential. These aspects prove that the two extracts are deprived of toxicity and do not induce undesirable side-effects on renal function and structure. At the adrenal level it has been observed an inhibition of glucocorticoid secretion, more pronounced in the SLP group. This inhibition of the adrenal glucocorticoid hormones secretion highlights the protective effect of the two extracts against the induced stress.The thymus changes following administration of both extracts have highlighted the immunostimulating effect on the thymus lymphocytes population, due to the presenceof active principles from the *Lamium* extracts (flavonoids and polyphenols). The most powerful protector effect against changes induced in the stress condition was manifested more strongly in the SLA group.
Conclusion. The results of our study suggest that the vegetal extracts of *L. album* and *L. purpureum* at the concentration used in the study (20 mg/100 g b.w.), induced protective modulator effects on the dynamics of the entire set of functional and morphological analyzed parameters.

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Phytotherapyneurodegenerativediseases due to poisoning by heavymetals "Experimentalstudy"

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More than 600 disordersafflict the nervous system. Neurodegenerative diseases are defined as hereditary and sporadic conditions which are characterized by progressive nervous system dysfunction. They included is eases such as Alzheimer's Disease, Parkinson's Disease and other dementias, Brain Cancer, Degenerative Nerve Diseases, Epilepsy.
The major neurodegenerative dieases are Alzheimer's disease (AD) and Parkinson's disease (PD), associated with deficiencies in the central nervous system of the neurotransmitters acetylcholine and dopamine respectively. Factors are considered which appear to increase or descreas the risk of contracting these diseases since plants which inhibit or enhance such effects respectively may be useful in prevention of these diseases.The continued occurrence of occupational Aluminum overexposure and Aluminum poisoning in the World remains a serious problem despite awareness of its adverse healtheffects. Aluminium is suspected to play a role in the emergence of some forms of dementia and degeneration of the central nervous system. Parkinson'sdiseaseis the second most common neurodegenerative disorder and the most common movement disorder. It ischaracterized by progressive loss of muscle control, which leads to trembling of the limbs and headwhile at rest, stiffness, slowness, and impaired balance. As symptoms worsen, it may become difficult to walk, talk, and complete simple tasks.A substance called dopamine acts as a messenger between two brain areas. Lead poisoning is, and for centuries has been, one of the most significant preventable causes of neurological morbidity from an environmental toxin. As a heavy metal, lead is ubiquitous in our environment, yet it has no physiologic role in biological systems. Lead affects the central nervous system by multiple different mechanisms, most of which are unexplored.Chronic lead exposureincreases of PD. With dopamine production inhibited, the underlying cause of Parkinson's disease development is attributed to the deterioration of the gland in the brainknown as the substantianigra. Plants from Word traditional medicine systems have been investigated and found to yield compounds and extracts which give symptomatic relief and help control both of these diseases. In some cases the plants have been used for conditions very similar to AD or PD but in other cases they have been used for other ailments, and their value in treating neurodegenerative effects has been found as a by-product of other investigations. The knowledge of the mode of action of the active constituents of plants used as poisons has been applied in treating the condition. Plants of use include those which are the source of single compounds and those where a variety of compounds and activities all contribute to an overall effect. Individual compounds are mainly used to increase the levels of deficient compounds, either by acting on the same receptors or by inhibiting enzymes which causes a drop in levels of the transmitters by metabolizing them. Acetylcholinesterase inhibitors which have been exploited in this way are huperzine. The present work constitutes a review of some Plants extracts with a variety of activities; one of those ingredients is curcumin, sideretis and is in the curry spice turmeric.

The influence of the harvesting phenophase on the dried herba yield and the content of triterpenic acids in the *Ocimum basilicum* L. species (sweat basil) at the conditions of A.D.R.S. Secuieni

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The basil is an annual species which is cultivated in therapeutic purposes for the aerial part (herba) which contains volatile oil (0.5-1.5%), with different chemical composition depending on the chemotype [1, 2]. The compounds from the aerial parts have intestinal antiseptic, carminative, digestive and expectorant stimulant effects. The basil essential oil has antibacterial and antimitotic action, being, also used in food, perfumes and cosmetics industry [3]. The researches were made at S.C.D.A. Secuieni and aimed to evaluate the influence of the harvesting phenophase on the herba yield and on the content of the triterpenic acids of the *Ocimum basilicum* L. species. As a result of the determinations made on the basil culture in the 5 phenophases, we noticed that in the conventional culture, fertilized with chemical fertilizers, the plants were higher, had a greater number of branches, mature and immature inflorescences and fruits compared to the plants cultivated ecologically. In the ecological experiment, the average yield of dry vegetal raw material was the greatest (2895 kg/ha) obtained by the variant harvested in phenophase number 3 (the formation of immature fruit in the verticils of the basal third). Out of the variants of the conventional culture, the greatest yield was obtained by the variant harvested in phenophase 3 (immature fruit formation in the verticils of the basal third), the average yield obtained was 2924 kg/ha, and the difference compared to the control – F1 (floral bud growth in the verticils of the basal third) being of 1618 kg/ha. The content of triterpenic acids (3.16%), the greatest yield, respectively, (216.03 kg/ha), was determined in case of the plants harvested in phenophase 5 – the formation of the mature fruit at the central inflorescence in the experiment that used ecological cultivation technologies (tab.1). In case of the experiment using conventional cultivation technologies, the same variant had a triterpenic acid content of 2.97% and the yield was 218,6 kg/ha.

Tab. 1. Triterpenic acid content (%) and dried herba yield (kg/ha) determined for *Ocimum basilicum* varieties cultivated at *Agricultural Research and Development Centre Secuieni* in 2009

Quantity	Variety Ecological (E.) and Conventional (C.)									
	E.	C.	E.	C.	E.	C.	E.	C.	E.	C.
	F1		F2		F3		F4		F5	
Triterpenic acids content (g/100g dried herba)	1.27	1.24	1.74	1.05	1.37	1.69	2.89	2.19	3.16	2.97
Dried herba yield (kg/ha)	32.46	28.47	61.91	35.32	69.46	60.44	173.98	144.47	216.03	218.60

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Polyphenols, Radical Scavenger Activity, Short-Chain Organic Acids and Contaminants from several Plants Extracts from `Bucharest Delta`

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In the last years, an outlandish habitat was developed in Bucharest, the so-called `Bucharest Delta`. The aim of our study was to identify the active compounds and contaminants in plants collected from `Bucharest Delta` and to evaluate the influence of urban polluted environment on the plants composition profile, with the intention of potential exploitation of the habitat and of widening the knowledge about such humans-modified ecosystems.
The studied plants were *Mentha aquatica* and *Bidens tripartita* from close proximity of the water and *Ambrosia artemisiifolia* from the access pathways of the `Delta`. Moreover *Mentha aquatica* was compared with *Mentha piperita* (from the garden, bordering Bucharest). The collected plants were analyzed for polyphenolic compounds, radical scavenger activity and content of short-chain organic acids and heavy metals. Spectrophotometric, chromatographic (high performance liquid chromatography with mass spectrometry detection), electrophoretic (capillary electrophoresis with UV detection) and electrochemical methods were used. The analysis of total polyphenols and flavonoids content in plant samples was correlated with their anti radical activity; *Mentha aquatica* extracts presented the highest anti radical activity and *Mentha piperita* the smallest. Also, *Bidens* and *Ambrosia* presented both noticeable radical scavenger activities as determined by DPPH method. In addition, 11 polyphenolic compounds were quantified by HPLC-MS and other 25 compounds were identified [1]; *Mentha aquatica* extracts presented the highest concentration levels of rosmarinic and ferulic acids and *Bidens* extracts presented the highest concentrations of chlorogenic acid, phenolic acids with important radical scavenger activity; the same extracts contain the most important levels of apigenin, luteolin or their derivatives (flavonoids).
The quantities of oxalic, succinic, malic, tartaric, citric and lactic acids found in plant extracts by CE [2] were those usually known from other studies and comparable with plants from other habitats. The same statement we could say about the levels of heavy metals detected in studied plant samples. Low concentration levels of Cu (II) and Pb(II) ($\mu g\ Kg^{-1}$) were detected in all samples, while only *Bidens* contain very low amounts of Hg(II).
It was concluded that the studied plants are not affected by anthropic modified environment and could be used for their biologic activities. We emphasized the highest radical scavenger activity and adaptation potential of *Mentha aquatica,* and apparently higher efficacy when compared with *Mentha piperita.*

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Dynamics of phenolic compounds content changes in aromatic plants seeds during germination

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It is known that germinated plants seeds and grains have increased biological and nutritional value. The objective of this study was to determine phenolic compounds content and their antiradical activity during germination of selected aromatic plants seeds, which are widely used in food industry. The antioxidant properties of these seeds can be used as a new source for food supplements.

Seeds of dill (*Anethum graveolens)*, fennel (*Foeniculum vulgare* MILL.) and black cumin (*Nigella sativa*) were cleaned and disinfected with 1% potassium hypochlorite for 5 min, watered by distilled water, swollen for 4 h in germination plates at 22 º C and germinated in dark for 10 days. Seeds were kept moist by spraying them with distilled water. The germination process was evaluated by the percentage of germinated seeds. Seeds extracts were assayed for phenolic content and antioxidant properties every day during period of germination.

Seeds and sprouts were subjected to extraction using water, 70% aqueous ethanolic solution separately. The ratio of raw material: extractant was 1:10. Before extraction seeds were dried and milled. The extraction process was continued for 4 h at room temperature. The extracts were centrifuged at 4000 rpm for 15 min and filtered through filter paper. The supernatants were used for further analysis. All samples were analysed in triplicate and averaged.

The total phenolic content was determined using Folin-Ciocalteu assay [1], free radical scavenging activity - using DPPH assay [2].

It was shown that the content of phenolic compounds has been decreased in dell and fennel seeds since the 1^{st} till the 3^{th} – 4^{th} days of germination. Significant increase of the content of phenolic compounds was associated with appearance of the first sprouts (on the 3^{nd} day of germination for black cumin, the $5\text{-}6^{th}$ - for dill seeds, on the 7^{th} day for fennel seeds). The maximum content of phenolic compounds (2-3 fold compared with intact seeds) was observed when a germ length was 3-4 mm. The maximum time of germination was fixed in accordance with achieving 96.5% sprout seeds of black cumin, 92.1% sprout seeds of dill, 83.4 % sprout seeds of fennel.

The results showed that germination process significantly increased the content of phenolic compounds. Relationship between total phenolics content and free radical scavenging activity of germinated seeds was also demonstrated. Germinated seeds can be used as a source of natural antioxidants in functional foods.

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Influence of *Helleborus purpurascens* Waldst. & Kit. extracts on haematological parameters of immunodefficient rats

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In empiric veterinary medicine, the rhizome and root of *Helleborus* sp. used as an implant through the animal skin were considered a sort of "panacea"; this tradition has a scientific base as the plant induces a non-specific immune effect with benefic results in many diseases.

Aim: The objective of this research was to study the haematological effects that the extracts of rhizome and root of *Helleborus purpurascens* Waldst. & Kit. (Ranunculaceae) can have on immunosupressed Wistar rats.

Methods: Two types of extracts were used, one obtained by maceration of dried plant in methanol (H1) and one obtained by extraction of raw material under reflux in 30% ethanol (H2). HPTLC fingerprint confirmed the presence of polyphenols, ecdysteroids and cardiac glycosides, compounds involved in modulation of immune cells activity. The extracts contained caffeic acid: 56.48mg% (H1) and, respectively, 36.41mg% (H2) and β-ecdysone: 216.64mg% (H1) and, respectively, 143.16mg% (H2) as it was showed by HPLC. The single-dose toxicity test on rats proved that the extracts are non-toxic at the dose of 250mg/ kgw and are safe to use. The *in vivo* trial was conducted on 12 male rats divided into 4 groups with 3 animals in each group: I – untreated control (C); II – dexamethasone (D) 5mg/ kgw; III – dexamethasone 5mg/ kgw + levamisole (L) 7.5mg/ kgw; IV, V – dexamethasone 5mg/ kgw + hellebore extracts H1 and H2 12.5mg/ kgw, respectively. Dexamethasone (as immunosuppressing agent) was administered intraperitoneally twice a day for three days, while levamisole (as immunostimulant control) and hellebore extracts were given subcutaneously in two doses in an interval of 24 hours. The blood samples for analysis were taken at 24h after last administration.

Results: The model used dexamethasone as agent for inducing immunodefficiency and susceptibility to infections and it is correlated to the low values of white blood cells (group II). The consequence of subcutaneous application of hellebore extracts in immunodefficient rats was an increased count of white blood cells. The effect was obvious in the case of H2 where the white blood cells population almost doubled compared to group treated only with dexamethasone. The percent of granulocytes was statistically significant ($p<0.05$) higher in groups that received hellebore extracts (52,4%±3,56 for H1 and 63,3%±18,6 for H2) comparing to the control group of imunosupressed rats (41,8%±1,26). The mechanism of action is different from levamisole which acts slowly towards increasing the number of lymphocytes instead of granulocytes. As regards the correlation between chemical composition of the two extracts obtained by two different methods and pharmacological effect, further analysis are needed, but it can be hypothesized that this immunostimulatory effect is due to the cardiac glycosides.

Conclusion: This study contributes to the knowledge of *Helleborus purpurascens* Waldst. & Kit., adds a scientific proof of the known traditional uses and opens in the same time the way for further analytical and pharmacological investigations.

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Induction of Nrf2 Activity by Ethyl Acetate Extract of *Lagenaria breviflora*

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The field of cancer chemoprevention is based upon the ability of chemical compounds from any source (synthetic and natural) to prevent cancer. Phytochemicals with the ability to increase the activity of the transcription factor Nrf2 (NF-E2-related factor 2) are central to this field. Nrf2 has been reported to modify the expression of up to 200 genes, many of which are involved in cellular defence against toxic insult including heme-oxygenase 1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione S-transferases (GSTs). Interestingly many plant species which are known possess direct antioxidant activity through mechanisms such as free-radical scavenging have not been investigated for their ability to modify the expression of such crucial cellular defence genes. This study was consequently designed to investigate the Nrf2 induction capability of the medicinal plant *Lagenaria breviflora* Roberty (LB), which is documented to possess both *in vivo* and *in vitro* antioxidant and anti-inflammatory activities.

Nrf2 induction was investigated using the AREc32 cell line, (Nrf2 luciferase assay reporter cell line) and by activation of the Nrf2 regulated gene NQO1 in HepG2 cells by Western blot. Exposure of AREc32 cells to graded concentrations of LB ethyl acetate extract (4 mg/ml, 6 mg/ml, 8 mg/ml and 12 mg/ml) significantly increased luciferase activity by 3 - 7.5 fold relative to control. Higher concentrations (8 mg/ml and 12 mg/ml) were however toxic to the cells (by MTT assay). Kinetic studies revealed significant induction of luciferase activity in AREc32 cells treated with 6 mg/ml of the extract for 24 h (4 fold) and 72 h (3 fold). LB extract (6 mg/ml) also significantly increased the expression of NQO1 protein in HepG2 cells, relative to control, following 24 h exposure. These results indicate that the ethyl acetate extract of LB contains Nrf2 modulating activity and as such indicates that LB is worthy of further investigation into its potential to act as a cancer chemopreventative agent.

EVALUATION OF THE ANTIDIABETIC ACTIVITY OF *Gnetum africanum* AND *Gnetum buccholzzianum* LEAVES EXTRACT

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Background / Objective: According to WHO statistics, there are currently more than 100 million diabetic patients in the world and the number could reach 438 million by 2030 [1]. Various types of drugs have been developed to facilitate physiological use of glucose or stimulation of insulin secretion. But these drugs are most often away from vulnerable social groups especially in rural areas. In Africa, traditional medicine offers alternative based on plant extracts [2]. Leaves of african species of *Gnetum* are therefore used in some countries of Congo Basin to control diabetes [3].This study aimed to evaluate antidiabetic activity of methanol, hexane and ethyl acetate extracts of *Gnetum africanum* and *Gnetum buchholzianum*. **Methodology**: Leaves of both *Gnetum* species were collected in Limbe botanical garden (Cameroon) and in CENDEP (Centre for Nursey Development and Eru Propagation) garden of the same city. They were dried, ground and subjected to successive extractions using methanol, hexane and ethyl acetate as solvents. After chemical screening, extracts (180 mg/Kg) were administered to normoglycemic Whistar albinos rats and to diabetes induced rats following subcutaneous injection of streptozotocin at a dose of 55 mg/kg body weight. **Results:** Chemical screening revealed presence of tannins, flavonoids, saponins, steroids and cardiac glycosides in the methanol extracts of both plants. Ethyl acetate extract of *G. buccholzianum* contained steroids and cardiac glycosides whereas the one of *Gnetum africanum* contains flavonoids and steroids. All extracts significantly reduced basic blood sugar within 2 hours ranging from 0.98 ± 0.02 to 0.46 ± 0.17 g/L ($P < 0.05$) with a reduction rate of 53.06% for methanol extracts of *G. buchholzianum*. Reduction rate were 18.75% when the reference drug glibenclamide was administered. In the case of *G. africanum*, a significant ($P < 0.05$) decrease of 74.8% in blood glucose was obtained with ethyl acetate extract. These results were confirmed by a significant ($P < 0.05$) decrease of blood glucose in diabetes induced rats after 7 days treatment with methanol extract of *G. buccholzianum* leaves or with ethyl acetate extract of *G. africanum* leaves.
Conclusion: Methanol extracts of *Gnetum buchholzianum* and ethyl acetate extract of *Gnetum africanum* may have insulin secretor-like activity. Prior to further analysis, leaves of these two plant species could be used to lower blood glucose.

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Wild *Thymbra capitata* from Western Rif (Morocco): Essential Oil Composition, Polyphenol Contents and Antioxidant Activities of its Methanolic Extract

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Essential oils (EO, 15 collective samples and 47 individual samples) of *Thymbra capitata* collected from Moroccan Western Rif were analyzed using gas chromatography (GC) in combination with retention indices (RI), gas chromatography-mass spectrometry (GC-SM) and 13C NMR spectroscopy. The methanolic extracts (1:15 g/w) were analyzed for the total phenolic and flavonoid contents by spectrometric methods. Their potential antioxidant activities were evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH·) and 2, 2`-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) systems. The free radical scavenging activity was expressed as Trolox equivalent antioxidant capacity (TEAC). The individual compounds content of methanolic extracts were determined by the analytical RP HPLC method.
Concerning the EOs, twenty components were identified. Carvacrol was by far the major component of all the samples (68.2%-85.9 %), while the thymol content was very low (0.1-0.3%). Other components were present at substantial amounts: γ-terpinene up to 8.9%, p-cymene up to 7.1%, linalool up to 4.4% and (E)-β-caryophyllene up to 4.1%. In contrast, the EO yield varied drastically from sample to sample (0.5-3.7%). No correlation could be established between EO yield and altitude, the soil pH, chemical composition and granularity.
The antioxidant activity data showed that all the CME had a strong antioxidant activity and were always accompanied by high total polyphenol and flavonoids contents, respectively 30.2 to 41.4 mg/g DW gallic acid equivalents and 6.1 to 9.7 mg/g DW quercetol equivalents. The antioxidant activities of CME measured by the DPPH method ranged between 94.97 and 138.93 mM Trolox/DW. The radical scavenging activity of CME in ABTS reaction varied from 156.72±0.21 to 183.35±0.20 mM Trolox/DW. The polyphenolic compounds (chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, rosmarinic acid, luteolin, apigenin, rutoside, kaempferol, quercetol) quantified in CME showed variation in their concentrations.
The present study established that cultivation under controlled conditions is suggested to improve the quantitative characteristics of carvacrol-rich Moroccan *T. capitata* and its alcoholic extracts are a valuable natural sources of polyphenols that have strong antioxidant activity.

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Application of bryophytes in ethnomedicine

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Bryophytes are the second largest division of plant kingdom; however they are usually neglected as a study object for biologically active substances. Due to their complicated identification and small size, biologically active substance extraction is difficult [1,2]. Active research of bryophytes has started only in last 30 years and currently only 10 % of all bryophytes species have been studied concerning their chemical composition and biological activity. At this point approximately 400 new and unique substances have been detected [2]. Many of the identified substances show potentially practically applicable biological activity such as anti-inflammatory activity. *Rhodobryum giganteum* moss in Asia is used as homeopathic drug for heart diseases [1] and it has shown anti-hypoxia activity when tested on mice [3]. The most studied class of bryophytes is *Hepaticea* due to their physiological and chemical differences when compared with *Musci* and *Anthorcerotae*. have oil bodies that are rich in hydrophobic substances such as terpenoids, steroids and fatty acids [2], nevertheless also in mosses these constituent groups can be found but in lower concentrations and diversity. This leaves moss chemical composition and biological activity even less known in comparison with liverworts.

In current study bryophytes characteristic for Northern Europe have been analysed for their chemical composition and biological activity. Bryophytes chemical composition have been analysed with spectrometric methods, GC/MS as well as UPLC. Extracts with different solvents have been prepared and their total polyphenol, flavonoid and carbohydrate content as well as antiradical activity have been detected. To have some idea about biological activity of bryophytes, antimicrobial and anticancer activity of extracts was measured.

Obtained results show that bryophytes extracts have antimicrobial activity as well as extracts have antiproliferative activity on cancer cell lines.

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GENETIC VARIATION IN MEDICAL AND AROMATIC PLANTS BASED ON MOLECULAR MARKER ANALYSIS

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Breeding programs aim is to create a genetic variability, allowing more efficient production of new genotypes. In this context, the study of medicinal and aromatic plant species focus on increasing production and getting essential oil with high concentration of main components. Molecular polymorphism in the amplified DNA sequences that identify sequences provides valuable information, particular to the association of the different character of interest which can be used in molecular assisted selection markers.

This paper aims to reveal genetic particularities of ***Hyssopus officinalis,*** based on RAPD-PCR method. The material studied is composed from six genotypes, the selection criterion being the color of the flowers blossom: white, pink and blue collected from three locations: *Botanical Garden* (Institute), Chisinau, Moldova (GB), *CCB Stejarul Piatra Neamt, Romania* (PN) and *SCDA* Secueni, Romania (S).

Electrophoresis analysis obtained under RAPD technique allows highlighting the most informative primers for hyssop, which are **UBC215, OPA9** and **OPB01.** These revealed amplification products of all genotypes that were supposed to analyzes. Also the repeatability and number of amplicons reveals the results for 89% of the tested primers and the average of recorded amplicons for each primer is 5-9 of genotypes.

Selection criterion analysis has revealed that the largest number of results – 96 % was obtained for the hyssop with white flowers - *Piatra Neamt, RO;* this is followed by hyssop form with pink flowers - *Botanical Gardens, RM* and hyssop with blue flowers - *Secuieni, RO* with 92% (22 primers).

In comparison with the forms of the **blue** flower of *H. officinalis* the genotype of the Secueni and GB has higher results - 19 and 18 primers and in comparison with the genotype of NP it is evidencing the 13 primers. Analysis of data on **white** flowers highlights the NP and S genotypes with the highest results - 20 and 21 primers and in case of **pink** flower forms the genotype results for 22 of GB tested primers is PN - 21 and S 20 primers. Analysis of the total number of fragments revealed by each primer, presents values in the range of 0-20. It is noticeable that the most of the amplified fragments were obtained with the primer OPJ01 (20 amplicons), followed by the A2 (14). However, the few bands - 3, were attested for UBC215 and OLIGO28 primers.

Regarding the results obtained for each separate primer from a total of 203 fragments, there are 52 specific bands and 145 polymorphic ones. All data for hyssop forms provides the possibility of detection of the major genes that determine quantitative or qualitative characters. Also it provides some minor genes closely linked to the groups of major genes that affect these traits. Further, the obtained joint amplicons are going to be interpreted as a genetic similarities and the rest of the differences on a genetic level.

Comparative studies of leaf morphology in different species belonging to the genus *Ranunculus* using electron microscopy tehniques

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In traditional medicine species belonging to the genus *Ranunculus* are commonly used for their antirheumatic, antifebrile and rubefaction effects. *Ranunculus ficaria* L. is widespread in Romania. The plant grows in woods, bushes, groves of plain pan and in mountain areas. *Ranunculus bulbosus* L. presents a distribution area which includes orchard grass, dried meadows, rocky coasts and plains and mountain areas. *Ranunculus sardous* Crantz is growing on wet plowing between the cultures of the plains and mountain meadows.
In this study we performed a morphological and ultrastructural characterization of the leaf in a comparative study of various species belonging to the genus *Ranunculus.* For this purpose we used scanning electron microscopy investigation using HV(high vacuum) mode and also ESEM mode (Environmental scanning electron microscopy). It was described both surface (upper and lower epidermis) and transverse fracture of foliar lamina. Adaptive structural changes were observed in the leaf lamina of these species correlated with their habitat and climate. Specific ultrastructural characterization was performed at each of the studied species *Ranunculus ficaria* L., *Ranunculus bulbosus* L.*, Ranunculus sardous* Crantz.

Contributions to the Phytochemical Study of the Polyphenolic Fractions Separated From *Thymus pulegioides* L. Natural Populations Harvested in Northern Romania

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Thymus L. is one of the largest genus of the *Lamiaceae* family, that based on its high polymorphism and its large variety of culture sorts, it still presents some important taxonomic challenges. Some publications [1,2] estimated the total number of species for this genus to be about 150 out of which 75 species belong to the European flora. *Thymus pulegioides* L. represents one of the plant species that are part of *Serpylli herba*, a drug used in therapeutics as a stomachic, carminative, expectorant and diuretic and also in nutrition as a flavouring agent [3]. In our study we evaluated the quantitative determination of phenolic acids and flavonoids from 26 samples of *T. pulegioides* collected from spontaneous flora in North-East Romania. We also aimed for the identification of some components by means of high-performance liquid chromatography. In order to determine the nature of some of the polyphenols present in the plant material we applied HPLC analysis, by which we could determine, based on available standards, the presence of rosmarinic acid (quantitatively prevalent), chlorogenic and caffeic acids, and also the flavonoids, apigenin, apigenin-7-O-glucoside and luteolin. Our results indicate that rosmarinic acid is the acid component most present in the plant product (from 4 to 20 mg/g dried herba), while apigenin-7-O-glucoside is the major flavonoid (max. 0.42 mg/g dried herba). Our determinations could not reveal the presence in the plant material of rutoside or luteolin-7-O-glucoside. Our data confirm our previous observation that the biosynthesis level of polyphenolic derivatives varies within the *Thymus pulegioides* species (probably depending on pedoclimatic conditions of the place of origin), yet in the frame of the same population, it proceeds to the level of each individual component.

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Acknowledgments
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The Oleaceae extracts – comparative study between the gemmotherapic and phytotherapic extracts

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The Oleaceae plants belong to a family containing 24 genera and about 600 different species, being mainly shrubs and trees. These species are widely spread on the Earth from tropics to subtropics and temperate climate. The most known genera of this family are *Chionanthus, Forsythia, Fraxinus, Jasminum, Ligustrum, Olea* and *Syringa*. In gemmotherapy there are used a few species from the *Oleaceae* family: the common ash - *Fraxinus excelsior* L. (buds and seeds), the primwort - *Ligustrum vulgare* L. (young shoots), the olive - *Olea europaea* L. (young shoots) and the lilac – *Syringa vulgaris* L. (buds). These extracts have similar but also significantly different recommendations that should be correlated with the compositional differences. In phytotherapy the olive and common ash leaves are more used, having similar indications with the corresponding gemmotherapic extracts. All these considerations have opened our interest for performing a comparative study of the active compounds of Oleaceae gemmotherapic and phytotherapic extracts [1-5]. The study shows a comparative evaluation of the mentioned extracts using different chromatographic (TLC, HPLC) and spectral (UV-Vis) methods. There was investigated the presence of different active compounds such as: polyphenols (caffeic acid derivatives, coumarines, flavonoids, tannins), triterpenes, etc. It was also determined the antioxidant activity of these extracts using the DPPH and FRAP methods. There were identified the following compounds: in the common ash buds (9 compounds) and leaves (11 compounds) - coumarines and caffeic acid derivatives, oleanolic acid in all 3 studied extracts (buds, seeds and leaves); in the olive young shoots and leaves - quercetine derivatives, gallic acid and oleanolic acid; in the primwort young shoots extract – polyphenols - quercetine and caffeic acid derivatives; in the lilac buds - oleanolic and ursolic acids. The HPLC analysis identified in all gemmotherapic extracts a compound at 3 min and another one at 15,3 min having similar UV-Vis spectra. These compounds could demonstrate the same origin of these species, belonging to the same plant family. The total polyphenol and total flavonoid content vary from 0,68 mg/ml to 6,65 mg/ml and from 0,12 mg/ml to 1,79 mg/ml, respectively. In case of gemmotherapic extracts these values vary from 0,68 mg/ml to 1,96 mg/ml and from 0,12 mg/ml to 0,49 mg/ml, respectively. The phytotherapic extracts are generally more concentrated in polyphenols. The lilac and common ash buds extracts have the highest content in polyphenols, and the primwort young shoots and the common ash buds extract are the most concentrated in flavonoids. The antioxidant studies indicate that from the gemmotherapic extracts those obtained from the common ash buds are the most potent, followed by the olive young shoots, lilac buds, primwort young shoots and common ash seeds extracts. These results can not be correlated with the polyphenol and flavonoid contents of these extracts, because the maximum polyphenols concentration is in the lilac buds extract. Another unexpected result is the lower antioxidant effect of the common ash leaves extract in comparison with those obtained for common ash buds extract. This result is unexpected if considering the extraction ratio of leaves extract 1:5 in comparison with that of the buds extract of 1:20. The results indicate that the Oleaceae gemmotherapic extracts contain polyphenols, probably responsible for their diuretic effect. In those gemmotherapic extracts there were identified mostly the triterpenic acids – oleanolic and ursolic acids. The extracts have mainly recommendations on cardio-vascular system, as in the case of lilac buds extract and olive young shoots extract. On the cardio-vascular system some compounds having similar structure (ex. cardiac glycosides) have evidence-based effect, being possible that these compounds to be responsible for the effect of these extracts on the heart and vessels. The antioxidant studies show that not only the polyphenols can be responsible for this effect. A higher concentration of polyphenols does not mean obligatory a better antioxidant effect. These results show again that a phytocomplex effect is more powerfull in comparison with its components effects and not only the main compounds have importance for the effect of an extract. The better antioxidant effect of the gemmotherapic extracts indicates also their increased therapeutic value in comparison with that of the phytotherapic extracts.

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Screening of Suspected to be Adulterated Herbal Food Supplements used for Improving Sexual Performance

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Herbal food supplements are special borderline products, whose place is between traditional herbal medicines and conventional food, due to their composition, presentation and use. In the last ten years, this category has constantly increased as number of products, ingredients and their combination. The worldwide market of consumption has also increased, because consumers' trust in natural products, believed to be safer and free of side effects [1]. Phosphodiesterase type 5 (PDE-5) inhibitors such as sildenafil (Viagra®), tadalafil (Cialis®), vardenafil (Levitra®) and most recently avanafil (Stendra™) are used in the treatment of erectile dysfunction. But certain herbal food supplements which claim to enhance the sexual performance have been found to be adulterated with these synthetic drugs, their related analogues or other pharmaceutical substances [2].

This study aims to develop a useful method of gas chromatography-mass spectrometry (GC-MS) for the screening of herbal food supplements supposed to be adulterated. The identification of pharmacological active principles was done by detection of specific molecular ions for each compound, comparing them to the Nist library of the device.

GC-MS system consists of GC 6890N, MS 5973N and fused silica capillary column, TR-5MS (30 m x 0.25 mm i.d. 0.25 µm film thickness). A 1 µL injection using splitless mode was performed on 250^{o}C injector port with helium flow at 1.0 ml/min. The oven ramping temperature was programmed at 135-200^{o}C (1 min hold) at a rate of 13 ^{o}C/min, and 200-315^{o}C at a rate of 6 ^{o}C/min, at 315^{o}C holds for 20min. Identification was done on full scan mode (50-500 a.m.u). All the analyzed products provided by the National Office of Medicinal, Aromatic Plants and Bee Products were solid powders compacted in tablets (11 products) or encapsulated (15 products). Each capsule was emptied and each tablet was crushed. Every 100 mg of fine powder was mixed with 1 ml of absolute methanol. Samples were mixed by vortexing, followed by 15 minutes of sonication and 5 minutes centrifugation at 4000 rpm. The supernatant was collected and filtered by 0, 2 µm membrane filters for GC-MS analysis. By GC-MS, sildenafil, tadalafil and vardenafil were successfully identified in different herbal food supplements, as well as others hidden compounds, such as phenolphthalein. The results of this study showed that 5 of the 26 analyzed herbal food supplements from the Romanian market (19.23%, respectively) were found to be adulterated with commercial PDE-5 inhibitors, their analogues (homosildenafil, thioaildenafil, thiosildenafil) or other forbidden substances. Herbal food supplements advertised as "all natural products" could be dangerous when pharmaceutical substances, undeclared on the label, are hidden in their composition, because they may interfere with the consumers' diet or medical treatment and could result in adverse reaction or side effects.

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Phytochemical profile of *Araiostegia yunnanensis* (Christ) Cop with antioxidant, anticancer and acetylcholinesterase inhibition potential

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Ferns are well-known traditional Chinese medicinal herbs and Yunnan province of China has about 1500 species ferns. The total flavonoid contents in 50% ethanol extracts of ferns were ranged from 0.055% to 30.642% [1-2]. The total flavonoid contents in 15 species of ferns were beyond 5.0% and the total flavonoid contents in 10 species were less than 1.0%. The antioxidant activities of these ferns extracts showed a significant reciprocal proportion to the total flavonoids contents [3-5]. *Araiostegia yunnanensis* (Christ) Cop is a Chinese medicine used mainly by minorities in Yunnan province of China. There are several traditional medical effects of *A. Yunnanensis* including heat clearing, detoxifying, healing sore and relieving pain. Moreover, *A. Yunnanensis* exhibited strong antioxidant and anticancer activities [4]. However, the major bioactive compounds in ferns are not clear and there are few experimental data about the bioactivities of *A. yunnanensis*. The phytochemicals and bioactivities of *Araiostegia yunnanensis* (Christ) Cop were investigated. The total flavonoids content in *A. yunnanensis* is about 84.90 mg/g. By means of HPLC-DAD-ESI-MS, the main flavonoids in *A. yunnanensis* were tentatively identified as myricetin 3-O-rhamnosylglucoside, eriodictyol-7-O-rutinoside, quercetin-3-O-rutinoside, luteolin-7-O-apiosylglucoside, quercetin 3-O-rhamnosylgalactoside, and luteolin 7-O-glucoside. The extract (0.268 mg/ml total flavonoids) from *A. yunnanensis* showed very strong superoxide anion radical scavenging potential and reducing power, which are higher than that of rutin (0.25 mg/ml). The extract (0.268 mg/ml total flavonoids) from *A. yunnanensis* exhibited similar DPPH• scavenging activities with that of rutin (0.25 mg/ml). However, rutin (0.25 mg/ml) appeared a significantly higher ABTS radical scavenging effect than that of the extract (0.268 mg/ml total flavonoids) from *A. yunnanensis*. The methanol extract from *A. yunnanensis* showed obviously cytotoxic effects on A549 cells and it had no effect on acetylcholinesterase.

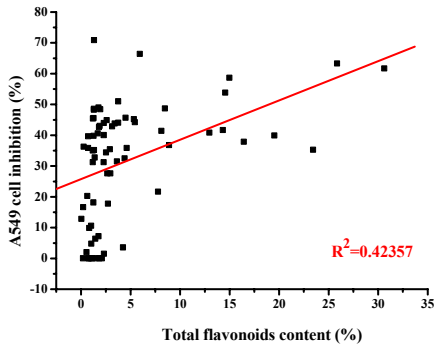

Figure 1. Relationship betwwen the total flavonoids content and the cytotoxic effects on A549 cells

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Nelumal A from *Ligularia nelumbifolia* is an aromatase inhibitor

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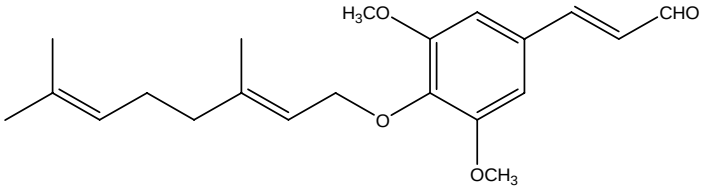
Aromatase is a member of the cytochrome P450 family (EC 1.14.14.1) and is the enzyme that catalyzes the main step along the biosynthetic pathway of estrogen hormones. In particular it promotes the aromatization process of ring A of the steroidal core converting androstenedione to estrone and testosterone to estradiol. Aromatase is found in several organs and tissues like gonads, brain, adipose tissue, placenta, blood vessels, skin, and is involved in many physiological and pathological processes [1]. Although therapeutic means (e.g aromatase inhibitors) are nowadays available for the management of syndromes associated to aromatase dysfunctions, the search for novel agents able to target this key enzyme with minimized side effects is a field of current and growing interest. In this work we synthesized and investigated the effects of nelumal A [(2E)-3-(4-((E)3,7-dimethylocta-2,6-dienyloxy)-3,5-dimethoxyphenyl)acrylaldehyde] **1**, the active principle of the Chinese medicinal plant *Ligularia nelumbifolia* Hand. Mazz. (Asteraceae) [2] on aromatase using HEK and KGN cells, that was previously reported to exert *in vitro* anti-cancer effects in KB cells [3].



1

The title natural product was preliminarly tested in HEK293 cells transfected with aromatase cDNA and using anastrazole as the reference drug. This assay revealed that nelumal A showed an appreciable level of inhibition. Subsequent experiments carried out with the scope of evaluating the aromatase activity and expression in KGN cells confirmed that this oxyprenylated cinnamaldehyde derivative, after an incubation of 48 h, compared favourably to anastrazole (1 μM) in the concentration range 10 – 30 μM. Moreover nelumal A (30 μM) abolished the aromatase mRNA expression in the same cell line. The findings described herein, together with already described effects, may contribute to shed light on the anti-cancer properties of nelumal A. In this context the results of the present study could be considered as a topic for future studies aimed at better defining the pharmacological profile of this and other oxyprenylated secondary metabolites, and to develop a novel series of lead natural or semi-synthetic compounds. Italian Authors wish to thank Biosline S.p.a., Ponte San Nicolò, Padua, Italy, for the financial support to the chemical synthesis of nelumal A.

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Synthesis and biological activity of oxyprenylated diketopiperazines of fungal origin

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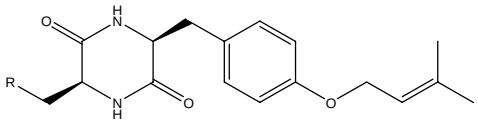
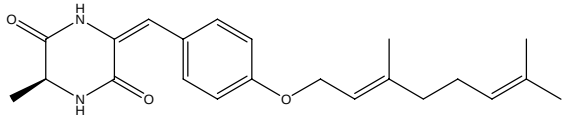
Diketopiperazines represent a class of natural products deriving from the linkage of two aminoacids to yield a lactam. Such secondary metabolites are biosynthesized by several organisms, comprising mammals, by the activity of a specific group of enzymes, namely the dipeptidyl peptidases, that are able to hydrolise the bond between the last two aminoacids and the remaining peptide providing a dipeptide that in turn spontaneously cyclise to give the diketopiperazine ring. During last years the title products have shown great potentialities as anti-cancer, anti-viral, anti-fungal, anti-bacterial, and neuroprotective agents [1]. In this context recent investigations led to the isolation and structural characterization of oxyprenylated diketopiperazines having dimethylallyl- or geranyl *O*-side chains. As a continuation of our ongoing studies aimed to get further insights into the phytochemical and pharmacological properties of oxyprenylated secondary metabolites, in this communication we wish to report the synthesis and preliminary data about the cancer cells growth inhibitory activity of three prenyloxydiketopiperazines of fungal origin, namely phomamide **1**, isolated from the Ascomycetes Leptosphera maculans, a pathogen of Brassica spp., and Leptosphera biglobosa, cyclo-(glicyl-L-tyrosyl)-3-methylbutenyl ether **2**, extracted from Gliocladium virens, parasite of other fungi, and finally deoxymycelianamide **3**, isolated from different species of the genus Gliocladium.



1 R = OH
2 R = H



3

Diketopiperazines **1** and **2** have been synthesized in 46% and 21% overall yields respectively starting from commercially available Boc protected tyrosine. Deoxymycelianamide **3** have been obtained from the coupling of suitably protected alanine and glycine followed by reaction of the resulting diketopiperazine with 4-geranyloxybenzaldehyde in 61% overall yield. All the three phytochemicals have been tested as anti-tumor agents evaluating their growth inihibitory capacities against a panel of six human cancer cell lines with different sensitivity to pro-apoptotic stimuli by the MTT colorimetric assay. Compound **3** was seen to be active against human lung and breast cancers, human oligodendroglioma, and murine melanoma with IC_{50} values ranging from 2 to 7 μM.

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A validated HPLC-UV/Vis methodology for the quantification of oxyprenylated cinnamic acids in *Citrus* spp.

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4'-Geranyloxyferulic (herein indicated with the acronym GOFA) and boropinic acids have been shown during the last decade as interesting phytochemicals having valuable pharmacological effects as cancer chemopreventive, anti-inflammatory, neuroprotective, and anti-*Helicobacter pylori* agents. A RP HPLC-UV/Vis method for the separation and quantification of the title oxyprenylated secondary metabolites in extracts obtained from the skin of selected edible *Citrus* fruits, namely lemon (*C. limon* (L.) Burm. f.), orange (*C. sinensis* (L.) Osbeck), kumquat (*Fortunella japonica* Swingle), mandarin orange (*C. reticulata* Blanco), lemon citron (*C. limoni medica* Lush.), lime (*C. aurantiifolia* (Christm.) Swingle), white and red grapefruits (*C. paradisi*), and finally citron (*C. medica* L.)was successfully applied. The set-up methodology was shown to be useful to selectively quantify both title natural products. Triturated skins (5 g) were initially suspended in MeOH (20 mL) and preliminarily ultrasonicated for 40 min. An overnight maceration at room temperature in the dark under magnetic stirring in the same solvent (1 g / 3 mL ratio) followed. Extracts were then filtered on 0.45 μm polyamide filters. The filtrate (5 mL) was then extracted three times with *n*-hexane, this latter recovered and evaporated to dryness under vacuum. The dry powder so obtained was finally stored at – 20 °C in the dark before analyses. These were performed using a Waters liquid chromatograph equipped with a model 600 solvent pump and a 2996 photodiode array detector. Empower v.2 Software (Waters Spa, Milford, MA, USA) was used for data acquisition. A C18 reversed-phase packing column (GraceSmart RP18, 4.6 mm × 250 mm, 5 μm; Grace, Deerfield, IL, USA) was employed for the separation. The column was thermostated at 10 ± 1 °C using a cool pocket chiller (Thermo Scientific, Waltham, USA). The UV/Vis acquisition wavelength was set in the range of 200 – 600 nm. 316 nm was used as the wavelength for the quantitative analyses and 288 nm for the qualitative one The injection volume was 20 μL. The mobile phase was directly on-line degassed using a Degassex, mod. DG-4400 apparatus (Phenomenex, Torrance, CA, USA). Mobile phase composition consisted in double distilled water (solvent A) and methanol (solvent B) at a flow rate of 1.2 mL/min. following a gradient elution program (A/B ratio 60:40 from 0.01 to 3 min, 10:90 from 3.01 to 8.60, then back to 60:40 from 8.61 to 15 min). Results about the concentration of GOFA and boropinic acid in citrus skin extracts are reported in the Table.

***Citrus* spp.**	**GOFA (mg/g ± SD)***	**Boropinic acid (mg/g ± SD)***
Lemon (*C. limon*)	0.015 ± 0.002	0.063 ± 0.009
Orange (*C. sinensis*)	0.141 ± 0.011	0.028 ± 0.001
Kumquat (*F. japonica*)	N.D.	0.206 ± 0.002
Mandarin orange (*C. reticolata*)	0.069 ± 0.003	0.090 ± 0.007
Lemon citron (*C. limonimedica*)	0.080 ± 0.005	N.D.
Lime (*C. aurantiifolia*)	N.D.	0.133 ± 0.003
White grapefruit (*C. paradisi*)	0.038 ± 0.002	0.058 ± 0.002
Red grapefruit (*C. paradisi*)	0.075 ± 0.002	0.034 ± 0.003
Citron (*C. medica*)	0.010 ± 0.001	0.020 ± 0.001

*Values expressed as mg/g of exocarp fresh weight. Data are reported as mean ± standard deviation (n=10); N.D. = Not detected

Extraction of anthraquinone compounds of *Rhamnus frangula* L. (Fam. *Rhamnaceae*)- preliminary study for their utilization as anti-patogenic agents

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Frangula alnus Mill. (*Rhamnus frangula* L., *Rhamnaceae* family) (alder buckthorn) is used traditionally as a medicinal plant and accepted by the EMEA (European Medicines Agency) and HMPC (Committee on Herbal Medicinal Products) for internal use [5]. The most studied compounds are the derivatives of 1,8 dihydroxyanthraquinones (1,8 DAD/form of aglycones or glycosides), their therapeutic use being for a long period only laxative and purgative [2,5]. For about 25 years, numerous scientific studies have also indicated the antimicrobial, antifungal and insecticidal activities after exhaustive pharmacological investigations, with applications in agriculture [1,3,5].

The study presents different extraction processes of the main constituents in alder buckthorn bark (*Frangula cortex*), in order to obtain an extract enriched in anthraquinones with potential use in crop phytopathology. It was performed the extraction at room temperature and by reflux using selective solvents for the extraction of trace active principles: alkalized distilled water , 30%, 50%, 70% and 95% v/v ethanol (E_{30}, E_{50}, E_{70}, E_{95}). By specific extraction procedures in established conditions (temperature, time, specific extraction solvents, etc..) there were obtained extracts enriched in anthraquinone compounds with high content of emodin (1,8-dihydroxyanthracene) which where analyzed by different chemical and instrumental methods: qualitative (phytochemical screening and spectroanalytical profile by HPTLC and UV/VIS absorption spectrophotometry) and quantitative (densitometric determination of emodin and dosage of hidroxymethyl-antraquinones). In extractive solutions E_{50} and E_{70} there were identified and quantified important quantities of emodin (5.3 to 6.8 mg/100 ml extract solution) and total hydroxymethylanthraquinones expressed as 1,8-dihydroxyanthraquinone (0.09 to 1.33 g/100 g extract solution), compounds that are mentioned in the literature as having anti-pathogenic activity [1,4]. These analyses revealed the highest content of emodin in 70% v/v ethanolic extract (6.7 mg/100 ml);

This is a preliminary study of anthraquinones in *Rhamnus frangula,* active principles with anti-pathogenic activity. Next step is to demonstrate this activity by biological screening.

Keywords: **Rhamnus frangula*, extraction, phyto-chemical screening, anthraquinones***

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Comparativ profile of antioxidant activity of two *Nigellae* seeds species

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Nigella sativa L. (*Ranunculaceae*), commonly named black cumin, and *Nigella damascena* L. (*Ranunculaceae*), commonly named love-in-the-mist are two spontaneous plants in the North Afrika, Maghrebian Region. This plants grows also in Romania. *Nigellae sativae semen* has been used for a long time in the Arabian ethnopharmacological field for its tonic effect. The scientific basis for using this plant was confirmed by phytochemical studies. These studies represent the scientifical confirmation of the empirical use of this vegetal product and give the opportunies to use also *Nigellae damascenae semen* with the same indications due to the similarities in chemical profile. The lipophylic fraction from *Nigellae sativae semen* but also *Nigellae damascenae semen* contains, besides essential oils, fatty oils. The hydrophylic fraction from both species seeds contains polyphenolic acids, flavonoids, etc. The qualitative and quantitative analysis of fatty acids from mentioned oil were performed using GLC (Gas- Liquid Chromatography). The obtained fatty oil was found highly unsaturated, 84.35% from the total fatty acids representing the mono- and polyunsaturated fatty acids.
The main fatty acid of the *Nigellae sativae seeds* was found the linoleic acid (C18:2) representing about 63.71% of the total fatty acids.
That study supposes a discussion about chemical profile versus antioxidant activity of the two *Nigellae species*.

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Terpenoid biogenesis in *Artemisia alba* is related to the structural organization of thylakoid membranes *in vitro*

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Introduction: Terpenoids comprise over 30‘000 different molecular structures isolated so far (1).Terpenoids such as plant hormones, photosynthetic pigments, signal transduction components, defensive molecules, and structural components of the plant cell wall play a major role for the survival of the plant organism (2). The pharmacological activity of many terpenoids imposes the challenge of better understanding the fundamental aspects affecting their biogenesis in the plant organism.The essential oil profile of the fragrant shrub *Artemisia alba* Turra has shown high variability in its terpenoid profile, attributed by different authors to environmental, climatic, as well as to genetic factors (3). Our previous research has shown that the terpenoid profile of the essential oils is strongly affected by the morphogenetic changes brought by auxin and cytokinin supplementation to the plant *in vitro* (4). As a part of the PhytoBalk project, financed by the Swiss National Science Foundation a complex study is ongoing for the better understanding of the fundamental aspects of terpenoid biogenesis in this plant.

Aim of the work: This work aims at the study of the structural and functional changes occurring in photosystem II of *A. alba in vitro* cultured plants, as well as in the area and height of thylakoid membranes in relation to the terpenoid profile of essential oils of the plant in the different model systems obtained by plant growth regulators treatment.

Methods: *In vitro* culturing techniques; micro-steam distillation; GC-MS identification and quantification of terpenoids in the essential oil; 77 fluorescence spectroscopy that gives information about the light energy utilization by the photosystems; circular dichroism spectroscopy that probes the macroorganization of the pigment-protein complexes and atomic force microscopy which probes the topography and morphology of the thylakoid membrane.

Results: Our data indicate that PGR treatment resulted in major structural changes in the thylakoid membranes ─ possibly altered macroorganization of the photosynthetic complexes and/or smaller photosystem II supercomplexes. Predominance of sesquiterpenoids in the essential oils was related to most prominent manifestation of this effect and was apparently related to a high diversity in the morphological parameters area and height of the thylakoid membranes, implying possible disturbance of chloroplast structure in this plant *in vitro* culture.

Conclusion: As it is known, the activity of the mevalonate-independent pathway of terpenoid biogenesis is involved in the biosynthesis of plastidic terpenoids, including monoterpenes, diterpenes and carotenoids. The predominance of sesquiterpenoids in the essential oils of *in vitro* cultured plants with impairment of chloroplast structure might possibly be explained with affecting the functionality of this pathway in *A. alba in vitro* model. The understanding and utilization of this effect might further be used as a practical tool for the delivery of essential oils with pre-determined chemical composition in this plant species.

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Partial results regarding the transfer into culture of the *Eupatorium cannabinum, Helleborus purpurascens* si *Inula helenium*, species in the pedoclimatic conditions of the Dacia Plant SRL company production unit

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Ecological agriculture atempts the transfer of medicinal plant species into culture trying to approach the cultivation conditions to those characteristic to the wild flora and providing friendly technological solutions to protect the environment and to obtain original homogene raw material without chemical residues.

Our experiment is achieved on the basis of a thorough and comprising botanical study for the systematic framing of the species, the study of the biological peculiarities, of the climate and soil [1] characteristics and a comprising investigation to establish all the technological links to favour the cultivation of a species (establishing the method and the optimum moment to initiate the culture, deciding on the optimum nutrition space and the seed norm as well as the plant density on m^2, establishing the optimum harvesting time in order to obtain high raw material yields and an increased active principle ratio [2].

Objectives of the research:

Identying the natural areal of the species; harvesting the initail multiplication material;

Multiplication of the material in the collection of genetic resources of our laboratory and the study of the species biological peculiarities;

Achieving the mirocultures in the experimental fields to elaborate the cultivation technology.

Conclusions after the first experimental year:

The multiplication methods of the three species are based on a common characteristic regarding the presence of the dormant buds at the level of the neck;

After two years from the expeeriment initiation, the development of the *H. purpurascens* roots registered significant levels while in case of the other species there was a development of 7-12 flotiferous stems/ plant (*E. cannabinum*) and 3-4 stems/ plant (*I. helenium*);

We did not register incompatibility symptoms wit the basic species (*Ribes nigrum*).

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Rapid identification of antioxidant compounds of *Genista saharae* Coss. & Dur. by combination of bioautography and HPTLC-MS

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Genista species are sources of antioxidant phenolic compounds such as *O*- and *C*-glycosylflavonoids and isoflavonoids. Combination of bioautography with HPTLC-MS, a fast and efficient method for identification of bioactive compounds, has been applied for evaluation of radical scavenging activity of metabolites from *Genista saharae* Coss. & Dur. Different organs, collected at various periods have been compared. Identification of antioxidant compounds was obtained by elution of the major DPPH-inhibition zones. The resulting HPTLC-MS analysis in moderately polar conditions, coupled to DPPH revelation led to the putative identification of two antioxidant isoflavone aglycones: 3', 4', 5, 7-tetrahydroxyisoflavone (**1**) and ficuisoflavone (**3**), whereas polar migration conditions conduced to identification of glycosides 5-methoxy-4',7-trihydroxy-8-glucopyranosyl (**4**) and 4',5-dihydroxy-7-methoxyisoflavone-4'-O-β-D-glucopyranoside (**5**). Evaluation of percentage of inhibition of DDDH radical by the purified isoflavone (**4**) from the root extract showed that it affords a moderate contribution to the total radical scavenging activity of the extract.

Research study on testicular cell cultures treated with hormonal substitute based on herbal extracts

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The aim of this study was to investigate the potential use of herbal extracts as hormonal substitute in testicular cell cultures.
For this study, piglet testicles at birth and adulthood were collected, the albuginea was removed and testicular parenchyma was sectioned and allowed to incubate. Cultures were monitored under a microscope to track cell morphology until a monolayer culture was formed.
The cultured cells were pretreated with a hormonal substitute based on herbal extracts called MANYPHIT. A culture medium for Sertoli cells obtained from piglets at birth and adulthood was prepared in the laboratory. Cells were then cultured and increasing doses of herbal hormonal substitute in different dilutions were applied. Primary cultures derived from adult normal cells can be subcultured for 4-6 passages; after that, cells dye (apoptosis). Cytopathic effects, cell detachment from the surface and additional changes were strictly monitored. After 48 hours, cell cultures were stained and examined under microscope.
It was observed that Sertoli cells derived from piglets testicles at birth are less sensitive to Maniphyt. The use of trypsin for testicle cell isolation does not affect Sertoli cell receptors. Fibroblasts are free to form homogeneous layers scattered over the entire substrate. Cylindrical, triangular, star-shaped to polyhedral shapes can be observed.
Endothelial cells are grouped in colonies consisting of small areas of polygonal cells. Their shape can vary from round to polygonal with flat borders and protuberant centers. Sertoli cells are scattered and juxtaposed in a single layer; cell borders are not always defined. Residual germ cells may remain attached to the free surface during the initial culture.
In conclusion, herbal extracts can be used as hormonal substitutes for *in vitro* tests on testicular cell cultures.

Preliminary research on the immunomodulatory effect of an herbal extract administered to cows with chronic diseases (case study)

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Chronic diseases start with a disease affecting one organ; because all organs are interconnected, the original disease will change into a variety of problems sprouting from the various affected organs. Modulation of immune system improves the general status and body's defences, providing the necessary support to counteract any disorder.
In our case study we used an herbal extract (under test) with immunomodulating potential administered subcutaneously in two cows with chronic diseases. The administration of herbal extract resulted in a substantial neutrophilic leukocytosis: we can say that we are dealing with a net-reactive leukocytosis that was easily correlated with the clinical diagnosis of cows studied (arthritis and foot pad dermatitis, mastitis), accompanied by significant leukocytosis and significant changes in the profile of leukocyte formula.
Comparing to initial phase, the number of white blood cell count doubled after 14 days of administration of the extract. Leukocyte lines involved suffered significant deviations from normal, generally similar to Schilling's descriptions: neutrophil combat phase, monocytic combat phase and lymphocytic healing phase. Left deviation of Arneth nuclear index occurred is a regenerative reaction. At 24 hours after the administration of the extract lymphocytes-neutrophils ratio is inverted, together with the values of phagocytic index measured by embedding carbon particles test which suggest a non-specific stimulation of the phagocytic functions of neutrophil population. The ratio of T and B lymphocyte populations shows that during the entire experiment it was maintained at normal levels described in cattle. Lymphoblastic transformation test using phytohemagglutinin as mitogen demonstrates that non-specific reaction was mainly expressed by the population of T lymphocytes suggesting an activation of the cellular mechanisms of the immune response.
Finally, the ratio of T helper and T-suppressor subpopulations of lymphocytes reached to 3.8 (baseline 2.1) which indicates the activation of helper T-cell clones, the cells responsible for coordinating the immune response and it is a proof of the activation of cellular immune response based on neutrophil phagocytosis and functional activation of T-helper lymphocyte clone with all the immune mechanisms controlled by these cells.
In conclusion, herbal extracts are a viable therapeutic alternative for immunomodulation in cattle with chronic diseases.

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The therapeutic efficacy of *Gleditschia triacanthos* extracts on diarrheal syndrome of calves

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Treatment of calves with diarrhea syndrome is expensive and complex and it is not possible to use only one drug. Clinical diagnosis of diarrhea requires the pathogenetic evaluation in order to establish the main therapeutic targets: rehydration, stopping diarrhea, supporting the major biological functions, combating acidosis, hypovolemic shock and uremia, antibiotic protection and nonspecific stimulation.
It is clear that a single preparation cannot solve all these issues and there is a need for an association of products for covering the therapeutic effect sought. The aim of our study was to provide a natural, safe and efficient solution to be used as adjuvant to the conventional therapeutic scheme.
In our study, for stopping diarrhea, we used several extracts – tincture and decoction of *Gleditschia triacanthos* L. (Caesalpiniaceae). The experiment was conducted on three batches of four calves, each having mild, moderate and severe dehydration. Calves were treated following a therapeutic scheme established by farm specialists and as adjuvant therapy to stop diarrhea we have used the extracts in comparison to other similar preparations on Romanian pharmaceutical market. Calves were observed clinically and the paraclinic evolution was assessed by blood tests. Only two calves in the group with moderate dehydration treated with herbal extracts had a difficult recovery. By using only conventional therapy established in farm, they surely could not have been saved.
We believe that the use of these extracts in the treatment of diarrhea in new-born of all domestic species is benefic, and it can be a cheap and effective alternative in the treatment of this expensive syndrome.

The use of Fenugreek seed mucilage as excipient in freeze dried orodispersible tablets

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Introduction. The use of orally disintegrating tablets has developed in the last years due to the ease of administration, patient compliance, fast onset of effect. Finding new methods and excipients is a constant challenge for the pharmaceutical industry. Natural materials are valuable resources, being cost effective, easily available and non toxic.
Objective. This study aimed the formulation and *in vitro* characterisation of lyophilised orodispersible tablets, based on colloidal dispersions of Trigonella foenum graecum seed mucilage.
Materials and methods. The isolation of mucilage was performed according to a literature reported technique, that involves water dissolution and acetone precipitation, followed by room temperature drying [1]. Furthermore, water dispersions of mucilage were prepared and their rheological characteristics were investigated using a Brookfield DV III Ultra viscosimeter. For the freeze drying process, one mucilage dispersion was chosen, as binder and 15%(w/v) mannitol was added, as cryoprotectant and diluent. 0,5 ml stock solution were poured in each of the 20 tablet moulds and were progressively frozen to -25°C, for 10 h, then freeze-dried for 60 h at a shelf temperature of -40°C, followed by secondary drying for 10 h at 5ºC. The tablet characterization consisted of uniformity of weight, strength, disintegration time, wetting time and water absorbtion ratio[2].
Results. The percentage yield of mucilage extraction from fenugreek seeds was 19%. A series of eight colloidal dispersions of the fenugreek mucilage were obtained, with concentrations that ranged between 0.1% and 2%. "Shear stress vs. shear rate" tests were applied; the data were fitted to the Herchel-Burkley model. The influence of mucilage content in viscosity was evaluated. The viscosity of the 8 dispersions increased with concentration of fenugreek seed mucilage and the viscosity data showed a good correlation with the Herchel-Burkley equation. Having a convenient viscosity, the 1% mucilage dispersion was chosen for the freeze-drying process. The tablets had very low disintegration times (15,8s±3,89), wetting times (1,3s±0,5) and high water absorption ratios (298,35%±44,76). The crushing strength was 19,23N±3,67.
Conclusion. The fenugreek seed mucilage can be successfully used as a binder in fast disintegrating oral dosage forms. Further research will concentrate on improving the crushing strength of tablets.

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Contributions to the Biomimetic Synthesis of Terpenoids

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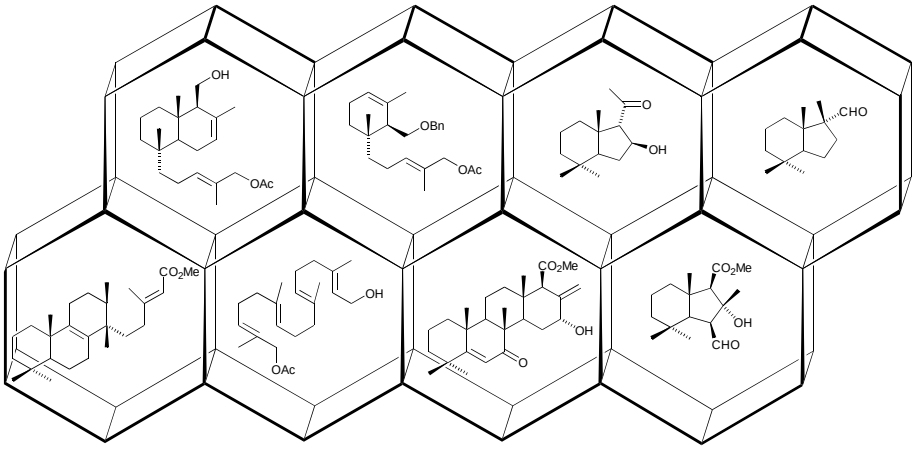
Terpenic compounds represent the most numerous family of natural secondary metabolites and are widely distributed in plants. Therefore, their isolation and practical application is connected to a continuous process of plant material screening and evaluation. On the other hand, a deep investigation of the properties of new metabolites is not always possible without the support of modern synthetic organic chemistry. Elaboration of new pharmaceuticals, crop protection agents, flavoring and cosmetic ingredients require multigram quantities of individual active substances that can be made available only synthetically.



Organic chemistry transformations have been frequently designed with the focus on similar biosynthetic pathways that occur in living cells. This biomimetic strategy proved to be always an efficient tool for assembling very complex organic molecules. The current communication outlines a set of biomimetic approaches, implemented successfully for the synthesis of polyfunctionalized terpenoids on the basis of available plant derived materials. These procedures are based on oligomerizations of available monoterpenes, controlled cyclizations by selective activation of internal double bonds of open chain terpenic substrates, molecular rearrangements of optically active polycyclic scaffolds, radical relay heterofunctionalizations and oxidative degradations followed by aldol cyclization. The resulting products are complex terpenes belonging to different families, including *seco*-eudesmanes [1], sacculatanes [2], scalaranes [3], cheilanthanes [4] and also an emerging class of perhydrindanes with promising properties [5].

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Methylglyoxal - a causative agent of detrimental glucose oxidase modification in manuka honey

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Honey is considered to be an important therapeutic agent possessing significant antibacterial properties. Hydrogen peroxide is one of the major antibacterial components, and is produced by glucose oxidase (GOX)-mediated conversion of glucose under aerobic conditions in diluted honey. However, it does not accumulate in medical-grade manuka honey, and the pronounced antibacterial activity directly originates from methylglyoxal (MGO, $C_3H_4O_2$). Manuka honey is produced in New Zealand by domesticated European honeybees from the nectar of the manuka tree (*Leptospermum scoparium*). MGO originates from dihydroxyacetone (present in the nectar) upon storage of freshly produced honey.

Recently, destructive effects of MGO on some proteins in manuka honey have been documented [1]. Based on this knowledge, we hypothesized that the inability to produce high levels of H_2O_2 could be associated with high reactivity of MGO with GOX enzyme. Accordingly, we investigated the effect of artificially added MGO on H_2O_2 accumulation in natural non-manuka honeys, which are capable to generate high levels of H_2O_2. Furthermore, we examined the effect of MGO on *in vitro* cross-linking of purified GOX and its biological activity.

To evaluate the production of H_2O_2 we employed fluorometric resorufin assay. Among several tested honeys, the most potent producers were the two honeydew honeys, whose 50% (w/v) solutions accumulated up to 495.8 ± 9.1 μM H_2O_2 in 24 h. Contrary to this, the MGO-treated honeys generated significantly lower amounts of H_2O_2. The untreated honeydew honeys contained up to 5-fold higher concentrations of H_2O_2 than honeys treated with 1000 mg/kg MGO. Furthermore, we observed formation of high molecular weight adducts on SDS-polyacrylamide gels when GOX was treated with MGO. This observation was confirmed by ultra high-performance liquid chromatography (UHPLC) analysis. In addition, formation of high molecular weight adducts was accompanied by significant loss of its enzymatic activity (up to 70% reduction).

In conclusion, our observation suggests that high levels of MGO in manuka honey are responsible for suppressing H_2O_2 generation. These data highlight the detrimental effect of MGO on proteinaceous components, including those contributing to the antibacterial activity of honey. However, comprehensive clinical trial study should be performed to assess the advantageousness of various selected honeys (manuka, other floral, honeydew) for specific types of wounds.

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Osteogenic Activity Evaluation of *Salvia officinalis* L. in a Human Bone Cell Line

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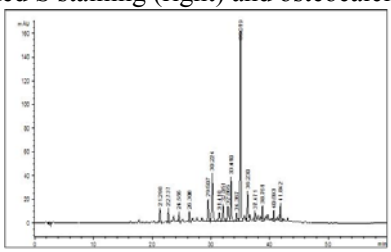
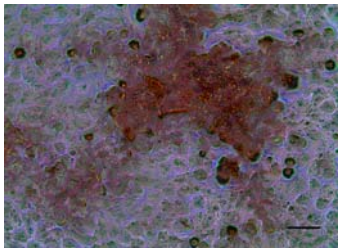
Plant derived compounds and extracts have been reported to affect the osteogenic differentiation pathway in diferent cell lines [1, 2]. *S. officinalis* is known to have a wide range of biological activities including anti-bacterial, anti-inflammatory, anti-fungal, antioxidative or hypoglycemic effects [3], but its osteogenic potential has not been studied.

In the present study, it was investigated the potential of *S. officinalis* leaves hydroalcoholic extract and rosmarinic acid to induce osteogenic differentiation of human osteosarcoma (U2OS) cell line.

S. officinalis hydroalcoholic extract was obtained by ultrasound-assisted extraction and characterized by HPLC analysis. Extract cytotoxicity was evaluated in U2OS cells by MTT test. Its osteogenic activity was analyzed by determination of alkaline phosphatase (ALP) and calcium content secreted in the culture medium after 5 and 7 days of cultivation. Calcium secretion was also observed using Alizarin Red S specific staining and osteocalcin secretion was immunocytochemically analyzed. HPLC analysis revealed high amounts of polyphenolic compounds like ferulic acid, quercetin-3-O-rutinoside or syringic acid. The major polyphenolic constituent was rosmarinic acid (left). The *in vitro* cytotoxicity test allowed selection of plant concentration (30µg/mL) with the highest biocompatibility for further experiments. U2OS cells grown in culture media supplemented with *S. officinalis* or rosmarinic acid secreted a higher amount of ALP and calcium compared to untreated cells. Also, the treated cells were positive for Alizarin Red S staining (right) and osteocalcin at the end of the treatment.





In conclusion, *S. officinalis* and its main component, rosmarinic acid, promoted osteogenic differention towards osteoblasts and could have a therapeutic potential in bone tissue regeneration.

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Screening by HPLC-UV of legal substances (caffeine and yohimbine) and illegal substances (ephedrine and sibutramine) from weight loss dietary supplements for athletes

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A HPLC –UV method for the identification of ephedrine (EPH), sibutramine (SB), yohimbine (Y) and caffeine (CF) was developed. Separation was performed on a Kromasil 100-RP8, 150 mm x 4.6 mm, 5 mm column equipped with a precolumn Kromasil RP 8. Mobile phase was a gradient of 80-35 % sodium dihydrogen phosphate pH=5 with NH_4OH and acetonitrile over 15 minutes time of analysis. Based on the responses of 113 athletes about dietary supplements (DS) consumed for " fat burning " and weight loss which have a legal status in Romania, 28 supplements have been selected and investigated for their content in CF, Y, (legal substances) and SB, EPH (prohibited substances in DS). The method allows quantitative determination of the four substances in a short analysis time and with minimum cost. The presence of SB and EPH in the analyzed DS was not detected while the content in CF and Y considering the dosage recommended by the manufacturer does not affect the health of the consumers. DS labeling (plant extracts with CF and Y content) allows manufacturers to avoid declaring correct and exact amounts per pharmaceutical form (pure CF or equivalent and Y, respectively).

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Biotechnological approaches for the targeted delivery of volatile and non-volatile biologically active secondary metabolites in *Artemisia alba* Turra

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Introduction: Decoctions of the aerials of *Artemisia alba* have been traditionally utilized as stomach digestive and tonic and its essential oil has been shown to possess spasmolytic and antimicrobial activities(1-3). However, the essential oil profile exhibits high variability depending on environmental and climatic impact, as well as to genetic factors (4). Also little is known on the non-volatile constituents present in the aerial parts of this plant. The main objective of the PhytoBalk project, financed by the Swiss National Science Foundation strives for the development of standardized biotechnological protocols, on the one hand to serve for *in vitro* conservation of valuable medicinal plants germplasm *ex situ*, and on the other to provide for the biotechnological delivery of pharmaceutically relevant raw plant material with standardized quality.

Aim of the work: The interplay between plant growth and development, production of essential oils, antioxidant polyphenolics, as well as the structural organization of the photosynthetic apparatus of the plant has been studied in a model system of exogenous plant growth regulators treatment *in vitro*.

Methods: Pharmacognostic methods combined with *in vitro* culturing techniques, subsequent analysis using adapted well established methods from Ph Eur HPLC and HPTLC methods as well as radical scavenging and antioxidant tests and preparative chromatographic isolation techniques, colorimetric assays and 77 K steady state fluorescent emission spectroscopy.

Results: Predominance of sesquiterpenoids in the essential oils was also related to stimulation of polyphenolic production and impairment of the structure and function of the photosynthetic apparatus in the plant. On the contrary, monoterpenoid dominated essential oils were produced by plants with lower polyphenolic productivity and was associated with a higher degree of aggregation of PS II peripheral antennae.

Conclusion: An *in vitro* system has been developed for the targeted production of raw plant material with the predominance of either monoterpenoids or sesquiterpenoids in the essential oils. Simultaneous stimulation of polyphenolics and biomass production has been achieved, as result of benzyl adenine treatment.

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'Mattmark', a high yielding *Rhodiola rosea* cultivar launched in Switzerland

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Rhodiola rosea L., also called Golden Root or Roseroot, is an adaptogenic medicinal plant from alpine and arctic regions and has robust traditional and pharmacological evidence of use in fatigue, and emerging evidence supporting cognition and mood [1]. Breeding of a *R. rosea* cultivar is an important step to preserve natural populations of this species, ensure supply of standardized raw material and prevent frauds. In this study, the phytochemical variability of salidroside and total rosavins in five natural populations in the Swiss Alps are tested in order to select the most interesting genotypes for a polycross to get a new cultivar.

The five populations from the Swiss Alps were screened for their salidroside and rosavins contents. With an average content of 1,49% (± 1,15) for salidroside and 1,57% (± 0,74) for rosavins, the population in Mattmark (Saas Fee, Valais) turned out to have the most most productive and vigorous genotypes. 4 male and 4 female genotypes of this population were choosen. A random polycross was performed with these 8 genotypes to produce seeds of 'Mattmark', the first cultivar of *R. rosea*.

The new cultivar 'Mattmark' showed high rhizome production and high salidroside and rosavins contents in the roots. The seeds of this cultivar are available through the company mediSeeds (www.mediseeds.ch).

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Green synthesis of gold nanoparticles using propolis aqueous extracts and its characterization

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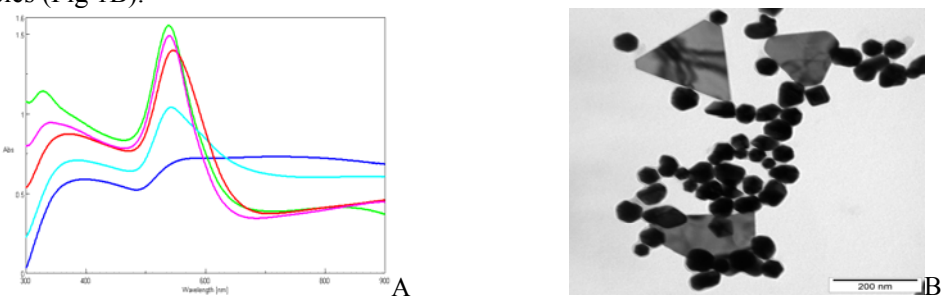
The present study reports the green synthesis of gold nanoparticles (AuNps) from gold precursor using aqueous extracts of propolis without the addition of any external surfactant, capping agent or template. In recent decades, gold nanoparticles synthesized by various techniques have received special attention because they have found potential application in many fields such as catalysis, biosensors, electronic and magnetic devices, biomedicine and drugs delivery system [1-3]. The aqueous propolis extracts (P1-P4) used for the synthesis of nanoparticles were characterised in terms of total polyphenol content by UV-Vis spectrometry and individual polyphenol content using RP-HPLC DAD. When the ratio of the reactants was varied with respect to 1.0 mM chloroauric acid solution a various shape and size of gold nanoparticles were formed. A preliminary characterization of the gold nanoparticles was carried out using UV–Visible spectroscopy. Absorption spectra of gold nanoparticles synthesized with propolis aqueous extracts showed an absorption band ranged from 525 to 558 nm (Fig.1A). TEM (Transmission electron microscopy) studies showed the particles to be of various shapes and sizes, the morphology consists of a mixture of prism and spherical like particles (Fig 1B).



A B

Fig.1. UV–vis spectra of gold colloids obtained for different concentrations of P3 sample (A); TEM images of colloid P3 (B).

Dynamic light scattering (DLS) studies revealed that the average sizes for colloids of P1-P4 samples are 70.76.2 nm, 84.04 nm, 50.30nm and 55.61 nm respectively. The DLS graph showed that the particles size was larger and more polydispersed compared to the one observed by TEM. Zeta potential value for gold nanoparticles obtained from colloids of P1-P4 are 37.23, 58.68, 29.16 and 30.94 respectively indicating the stability of the synthesized nanoparticles. These methods used for obtain gold nanoparticles do not use any chemicals and thus has the potential for exploit in biomedical applications.

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SAR of Naphthoquinone analogues against bacteria and fungi

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Keywords: Naphtoquinone, Antifungals, Antibacterials, Minimum inhibitory concentration

Naphthoquinones are natural pigments widely distributed in plants, fungi and some animal. They are privileged structures in medicinal chemistry due to their characteristics, structural properties and biological activities on prokaryotic and eukaryotic. 1,4-naphthoquinones exhibit strong action as antimalarial, antibacterial, antifungal, and anticancer agents.
The aim of this work is to perform a Structure-activity relationship study (SAR) of 52 naphthoquinone analogues looking for new natural product based drugs. 52 compounds were prepared and evaluated for its antimicrobial and antifungal activity on *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus*, *Candida krusei* ATTC 6258, *Candida parapsilosis* ATCC 22019 and *Cryptococcus neoformans* using broth microdilution method.
The activity was expressed as the minimum inhibitory concentration (MIC) in µg/mL. MIC values obtained varied from 16 to 1024 µg/ml for bacteria, and 2 to 512 µg /ml for fungi. Only 5 compounds exhibited activity against *Pseudomonas aeruginosa* at MIC≥64 µg/mL. *Candida spp* were the microorganisms more susceptible.
Halogen derivatives of the 1,4-naphthoquinone presented strong activity as 2-chloro-5,8-dihydroxy-1,4-naphthoquinone which exhibited inhibition at MIC of 16 µg/mL in *Staphylococcus aureus* and 2 µg /ml in *Candida krusei*, similar to that of pharmaceutical concentrations currently used in antimicrobial treatment. These compounds showed excellent profile of activity against fungi.

Cytotoxic Activity of the Extracts of *Flagellaria indica* L. against Breast Cancer Cell Lines, MDA-MB-231 and MCF-7

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Flagellaria indica L. (Flagellariaceae), commonly known as 'Rotan tikus' or 'Supple jack',is a perennial herb that is found in tropical and subtropical regions of the world including, South-East Asia, Polynesia,Australia and some parts of Africa. The root of this plant is known for its use in the treatment of wounds, cholera and headache, whilst the leaves and shoots can prevent premature hair loss. In this study, various extracts of the dried ground parts of *F. indica*were assessed for cytotoxicity against two breast cancer cell lines (MDA-MB-231 and MCF-7) and one normal cell line(HaCaT)by the MTT assay. MDA-MB-231 cells were found to beconsiderably moreresistant to extract-mediated cytotoxicityin comparison toMCF-7 cells.
The dicholoromethaneand the petroleum ether extracts obtained from the cold extractionsshowed similar level of cytotoxicity (IC_{50} values: 21.33-94.53 µg/ml), which was more potent than that of the ethyl acetate extract (cold extraction, IC_{50} values: 68.51-186.0µg/ml), againstthe HaCaT, MCF-7 and MDA-MB-231 cell lines after 48 h. Generally, extracts obtained from the Soxhlet extractions were much less potent than those obtained by the cold extractions using the same solvents. The cytotoxic effect of the extracts increased in a concentration- and time-dependent manner, except against the MDA-MB-231 cells.

Keywords:Flagellariaindica, MDA-MB-231, MCF-7, HaCaT, MTT, cytotoxicity

VISCUM ALBUM - AMINOACIDS AND PEPTIDE ANALYSIS AND THEIR ANTITUMORAL ACTIVITY

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Plants and herb extracts have been used for centuries as traditional medicines, throughout the entire world. One of the most renowned is *Viscum album* L., which has very different applications: tonic, cardiotonic, antiviral, cancer, etc. Initially, was considerated as the plant active compound was viscine, a mixture of esthers, alcohols, choline, acetilcholine, serpene and polipeptide. Recent studies shown shown that viscotoxine, another compound isolated from viscum present the cytotoxic activity.[1-4]

Over time, many studies have been carried out for determination the outstanding biological effects, but the extremely complex chemical composition of this plant has not yet been not precisely determined.

The attempts to establish the compounds responsible for biological, immunomodulating and cytotoxic activity had targeted especially the lectins and viscotoxins as active components, but these represent only a small content of percent from the entire plant peptide content which is not fully understood in terms of chemical structure and biological activity.[2-4]

Althrough the antitumoral activity of the viscum is still a controversial issue.

The paper investigates the biological activity of amino acids and small peptides identified from an alcoholic extract of *Viscum album* L. (*European mistletoe*) through chromatography (GC-MS analysis) and spectrometric methods (Fully automated chip-nanoEIS –QTOF-MS).

By positive mode nanoESI chip QTOF-MS we were able to identify peptide chains encompasing up to 4 amino acids with an experiment sensitivity situated at subpicomolar levels.

In our work was developed an original, simple and rapid technique using tumor angiogenesis on CAM assay *in ovo* for the investigation of biological, especially antitumoral activity of these compounds.

The results provide important information about the about these highly bioactive compounds isolated from plant extracts.

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Ecological Peculiarities and Phytochemical Studies on *Lamium album* L. (*Lamiaceae*)

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Lamium L. genus is a member of *Lamiaceae* family with four species in spontaneous flora of Republic of Moldova (*L. album* L., *L. amplexicaule* L., *L. maculatum* (L.) L. and *L. purpureum* L.). The present research refers to *Lamium album* L. (White Dead Nettle) and started from the necessity of assessing this indigenous medicinal species, insufficiently studied at national level. *L. album* extracts mainly include the phenylpropanoid glycosides verbascoside and isoverbascoside, and some phenolic acids and flavonoids [3] responsible for cytotoxic, anti-inflammatory, antioxidant and other notable therapeutic activities of this species. *Lamium album* L. is a perennial species naturally distributed throughout the country except the southern parts. The field studies were conducted at three forested sites: Landscape reserve "Saharna", South of Soroca town, and north of Kamenka town, Transdniester region. In order to determine the abundance of the species in studied locations the DAFOR scale (internationally recognized abundance scale for counting wildlife populations) were used. Regarding the ecological growing conditions it is frequent in the edges of fields, hedgerows, woodland edges and clearings. The habitat for *L. album* was categorized as Euro-Siberian steppic woods with *Quercus* sp. with code number 91IO (Habitat Directive 92/43/EEC). The abundance of the species in all recorded sites was determined as frequent in one location and abundant in others two. This abundance index indicates the possibility for this species to be harvested in the wild without any damage to the species subpopulations. For the comparative phytochemical study, the methanolic extracts of *herba* (harvested in June-July and October) were analyzed by means of TLC and HPLC. The phenolic acids and flavonoids are the main classes of bioactive secondary metabolites envisaged in this study. The TLC analysis highlighted the chlorogenic ac. as the main phenolic ac., observation which was confirmed by the HPLC analysis (57.96 – 220.04 mg/100 g d.w.). The rosmarinic ac. was found in amounts of 40.11 – 83.41 mg/100 g d.w. This is in accordance with the literature data, which states the chlorogenic ac., rosmarinic ac. and caffeic ac. as the main polyphenols compounds present in *L. album* [2]. The rutoside content varied between 31.73 and 40.03 mg/100 g d.w. The phenolic acids were found in higher amounts (chlorogenic acid and rosmarinic acid), followed by the flavonoid rutoside, ratio which was in all samples. The phytochemical intraspecific variation (particularly for the chlorogenic ac.) for the *Lamium* species harvested from various habitats can be correlated with the plastic response to the ecological peculiarities as phenotypic expression, which can be also observed for the morphological traits [1]. The higher content of chlorogenic ac. in the samples harvested in the autumn can be considered an adaptative response to the environmental features of the seasons.

The final aim of the study is the identification of *L. album* populations with high contents of bioactive secondary metabolites, respectively with high antioxidant potential.

The work was supported by Bilateral Collaboration Project MD-RO (Nr. 04/RoA/2013) and the project: PN 09-360401 (BIODIV) financed by National Agency for Scientific Research Romania.

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Bio-morphological Studies on *Satureja* L. Species Cultivated in the Botanical Garden (Institute) of ASM

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The genus *Satureja* L. (fam. *Lamiaceae*) comprises about 25 species distributed from the Mediterranean region of Europe and south-west of Asia [1]. As a medicinal plant, savory has been traditionally used as a stimulant, carminative, aphrodisiac and in the treatment of colds. Due to its aromatic properties it is widely used as a culinary herb. Savory has also impressive ornamental value being used in landscape architecture. Numerous studies have been conducted on these species, growing in different regions of the world, particularly on their volatile oil compounds. In recent years, antiviral, anti-inflammatory, antibacterial, antifungal, antispasmodic, antidiarrheic, vasodilatory and antioxidative [2] effects have been reported for different *Satureja* L. species.

In the Botanical Garden (Institute) of ASM *Satureja* L. genus is represented by seven species (*Satureja montana* L., *S. kitaibelii* Wierzb**.**, *S. parnassica* Heldr. et Sart. ex Boiss., *S. thymbra* L., *S. hortensis* L., *S. calamintha* (L.) Scheele. and *S. subspicata* Bartl. ex Vis) obtained exclusively by seed exchange with other institutions in the world [3]. Four aromatic and medicinal *Satureja* L. species (*Satureja montana*, *S. kitaibelii*, *S. parnassica* and *S. subspicata*) were studied. The propagation by seedlings, cuttings and division of the plants has been investigated. The phenological stages and bio-morphological peculiarities of the plants under conditions of Republic of Moldova were highlighted. An advantageous way of multiplication of these plants in our conditions is vegetative propagation (by cuttings and division of the plants). In the case of the propagation of plants through cuttings best results (85% rooted cuttings) were obtained from the *S. kitaibelii* species. Phenological observations demonstrated that the vegetation period of plants in local conditions lasts 158-165 days. Species of *S. montana* start flowering stage 14-15 days earlier than *S. kitaibelii, S. parnassica* and *S. subspicata*. The flowering phase of *S. montana* lasts 70-75 days, and 60-68 days for *S. parnassica, S. kitaibelii* and *S. subspicata*. At the end of vegetative period *S. montana* adult individuals reach a height of 70-75 cm and 50-60 cm in diameter. Impressive biometric parameters were also registered for *S. kitaibelii*. The comparison of the preliminary results of essential oil analyses of studied species with the literature data showed some differences for oils, which can be attributed to ecological factors, pedo-climatic conditions or other factors that can influence the oil composition. Nevertheless, the high concentration in carvacrol and thymol as main components in *S. montana* and *S. parnassica* essential oil, makes the savory essential oil obtained from the plants cultivated in our conditions very valuable.

The research regarding plant biology features, ontogenetic cycle, growth and development during the vegetative period showed that investigated species grow and develop normally in the local climatic and soil conditions, completing the entire ontogenetic cycle. The high level of morphological variability among the studied populations suggests a breeding approach to obtain new and promising cultivars for local agriculture.

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An easy HPLC-UV/VIS methodology for the quantification of polyphenols in propolis, grapefruit seeds, and elder fruits extracts

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Due to their high content of bioflavonoids and anthocyans, herbal products having propolis, grapefruit seeds and elder fruits extracts as ingredients are often used as remedies to cure bacterial and viral diseases affecting the upper ways of the respiratory apparatus. Being marketed all over the world, it is nowadays well recognized how the commercial value of such phytopreparation strictly rely on the total polyphenols content. This quantification is typically achieved by means of UV/Vis analysis (e.g. the Folin quantitation method) in which the polyphenols concentration is expressed in terms of only one reference compound (e.g. gallic acid). However the sensitivity of these methodologies can be affected by the presence of interfering substances. In this communication we wish to report an alternative and efficient HPLC-UV/Vis method for the quantification of individual polyphenols (flavonoids expressed as naringenin, galangin, and apigenin, anthocyans expressed as oenin, catechins expressed as (+)-catechin, benzoic and cinnamic acids expressed as gallic and caffeic acids, respectively) in propolis, grapefruit seeds, and elder fruits extracts. Analyses were performed using a Waters liquid chromatograph equipped with a model 600 solvent pump and a 2996 photodiode array detector. Empower v.2 Software (Waters Spa, Milford, MA, USA) was used for data acquisition. A C18 reversed-phase packing column (GraceSmart RP18, 4.6 mm × 250 mm, 5 μm; Grace, Deerfield, IL, USA) was employed for the separation. The column was thermostated at 25±1 °C using a cool pocket chiller (Thermo Scientific, Waltham, USA). The UV/Vis acquisition wavelength was set in the range of 200 – 600 nm. The qualitative analyses were achieved at a wavelength of 288, 340, and 365 nm for flavonoids, 520 nm for anthocyans, 271 nm for benzoic acids, 280 nm for catechins, and finally 323 for cinnamic acids. The injection volume was 20 μL. The mobile phase was directly on-line degassed using a Degassex, mod. DG-4400 apparatus (Phenomenex, Torrance, CA, USA). Mobile phase composition consisted in double distilled water / CF_3COOH 0.4% v/v (solvent A) and acetonitrile / CF_3COOH 0.4% v/v (solvent B) by gradient elution with different A/B ratios at a flow rate of 0.6 mL/min. Results are reported in Table 1. In each case the concentration values recorded are in the range of ± 2% when compared to the ones obtained by the Folin quantitation method applied to the same extracts.

Table 1

Total content (mg/g dry extract)								
	Flavanones	**Flavones**	**Flavonols**	**Anthocyans**	**Benzoic acids**	**Catechins**	**Cinnamic acids**	**Total**
Propolis	0.044	0.007	0.011	-	0.0096	0.032	0.005	**0.11**
Grapefruits	0.50	0.007	0.005	-	-	-	-	**0.51**
Elder	-	-	-	0.13	-	-	-	**0.13**
% Content								
Propolis	4.4	0.71	1.17	-	1	3.3	0.48	**11.01**
Grapefruit	49.7	0.74	0.55	-	-	-	-	**51.00**
Elder	-	-	-	13.74	-	-	-	**13.74**

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Genetic variability in natural populations and cultures of *Origanum vulgare* subsp. *vulgare*

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Studies of genetic diversity are very important for designing cultivar-improvement programs in the management of germplasm and in conservation strategies (Joshi et al. 2004). Limited numbers of studies have investigated genetic variability within species or correlation between genetic structure and essential oil composition (Tonk 2010).
This study is the first to use RAPD markers and essential oil composition and content to investigate the genetic and chemical structure of intrapopulational differentiation of *Origanum vulgare* subsp. *vulgare* in RMoldova.
Genotypic variations were assessed across various populations by means of analysis of molecular variance (AMOVA) using GenALEx 6. This analysis not only allows the partitions of the total variation into within-group and among-group variations components, but also provides a measure of inter-group genetic distances as the proportion of the total variation residing between accessions. The significance of the resulting variance components and inter-population genetic distances was tested using 999 random permutations.
Random Amplified Polymorphic DNA (RAPD) marker data were obtained and analysed with respect genetic diversity, population structure and gene flow. Twelve primers generated total of 298 discernible and reproducible bands in the analysed population, out of which 31 were polymorphic.
Variation of essential oils in the populations was subjected to cluster analysis, and one chemotype with *sabinen* / 4(10)-*thujene*, trans-β-*ocimene, γ-terpinen, β–caryophyllene, germacrene d* was identified.
The UPGMA cluster analysis permitted the discrimination of all the genotypes and their sorting into 3 main groups.
This study indicated there was no correlation between the RAPD data and chemical composition data. This result is evidence that genetic similarity may not always reflect similarity or difference in phenotypic traits, for instance oil composition; this has previously been reported by Tonk (2010). Also, effects of environmental conditions, the extraction method used, and plant growth stages on essential oil of medicinal and aromatic plants should be considered. In contrast, DNA genotyping is not affected by plant development stages and other environ-mental factors (Masi et al. 2006).
Use of RAPD markers has enabled discrimination of oregano populations and can be used successfully for the selection and improvement of cultivars in future studies. In addition, knowledge of chemical composition, revealed in this investigation, gives the opportunity to select genotypes containing different types of essential oil.

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