Association for the Medicinal and Aromatic Plants
of Southeast European Countries (AMAPSEEC)

Slovak University of Agriculture in Nitra
Faculty of Agrobiology and Food Resources

Department of Sustainable Agriculture and Herbology

III CMAPSEEC

3rd CONFERENCE ON MEDICINAL AND AROMATIC PLANTS OF SOUTHEAST EUROPEAN COUNTRIES

BOOK OF ABSTRACTS

SLOVENSKÁ POĽNOHOSPODÁRSKA UNIVERZITA
NITRA

5 - 8 September 2004

Nitra, Slovak Republic

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Rector of the Slovak University of Agriculture in Nitra

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PREFACE

Dear members of AMAPSEEC and other participants of this conference,

let me welcome you in the heart of Europe, Slovakia, in an old (but in the same time young) Nitra town, the town of universities. I speak to you from the position of the Rector of the only agricultural university in Slovakia, which has educated more than 30 thousand professionals. Today, more than 10 thousand students of all study forms are studying at our University. The Slovak University of Agriculture belongs to the universities, which are largely engaged in the grant and research projects, meeting the European research area requirements. The results of the research projects are presented by the researchers at congresses and conferences all around the world. Today we are happy to host the specialists in the field of medicinal, aromatic and spicy plants not only from the whole Europe but from other continents, too. Slovakia has a long-established tradition and considerable experience of growing and processing medicinal, aromatic and spicy plants. The growing sites and processing capacities are nowadays even increasing. Whereas in the year 1989 these special plants were grown on the area of 408.3 ha, in the year 2003 it was 1,316 ha, and the prognosis for the year 2010 is 2,500 ha. Support for growing medicinal, aromatic and spicy plants is declared by the Ministry of Agriculture of the SR in 'The Development Programme of Medicinal, Aromatic and Spicy Plants Cultivation and Processing in the Slovak Republic', that was developed in the year 2000 with the development prognosis up to the year 2010.

Growing medicinal, aromatic and spicy plants, their exploitation in pharmaceutical, food, cosmetic, and other types of processing industry belong to the most significantly developing fields in highly developed European countries, as well as on a world scale, that generates lively interest in natural products. Up-to-date scientific and specialist knowledge presented at this conference will no doubt contribute to its enlarging, that will help facilitate the progress of this field in future.

The Slovak University of Agriculture hosts the third European conference yet, and I believe, that our Congress Centre will create favourable conditions not only for evaluation of the AMAPSEEC activities but also for the exchange of scientific and specialist knowledge of medicinal, aromatic and spicy plants. In addition to the official scientific part of the conference, social programme is prepared to show you the beautiful historical sites of the town Nitra and its surroundings.

Prof. Dr. Ing. Imrich Okenka, PhD.
Rector of the Slovak University of Agriculture

FOREWORD

Dear Colleagues,

Since official launching of idea for keeping regularly regional conferences on medicinal and aromatic plants of Southeast European countries in 1998, two such conferences were successfully organised. These meetings were imagined as wide profiled international (regional) scientific and professional meeting, open for all those working in the field of medicinal and aromatic plants (MAPs). The first conference was held in the year 2000 in Aranđelovac (FR Yugoslavia) and the second one in Chalkidiki (Greece) two years latter. According to the number and quality of presented contributions, these conferences were very successful. For the 1st Conference 331 author from 16 countries prepared 170 contributions, whose abstracts were published in the book of abstracts from that meeting. For the second one, 468 authors from 26 countries prepared 204 such contributions. Main and additional programs at these meetings surely fulfilled expectations of the majority of participants, fully approving purpose of keeping such conferences. Subsequently, expected profile of the 3rd Conference should be very similar to that of those previous.

Leadership in the organisation of the 3rd Conference on Medicinal and Aromatic Plants of Southeast European Countries (3rd CMAPSEEC) has been given with trust to our colleagues and members from Slovakia, Dr. Miroslav Haban and Dr. Ivan Šalamon. Their nomination of the Slovak University of Agriculture (SUA, Nitra) for the host of the 3rd Conference was fully accepted and approved by all relevant managing bodies of the Association for Medicinal and Aromatic Plants of Southeast European Countries (AMAPSEEC). Now, after two years of preparation, there is no doubt that the 3rd Conference will at least maintain, or even improve, scientific and professional level achieved in previous ones. Thanks to the great effort of our host to provide best conditions for the keeping of the 3rd CMAPSEEC, we are now in position to fulfil our obligations in calm cheerfulness. For all those, which will come here in Nitra, in the heart of the Europe, 3rd Conference would be an extraordinary opportunity to exchange and enrich their experience in the field of medicinal and aromatic plants (MAPs), to refresh existing and establish new contacts.

At the 3rd Conference, which is the official meeting of the AMAPSEEC, one session will be reserved for keeping the 4th Members meeting of the Association, which could be highly important, even detrimental for its further destiny. At that meeting report about activities of the AMAPSEEC, Executive Council and Board of Directors in the period between the third and the forth Member's meeting will be given, as well as report on the membership and available funds of the Association. Moreover, decision about the host of the 4th CMAPSEEC will be made. Simultaneously, at the 4th Members meeting, election of new managing bodies of the AMAPSEEC, as well as selection of new location for the AMAPSEEC's headquarter should be accomplished too.

AMAPSEEC is extremely thankful to all those, which made possible keeping of the 3rd Conference on Medicinal and Aromatic Plants of Southeast European Countries. First of all to our host, Prof. Dr. Ing. Imrich Okenka, rector of the Slovak University of Agriculture in Nitra, Dr. Miroslav Habán and Dr. Ivan Šalamon, who leaded whole organisation, as well as to sponsors and all others being involved in the organisation of the conference.

Knowing that distant goals of the AMAPSEEC could be reached only through effective and long-lasting co-operation, between all those engaged in MAP problems from many different aspects, the 3rd Conference will surely make an additional momentum in that sense.

Mihailo Ristić MSc.
President of the AMAPSEEC

PLENARY LECTURES

TOPICS OF CONFERENCE:

* Ecobiology of Medicinal and Aromatic Plants (MAP)

* Biodiversity, Genopool Protection and Conservation

* Pharmacognosy

* Cultivation and Industrial Processing

* Pharmacology and Toxicology

* Phytochemistry

* Quality Control of MAP and MAP Products

* Environment and Its Influence on MAP

* Traditional Medicine and MAP Use

PL 01

HARMONY AND CONTRADICTIONS IN ECOLOGICAL AND PHYSIOLOGICAL OPTIMUM OF MEDICINAL AND AROMATIC PLANTS

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The regional specialisation in Hungary according to production of medicinal and aromatic plants is known from the beginnings of the 20th century. As a result of spontaneous process seven well-defined production areas were developed. The relationship between regions, their climatic conditions and spectrum of species produced there are compared (1).
Ecological requirements of 97 collected and 55 cultivated medicinal and aromatic plants are characterised. Based on the analysis of `T` (temperature regime values) about 63 per cent of cultivated species came from Sub-Mediterranean and Mediterranean type of habitat, originally, while the majority of collected plants (61,8 per cent of them) prefer the deciduous forest conditions. The differences between collected and cultivated species are appreciable too, if the distributions of their characteristic water regime (`W` values) are compared. The majority of cultivated species require dry (moderate dry) and fresh (moderate fresh) habitats, while the amplitude of water requirement of collected species is much more wide-ranging.
However, the examples of many species including camomile (Matricaria recutita), valley lily (Convalaria majalis) etc. prove there are large differences between the condition of original growing site and the real physiological requirements of the species (2). If we want to optimise the collection practice or the cultivation technology (3) we have to analyse the effect of individual environmental factors on both biomass and active agent production (4).

Key words: regional specialisation, physiological and ecological requirements, active agents

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PL 02

ECOBIOLOGY OF THE MEDICINAL AND AROMATIC PLANTS

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The plant ecobiology studies the biological and ecological properties of species in connection with their life strategies. The life strategy of all plant species is a system of co-evolutionary linked adaptable features ensuring the plants to exist in certain environmental conditions and to occupy position in biocenoses.
In regard to the research, development and production of each medicinal and aromatic plant species its ecobiology has a significant importance. The presentation and deep study in the fields: taxonomy, nomenclature, ecophytogeography, ecophytocenology, ecomorphology, ecophysiology, bioperiodicity,

reproduction, biotic and abiotic factors, ecodemography, environment, economical importance, legislation etc. (eco-biological monograph [1]) of single medicinal and aromatic plants play a decisive tasks in order to understanding of primary or secondary metabolite biosynthesis, their highest component accumulation, large-scale herb cultivation, their technology isolation and using in the food, flavor, and perfumery, pharmaceutical and healthcare industry.

Key words: plant ecobiology, life strategy, eco-biological monograph, medicinal & aromatic plants

References:
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PL 03

CONSERVATION OF MEDICINAL AND AROMATIC PLANTS IN EUROPE – STRATEGY AND DIRECTIVES

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WHO is concerned about the natural resources of MAP because over 80% of the world population still depends on traditional preparation, which are prepared on the basis of wild-collected plant material. Uncontrolled over-exploitation of wild plants in trade, their habitat-loss and alteration are the main reasons why medicinal plants, their study, evaluation, utilisation and conservation have become essential parts of the programs of international organisations such as WHO, IUCN and WWF. These organisations agree that a co-ordinated approach at a national or international level is necessary to conserve the large number of species used by man, that are at risk of extinction. As a result of the national developmental programs in some European countries the acreage covered by MAP is gradually increasing. Although a few years ago the cultivation area of MAP at European level was estimated at 70,000 ha, the current data reveal that MAP are cultivated already on more than 100,000 ha. Monitoring and characterization of MAP natural habitats as well as introduction of "wild" plants into cultivation count for the main strategic priorities in European countries. The conservation strategy should foresee also estimation of possible risks that might endanger the native populations of selected species. When natural populations are not endangered, their abundancy changes should be followed and monitored in natural habitats (*in situ* conservation). Seed material from rare and vulnerable plant species should be collected and transferred to the Genebanks for Medicianl and Aromatic plants at appropriate institutions, where MAPs are conserved *ex situ*. The Main directives of MAP conservation program in Europe are presented.

PL 04

CULTIVATION AND INDUSTRIAL PROCESSING OF MAP

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We still witness an increase of MAP use in European market. It is not restricted only to pharmaceutical sector but also cosmetics, speciality foods, nutraceuticals and others. Till late 80ties of last century most south eastern European (SEE) countries were considered suppliers of MAP raw material harvested in the nature. But also cultivation took part in most of them.

In documents about biodiversity and gene resources management of MAP cultivation is now generally accepted as one of ways of biodiversity conservation. In last ten years the demand for high quality raw material with known provenance and tractability increased. This can be achieved in most cases only by cultivation. The result is development of Guidelines for good agricultural practice in MAP cultivation. They are connected with GLP and GMP practices, EU and WHO regulations.
Besides big pharmaceutical companies in developing economies of SEE countries small and medium size enterprises should take a leading role in inovative herbal products. In this concept companies producing semi products as extracts, essential oils and special plant products should be equally promoted.
SEE countries have tradition, knowledge and natural sources to promote sustainable cultivation of MAP and harmonize it with regulatory issues to make the raw material, processing and end products safe, efficient and of high quality

Key words: medicinal and aromatic plants, cultivation, processing, south eastern European countries

PL 05

HIGH PERORMANCE LIQUID CHROMATOGRAPHY FOR INDENTIFICATION AND DETERMINATION OF FLAVONOIDS: INVESTIGATION OF FLAVONOIDS IN THE REPRESENTATIVES OF MACEDONIAN FLORA

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The results of the work on development and validation of HPLC methods for determination of frlavones, flavonols and flavanones in plant matertial from species growing in the Macedonia are presented. The HPLC methods for determination of flavones, flavonols and flavanones were developed using a liquid chromatograph (Varian) equipped with a ternary pump (Model 9012) and the ultraviolet diode-array detector (UV-DAD) (Model 9065 Polychrom), which eables monitoring of the eluent in the whole UV-range and therefore characterization of the flavonoids by their UV-spectra and quantification at the corresponding absorption maxima thus producing better selectivity and sensitivity. A C18 reversed phase was used as a stationary phase (column dimensions: 25 x 0.46 cm, 5 mm) and the mobile phase consisted of water acidified with acetic or formic acid and methanol and/or acetonitrile. The column was thermostated for providing reproducible retention. The analuses were performed in ethylacetate extracts obtained after acid hydrolysis of the heterozyde forms using concentrated HCl. These experimental conditions were used for establishing reliable, accurate and reproducible HPLC methods for: determination of flavones in samples of Origanum vulgare and in samples of various taxa of species Thymus (quantification of luteolin) [1, 2], determination of flavonols in samples of Hyperici herba, Uvae-ursi folium, Pruini spinosae flos, Sambuci flos, Betulae folium, Primulae flos, Herniariae herba, Centaurii herba, Tiliae flos, Bursae pastoris herba [3] (quiantification of quercetin, simultaneous quantification of quercetin, kempferol and myricetin and simultaneous quantification of flavones, flovonols and flavanones in samples of Helychrisum plicatum (simultaneous quantification of luteolin, apigenin, quercetin, kaempferol and naringenin) [4].

Key words: flavonoids, identification, determination, HPLC

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PL 06

PHARMACOLOGY OF ST. JOHN´S WORT. SCREENING FOR BIOACTIVITY OF GREEK *HYPERICUM* SPECIES

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The genus *Hypericum* L. (Guttiferae) comprises 400 species widespread in warm-temperate regions and tropical mountains [1]. The most well known species of this genus is *H. perforatum*, which has been used, in traditional medicine for a long time, as anti-inflammatory and healing agent. Plants of this genus are widely used in folk medicine and important pharmacological properties have been attributed to extracts of *Hypericum* spp. [2].
Methanolic extracts of *Hypericum* samples collected from different areas in Greece were screened on brine shrimps, human colon carcinoma and human hepatoma cell lines for cytotoxic activity. The antioxidant properties of these extracts were also evaluated [3].

Key words: Hypericum species, antioxidant activity, cytotoxic activity

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PL 07

PHYTOCHEMISTRY AND MEDICINAL AND AROMATIC PLANTS

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Medicinal and aromatic plants (MAPs) have played a changing role through history. Nowadays, they continue to be used world-wide in prevention and treatment of disease and novel medicinal products entities continue to be developed through research into their constituents. Moreover, constantly increasing interest in ”Natural” has revolutionized the use of MAPs by different industries related to the human wellbeing. To cover all needs, modern research of MAPs has multi-disciplinary approach and this includes expertise in phytochemistry.
In this presentation, the impact of phytochemistry on MAPs research will be reviewed, both from selected research in literature and from the authors’ own experiences. Special attention will be paid on

the role of phytochemistry in modern international harmonization policy and quality control of MAPs and their preparations.

PL 08

SECONDARY PLANT METABOLITES AND THEIR POTENTIAL FOR NOVEL BIOACTIVE AGENTS

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Plants have been a source of bioactive agents for thousands of years and both their discovery and use are increasing in pharmaceutical, herbal, cosmetic, fragrance, food and drink industries[1]. Detailed analyses of active natural product skeletons have led to the identification of an assembly of precursor molecules and the complex biosynthetic schemes[2]. This has enabled studies of structure-activity relationships and has led to the generation of various analogues for synthetic production[3]. Recent advances in the role of plants in drug discovery will be discussed[4]. Study cases from our research activities will be presented: *Mentha aquatica* and *Myrica gale* chemotypes from natural population in Scotland; frankincense resin [*Boswellia* sp.] harvested from the local tree population in Somalia (intensive investigation of terrestrial flora is important for biodiversity and exploration of new resources)[5]; *Arnica montana* and *Arnica chamissonis* (an investigation of volatile oils and carotenoids); the use of selected essential oils for their antimicrobial and antifungal activities (including skin bacteria in humans, mastitis in dairy cows, clostridial infections in poultry, important fungal pathogen in agricultural crops). As well as the volatile oils, polyphenolic tannins have long been used for industrial processes (tanning) and recent research has highlighted their potential for benefit to the livestock industries and for human health[6].

Key words: essential oils, tannins, carotenoids, biosynthetic pathways, antimicrobial/antifungal activities

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PL 09

QUALITY ASSURANCE IN THE PRODUCTION OF MEDICINAL AND AROMATIC PLANT DRUGS

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Quality assurance through the production of medicinal plant drugs and their processed products is already a prerequisite both in agriculture and industry. The lecture presents the latest aspects of this

question. Quality, safety and efficacy should be assured as main requirements and are the bases of each quality control procedures. Quality assurance is based on documentation and control at regular steps of the whole production.
The need for the introduction of a quality assurance system emerged already at the second half of the eighties (1). At the beginning it was called GAP (Good Agricultural Practice) (2), later on as GHP (Good Harvesting Practice), after it GSP (Good Sourcing Practice). Recently, the material of the EMEA and HMPWP furthermore that of the WHO (3) have been accepted and serve as general guidelines assuring proper bases for specialised systems in each crop. They include biological bases, cultivation technics, personnel criteria, buildings and facilities, equipments, primary processing and aspects of nature preservation. Numerous difficulties arise in adaptation of quality assurance for drugs of wild harvested plants because of false identfication, pollutions and uncertain spectrum and level of active ingredients. Introduction into the culture is therefore not only a possibility but even a necessity for several species in the XXI.century.
The quality examination of the drug –which is a step of GSP - is based on national/European Pharmacopoeia and focuses on identity, purity and content of effective materials.

Key words: GAP, GSP, cultivation, safety, efficacy

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PL 10

QUALITY CONTROL OF MEDICINAL AND AROMATIC PLANTS AND THEIR PRODUCTS

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Quality control (QC) of medicinal and aromatic plants (MAPs) and their products assumes procedure of determination and comparison of certain (selected) characteristics of these products, with requirements which can be supplied by different sources (pharmacopoeias, ISO and other standards, etc.). Number of mentioned characteristics, which should be determined by QC, is strictly defined by requirements taken from selected source. Since MAPs are roughly always used as raw material for production of different products, such as herbal remedies, cosmetics, beverages of food products, quality requirements may vary in some extent. It should be noticed that requirements given by pharmaceutical industry are surely the most sophisticated and the most restrictive. QC report is always detrimental in making decision about further destiny of tested MAPs and related products. In the final instance, reason of existence of QC is to protect consumers, as well as producers, processors and traders. Stable and homogeneous production and processing of MAPs assume implementation of certain generally adopted practices (GAP, GCP, GSP, GMP, GLP, etc.), approaches (such as HACCP), and standards (for example, ISO 9000), as the main precondition. Main effort in Europe in this field is focused to harmonisation and improvement of national and common policies, as well as foundation on new international standards [such as ISO 22000 (FSM)]. The actual procedure for marketing authorisation of herbal medicinal products, according to the newest directives supplied by EEC, and other relevant sources will be given in details.

Keywords: quality control (QC), medicinal and aromatic plants (MAPs), MAP products, standards, legislation.

PL 11

INFLUENCE OF SOIL CHARACTERISTICS AND NUTRIENT SUPPLY ON MEDICINAL AND AROMATIC PLANTS

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Soil characteristics and nutrients supply are of great importance for growth and development of any plant species. Apart from the influence on yield of medicinal and aromatic plant species, soil factors are also responsible for the MAPs quality.

Physical characteristics of soil (texture, porosity, air and water capacity, water permeability) in great incidence determine suitability for growing particularly of species that are grown for their roots (angelica, marsh mallow, valerian). Soil reaction (pH) and parent material are the most responsible factors moderating the content and availability of mineral elements in the soil. In soils with high pH reaction and high content of lime, availability of most mineral elements decreases, what usually causes chlorosis and retards development of certain plants (arnica). On the contrary, in strong acid soils, the availability of mineral elements increases so, that it can cause harmful accumulation of heavy metals (Cd, Ni, etc.), especially in certain plant species that are prone to such phenomenon (St. John's Worth, Yarrow, etc).

Dose of the applied macronutrients (in form of fertilizers) in MAP production depends primarily on the content of these nutrients in the soil as well as of the requirements of grown MAP species. Nitrogen has the greatest influence on growth and development of the aboveground part of plant (the yield) and in certain cases also has a positive influence on plant quality. However, too much of nitrogen in the soil may cause negative effects in species that are grown for their flowers (chamomile) or their roots (*Echinacea angustifolia*). Actually, excess of nitrogen in the soil may cause delay in flowering and vigorous development of aboveground part of the plants instead of desirable root development. Potassium most often has positive effects on development of MAP cultures grown for its roots (angelica, valerian, *E. angustifolia*), while for phosphorus it is characteristic to have positive influence on development of generative organs and stimulation of flowering. Such a global division has an array of deviations in practice, depending on numerous other ecological and physiological factors, among which the most important one is availability of other nutrients in the soil (Ca, Mg, B, Fe, Zn, and Mn).

Last decades, under the strong anthropogenic influence, some soils have been additionally loaded with heavy metals (Pb, Cd, Cr, Ni, Hg) as well as with some other potentially harmful substances. Big industrial centres, highways, Cu, Zn and Pb smelters, steam power plants and communal sladge are the main sources of heavy metal contaminations. In case of MAPs, this problem may be solved by avoiding such localities, weather it is a word about growing or collection of MAPs from the nature. Strongly defined principles of Good Agricultural Practice (GAP) and/or organic collection and production of MAP, promote avoidance of any kind of pollution. Consequently, it is expected in the future that these kinds of MAP production and/or exploitation from the nature will be intensified.

Key words: medicinal and aromatic plants, soil characteristic, nutrients supply, heavy metals

PL 12

USE OF MEDICINAL AND AROMATIC PLANTS IN THE SOUTHEAST PART OF THE PANNONIAN PLAIN THROUGH THE CENTURIES

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Prehistoric use of medicinal and aromatic plants in the southeast part of the Pannonian plain have been investigated by archaeobotanists at archaeological sites: Feudvar (Bronze and Iron Age), Gomolava (Neolithic, Eneolithic, Iron Age, Roman period), Gradina/Bosut (Iron Age), Kalakača (Iron Age), Opovo (Neolithic), Petrovardin (excavation in progress), Starčevo (Neolithic) and Židovar (Bronze Age). Silent witnesses of plant husbandry and gathering economy, carbonised seeds and fruit stones, lay scattered in the cultural layers of prehistoric settlements. Macrofossil plant remains consist mostly of cultivated plants (cereals, pulses and oil/fibre plants) and accompanying weeds. Woody plants used for building and other technical purposes, wild fruits, vegetables, spices and plants used for fibre complete the spectrum of ancient settlements plant economy. The existence of medicinal plants in prehistory as such can be hardly proven. Parts of many plant species were gathered and used for diverse purposes that we will probably never be able to find out. A concentrated find of seeds of many-seeded goosefoot *Chenopodium polyspermum* L. in the fortified settlement of Feudvar is a nice example for this. However, today's traditional herbal medicine is still using some plants which were found during archaeological excavations: wall germander *Teucrium chamaedrys* L., vervain *Verbena officinalis* L., high mallow *Malva sylvestris* L., dwarf mallow *Malva neglecta* WALLR., black henbane *Hyoscyamus niger* L., St. John's wort *Hypericum* sp., white mallow *Althaea officinalis* L., mint *Mentha* sp. and camomile *Matricaria* sp. The list of possible drug/medicinal plants can be extended with poppy *Papaver somniferum* L. - in the prehistory common cultivated oil plant. The hemp *Cannabis sativa* L. was archaeobotanicaly recorded in the central part of the Pannonian plain in Iron Age. The inhabitants of Feudvar were using barley *Hordeum vulgare* L. and emmer *Triticum dicoccon* SCHRANK, an prehistoric wheat-crop, for making beer. Use of hops *Humulus lupulus* L. as beer-spice was not proven. Dill *Anethum graveolens* L., wild carrot *Daucus carota* L., wild celery *Apium graveolens* L. and wild parsnip *Pastinaca sativa* L. are species that could have been used for spicing seasoning food.

PL 13

TRADITIONAL MEDICINE AND MEDICINAL PLANT USE IN BULGARIA

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Medicinal and aromatic plants have been used in maintenance of health as well as in the prevention and improvement of physical and mental illness in Bulgaria. Earliest archeological evidences were dated to ancient Thracians. Until the end of 19th century herbal medicine was almost the only available healing practice and its traditions are been saved until now. Bulgarian flora is rich and diverse with 7835 species. Ethnographic data show that 750 plant species are used as 250 have been applied mainly in phytotherapy [1, 2]. Preservation of natural resources were set up during the economic crisis after the 1st World War and resulted in establishment of the Medicinal Plants Act in 1941 thus regulating gathering of wild herbs and state responsibilities. Recently 120 of herbs have been traditionally gathered from their natural populations, 47 are under protection, 38 are included in

the Red Data Book of Bulgaria, 60 have been cultivated, 35 are been main industrial crops [3, 4]. Main steps in protection of natural resources of MAP combined with their sustainable use were: National Strategy of Biodiversity Protection (1990-1994), new nature-protection legislation (Medicinal Plants Act, Protection Areas Act, Biodiversity Act, Forests Act), Branch Strategy for Medicinal Plants until 2015 (2003). Bulgaria is an important exporter of herbal materials [5] and has developed scientific background in this field. Numerous herbal preparations are been produced: patented medicines, food additives, herbal teas, extracts, essential oils, etc. New prospects in research and practice is to improve quality by means of GGP, GAP, GLP, GMP.

Key words: medicinal plants, traditional medicine, phytotherapy, herbal products

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PL 14

THE DEVELOPMENT PROGRAMME OF MEDICINAL AROMATIC AND SPICY PLANT CULTIVATION AND PROCESSING IN THE SLOVAK REPUBLIC

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The medicinal, aromatic and spicy plants in Slovak Republic are originated into the natural raw material resources of plants, which are the genepool of these plants and an introduction of new plants from abroad. The Slovak Ministry of Agriculture mandated the Research Institute of Agroecology in Michalovce to elaborate "The Development Programme of Medicinal, Aromatic and Spicy Plant Cultivation and Processing in the Slovak Republic" in the first half of the year 1999 [1]. I more than 60 specialistts from the sector of medicinal plant cultivation, processing, business and science were contacted. All materials presented to the programme and table 1.

Table 1: The cultivation of medicinal, aromatic and spicy plants in Slovak Republic (2000 -2003)

Plant group/species	Cultivate area [ha]			
	2000	2001	2002	2003
Medicinal plants	783.61	696.45	678.02	1 316.00
Chile pepper	564.78	518.97	289.72	369.00
Caraway	82.00	142.50	287.82	279.00
Others spicy plants	58.50	18.00	12.88	34.80
Poppy (farmaceutical use)	2 384.46	2 122.71	372.20	430.70
Total	3 873.35	3 498.63	1 640.64	2 429.5

Slovakia has a long-established tradition and considerable experience of growing and processing medicinal, aromatic and spicy plants. The growing sites and processing capacities are nowadays even

increasing. Whereas in the year 1989 these special plants were grown on the area of 408.3 ha, in the year 2003 it was 1,316 ha, and the prognosis for the year 2010 is 2,500 ha [2].
New development programme should contribute to the solution of the following task: to decrease a risk of production; to improve variable profitability of the single medicinal plant species; to propose and develop suitable species mechanization, chemical protection, high quality of seeds, determination of radiation and pollution, etc.; to desing progressive trimming lines, store-rooms, extraction and distillation equipment – post harvest technologies; to decresae a ration of manual labor; to establish functioning relation between suppliers and customers; to improve the skill of people working in the herb production.
For the perspective conception of medicinal, aromatic and spices plants cultivation and processing in Slovak Republic there is necessary to consider economical aspects of their production as well as the demands of industry [2,3]

Key words: medicinal, aromatic and spices plants, crop production, development programme

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ORAL PRESENTATIONS (LECTURES)

L 01

ECOBIOLOGY OF MEDICINAL PLANTS OF SOME PARTS OF SERBIA

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Ecobiological studies of medicinal plants included the study of soils and the study of ecological for medicinal plants. The investigations were carried out in Kosmaj (Šumadija) and Kopaonik (central part of Serbia). The aim of this investigations is to define the possible, limiting and hazardous factors for the development and exploitation of medicinal plants. The assesment of soil quality was based on the on the calculation of indicator values of the availability of nitrogen (N), phosporous (P), base cations and havy metals and sensitivity to acidification. By the regressive succesion of beech and oak forests, degrading phases with meadow species *Festuca vallesiaca* and *Holcus mollis* were developed. On both localities, about 45% plant species have medicinal characteristics. The most numerous are submediterranean and Eurasian species such as: *Galium verum, Origanum vulagre, Teucrium chamaedrys* etc.

Key words: soil quality, medicinal plants, plant ecological index, exploatation possibilities

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L 02

MOLECULAR TARGETS IN PHARMACOLOGICAL EVALUATION OF PLANT EXTRACTS

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Screening for specific activities in drug chemicals, including plant extract, can be performed at high efficiency and accuracy using in vivo and in vitro testing. Classical in vitro testing – such as cell culture, may gain sensibility and provide more information on mechanisms of action when signal transduction targets are involved. Such molecular targets may be built around cytokines production by competent cells, and further refinement of the model may be achieved by evaluation of cellular responses to cytokines in the same or in complementary test systems, including modified systems

expressing a reporter gene in relationship to the primary signal [1]. Such an experimental system, based on the regulation of luciferase by NfkB promoter permits good evaluation of dose-dependent reactions, and also, in association with other test systems, offers valid information on mechanisms of actions [2]. The luciferase modification can be performed on several established cell lines – macrophages, limphoblastoma, and hepatoma, thus providing valuable instruments for pharmacological assays [3,4].
Several plant extracts – complexes or pure compounds, were tested concerning their ability to regulate cellular reactions involving TNF-alpha signaling cascade (TNF-alpha synthesis, TNF-alpha dependent NFkB activation, apoptosis).

Key words: plant extracts, TNF-alpha, NFkB, luciferase

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L 03

STIMULATION OF THE YIELD OF CORIANDER (*CORIANDRUM SATIVUM* L.) BY MILD ABIOTIC STRESS UNDER FIELD-LIKE CONDITIONS

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Coriander, seeds of *Coriandrum sativum* L. (*Apiaceae*), has been used as spice and in herbal medicine since the ancient times. The main active constituent is essential oil (0.8 –1.8 % in most commonly planted *Coriandrum sativum* L. var. *Microcarpum* D.C.). Coriander essential oil, as many other secondary metabolites in plant kingdom, probably serves as a defense of the plant against herbivores and infections. Contents of numerous such substances could be elicited in *in vitro* plant cell cultures by the addition of stressing factors, so-called elicitors, that forces the plant cells to increase it´s „arsenal of warfare“. More, stress that does nor harm too much causes stimulation of growth and yield (the effect called *hormesis*).We have thus tried to elicitate the essential oil content and yield of whole intact plants of coriander by leaf sprays containing elicitors, namely titanium ascorbate complex and plant stress hormone salicylic acid in comparison with untreated controls. The yield was increased by up to 24% (by 10^{-3} mol.L^{-1} salicylic acid) while the essential oil content was increased by up to 7% (by 10^{-4} mol.L^{-1} salicylic acid) so the total yield of essential oil could be increased by up to 27% (by 10^{-3} mol.L^{-1} salicylic acid). This work was done with a govermental financial support as the part of the grant number 521/02/1378 of the Grant Agency of Czech Republic; grant KONTAKT ME704 and grant MSM 122200002 both of the Ministery of Education of Czech Republic.

L 04

ANTIOXIDANT STATUS OF RARE AND ENDANGERED PLANTS WITH MEDICINAL PROPERTIES FROM THE PANNONIAN PART OF SERBIA

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Many plant species belonging to the group of rare and endangered, show some medicinal properties. In northern parts of Serbia a great number of plant taxa from endemic and subendemic groups of the Pannonian plane is still present today, but gradual deterioration of natural habitats due to forest management, plant collecting, plowing, irrigation etc., resulted in decrease in their number [3]. In this study, we investigated *Lepidium cartilagineum* Thell., *Plantago schwarzenbergiana* Schur. and *Acorus calamus* L.

It is known that many environmental factors provoke stress in plants which may lead to reduction and activation of O_2 on a cellular level [1, 2]. In order to establish the level of oxidative stress in plants in their natural habitats, we evaluated plants antioxidant status. We determined the quantity of reactive oxygen species (ROS; $O_2^{\cdot-}$, $^{\cdot}OH$) and malonyldialdehyde (MDA), the activities of antioxidant enzymes superoxide dismutase (SOD) and guaiacol peroxidase (GPx), as well as the content of a non-enzymatic antioxidant reduced glutathione (GSH). In addition, the content of total flavonoids and DPPH-radical scavenging activity of plant extracts were measured as well.Results obtained showed the presence of ROS in plant material, as well as corresponding level of antioxidant defence mechanisms. Considering the activity of antioxidant enzymes and lipid peroxidation, it seems that plants were only partially susceptibile to the action of ROS. Thus, plants investigated were not exposed to significant action of negative environmental stimuli. Bearing this in mind, we may conclude and confirm that anthropogene influence is still the leading factor responsible for reduction in plant genotype fund, including both plants with medicinal properties and those who have not been phytopharmacologically evaluated.

Key words: endangered species**,** oxygen stress, antioxidants

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L 05

EFFECTS OF DIFFERENT COMPOUNDS FROM *DURANTA ERECTA* FRUITS

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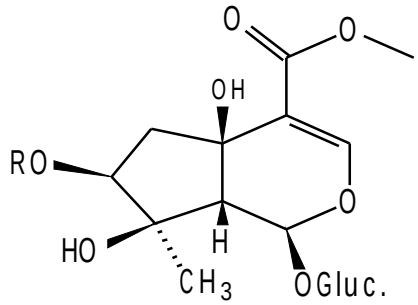
Duranta erecta (*Verbenaceae*), locally known as Miguelito, is a popular ornamental plant in Costa Rica for its perennial floration and beautiful golden fructification, located in groups. The fruits of this plant has been used in folk medicine, in China, for the treatment of malary and aczema. In Central

America, the decoction of the flowers and fruits are recomended as stimulant and febrifuge, respectively. The leaves are used as food to something larval stages of butterfly, which taking iridoids as mechanism of defense. However, the fruits are rejected for the commons depredator of the tropical forest, birds, insects and rodents. Also, is observed that not many plants growth around it. This suggest, that this plant contain possible alelopatic compounds.
Aqueous and ethyl acetate extracts obtained from fruits showed, *in vivo*, important antimalaric activity against *Plasmodium berghei*, and partial neutralization of antihemorrhagic effect induced for *Bothrops asper* snake venom.
From both extracts were identified, the lamiide iridoid 1, together verbascoside and four C-7 aryl lamiide derivatives (2-4), respectively. Also, hemolytic properties observed in aqueous extract indicate the presence of saponins.
The structural studied based on ^{1}H and 2D NMR experiments and pharmacological potential of this active constituents, will be discussed in comparison with similar properties publishing to *Duranta repens.*



1 R = H Lamiide

2 R = cinnamoil

3 R = (p-OCH3) cinnamoil

4 R = (3,4-di OCH3) cinnamoil

L 06

PHYTOCHEMICAL RESEARCH ON *ORIGANUM VULGARE* L. (LAMIACEAE) FROM ROMANIA

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The chemical composition of Origanum vulgare L. (Lamiaceae) from România was studied. A qualitative and quantitative analysis of essential oil, flavonoids, anthocyanins, phenyl-propanoid compounds of caffeic acid type and tannins were performed.
In "Origani herba" harvested from Drăgan Valley - West Carpathian Mountains in the middle of Transylvania a content of 0,58 ml essential oil/100g dried material was determined. A GC-MS analysis of essential oil showed that the phenolic compounds (thymol, carvacrol) are absent, even though the literature data show a high concentration in this oil; in Romania is the first experimental work on this subject.
The main components of the essential oil, among the 40, are sabinen (18,13%), b-cariophyllen (17,81%), D-germacren (15,63%), Δ-4-caren (5,83%), linalool (3,63%) and α-farnesen (3,25%).
In addition, in Origani herba 1,36% flavonoids, 5,1% phenylpropanoid compounds, 46mg/100g anthocyanins and 4,65% tannin were determined.

For flavonoids 19 fractions in HPLC, 5 in TLC (unidimensional) and 10 in TLC bidimensional were found.

Key words: Origanum vulgare, essential oil, flavonoids

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L 07

THE CHANGES IN APIGENIN, LUTEOLIN AND THEIR -7-O-GLUCOSIDES CONTENT DURING *HERBA MILLEFOLII (ACHILLEA COLLINA* BECKER EX. RCHB. VAR. ´ALBA´) ONTHOGENESIS

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The spasmolytic and antioxidant activity of *Achillea collina* Becker ex. Rchb. Var. ´Alba´(yarrow) has been attributed to flavonoids. The screening of flavonoids content in ten developing stages of yarrow flowering top *(Herba millefolii*) is the aim of this paper. The methanol extract of plant material (tops in stages from the beginning of flower bud differentiation, throw full flowering, until the stage of ripe seeds) has been used for HPLC analysis with column Nova-Pak C_{18} and acetonitrile / 0,1% TFA as the mobile phase with gradient program. Apigenin, luteolin and their 7-O-glucosides were found as the main flavonoid constituents in all developed stages of drug. Luteolin, apigenin and apigenin-7-O-glucosid amount have a similar trend during onthogenesis – their content is fluently increased until full flower phase and after than turns down. The maximal amount of this flavonoids riched about 600 $ng.g^{-1}$ of dry material. The luteolin-O-glucosid on the other hand has the highest content at the stage of small flower bud – it is more than 900 $ng.g^{-1}$ of dry material. The curve of luteolin-O-glucosid amount decrease later on and second top is riched together with the others flavonoids at the full flower phase on the level 600 $ng.g^{-1}$of dry material.

Key words: Achillea L., flavonoids, apigenin, luteolin, HPLC

L 08

A COMPARATIVE STUDY OF SOME VERONICA SPECIES

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The *Veronica* genus belongs to the *Scrophulariaceae* family and it includes 41 species in the Romanian flora. From the 41 species we can remark with demonstrate phyto-therapeutic activity *Veronica officinalis* (speedwell).*Veronica officinalis* has a specific areal and it is often substituted with another species of *Veronica* genus especially with *Veronica chamaedrys* We have tried to establish

some criteria to avoid the substitution of *Veronica officinalis* with other species of the genus *Veronica*, especially *Veronica chamaedrys*, widely spread and without therapeutic action. We have studied the differential histological, anatomical and phytochemical characters, for the two species. A rapid method for the identification of the two species is the TLC for flavonoids and phenyl-propanic compounds was done. We have done also a HPLC study wich has permited the detection of acteoside in *Veronica chamaedrys* and isoacteoside in *Veronica officinalis.* In order to confirm the supposed hypocholesterolaemic effect of *Veronica officinalis* (used in ethno-pharmacy like an hypocholesterolaemic agent) we have done an experiment in two steps for the two species of *Veronica: Veronica officinalis* and *Veronica chamaedrys*. *Veronica officinalis* in the diet showed no significant effect on the levels of cholesterol and triglycerides in the serum of the cholesterol free diet animals. *Veronica officinalis* also had a lowering effect on triglycerides and cholesterol level in the serum of high cholesterol diet animals.

Key words: Veronica officinalis, Veronica chamaedrys, hypocholesterolaemic effect, flavonoids, phenyl-propanic compounds

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L 09

ALKALOIDS BIOSYNTHESIS IN *PAPAVER SOMNIFERUM* L. SUSPENSION CULTURES TREATED WITH FUNGAL ELICITORS

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For *Papaver somniferum L.*, a very important pharmaceutical species, remain unknown problems linked to its “in vitro” operation. The very important one is alkaloid production, unsolved till now in cell or tissue cultures. The induction of biochemical responses of defence to the fungal elicitors treatment in cell cultures is accompanied by an activation of alkaloid production involved enzymes. The investigations on two elicitors (glucan fractions extracted from *Phythophthora megasperma* and *Alternaria cathami* cell walls) revealed their ability to stimulate the morphinan alkaloids production, depending of both the elicitors level and the tratment duration.. The cell suspensions were maintained in Gamborg medium, on shaker, without light.. The medium was changed at every 7 days, the suspensions were analysed after 24 and 48 hours of incubation. The calitative and cantitative samples analyses, by TLC and spectrophotometry, pointed out the presence of sanguinarine both in the control and treated variants. But, under the elicitor influence it was identified on other compound, which displaied a grey- blue spot with a low speed of migration and Rf belonging to thebaine.

Key words: Papaver somniferum, alkaloid, elicitor

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L 10

STUDIES ON *SCOPOLIA CARNIOLICA* JACQ. FROM THE SPONTANEOUS FLORA AND IN VITRO CULTURES

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Scopolia carniolica Jacq. (*Solanaceae*) is a herbaceous plant, 60-80 cm tall, with brown-red flowers, that grows at the basis of the forests in the Central and South-east Europe. In Romania it has few areals in the spontaneous flora and from some of them the plant is harvested in order to be used in the industrial extraction of scopolamine and atropine, alcaloids that are present in the rhizomes (Scopoliae rhizoma). Our study concerned two directions: the analyse of the vegetal mass harvested from the spontaneous flora and the study of possible in vitro cultivation of the plant for the protection of the environment from the excesive harvesting.
A morfological, anatomical and phytochemical analyse of the plant harvested in three different regions from the spontaneous flora was made. The medicinal product was investigated for its alcaloid content by TLC-densitometry and titrimetric means. The alcaloid content was established at 0,26-0,35% (g/g d.w.), the atropine and scopolamine being in 1: 0,43 ratio. The study of in vitro cultures concerned the possibility of cultivation, the optimal culture medium and conditions for the plant growth and the alcaloid synthese. We have investigated cultures of regenerated plants, calus and roots. The atropine and scopolamine content was determined by HPLC means. The root cultures developed in Gamborg medium could produce higher levels of alcaloids (atropine and scopolamine) than the others, also increased in the presence of putresceine. The atropine synthese was favorised by the light-dark exposure, while the scopolamine one was favorised by the dark exposure.

Key words: Scopolia carniolica, atropine, scopolamine, root cultures

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L 11

MORPHOGENETICAL, PHYSIOLOGICAL AND BIOCHEMICAL STUDIES OF *MENTHA VIRIDIS* L. IN *IN VITRO* AND *EX VITRO* CULTURES

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The study presents the results of the investigations concerning the morphogenetic reaction of some explants of *Mentha viridis* micropropagated *in vitro* as well as some aspects of the in field-grown regenerants behaviour. *In vitro* culture initiation was made with biological material originating from Chalkidiki (Greece), using shoot tips and nodes from the upper stem part. The explants were sterilised with a 5 % chloramine-T solution for 25 minutes and were inoculated on Murashige-Skoog medium (hormone-free or supplemented with 0.2 mg/l BAP).

The morphogenetic reaction of shoot nodes and tips was unusually uniform on numerous tested hormonal formuli in the respect of giving rise to neoplantlets. Only when we introduced 2.4-D (single or combined with citokinins) into the culture medium, the nodes, root fragments, internodes and leaves produced a friable, low proliferating, cream coloured callus tissue which does not differentiate and finally degenerates.
The in vitro regenerated neoplantlets registered higher values respectively: root length - on MS supplemented with BAP (0.2 mg/l), IBA (2 mg/l) and hormone free MS; stem length on MS containing BAP and NAA (2:1), BAP and IBA (2:1), BAP and GA (2:1) and the individual biomass on MS supplemented with BAP (0.2-0.5 mg/l), BAP and GA (2:1). Neoplantlet accomodation to the septic medium was easily achieved in a hydroponic system and the regenerants had no significant losses when transfered in field. At present we are developing biochemical, and morpho-physiological analyses of the field-grown regenerants.

Key words: Mentha viridis L., in vitro culture, micropropagation

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L 12

DEVELOPMENT OF FIELD GROWING TECHNIQUES OF NETTLE (*URTICA DIOICA* L.)

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A series of experiments were carried out during 1997-2003, aiming to elaborate field cultivation techniques for stinging nettle (Hakkarainen, 2000, 2004). Due to the high risk of direct sowing, we propagate nettle by transplantation of seedlings in spring or in September. The plant density is 5 seedlings/m. The life cycle of the nettle plantation is 6-7 years. Potato ridges seemed a very suitable mechanical weed control method for large-scale cultivation of nettle. Weedings are carried out mechanically by 2-3 times per growing seasons. Hand weeding is necessary during the first year only. During the first growing year one leaf harvest can be made with three consecutive leaf harvests during the following years. The leaf yield can be can be harvested by hay harvesters, when the nettle shoots are 35-45 cm high. The total leaf yield from the second year ranges between 15-30 t/ha. The optimum harvest time for stem yield was during the green seed phase, in early September. Harvesting of the 120-150 cm high stems can be carried out mechanically, using an old grain binder or modern big bale technique. The dried stem yield ranged between 4-6 to/ha.
The following questions need additional studies: direct seed sowing, chemical weed control, plant protection, improving the stem fibre content by breeding and the economical valuation of field costs.

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L 13

ASCERTAINING THE GENETIC VARIABILITY OF *ACALYPHA INDICA* L. IN TIRUNELVELI HILLS IN TAMILNADU IN INDIA

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In the wake of the current pace of habitat loss and depletion of plant genetic resources in the tropics, it is essential for the developing countries, which are rich in biological diversity, to evolve strategies and conscious efforts to scrutinize their resources and identify the variants of economic value for conservation and utilization. Although reliable estimates of genetic variability are now available for many plant species, they do not include medicinal plants. *Acalypha indica* L., a known herb in South India is widely used as a potent medicinal plant for skin diseases, toothache, earache, cough, and ring worms and burns. A cyanogenic glucoside, acalyphin, is obtained from the aerial parts of *Acalypha indica.* There is wide range of morphological variation observed. In order to ascertain the genetic variability, plants were collected from different populations at various localities and Random Amplified Polymorphic DNA fingerprints were analyzed by Polymerase Chain Reaction of genomic DNA using random primers. The RAPD fragments were scored for presence/absence to calculate Jaccard's similarity index. Clustering based on similarity index was done following unweighted pair group with arithmetic mean method and a dendrogram was constructed and analyzed.

Key words: Acalypha indica L., RAPD, PCR, Genetic variability.

L 14

INVESTIGATION ON DIFFERENT SAGE (*SALVIA OFFICINALIS* L.) CULTIVAR GENEPOOL

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There were introduced three sage cultivars of indigenous and allochthonous provenances to the field experiments realised in the Experimental station of the Research Institute of Agroecology in Michalovce. The influence of selected factors (cultivar, ecological characteristics over the years of cultivation) on the changes of sage dry herba qualitative – quantitative parameters was performed.
Statistically expressive significance of genotypes and ecological factors over the year of cultivation was confirmed on the productive potential of sage and essential oil biosynthesis respecting the required qualitative-quantitative parameters. The most effective influence of genetic factors was expressed as an important endogenous factors of herbal yield production, production of sage essential oil and its qualitative composition. The highest yield of dry herba were obtained at Italian cultivar Comune (increase about 0,5 $t.ha^{-1}$ in comparison of Slovak cultivar Krajova). The highest yield of essential oil was found out at Yugoslav cultivar Primorska. The most optimum values of sage dry herba qualitative – quantitative parameters were found out at cultivars Primorska and Krajova, at the same time the composition of their essential oils respond to the prescribed parameters.
Cultivation of sage and introducing new cultivars to the assortment grown in Slovakia has high perspective for demand expansion of this medicinal plant.

Key words: sage, cultivar, dry herba, essential oil

L 15

ELICITATION OF PHARMACOLOGICALLY ACTIVE SUBSTANCES IN WHOLE INTACT PURPLE REDCONE PLANTS [*ECHINACEA PURPUREA* L. (MOENCH)] UNDER FIELD-LIKE CONDITIONS

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One of the currently most used medical plants is *Echinacea purpurea* L. (Moench), a potent immunostimulant. As every organism, the plant can get into stressing situations – due to dryness, lack of mineral nutrients, bacterial, fungal or viral infection etc. A system of defense reactions has thus developed, involving changes in physiology and metabolism, that enable to overcome these situations. One of the key steps in this system is the induction of secondary metabolism. This principle is being widely used in plant cell culture (the stressing agents, so-called elicitors, are being added to the nutrient medium in order to increase the production of secondary metabolites). Despite this, up to our knowledge, no such approach was applied in the case of the whole intact plants grown in hydropony or on soil. Very promising results in the induction of contents of pharmacologically active substances (up to 10-times increase) in this plant were obtained with several organic and inorganic elicitors applied on *Echinacea purpurea* L. (Moench). This work was done with a govermental financial support as the part of the grant number 521/02/1378 of the Grant Agency of Czech Republic; grant KONTAKT ME704 and grant MSM122200002 both of the Ministry of Education of Czech Republic and Research Intention of ILE AS CR: AV0Z6087904 “Ecology of the man´s influenced landscape“.

L 16

THE RATIONALISATION OF SELECTED MEDICINAL HERBS PESTICIDAL CONTROL

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During 1999 – 2001, field trials were established and managed to observe pesticidal control on German Camomile (Bona variety) and St.Johnswort (Uperikon variety). Special trials were established at experimental research station of RIA – locality Vysoká nad Uhom and at experimental-breeding station PD “Roykvet” – locality Nová Ľubovňa. Ten herbicides, four insecticides and twelve fungicides were tested. The pesticide use were differentiated according to curing crop.
It was found that the weed infestation rate of medicinal herbs were strong to very strong. We registered 10 monocotyledonous and 32 dicotyledonous weed species. Graminicidal herbicides Gallant, Fusilade and Pantera had efficiency on the monocotyledonous weeds, especially Gallant. Fusilade and Pantera had soft low efficiency in comparison to Galant. Graminicides had no efficiency on dicotyledonous weeds. Opposite action was recorded in case of herbicides Gesagard, Afalon, Duplosan and Lontrel, they act on dicotyledonous weeds. Tested herbicides had different biological efficiency on present weeds. The most effective, but highly selective action had herbicide Lontrel. The

herbicide Afalon must be emphasize in term of biological efficiency evaluation. Treflan effected on mono- and dicotyledonous weeds. We registered, in action on monocotyledonous weeds, more favourable effect of specific granimicides in comparison to the wide-spectral Treflan. Herbicides Solar and Starane manifests itself in a high selectivity with biological impact against the cleavers (*Galium aparine* L.) mainly. We registered fytotoxic effect of herbicides on plants, in the course of tested amount, at german camomile stand only. The toxic effect on plant had Duplosan (5 points according 9-point EWRS scale) and Solar, Starane (2 points). During 2 – 3 weeks, the toxic symptoms has subsided. The toxic effect of tested herbicides was not registered on St.Johnswort. It is predictable the fytotoxicity of tested herbicides on other varieties of St.Johnwort. The higher efficacy of tested insecticides was decreasing in following order: Regent (9 points) Condifor (9 points) Nurelle D (8 points) and Karate (7 points, according 9 point = max., 1 point = min.). The efficacy we registered against *Cnephasia wahlbomiana* at German Camomile stand and *Byturus aestivus* at St.Johnsonwort stand.

On the strenght of results we can make the following downward order of tested fungicides, decrease efficacy: Hattrick, Juwel, Tango – 9 points; Bravo, Ripost – 8 points, Baycor – 7 points, Acrobat, Euparen Multi, Sandofan, Tatto – 6 points; Dithane, Champion – 5 points. The powdery mildew presence was medium to strong in spotted localities and years.

For definitive recommendation to put pesticides into practise it is necessary take into consideration results of residual tests, eventually standpoint of register authorities, criteria done by State Institute for Drug Control.

Key words: German Camomile, St.Johswort, herbicides, insecticides, fungicides

L 17

UTILIZATION OF TRAITS' VARIABILITY IN GERMPLASM OF FLAX (*LINUM USITATISSIMUM* L.) FOR PHARMACEUTICAL USE

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In our experimental work we evaluated 265 genotypes of flax in 23 quantitative and in 19 qualitative traits, according to evaluation system of descriptors which are situated into ours innovated list of descriptors. Low coefficient of variation (V < 10 %) was determined for these traits – length of petals (9,0 – 16,2 mm), width of ball (5,17 – 7,55 mm), width of seed (1,98 – 2,72 mm), length of seed (3,56 – 5,28 mm). Medium degree of variability (V = 10 – 20 %) was detected at these traits total length of stem, thickness of stem, length of leaves, diameter of opened flower, width of petals, length of sepals, length of ball, weight of thousand seeds. High degree of variability (V = > 20 %) was found at traits technical length of stem (187 – 516 mm), length of panicle (48 – 215 mm), total number of balls per plant (3 – 18 no), weight of seeds per plant (0,08 – 0,75 g), number of seeds per ball (2 – 9 no), total length of seeds per plant (12 – 96 no). We compared variability of experimental set with variability of world (n = 2320) and Canadian (n = 19) collection by the basic statistical measures and by comparative-diversity index (D_{comp} %) in six quantitative characters (Diederichsen, Richards, 2001). By comparison of world collection with our experimental collection were detected the lowest index of comparative-diversity (D_{comp} = 23,9 %) at the trait length of flowering, and the highest value of comparative-diversity index (D_{comp} = 93,92 %) which was reached by the trait weight of thousand seeds. Significant ($p \leq 1\%$) and absolute correlation coefficient ($r \geq 0.5$) were observed as follows: the diameter of opened flower and length of petals are positively correlated with size of seeds. Negatively correlated are length and width of ball with total and technical length of stem.

Key words: flax, linear dependence, morphometric analysis, qualitative characters, and quantitative characters

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L 18

CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM *PELARGONIUM RADENS* AND EFFECTS IT PRODUCES UPON MICROORGANISM CULTURES

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Samples of Pelargonium radens have been taken before the flowering period, for anatomical identification of the secretor structures and essential oil extraction and analysis. The essential oil of P. radens before the flowering period has been found in amount of approximately 0,33% from the fresh material weight. Essential oil was extracted by steam distillation using a modified Clevenger apparatus, whereas the chemical composition was analysed by GC – MS, and for P. radens it was found to have 64 components. The main components of P. radens essential oil are: citrinelol: 28,7%; mentone 27,3%; citronelil formate 8,4%, β-endesmol 4,81%, feniletil caproate 1,6%, geraniol 1,6%, isomentone 1,58%, as well as other components, in a concentration of less than 1,5%. The microorganisms tested were strains of Escherichia coli, G(-) and Staphylococcus aureus, G(+) using the antibiogramme method. Essential oil was tested in a concentration of 1000ppm and 500 ppm, solved in DMSO. The effect of DMSO was tested and was found null. At these concentrations, the essential oil of P. radens was found to have no effect upon E. coli and inhibitory effect upon S. aureus.
Key words: *Pelargonium radens*, essential oils, chemical composition, effects upon bacteria

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L 19

ANTIOXIDANTS IN FRUITS OF *PHYSALIS ALKEKENGI* L.

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The fruits of Physalis alkekengi L. (Solanaceae), a species growing at borders of forests, in glades, bushes and osier-beds, are used in traditional Romanian folk medicine as a tonic, diuretic, analgesic and antirheumatic [1]. Due to its orange calyx surrounding the fruits like a lampion, the species is cultivated as an ornamental plant. The fruits were reported to contain vitamin C, sugar, organic acids, pectins, citric acid, carotenoids and fatty oil in the seeds [3]. In the course of our phytochemical investigation of Physalis alkekengi L. we now report on lipophilic and hydrophilic antioxidants of this fruits. Several extracts have been submitted to CC, TLC and HPLC analysis. From the petroleum ether extract there have been separated and identified alfa-cryptoxanthin, beta-

cryptoxanthin, cis-beta-cryptoxanthin, beta-carotene, lutein, polyhydroxyxanthophylls as well as xanthophyll mono- and diesters. Amongst the hydrophyllic antioxidants, extracted with 80% methanol and fractionated over a silica gel column, we identified luteolin-7-O-glucoside, chlorogenic and caffeic acid. The content of vitamin C (115 mg%) was determined by a spectrophotometric method according to the Ph.Hung. VII. The quantitative determination of the proanthocyanidins (735 mg%) was carried out by the proanthocyanidin (butanol/HCl) assay [2].

Key words: Physalis alkekengi L., lipophilic antioxidants, hydrophilic antioxidants

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L 20

MISTLETOE (*VISCUM ALBUM* L.) EXTRACTS IN PREVENTION OF *IN VIVO* CCL_4-INDUCED OXIDATIVE STRESS – BIOCHEMICAL STUDY

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In this paper effects of different extracts obtained from mistletoe berries and leaves on some biochemical parameters in rats liver homogenate and blood hemolysate were examined. Intensity of LPx in rats blood was increased after application of each extract of mistletoe berries, as well as combination of extracts and CCl_4. Et_2O and $CHCl_3$ extract inhibited LPx in rats liver. Mistletoe leaves extracts haven't shown statistically significant changes of the LPx values neither in rats blood nor in rats liver homogenate (except Et_2O extract that increased the LPx in a very high degree). We can conclude here that mistletoe extracts showed protective effect. As for CAT activity, it was reduced by two extracts (nBuOH and H_2O fraction). Intoxication with CCl_4 caused the increase of CAT activity, while the combinations extracts/CCl_4 significantly decreased its activity. Activity of XOD was especially reduced upon treatement with $CHCl_3$ fraction, as well as combination extract/CCl_4. H_2O fraction increased, while nBuOH decreased Px activity in a very high degree. It is interesting that combination nBuOH/CCl_4 significantly increased this activity. The content of GSH has not been changed upon teatment with mistletoe extracts (except small decrease with nBuOH fraction), but it has been significantly reduced after intoxication with CCl_4. Combination extract/CCl_4 increased GSH content. Activity of GSHPx was decreased in a very high degree after using H_2O fraction, but significant reduction was also obtained after treatment with combination $CHCl_3$ extract/CCl_4. We concluded that ethanol solutions of different extracts of mistletoe leaves showed some very good antioxidative and protective properties.

Key words: oxidative stress, Viscum album, antioxidants

L 21

DISTILLATION METHODS USED IN THE CZECH REPUBLIC FOR DETERMINATION OF ESSENTIAL OILS CONTENT

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Essential oil content is one of the main criteria for quality in medicinal and aromatic plants and spices used as raw materials for the pharmaceutical and food industry. The quality of the raw material follows guidelines and requirements as described in the Czech Pharmacopoeia 2002 and the Food Code (419/2000). However, several modifications to standard distillation methods are used, and the essential oil content for the same sample is often different in different laboratories. The differences can be several fold. Therefore, one sample of caraway (*Carum carvi* L.) was sent to several laboratories for essential oil determination. The essential oil content ranged between 2.35 and 6.40 %. The effect of distillation method, grinding intensity, size of element and grinder type was investigated. Even when the extreme values were eliminated, it was not possible to recommend any particular method. It is therefore suggested that a distillation method be used which provides the least variation in yield. This method would enable reliable comparison of samples from different origin. Consequently, it is proposed to use unground samples to eliminate one of the most significant factors that influences essential oil content.

Key words: distillation, essential oils, caraway

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L 22

THE EFFECT OF CADMIUM ON THE VOLATILE COMPONENTS AND DEVELOPMENT OF *ORIGANUM VULGARE* AND *THYMUS VULGARIS*

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Origanum vulgare and *Thymus vulgaris* were grown at 0, 10, 20, 40, 60, 80 and 160 ppm of Cadmium (Cd) for a period of 4 months. The accumulation of Cd in the roots of plants was much higher than in the leaves. At the maximum concentration of Cd, oregano plants had 19 times more Cd in the roots than leaves. With the same treatment thyme roots had 4 times more Cd than leaves. While at the begining of the experiment plants grew well in the presence of Cd, at the end of the experiment the growth rate was significantly lower than the contols. Chlorophyll content increased in both species at 10, 20, 40, 60 and 80 ppm of Cd in the soil, while a negative correlation between leaf area and chlorophyll content was observed. No significant change in content and composition of essential oils was found. Furthermore, no Cd was detected in the essential oils of either species.

Key words: oregano, thyme, cadmium, essential oils, chlorophyll

L 23

EFFECT OF ENVIRONMENTAL POLLUTION ON ANTIOXIDANT STATUS OF *EQUISETUM ARVENSE* L.

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Equisetum arvense is the most common and widespread species of the genus *Equisetum*. *Equisetum* species accumulate a highly complex pattern of phenolic compounds, i.e. various flavonoids, styrylpyrone glucosides and caffeic acid esters [1]. They have been shown to be of major importance in protecting plants against pathogens but also have pharmacological and antioxidant properties. The aim of our study was to compare the antioxidant statuses of two different *Equisetum arvense* biotypes. Biotype wich was free from environmental stress influences was used as a control. Contaminated biotype was piked up near oil rafinery. In this study antioxidant activity of above ground parts of *Equisetum arvense* L. was investigated. Next biochemical parameters were determined: activities of antioxidant enzymes (superoxide dismutase, catalase, peroxidase, glutathione peroxidase), quantities of malonildialdehyde (MDA), superoxide and hydrohyl radicals and reduced glutathione, contents of total flavonoids, soluble proteins and pigments, DPPH-radical scavanger activity and inhibition of nitric oxide production. Furthermore, scavanger activities of plant extracts were determined by ESR spectroscopy. Ecological stress provoked decrease of antioxidant enzyme activities, but quantities of nonenzymatic antioxidants increased. Scavanger activity of stressed plant extract, measured by ESR, increased by 25,2 % compared to the control. As a result, hydroxyl radical content and MDA quantity decreased in stressed biotype which showed better antioxidant properties than the control.

Key words: Equisetum arvense, antioxidant status, ESR.

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POSTER PRESENTATIONS

P 001

THE RESOURCE POTENTIAL OF MEDICAL PLANTS OF NORTH-EASTERN RUSSIA

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The flora of vascular plants of North-East of European Russia consists of 2000 species, 130 of which are included into the State Pharmacopoeia of Russia and are permitted to be used in medicine. The base of resource potential is made by typical taiga species: Vaccinium vitis-idaea L., V. myrtillus L., Rubus idaea L., Sorbus aucuparia L., Padus avium Mill. Their biological stock in the region is more than 900 thousand tons. In modern conditions the usage of the stock of this plants varies from 2 to 90%. The system of stocking in the region is not enough developed. The main volume is stocked up by local dwellers. The raw material is used for personal needs. More than 50 species of vascular plants used in people's and scientific medicine have insignificant stocks and are rare and are included into The Red Books of Russia and regions. The biggest quantity of such species is registered in family Orchidaceae, that is slightly presented in studied region. Significant damage is done to the populations of Rhodiola rosea L., Paeonia anomala L. To decrease exploitation press on this species their cultivation and protection are needed.

Key words: biological stock, usage, protection, cultivation

P 002

THE DYNAMICS OF BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS FOR SPECIES OF *PELARGONIUM* CULTIVATED IN IASI BOTANICAL GARDEN

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There have been studied three species of *Pelargonium*: *P. zonale* (L.), *P. radens* (H.E. Moore), *P. fragans* (Willd). Samples have been taken in two different ontogenesis periods, before and during flowering period, respectively. The biochemical and physiological parameters studied have been: the quantity and quality of the essential oils, photosynthesis and pigments. The extraction of the essential oils has been done by steam distillation using a modified Clevenger apparatus, whereas components have been studied by the gas –chromatography method. The essential oils of *P. zonale* have been found in amount of approximately 0,1%, with 49 components during the period before flowering, and approximately 0,15% with 161 components during the flowering period. For *P. radens* there has been extracted an amount of approximately 0,33% essential oils with 64 components during the period before flowering and approximately 0,38% and 44 components during the flowering period. *P. fragans* was found to have approximately 0,16% essential oils with 11 components before flowering and 0,23% amount with 69 components during flowering period. The photosynthesis process has been less intense before the flowering period, for all three species. The photosynthetic process has been found more intense during the flowering period.
Key words: Pelargonium, biochemical and physiological parameters, essential oils, photosynthesis, pigments.

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P 003

AGASTACHE RUGOSA FISCH – THE NEW PROSPECTIVE MEDICINAL PLANTS

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Medical property Lophant anise (*Lophantus anisatus* Adans) or Manispiker wrinkled (*Agastache rugosa*) is to reduce blood pressure and has strong bactericide property. In Mongol medicine over ground part of plant is used for regulation the metabolism, for generally strenghten and warning of getting old means infusion of flowers is used internally and externaly for treatment paralysis of face nerve and trembling of limbs.

This useful plants was investigated in Zakarpatian Institute of Agroindustrial Production from 1989 year. For years of research we taked out the variety named ´Pochatok´ (beginning), which had been by state variety – tast commisionin 2003 year. It can reproduce Agastache rugosa with seeds and by vegetative means by division of busch, by rootstocks or shoots. The most economical means especial under growing on big areas is sitting in to open soil. The preparation of soils is reasonable in autumn. The tilling on the depth is 8 – 10 sm after harvesting of crop precursor and after 15 – 20 days it is provided tilling on the ploughing horizont. Under the base cultivation it is reasonable to put 30-40 t/ha of organic fertilizers, 80-100 kg/ha nitrogen, 100-120 kg/ha of phosphorus and 30-40 kg of active substances of potassium. Under growing seeding in warm houses or film houses it is reasonable to use such microelements as Mn, B, Cu, stimulator of grows such as "Rostoc" manifest well.

For goods Agastache rugosa is mowed during period of budding and to the end flowering. Over ground green mass serves as raw material according to our research includes 20 – 25 % of flowers, 40 – 45 % leaves, 30 – 40 % stems.

Thus the growing Agastache rugosa as medicinal and spicy plants is rather prospective for conditions of West region of Ukraine.

Key words: Agastache rugosa, cultivation, fertilizers

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P 004

THE METODOLOGICAL PRINCIPLES FOR EVALUATION OF MEDICINAL PLANTS BIOMASS

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In the Romanian geobotanical literature (between 1970 and 2000), there are a lot of chorological and phytocoenotical data concerning the main spontaneous medicinal plants from different regions of the country. Generaly, herbal medicinal plants participate to the building up of phytocoenosises as companion species, achieving a covering of up to 35%. Each constitutive species presented in the phytocoenotical tables has been given a grade representing its covering in the stand table (relevé area). This grades were confered by geobotanists in accordance with the six-stage coverig scale drawn up by Braun-Blanquet (1964).
In order to be able to use this rich geobotanical material for the evaluation of medicinal plants biomass, we elaborated a simple and easy to apply methodology. To reach our goal, during our fields researches in Transilvania, we intended to emphasize the correlation between the covering degree of the medicinal plants species that are present in the phytocoenosises' structure and the species' populations' biomass on the unit of surface (g/m^2). For each species that has been object of a field research and has been given a grade in accordance with the real field covering, we sampled 3-5 vegetal biomass samples so as to characterise each covering coefficient. The material has been weighted directly in the field using an automatical scale. The results were statistically processed, by calculating the mean and the standard error for each grade given to the covering. As an example of the model there were presented the results obtained on the *Achillea millefolium* and *Hypericum perforatum*.

Key words: medicinal plants, biomass, evaluation method

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P 005

MEDICINAL PLANTS ON SERPENTINITE OF DIVCIBARE

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Divcibare is part of Mt Maljen, which is situated in the west of central Serbia. The geological layer of Divcibare, by most part, is serpentnite. The soils that form on this tipe of geological layer have such physical and chemical properties which unfavourably influence on plants. Because of that, vegetation on serpentinite is poor.
About 380 plant species are described on Divcibare. From this number about 30,50% species are medicinal plants. In this area *Lamiaceae* family is the reachest with medicinal species, then follow *Asteraceae, Rosaceae, Fabaceae* and *Ranunculaceae* families. Medicinal plants that are described in this area are not distinct indicators of serpentinite. They are facultative serpentinophytes, what means that these plants are able to adapt on edaphic condition of this site, but their populations are more numerous and denser on some other kind of geological layer. Among them are: *Erica carnea, Teucrium montanum, Primula veris, Achillea millefolium, Filipendula ulmaria, Anthyllis vulneraria, Anemone nemorosa, Centurium umbellatum, Hypericum perforatum* etc. The most of these species are very good adapt on serpentinite, what their abundance, presence degree and sociality confirm. It is very important to notice that on Divcibare grow species which are endangered taxa in Serbia. Some of them are medicinal plants, such as *Gentiana lutea* (endangered due to intensive exploitation).

Key words: medicinal plants, serpentinopyhte, serpentinite

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P 006

LEAF GLANDS OF THE SPECIES *THYMUS PANNONICUS* COLLECTED IN SERBIA

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Genus *Thymus* is represented in Serbian flora with the total of 31 species. *Thymus pannonicus* has sporadic distribution in Serbia, and it can be found both in the northern part of the country (Vojvodina) and on mountains of the East Serbia. *Th. pannonicus* is a chamaephyte, with shoots and leaves densely covered with trichomes. Leaves are sparsely glandular-punctate, whereas glands are peltate. The number of glands per leaf square mm was surveyed on populations collected from Vršačke mountains in Vojvodina. Plant material was taken in the flowering phase during three consecutive seasons, starting from 2002. Specimens collected in 2002 had averagely 5.33 ± 1.18 (minimum 1.18 and maximum 7.40) glands per square mm at the upper leaf epidermis and 7.88 ± 2.22 (ranging from 2.22 to 12.78) at the lower leaf surface. The average number of glands per upper leaf area of accessions collected in 2003 and 2004 was 5.02 ± 1.72 and 6.58 ± 1.65, respectively, and 6.19 ± 1.92 and 6.73 ± 1.39 of glands of the lower epidermis. According to the analysis of variance (ANOVA) there are statistically significant differences in number of leaf glands of the *Th. pannonicus* populations depending on the vegetative season, i.e. habitat conditions, suggesting the phenotypic plasticity in character such as the number of leaf glands.

Key words: *Thymus pannonicus*, glands, Vršačke planine

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P 007

ENDANGERED SPECIES OF SLOVAK FLORA AS POTENTIAL RESOURCES OF MEDICINAL PLANTS

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Territory of Slovakia belongs to the two different phytogeograpraphical regions in Central Europe. West Carpathian flora as upland (mountain) and Pannonian flora as lowland territory have included, whit very different geomorphological and climatic characteristics. More than three thousand autochtonous vascular plant species have been selected at least 20 000 years in this territory and clearly enriched the natural resources of Slovakia. Only these 5 –6 % species of the native flora have been used for directly exploitation for various human activities. Using some methods of botanical investigation it is possible to recommended 15-20 % species to exploitation as potential genetic resources for agriculture, horticulture, pharmaceutics and forestry. Related to solving scientific and technical projects we concentrated our attention to exploitation of native endangered plant species populations that are promising from the aspect of their exploitation in medicinal plant production. We analysed selected populations of some perspective threatened plant species: *Althaea pallida* Willd., Crataegus lindmani Hrabětová, *Empetrum nigrum* L., *Ephedra distachya* L., *Rosa gallica* L., *Teucrium scorodonia* L., *Tribulus terrestris* L. and *Verbascum speciosum* Schrad.

Key words: endangered medicinal plants, genetic resources, Slovakia

P 008

COMPLEX APPROACH FOR THE STUDY OF SOME ROMANIAN *CROCUS* SPECIES

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A survey of the already described *Crocus* species throughout the Mediteraneean region, up to W- China, revealed a number of 80 (Mabberley, 1998). According to this genus monograph (Mathew, 1980), 43 *crocus* species are dispersed through the Europeean regions. The analyses of this genus have been based on traditional approaches, comprising the morphological characters (presence of profiles, the nature of style, characteristics of corm tunica etc.). However, recent modern approach indicated the requirement of reconsideration of these classification, based on DNA markers.

The *Crocus* genus is represented in Romanian flora by 6 species (*C.vernus, C.banaticus, C.reticulatus, C.pallasii, C.flavus, C.chrysantus*). Among these, *C.pallasii* has received a special concern as a putative medicinal and aromatic plant, based on its use and similarities to *C. sativus* (soffran).This species, togather with the other two one (*C.chrysanthus*) need special attention for establishment of their endangerment degree in Romania.

Therefore, a complex biodiversity approach, imlying chorological and populational studies, as well as morphological ones, has been developed in our laboratory. RAPD markers based on two primers have been used also for estimation of DNA random amplicon polymorphisms and therefore the similarities between the species of aromatic interests.

P 009

PHENOTYPIC VARIABILITY DETERMINATION OF TRAITS OF HULL LESS SEEDED OIL PUMPKIN POPULATION (*CUCURBITA PEPO* VAR. *STYRIACA*)

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The aim of our research is detection of plants with seeds appropriate for another use and food processing technologies by morphometrical analyze. We analyzed 160 fruits of Styrian oil pumpkin population in 16 quantitative traits. Tested characters showed important degree of variability – weight of fruit (400 – 7870 g), volume of fruit (0,5 – 14,4 l), length of fruit (92 – 236 mm), width of fruit (100 – 313 mm), thickness of fruit skin (1 – 8 mm), thickness of pericarp (7 – 45 mm), total weight of fresh seeds (1 – 257,3 g), total weight of seeds after drying (0,5 – 158 g), number of matured seeds (8 – 800 no), number of non-matured seeds (2 – 494 no), width of seed (6,83 – 12,04 g), length of seed (11,20 – 32,73 g), thickness of seed (1,79 – 4,09 g). Also were observed 7 qualitative characters – color of pulp, shape of apical part of fruit, shape of stalk part of fruit, shape of fruit, intensity of orange color on the fruit skin, structure of fruit skin, color of seed. Simultaneously were prepared image documentation (960 detailed images) of shape of fruit from side, apical and stalk part, cross cut of fruit, and seed. We use international list of descriptors UPOV for species *Cucurbita pepo* L. (UPOV 2002) for evaluating of quantitative and qualitative characters. Data of morphometrical analysis were summarized and calculated by statistical and mathematical methods.

Key words: hull less seeded oil pumpkin, morpho-metric analyses, image documentation, and phenotypic variability.

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P 010

CATALOGUING OF GENOTYPES FROM EXPANDED POPULATION OF POPPY (*PAPAVER SOMNIFERUM* L.)

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Slovakia is characterized by long – time history of breeding and cultivating poppy (*Papaver somniferum* L.) and also its utilization for food and pharmaceutical industry.
Our objective is to insure systematic examination, valuation and preservation genotypes of poppy in the gene bank. Very significant for these activities is cataloguing of genotypes with the application of modern information and communication technologies. Basis cataloguing of genotypes is main characteristic of genotypes, valorization of morphology and economical descriptors and their classification accord of consolidated classificator. This characteristic of genotypes is possible to complete with a picture documentation, which allows to more complex detection of differences among genotypes. In the experimental study in all genotypes are important characteristics of flowers, leaves, capsules and seeds.

P 011

MEDICINAL FLORA OF LIMESTONE PLANT COMMUNITIES IN THE EASTERN SERBIA

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The participation of medicinal flora in various plant communities developed on limestone terrains of eastern Serbia, such as ass. *Danthonietum calycinae, Agrostetum vulgare, Arrhenatheretum elatioris, Potentilleto-Caricetum humilis, Poetum violaceae, Semperviveto-Seslerietum argenteae* i *Anthylleto-Seslerietum rigidae* was analyzed in order to comprehend the status of genetic resources of wild medicinal plants and, thus the opportunities and directions for their further exploitation.
Surveyed plant associations are spread at the altitude between 800 and 1500 m and in a range of water regime conditions.
In analyzed plant communities of eastern Serbia the total of 118 medicinal plants was registered, which accounts to 28.09% of the overall number of medicinal plants quoted for Serbia.
A need for preservation of these habitats may be illustrated by the presence of natural rarities, such as *Orchis militaris L.*, which has been categorized as a vulnerable plant species according to the Red Data Book of Serbia. Among the other species of the family *Orchidaceae*, the following species protected by the CITES were also noticed: *Orchis morio L., Orchis mascula L., Dactylorhiza sambucina(L.) Soo* and *Gymnadenia conopaea (L). R. Br.*
The species *Arctostaphylos uva-ursi L.*, which adhere to the group of the rare plant species, exhibits high abundance (4.5) in the ass. *Anthylleto-Seslerietum rigidae*, while significant populations of different species of the genus *Thymys* L. such as *T. jankae Cel., T. pannonicus, T. marschallianus Willd., T. montanus W. K.*, and *T. pulegioides L.* were found in the investigated limestone communities.
It was concluded that limestone plant phytocoenoses of the eastern Serbia are particularly rich in medicinal plants, of which of a number is considered as threatened and therefore important from the aspect of biodiversity maintenance, including both the natural rarities and endemic species.

Key words: medicinal flora, limestone, Serbia

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P 012

GENOTYPES OF BASIL (*OCIMUM BASILICUM* L.) IN SERBIA AND MONTENEGRO

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Basil is a multipurpose plant used as an ornamental, seasoning, medicinal and ritual plant (1,2). During several years of research (1995 – 2003.) a greater number of populations of basil was collected on the territory of Serbia and Montenegro out of which 10 typical genotypes were selected.

Their evaluation and characterization was conducted on the basis of which the first national descriptor for basil was formed. Morphological, chemical and microbiological characteristics of selected genotypes were analyzed. By means of statistical data processing, then based on cluster and factorial analysis, it was established that the material was divergent. On the level of all 10 genotypes genetic variance made up the greatest part of total phenotype variability. Heritability coefficient in broader sense ranged from 48,73% to 96,77%. By method of chromatographic analysis it was established that ether oils of the tested genotypes belong to the European chemotype with the dominant component lynalol which ranged from 53% to 76% and as small amount of estragol.
Based on the results of the research we grouped genotypes which could be used as seasoning (T-3, T-5, T-6) and as ornamental plant (T-1, T-2, T-4, T-6, T-8, T-9, T-10), and all ten could be used as as source of medicinal herbal raw material Basilici herba and Basilici aetheroleum.

Key words: basil, description, evaluation, descriptor,

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P 013

LATEST ADVANCES IN CONSERVATION OF MAP'S IN MACEDONIA

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Despite of its small territory (25,333 sq km), Macedonia is very reach in plants diversity, approximately 3500 vascular plant species and is at the top of the list of states called “European Hotspots” According to data from native and neighbors folks medicine there are more than 700 plants with medicinal features, but approximately only 200 species are applied more frequently. All these approximations are due to the fact inventarization on the flora in Macedonia is still not completed, therefore an inventarization of the medicinal and aromatic plant is yet to be done.
There are still laws that are up taken of the former Yugoslav legislative, most of them dating from early seventies. Therefore collecting, trade, cultivation, manufacturing, quality control, maintenance, preservation, conservation and evaluation of indigenous and introduced medicinal and aromatic plants in Macedonia is not regulated entirely.
Macedonian legislative body has ratified a number of international regulations and conventions. As a permanent member of CBD, Macedonia is liable to prepare national biological and landscape diversity conservation strategy and appropriate legislative background.
Recently, Ministry of Environment and Physical Planing has produced, first a Country Study on Biodiversity Status in Macedonia followed by a Biodiversity Strategy and Action plan which regulates, through a number of strategic principles and approaches, a lot of maters concerning MAP conservation in R.Macedonia.
There are several projects, concerning conservation of MAP's, ongoing now in Macedonia

Key words: MAP’s, conservation, Macedonia

P 014

INVESTIGATION A FAMOUS MEDICINAL PLANT - BEARBERRY (*ARCTOSTAPHYLOS UVA-URSI*) NATURAL POPULATIONS IN W. CARPATHIANS

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The results of population biology of *Arctostaphylos uva-ursi* (L.) Spreng. (bearbery) in Slovakia (West Carpathians) are presented. Size structure, population size, population density, spatial structure, ways of reproduction, natural and human risk factors were studied. Only non-destructive methods of population biology for clonal plants were used. Populations were monitored on 14 selected localities in 4 phytogeographical districts: Nízke Tatry (Low Tatras), Fatra, Slovenský raj (Slovak paradise) and Tatry (High Tatras). Populations are small or very small, included only a few individuals (1-23(-100)) polycormons). Population density is only 0,005 – 0,05 polycormons /m^2. Spatial structure is aggregated in all populations. Aggregated spatial structure is affected by microrelief and biology of this species. The average length of polycormons was 1,35 m and average width of polycormons was 1,06 m. Polycormons were 0,025 - 9,6 m^2 large. Vegetative reproduction of *Arctostaphylos uva-ursi* is only one way of reproduction at the time. Seed production was very low. No seedlings and juvenile plants was recorded during years 1998-2003. The most important human factors negatively determined surviving of most populations belong: pasture, collecting plants for rocky gardens and their medicinal effects, reafforestation and especially imission and tourism. Six concrete measures were recomended for protection of species, which eliminate this factors and range protection „in situ“, cultivation of species in the botanical gardens, arboretums and in „in vitro“ conditions.

Key words: *Arctostaphylos uva-ursi*, endangered species, population biology, reproduction, West Carpathians

P 015

THE SPONTANEOUS MEDICINAL PLANTS REGISTERING FROM THE TÂRNAVE PLATEAU

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This study presents in a rigorous research the medicinal spontaneous flora of Târnave Plateau. The research was accomplished from 2000-2004. The floristic inventory includes 210 vascular medicinal plants distributed in 63 families. In this work the right solutions were adopted as correct in accordance with the *Code de Tokio*, 1993. The mostly medicinal plants belong to the following families: *Rosaceae* (22 sp.), *Asteraceae* (19 sp.), *Lamiaceae* (17 sp.), *Apiaceae* (12 sp.), *Fabaceae* (9 sp.), *Scrophulariaceae* (9 sp), *Brassicaceae* (7 sp.) [3,6]. The medicinal plant species were chemically analysed, indicating the predominance of the tannins (16,113 %), followed by volatile oils (10,426 %), alkaloids (10,426 %), flavonols (9,478 %) saponins (8,056 %) and mucilages (7,109 %) [1,2,4,5]. We necessarily consider to acknowledge the medicinal flora from this area of the country, less investigated by the professionals, in order to highly turn to good account their phytotherapeutical effects.

Key words: medicinal plants, active principles, Târnave Plateau.

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P 016

VARIABILITY OF ECONOMIC TRAITS ON EXPANDED POPULATION OF SERVICE TREE (*SORBUS DOMESTICA* L.) IN SLOVAKIA

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Our aim is to present variability of selected economic traits on Service tree (*Sorbus domestica* L.). Genotypes are from five localities: Modrianskej, Moravsko Lieskovskej, Nitriansko Jeleneckej, and Zemplínskej. Evaluation of selected traits was done in years 2000 – 2003. The characters of fruits were observed: width of fruit, length of fruit, weight of fruit, and index of fruit shape. The most of fruits had medium wide fruit – 25 % (25 – 25,9 mm), length of fruit was mostly represented by short fruits – 29 % (24,9 – 26,1 mm), 46 % were fruits with medium weight (9,86 – 12,35 g) and index of fruit shape was represented by fruits with oval shape in 38 %. Obtained data were characterized by descriptive statistics. For quantitative characters were determined intervals of variability. Obtained information will use for preparation of list of descriptive traits our examined plant species.

Key words: sorbus domestica, characters variability, descriptor list

P 017

IN VITRO CLONAL PROPAGATION OF *LEUCOJUM AESTIVUM* L.

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Summer snowflake (*Leucojum aestivum* L.) contains the important alkaloid galanthamine used in the treatment of numerous diseases of the nervous system including Alzheimer's syndrome. Herbage has been gathered for years from the wild as crude drug for the Bulgarian patented medicine Nivalin which led to extreme decrease of the natural resources of the species. *Ex situ* strategy aiming the conservation of *Leucojum aestivum* wild populations in Bulgaria was developed including different parallel ways [1]. One of them is the biotechnological production of galanthamine. Earlier studies showed that bulb, leaf and stem fragments of *L. aestivum* have a high morphogenic potential and could be successfully used for *in vitro* micropropagation [2]. To compare the regeneration potential and the stability of the alkaloid biosynthesis in long-term *in vitro* cultures, plants of known origin and galanthamine content taken from 9 wild Bulgarian populations were cultivated on a same medium and equal conditions. Cycle procedure for clonal micropropagation was established: uniform (5-6 mm in

diameter) *in vitro* obtained bulblets were divided vertically into 4 equal parts and grown on MS medium with 2 mg/l BAP and 0.15 mg/l NAA, at about 25°C under long photoperiod. Adventitious bulblets appeared between the scales after 4 weeks of cultivation. Approximately 500 well formed bulblets could be obtained from a single one for a period of one year. Bulblets seem to be the best source of explants for rapid *in vitro* multiplication of *L. aestivum* biomass as they are free of contamination and available during the whole year.

Key words: Leucojum aestivum, in vitro clonal propagation, galanthamine

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P 018

POISONOUS PLANTS OF TURKEY

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Turkey is in a rich geographical area from the flora and fauna point of view. From the flora point of view, Turkey is the richest country in Europe and in the Middle East with more than 10 000 plant taxa, and more than 3 000 of which are endemic. The poisonous plant taxa among this higher number of plant taxa distributing in Turkey is expected to be higher. Therefore, in this study the poisonous natural plant taxa distributed in Turkey are identified and given in the list with appropriate explanations of each one of them.
Results of this study showed that 2 388 plant species belonging to 99 families are identified as poisonous in Turkey. The families with highest number of poisonous plant species are identified as *Fabaceae* (571), *Liliaceae* (243), *Ranunculaceae* (140) and *Asteraceae* (126). *Families* with the highest number of poisonous genus are *Fabaceae* (15), *Asteraceae* (14), *Liliaceae* (14) ve *Ranunculaceae* (11). Among them all of the members of 90 genera are identified as poisonous. Among these genera, the top three from the number of poisonous species point of view, are *Astragalus* (347), *Verbascum* (220) and *Allium* (141).

Key words: Poisonous plant, Turkey

P 019

PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS USEFUL AS THE MARKERS OF SUSCEPTIBILITY OF MEDICINAL AND AROMATIC PLANTS TO STRESS FACTORS

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Physiological methods may give to the researchers involved in the studies on medicinal and aromatic species the ability to assess the condition of plants influenced by non-optimal environmental

factors. In this paper we will show the usefulness of two popular methods: conductivity test (estimation of the permeability of cell membranes; electrolyte leakage parameter [1]), and Fv/Fm parameter of chlorophyll a fluorescence (given by various fluorometers, so-called potential quantum efficiency of photosystem 2 [2]).
Our studies on lemon balm, sweet basil, German chamomile, sage and maize revealed that electrolyte leakage parameter is a useful tool for estimation of condition of plants during drought, waterlogging, chill, elevated ozone concentration, and on recovery period.
Fv/Fm parameter should be used with the consciousness that its decline may reflect destructive influence of studied factor on PS 2, but in case of low-temperature stress the explanation may be the acclimatory processes leading to light energy dissipation. In this case, additional physiological (e.g. water content and relative water content, gas exchange), and biochemical assays (e.g. carotenoid content [3]) should be implemented to obtain the full information about plant's status.

Key words: stress, membrane permeability, electrolyte leakage, Fv/Fm

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P 020

INDIRECT CAULOGENESIS AT *CHRYSANTHEMUM MORIFOLIUM* RAMAT

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The technologies of vegetative multiplication "via callus" offer opportunities for obtaining and propagation of superior genotypes.
This paper aims to study the "in vitro" behaviour of single-node stem segments excised from two cultivars of Chrysanthemum morifolium Ramat: "Escorte" and "La Cagouille"., belonging to the collection of Botanical Garden from Iasi (Romania).
It were obtained and multiplicated the calli of Chrysanthemum morifolium Ramat, which have been subjected to regeneration. The process of callus formation was favoured by relatively high citokinine (2mg/l BAP) concentrations in the MS medium. This callus was generally friable, green-yellow in colour. After six weeks of incubation, one of these callus masses formed adventitious shoots buds from cells close to the callus surface.
The capacity of shoot differentiation was observed on MS medium with 2mg/l BAP and 0,002 mg/l NAA. Shoots of 2-3 cm, elongated "in vitro" were rooted on the MS medium in the absence of growth regulators. The autonomous regenerated plants displayed an vigorous growth and adapative capacity by transferring on soil.

Key words: Chrysanthemum morifolium Ramat, callus, organogenesis

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P 021

GENETIC TRANSFORMATION OF *ATROPA BELLADONNA*

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Plants are tremendous source for discovery of new products of medicinal value which nowadays are considered less risky then those of microbial or animal origin. The beneficial medicinal effects of plant material tipically result from the endogenous combination of their secondary products. The progress of the genetic engineering domain with medicinal plants has been ascribed however to the incresed yield in pharmaceutically important secondary metabolites. One of the model medicinal plant for such studies, *Atropa belladonna*, a member of the *Solanaceae* family, is abundant in tropane alkaloids, actually used as antimicrobial compounds in pharmaceutical drugs. The general aim of the genetic manipulation with medicinal plants is referred to as developing techniques for gene transfer into their genomes in order to improve the biosynthetic rate of the interest compounds. An essential concern in such strategies regards therefore the choice of the environment friendly transformation system (*Agrobacterium tumefaciens or A.rhizogenes mediated ones*) and the adequate marker genes for an efficient assistance of the practical gene transfer process, as well as for the regeneration and maintaining of the genetic manipulated plants.
This study represents the first Romanian approach to establishing the experimental and analytical conditions for an efficient indirect gene transfer into A.belladonna genome. A comparative study of the effect of the two *Agrobacterium* plasmids, Ti and Ri – wilde(oncogenes harboring) and modified (marker genes *nptII* and *gus* harboring), upon the biosynthetic rate of the alkaloids is described. In vitro and molecular (genotype and phenotype) assays are presented for characterization of the transformed lines. The evaluation of the alkaloid yields is presented by using biochemical (spectrophotometric and HPLC) methods..

P 022

COMPARATIVE STUDY OF DIFFERENT GERMPLASM OF *GLYCYRRHIZA GLABRA* AND *STEVIA REBAUDIANA* FOR EXPLOITATION OF THEIR SWEETENING CHARACTERISTICS

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Glycyrrhiza glabra and *Stevia rebaudiana* are extensively cultured medicinal plants species of interest for their sweetening chracteristics. These are the result of their secondary metabolites, respectively,. Their germplasm is of great value both from the point of view of the study of their amelioration and of in vitro and field conservation.
The protocol of induction of the in vitro culture of *G.glabra and S.rebaudiana* is presented. An *Agrobacterium rhizogenes* mediated genetic transformation method has been applied for both species in order to increase the yield of the two active sweetening principles. A comparative efficiency of such genetic manipulation is described in terms of the transforming capacity of a wild A.rhizogenes strain and of the spectrophotometric results of both glycyrrhizic acid and stevioside is presented. The possibility of amelioration of these plants and obtaining efficient root cultures is described.

P 023

RESEARCH OF ESSENTIAL OILS FROM PLANTS OF *ASTERACEAE* FAMILY BY GC-MS METHOD

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Essential oil-containing plants widely occurring in the flora of Kazakhstan are considered to be a renewable raw material for obtaining of new biologically active compounds which are used in the development of original phytopreparations.
In this regard, we are conducting systematic studies of essential oils from plants of Asteraceae family to identify perspective sources for new phytopreparations.
Asteraceae family is the largest one in the world's flora accounting for 10% of all highest plants. The flora of Kazakhstan which is especially rich with the representatives of this family (over 1000 species) is considered to be a potential source for the isolation of essential oils.
Using chromatomass-spectrometry, we've conducted complete characterization of constituents of essential oils from 31 species of Asteraceae family. The constituents of essential oils from *Achillea cartilaginea* L., *Achillea grandiflora* Bieb., *Achillea sudetica* Opiz., *Artemisia armeniaca* Lam., *Artemisia kasakorum* (Krasch) Pavlov, *Artemisia proceraeformis* Krasch, *Artemisia sericea* Web, *Artemisia Tournefortiana* Rchb. have been characterized for the first time.
It was shown that the essential oils from the studied plant species can be used as a renewable source of mono- and sesquiterpenoids in the synthesis of new biologically active compounds. For example, □tuion and spatulenol were major constituents of essential oils from *Artemisia proceraeformis* Krasch, *Artemisia armeniaca* Lam. and were found to occur in sufficient amounts for preparative processing and further usage in chemical modification.

P 024

DETERMINATION OF THE MAIN PHENOLIC ACIDS IN *AMARYLLIDACEAE* BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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The *Amaryllidaceae* comprises five species spread in Bulgaria: Pancratium maritimum L., Sternbergia colchiciflora W. K., Galantus nivalis L., Galantus elwesii Hook., Leucojum aestivum L. The phenolic acids (various benzoic and cinnamic acid derivatives) in the family might be a useful chemical marker in chemosystematic sudies (1, 2). In the present work the HPLC profiles of phenolic acids from the overground parts of each species were attained in order to contribute to the chemosystematic determination of the taxa. An improved high-performance liquid chromatography (HPLC) method was developed for the simultaneous determination of eleven phenolic acids.
The proposed HPLC separation was performed using Hypersil ODS, 5μm (250 x 4 mm) column on a Varian (USA) chromatographic system with tertiary pump Model 9012 and UV-VIS detector Model

9050 set at 280 and 310 nm. The mobile phase was composed of methanol - 20mM phosphate buffer, pH 2.92 and linear gradient elution mode within 40 min at flow rate 1.3ml/min was applied.
The relative standard deviations of the repeatability and reproducibility of the developed method were ≤ 1.82 % and ≤ 3.94 % respectively. The detection limits for the analyzed acids ranged from 0.3 ppm (e.g. p-coumaric acid) to 4.7 ppm (e.g. 3-hydroxybenzoic acid) and quantification limits ranged from 1 ppm (e.g. p-coumaric acid to 15.7 ppm (e.g. for 3-hydroxybenzoic acid).
The common constituents, occurring in the investigated five species are protocatechuic, 4-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric and ferulic acids. The obtained results revealed the tendency of higher content of ferulic and vanillic acids. The analysed samples have shown an identical HPLC profile with the exception of seeds from Sternbergia colchiciflora.
The determined protocatechuic, 4-hydroxybenzoic, vanillic and syringic acids in *Amaryllidaceae* are reported for first time.

Key words: Amaryllidaceae, HPLC, Phenolic acids

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P 025

STUDY OF *PODOCYTISUS CARAMANICUS* BOISS. & HELDR., A GREEK FABACEAE WITH PHYTOESTROGENIC ACTIVITY

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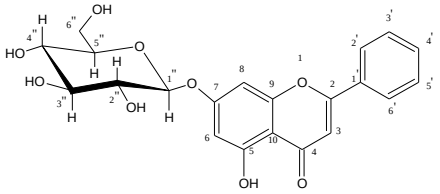
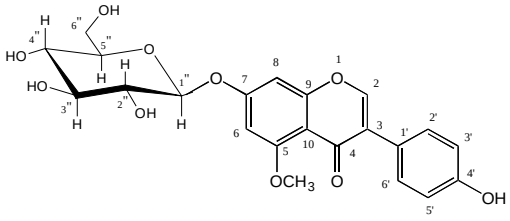
Phytoestrogens are natural compounds which are interesting for their hormonal action and their potentially protective effects.
Fabaceae are known to produce these kind of compounds, especially isoflavones. Thus, *Podocytisus caramanicus* Boiss. & Heldr., a greek Fabaceae, has been selected after preliminary biological tests on MCF-7 cells. *Podocytisus caramanicus* is a perennial, not climbing, shrub, distributed in Greece, Albania, former Yugoslavia and Turkey [3]. The plant material was collected near Ioannina (mountain Tymphi) in june 2001. A voucher sample has been deposited at Ioannina herbarium, under the accession number In 022.
Methanolic crude extract of the aerial parts shown a proliferative effect on human breast cancer cell line MCF-7.
Dichloromethanic and methanolic extracts were fractionated. Different chromatographic methods, including Centrifugal Partition Chromatography (CPC), led to the isolation of many compounds. Two of them were identified as chrysin 7-*O*-β-D-glucopyranoside [1] and 5-*O*-methylgenistein 7-*O*-β-D-glucopyranoside [2]. Their structure were established on the basis of their spectral data and by comparison with literature data.
Biological tests and structural identifications are in progress in order to identify bioactive fractions.



chrysin 7-*O*-β-D-glucopyranoside



5-*O*-methylgenistein 7-*O*-β-D-glucopyranoside

Key words : Podocytisus caramanicus, phytoestrogens, Fabaceae, chrysin 7-O-β-D-glucopyranoside, 5-O-methylgenistein 7-O-β-D-glucopyranoside.

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P 026

HPLC ANALYSIS OF THE SURFACE FLAVONOID AGLYCONES IN *VERONICA CHAMAEDRYS* L.

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V. chamaedrys L. is one of the most polymorphic species within the *Veronica* genus, widely spread in Europe. The populations of V. chamaedrys develop in variable ecological conditions from 0 to 2400 m above sea. Free flavonoid aglycones were shown to accumulate externally on overground parts of the plant species (1, 2).

Surface flavonoid profiles of two populations belonging to *V. chamaedrys* from natural habitats in Bulgaria (Black Sea region) and Romania (Transilvania) were analysed by high performance liquid chromatography. The separation of flavonoid aglycones in the acetone exudates of examined samples was achieved by isocratic elution mode using a reversed phase Hypersil ODS, 5 μm, 250 x 4.6mm (Shandon, England) column. The analyses were performed on a Varian (USA) chromatographic system with tertiary pump and UV-VIS detector set at 360 nm. The mobile phase was composed of *tert.*- butanol, methanol and 20 mM potassium dihydrogen phosphat (pH 2.98). The obtained results revealed the presence of common external flavones: luteolin, chrysoeriol, apigenin, 4'methoxyapigenin and 6,4'-dimethoxyscutellarein. Chrysoeriol and 6,4'-dimethoxyscutellarein were the main compounds in the Bulgarian population, whereas 4'methoxyapigenin was present in very small amount. The dominant level for the luteolin was detected in the population from Romania; in addition, 4'methoxy apigenin and 6,4'-dimethoxyscutellarein were also present in much lower concentration. The results obtained suggest that the distribution of surface aglycones in the species *V.chamaedrys* is quite different and the variety in flavonoid profile corresponds to the pattern of geographic variation within this taxon.

Key words: Veronica chamaedrys, HPLC, Surface flavonoid aglycones

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P 027

HPLC ANALYSIS OF NEPETALACTONE CONTENT IN SHOOT CULTURES OF *NEPETA RTANJENSIS*, GROWN UNDER DIFFERENT CARBOHYDRATE SOURCES

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The medicinal properties of catmints (*Nepeta spp.*) are usually attributed to their essential oils and flavonoids. The majority of *Nepeta* species are characterized by the presence of iridoid monoterpenoids-nepetalactones in its essential oils. Like other volatile compounds, nepetalactones are usually determined by GC after distillation of aerial parts. Recently, it has been reported that HPLC is a suitable alternative. HPLC method described in our study enables nepetalactone identification and quantification from small sample amounts (250 mg) and is useful tool for investigation of *in vitro* accumulation of nepetalactones in *Nepeta rtanjensis*, an endemic and critically endangered plant of Serbia.
The current investigation was carried out to determine potential for increasing the concentration of medically important biomolecules (nepetalactones) by exposure to elevated carbohydrate levels (sucrose, fructose and glucose) in culture medium. Changes in growth characteristics resulting from different carbohydrate supplies were also studied. The increasing concentration of sucrose, fructose and glucose led to an increased shoot growth and nepetalactone production. High concentrations of all carbohydrates inhibited growth of shoots and accumulation of nepetalactone as well.

P 028

QUANTITATIVE DETERMINATION OF MANGIFERIN IN VITRO PROPAGATED PLANTS OF *GENTIANA ASCLEPIADEA* L. BY HIGH PERFOMANCE LIQUID CHROMATOGRAPHY

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Gentiana asclepiadea L. is perennial herbaceous plant widely distributed in South and Central Europe, including mountain area of Serbia. Importance of this species, as well as genus *Gentiana*, is in production of bitter glucosides, xantones and other pharmaceutical active compounds.

The aim of this research was to establish *in vitro* propagation of *G. asclepiadea* and to analyse the effect of applied phytohormones on content of mangiferin.
In vitro propagation of *G. asclepiadea* was examined by shoot growth on WPM (woody plant medium) medium supplemented with various concentrations of cytokinine and different auxins. Various concentrations of gibberellic acid were used to investigate the effects of GA_3 on stem elongation and multiplication.
The content of mangiferin in different plant material was determined by high performance liquid chromatography (HPLC). The analysis revealed that the content of mangiferin in the shoots from *in vitro* culture varied with different concentration of applied phytohormones. There was no detectable content of mangiferin in roots obtained *in vitro.*

P 029

1,8-CINEOL AS A STANDARD SPECIMEN OF PHYTOPREPARATIONS BASED ON ESSENTIAL OILS FROM *ARTEMISIA*

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Essential oils present a mixture of volatile aromatic substances that are formed in plants and belong to different classes of organic compounds, mainly to terpenoids, possessing high biological activity which allows their medical and pharmacological applications.
The scientists of the Institute of Phytochemistry (Karaganda) have developed the inhalation form of 20% alcohol solution of essential oil from *Artemisia glabella* Kar. et Kir possessing wound-healing, antimicrobial and anti-injflammatory activities; and "Darmenin" ointment based on the essential oil from *Artemisia cina* Berg. which was shown to have a high anti-fungal activity against fungi causing onicho- and dermatomycosis in the course of screenings.
Gas-chromatography analysis showed that main constituent in the essential oils in question was 1,8-oxido-*p*-menthan (1,8-cineol). In this connection, we've developed the technology for the isolation of 1,8-cineol as a standard specimen including the following stages:
Extraction of essential oils by steam distillation;
Separation of oils to cineol-rich fractions by vacuum distillation;
Isolation of 1,8-cineol by column chromatography on neutral aluminium oxide at the purity up to 98.2%;
Freeze drying of obtained cineol at temperatures from 0 to $-5\ ^0C$ within 5-8 hours, purity – 99.9%. 1,8-cineol at the purity if 98% was included to the Standard Specimen List of European Pharmacopoeia-2000. The purity was verified by gas-liquid chromatography. The analysis was conducted on a column from stainless steel; the height of the column was 2 m and the diameter was 2 mm. Silicon elastomer SE-30 was used as a stationary phase, programmed temperature range was from 60 to $220\ ^0C$ at the rate $4\ ^0C$/min; evaporator's temperature - $200\ ^0C$; moving phase (a mixture of gases – inert argon : hydrogen : air = 1: 1 : 10); gas-carrier flow rate - 30 ml/min; the sample was loaded at the volume of 1 mcl.
Thus, the isolated compound was confirmed to be 1,8-cineol at the purity of 99.9% with the help of IR-, TLC, GLC, chromato-mass-spectrometry and by qualitative reactions.

P 030

COMPOSITION OF ESSENTIAL OIL FROM *STIZOLOPHUS BALSAMITA* (LAM.) CASS. EX. TAKHT.

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Stizolophus balsamita (Lam.) Cass. ex. Takht. is a high annual plant growing in Southern Kazakstan.
The composition of the essential oil from *S. balsamita* was investigated as a part of chemical studies of plants in Kazakstani flora.
The aerial part of *S. balsamita* was collected in South-Kazakstan region in 2003. Essential oil was obtained by water distillation procedure for 2 h using Clevendger-type apparatus. Yield of essential oil was 0,1%. Composition of essential oil was analyzed by GC/MS using Hewlett-Packard GCD system.
There were identified 64 components constituting 71% of the oil. Spathulenol, a sesquiterpene alcohol was found to be the main component of the essential oil from *S. balsamita*, and its content in the essential oil was 12,5%.Other major components were: phytol – 8,7%, β-ionone – 4,2%, carryophylene oxide – 3,4% and germacrene D – 3,3%.

P 031

ISOLATION AND IDENTIFICATION OF ACTIVE SUBSTANCES FROM *LEONURUS CARDIACA*

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The aim of this work is isolation and identification of active substances from lyofilisated tops of *Leonurus cardiaca* and investigation of their biological activity. *Leonurus cardiaca* L. (Lamiaceae) is a herb native to Eurasia. It is commonly used in folk medicine, though not much scientific investigation has been made on its active substances yet [1] . Extract obtained by extraction with 96% ethanole in the Soxhlet apparatus was further divided by partitioning between immiscible solvents. The ethylacetate part was separated by column chromatography. 9,1mg of light yellow crystals were isolated. M.p. 256-259 °C. Mass number is 578.53. UV and IR spectra indicate presence of a flavonoid aglycone. From preliminary NMR results we can conclude that the isolated substance consists of an apigenine aglycone joined to 6C sugar and p-coumaric acid. The isolated substance has weak antioxidant activity [2].

Key words: Leonurus, apigenin, p-coumaric acid

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P 032

SECONDARY PLANT METABOLITES AS ANTIMICROBIAL COMPOUNDS FOR THE LIVESTOCK INDUSTRIES

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Secondary metabolites represent a diverse group of natural products [1]. Although precise numbers are, at best, an estimate, at least 100,000 different compounds of natural origin have been described, of which at least 80,000 of those are derived from plants (PSM's). Many of these have been isolated and their structures have been elucidated by GC, GC-MS, NMR or x-ray diffraction [2]. However, it is likely that many more than these will be isolated in the future, as increasing numbers of novel plant species are being identified and studied for their potential use in the pharmacological, medical and agricultural industries. Of particular importance currently in the EU, is the restricted use of antibiotics as prophylactic feed additives, and the ensuing increased risk of disease, especially in the intensive industries. The biological effect of various PSM's on microbial populations is therefore of considerable interest. Tannins inhibit microbial activity by binding to macromolecules, especially cell—wall associated proteins involved in attachment, and are largely regarded as being antinutritional [3]. Volatile oils also inhibit microbial processes although precise mechanisms are not known. We have established a novel *in vitro* screening procedure to evaluate the effect of PSMs on anaerobic microbial activity in gastrointestinal samples from poultry, and on known pathogens. Results demonstrate selectivity of some PSM's against gut pathogens and suggest potential alternatives to in-feed antibiotics.

Key words: Plant secondary metabolites, volatile oils, tannins, antimicrobial activities, livestock

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P 033

DETERMINATION OF THE ESSENTIAL OIL CONTENT AND COMPOSITION OF SOME *ORIGANUM* SPECIES CULTIVATED IN GREECE

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The genus *Origanum* (Lamiaceae) consists of 38 species, widespread all over the Mediterranean area. The species *Origanum vulgare* L. and O. onites L. are used as condiment, while the prominent *O. vulgare ssp. hirtum* (Link) under the name "Greek Oregano" remains the only species systematically cultivated in Greece. *O. majorana* L., essential oil is involved in the food industry and the perfumery;

the herb, that is habitually garden - cultivated in Greece is used as spice and condiment. Because of the great industrial interest of *Origanum* species, we investigated the essential oils from *Origanum vulgare* L. *ssp. vulgare*, *Origanum vulgare ssp. hirtum* (Link) Ietswaart, *Origanum onites* L., and *Origanum majorana* L., cultivated in different sites of Northern and Central Greece. The water-distilled essential oils were analyzed by Gas Chromatography and Gas Chromatography – Mass Spectrometry. As main constituents of the essential oils resulted carvacrol (77.80%) and thymol (2.37%) for *O. vulgare ssp hirtum*, germacrene D (22.75%), β-caryophyllene (12.86%) and sabinene (10.49)% for *O. vulgare ssp. vulgare*, and carvacrol (22.83%), thymol (19.74%) and terpinene-4-ol (9.34%) for *O. majorana*. The essential oil content for the cultivated samples was determined at 4.65% for *O. vulgare ssp.hirtum*, at 0.28 % for *O. vulgare ssp.vulgare*, *4.4% for O. onites* and 4.8% for *O. majorana* correspondingly reaching the highest essential oil contents in correspondence to those scattered in the Literature for wild only growing populations.

P 034

TRITERPENE SAPOGENINS FROM *ASTRAGALUS CORNICULATUS* BIEB. (*FABACEAE*)

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Two oleanane-type triterpene were isolated from the ethanol extract of the aerial parts of *Astragalus corniculatus* Bieb. Sapogenins were obtained after acid hydrolysis of a saponin mixture, followed by column chromatography on Sephadex LH-20 and preparative TLC. The structures of the compounds were elucidated as 21α-hydroxyolean-12-ene-28-oic acid and 3β,19α-dihydroxyolean-12-ene-28,21β-olide – a new natural compound. Structure determination of these compounds was established on the basis of spectral data (IR, EIMS, NMR). The ^{13}C- and ^{1}H NMR spectra of sapogenins have been assigned using ^{13}C DEPT, HMBC and NOESY techniques.

P 035

PHYTOCHEMICAL STUDY OF *GYPSOPHYLA TRICHOTOMA* WEND. (*CARYOPHYLLACEAE*)

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Gypsophyla trichotoma Wend (*Caryophyllaceae*) is spread in the northeastern region of Bulgaria, along the Black sea coast. The work presents the results of phytochemical studies of the roots and the aerial parts of *Gypsophyla trichotoma* for flavonoids and saponins. The 80% methanol extracts are treated with different solvents and purified by column chromatography (Silica gel and Shephadex LH-20) and preparative thin-layer chromatography. After final purification on Shephadex LH-20, ten constituents have been isolated. Three of them were identified as hyperoside, vitexin and orientin. The identification of the remaining compounds is in progress.

P 036

PHENOLIC COMPOUNDS OF *SCLERANTHUS ANNUUS* L. (*CARYOPHYLLACEAE*)

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Scleranthus annuus L. (*Caryophyllaceae*) is widely spread in Bulgaria. In traditional Eastern medicine it is used for treatment of malignant tumours of the womb and in Bulgarian folk veterinary medicine as remedy against pasteurellosis. Triterpene saponins are only reported its biologically active compounds. The aim of this investigation is to study the phenolic acids and flavonoid composition in aqueous/alcoholic extracts from the overground parts of the plant. After purifying selected fractions are analyzed by HPLC. The analyses are performed on a Varian (USA) chromatographic system included UV-VIS detector Model 9050 set at 220, 280, 310 nm for the phenolic acids and 360 nm for the flavonoids. The separation of derivatives of benzoic and cinnamic acids is achieved using a reversed phase Spherisorb ODS-2, 5μm (250 x 4.6 mm) column by linear gradient elution mode. The mobile phase is composed of methanol and 20 mM potassium dihydrogen phosphate adjusted to pH 2.98 by *o*-phosphoric acid. The separation of several O-glycosylflavonols and C-glycosylflavons is achieved using a reversed phase Superspher RP18, 4 μm, (250 x 4 mm) column by isocratic elution mode. The mobile phase is comprised of *tret*.- or *iso*-butanol – methanol – 20 mM potassium dihydrogen phosphat adjusted to pH 3.22 by *o*-phosphoric acid. The obtained results reveal the presence of protocatechuic, 4-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric, ferulic, o-coumaric, salicylic and benzoic acids and luteolin, kaempferol, rutin, isoquercitrin, isoorientin, vitexin-4'-rhamnosid, isovitexin.

P 037

OPTIMALIZATION OF ANTIOXIDANTS EXTRACTION FROM WOOL MULLEIN *(VERBASCUM DENSIFLORUM)*

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Wool mullein (*Verbascum densiflorum Bertoni*) is one of the natural sources of antioxidants. Among the biologically active compounds we can mention flavonoids 1,5-4%, carotenoids 0,2-0,4%, phenolic acids 0,1-0,3%, iridoid aucubin, 6-β-xylosylaucubin, methylcatalpol, isocatalpol, verbascozid etc. [3, 5, 6].

The effect of different solvents (ethanol, methanol, acetone, petrolether, diethylether, tetrahydrofuran) at various temperatures (20, 40 and 60°C) on the yield of carotenoids, flavonoids, antioxidant and antiradical activity have been studied. The total carotenoids content was determined by the Britton method [2]. The content of flavonoids was determined by Christ-Müller method using quercetin as a reference compounds [4]. The $DPPH^{\bullet}$ antiradical activity of extracts was estimated according to the procedure reported by Brand-Williams [1]. Antioxidant potential of mullein extracts was estimated from their ability to reduce Mo(VI) to Mo(V) in the presence of pfosphor. The resulting green colour being linearly related to the amount of antioxidants content [7].

Methanolic extract obtained at 60°C was the most effective $DPPH^{\bullet}$ radical scavenger and showed the highest antioxidative power. The amount of carotenoids and flavonoids showed correlation with the inhibition of $DPPH^{\bullet}$ radicals only to 90 minute at 60°C. After this time radical scavenging activity decreased. The following order of antioxidant and antiradical activities of mullein extracts was found:

methanol > tetrahydrofuran > ethanol > acetone > diethylether > petrolether at each tested temperatures.

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Key words: antioxidants, extraction, Verbascum densiflorum

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P 038

ACCUMULATION OF RUTIN DURING FLOWER DEVELOPMENT IN CULTIVATED IN BULGARIA *SOPHORA JAPONICA* L.

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Quantitative changes in the accumulation of rutin have been investigated in flowers of cultivated in Bulgaria *Sophora japonica* during ontogenetic and seasonal development. A method for isolation of rutin with good recovery (98%) was developed. The subsequent high performance liquid chromatography (HPLC) separation of flavonoid glycosides was achieved using Hypersil ODS, 5μm (250 x 4 mm) column on a Varian (USA) chromatographic system. The mobile phase comprised methanol and 20mM phosphate buffer, pH 2.92 and linear gradient elution mode within 25 min at flow rate 1 ml/min was applied.
Accumulation of rutin started with initiation of the buds, having the highest content in mid – July and tended to have high level until mid – August. The amounts of rutin were five- fold higher in July as compared to September.
The amount of rutin increased during the earliest stages of bud development up to 266 mg/g and can account for up to 90% of flavonoid glycoside mixture in younger buds. A further decrease in the amount of rutin up to 44 mg/g was found proportionally with the decline of flavonoids throughout and after the flowering period. Rutin was the predominant glycosides in young buds, whereas sophorobioside dominated at later development stages.
No significant differences in rutin quantity between the analysis periods of two successive years were observed, this clearly indicated the ontogenetic dependence of rutin formation.

Key words: Sofora japonica, flowers, rutin, HPLC, accumulation

P 039

COMPARATIVE PHARMACOGNOSTICAL RESEARCH ON *ACHILLEA MILLEFOLIUM* L. AND *ACHILLEA DISTANS* W.ET K.

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Achillea distans W. et K. (Asteraceae) may substitute, in mountain regions, the officinal Achillea millefolium (yarrow), the former having a greater high leaves and inflorescences. Achillea distans was not been investigated till now in Romania and our preliminary results have indicated some important differences.
For chemical characterization of the two species the comparative qualitative and quantitative analysis were made. By means of GC-MS we have identified the main 30 components of the essential oils ; the most interesting observation was the low concentration of chamazulene and bisabolol, in the Achillea distans essential oil, important compounds for the pharmacological activity of this vegetable product.
The polyphenolic compounds (flavonoids, caffeic acid derivatives), were also analysed by HPLC for both species.
By the microbiological tests, the essential oils showed a better activity against Staphylococcus aureus, Bacillus subtilis, Candida albicans.
The antiinflammatory action was also tested by the plethysmometric method.

Key words: Achillea millefolium, Achillea distans, essential oil

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P 040

CONTRIBUTIONS TO THE PHARMACOGNOSTIC AND PHYTOBIOLOGIC STUDY OF THE SPECIES *ONOPORDON ACANTHIUM* L. (ASTERACEAE)

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The use of the species *Onopordon acanthium L.* (Asteraceae) is known only in the traditional medicine. The goal of our study was to perform phytochemical analysis on different parts of the plant using different methods, such us: cromatographical methods (TLC, HPLC, GC), spectrophotometrical (AAS, photocolorimetrical) and gravimetrical methods. Flavonoids were identified and quantitatively determined: 2,13 % expressed as rutosid, polyphenolcarboxilic acids 0,160 % expressed as caffeic acid, tannins 8,02 %, sesquiterpene lactones, alkaloids 0,8 %, proteins 5,40%, ascorbic acid and minerals.
Phytobiological study were carried on tissues with multiple divisions such as the radicular meristems. We noted the nuclear, cytoplasmic, and chromosomal changes, under the influence of the aqueous extract solutions of different concentrations of Onopordon acanthium L. The morphologic changes of the cells can be observed using the Feulgen coloration method, revealed an inhibitory effect. In

conclusion we may emphasize that Onopordon acanthium L. can be used as a potential souce of bioactive principles.

Key words: Onopordon acanthium L.,Phytochemical research, Inhibitory effect.

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P 041

MORPHOGENETIC AND PHYTOCHEMICAL CHARACTERISATION OF THE *IN VITRO* OBTAINED *CASSIA ANGUSTIFOLIA* VAHL. TISSUE CULTURES

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The investigation on *Cassia angustifolia* aimed to study the morphogenetic, physiological and biosynthetical peculiarities of the callus and shoots obtained on different hormonal variants of the culture medium. The explants were prelevated from plants selected on the basis of their raised bioproductive qualities.
The number of differentiated shoots varied with the cytokinine/auxine concentration. The combined action of BA and 2,4-D (1.5 : 0.5) favoured both the differentiation process and the biomass accumulation in callus, the explants of the stem nodes proving the greatest morphogenetical callusogene reaction.
To test the biosinthetycal capacity at callus and shoot level of *Cassia angustifolia* obtained *in vitro*, compared to the *herba* prelevated from the control, cultivated in a natural medium, we identified some pharmacologically active substances (polyphenols, flavons and triterpenes). Compared to the shoots, at callus level we noticed an important reduction of the biosynthetic spectrum for polyphenols and flavons (apigenin, luteolin and a coffeic acid derivate).
The phytochemical investigations reveal that the flavonoidic and polyphenolic aspect of the callus is different from that of the shoots (the number of spots is greater than in case of the callus), both presenting quantitative differences compared to *herba*. The secondary metabolites, evidentiated by TLC, in callus and shoots, are synthetised as in the original material with quantitative and qualitative differences from one sample to another, differences explained by the different degree of the analyzed vegetal tissue organization.

Key words: tissue culture, morphogenesis, active principle biosynthesis

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P 042

COMPARATIVE STUDY OF VOLATILE CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF JUNIPERI FRUCTUS SAMPLES FOR PHARMACEUTICAL USE

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Juniperi fructus is a medicinal product, which contains volatile oils known for its diuretic effect; particularly terpinen-4-ol, may cause an increase in urine volume without a loss of electrolytes such as potassium.
Chemical studies of volatile fractions, realized upon 5 types of medicinal tea, *Juniperi fructus*, were based on the isolation, identification and quantitative evaluation of volatile oils. Qualitative and quantitative quantification of these five volatile samples were performed by GC and GC-MS techniques.
It can be observed from the quantitative results that there is a comparative significant variation in the content of volatile fractions isolated form juniper berry (2,4-5,0%). In all investigated samples a relative high content of terpinen-4-ol was detected.
These five volatile samples exhibited strong antibacterial activities, in particularly against *B. subtilis* and *B. cereus*; moderate antibacterial activities against *Sarcina* and *Klebsiela*. The most resistant bacteria was *S. aureus*. Different antimicrobial activities of examined essential oils seem to be due to the differences in oil composition.

Key words: Juniperi fructus, essential oils, qualitative and quantitative quantification, antibacterial activities

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P 043

CHEMICAL INVESTIGATIONS ON *RHODIOLA ROSEUM* AND *SEDUM HYBRIDUM* OF ROMANIAN ORIGIN

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Crasulaceae Family includes many spontaneous and cultivated species very well known in Romanian folk medicine. In Europe, Rhodiola rosea L. is registered as a medical product in the category of botanical drugs. In our days, registered preparations of R. rosea are extensively used in Scandinavian and former Russian countries to increase mental work capacity during stress, as a psychostimulant and as a general strengthener.
In our comparative evaluation, we studied terpens and volatile compounds from rhizomes and roots of Rhodiola rosea and Sedum hybridum of Romanian origin (Ceahlau Mountain).
The chemical analyses of those two extracts (from roots of Rhodiola rosea and Sedum hybridum) were performed by GC and GC-MS. Notable differences were found in the qualitative and quantitative

composition of the oils, particularly for the geraniol, geranyl acetat, linalool and its oxides, important for floral notes and rose-like odor of R.rosea rhizomes. In both extracts the main chemical groups are: aliphatic alcools, monoterpene hydrocarbons, monoterpene alcohols

Key words: Rhodiola rosea, Sedum hybridum, terpens, volatile compounds

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P 044

QUANTIFICATION OF ALLANTOIN IN VARIOUS *ZEA MAYS* L. HYBRIDS BY RP–HPLC WITH UV DETECTION

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A RP-HPLC method for quantification of allantoin in silk of fifteen maize hybrids (*Zea mays* L., Poaceae) was described. Following extraction of the plant material with acetone-water (7:3, V/V) mixture, filtration and dilution, obtained extracts were analyzed without previous chemical derivatization. Separation and quantification was achieved using an Alltech Econosil C18 column under isocratic conditions at 40 ^{0}C. The mobile phase flow (20 % methanol - 80 % water with 5 mM sodium laurylsulfate added at pH 2.5, adjusted with 85 % orthophosphoric acid; pH of water phase was finally adjusted at 6.0 by addition of triethylamine) was maintained at 1.0 mL/min. Column effluent was monitored at 235 nm. This simple and rapid procedure (elution time of allantoin was below 5 min) afforded efficient separation and quantification of allantoin in plant material, without interference of polyphenols or other plant constituents of medium to high polarity, or similar UV absorption. Our study revealed that the silk of all investigated maize hybrids could be considered relatively rich in allantoin, covering the concentration range between 215.0 and 288.8 mg per 100 g of dry plant material. The quantity of allantoin appeared to be independent or, at least, slightly influenced by the biological source: the silk of all investigated maize hybrids contained close levels of allantoin.

Key words: *Maydis stigma;* Allantoin; HPLC

P 045

CHARACTERIZATION OF STANDARDIZED LYIOPHILIZED EXTRACT FROM *OLEA EUROPAEA* L. (OLEACEAE)

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A standard lyiophilized extract with 20% content of oleanolic acid was obtained from Olea europaea leaves (SLE – OE) [2]. The quality of the preparate was estimated by physico-chemical characterization after preparation at certain intervals of time along a period of an year. The parameter required by FR X for SLE – OE quality verification are mentained within admisible numerical limits [3].

The oleanolic acid content determined after preparation and sequencially during a years by means of a VIS spectrophotometric method (λ_{max} = 527 nm), practically remains constant, which shows a good stability in time for the active principle [1].
SLE – OE preparation presents the advantage of avoiding the degradation of active substances. Besides, as the results of lyiophilization a high degree disperse system is obtained, leading to a better absorbtion of active principles.
The antimicrobian activity of SLE – OE was tested by using the following test strains: Staphylococcus aureus ATCC 25923, Sarcina lutea ATCC 9341, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp, Candida albicans.
Against gram negative bacteria SLE – OE are inactive. SLE – OE have little activity against Bacillus subtilis, but good and comparable activity against Sarcina lutea and showed antistaphylococcal in vitro activity.

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P 046

IN VITRO MICROPROPAGATION AND ACCLIMATIZATION OF *LEUCOJUM AESTIVUM* L. (SUMMER SNOWFLAKE) IN BULGARIA

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Summer snowflake (Leucojum aestivum L.) is an economically valuable but rare medicinal plant included in the Red Data Book of Bulgaria on the category "endangered species" [2]. It's natural resources are unable to meet the needs of the pharmaceutical industry [1]. Application of in vitro micropropagation of L. aestivum with the aim to create industrial plantation could be an alternative of wild population use. Shoot clumps and bulblets were regenerated directly from bulb scales as well as from leaves and anther's stamens isolated from the bulb core, thus giving rise of genetically identical plants suitable for clonal propagation of valuable individuals. Formation of bulblets occurred in dark as well as at long light period on MS medium with or without growth regulators, although their addition enhanced the growth (best combination was 1 mg/l BAP and 0.5 mg/l NAA). Subcultures on the same medium for 3-4 months led to well formed bulblets with diameter 4-5 mm and with several leaves. Only hormone-free media appeared to be appropriate for normal rooting and when MS medium was supplemented with 4 g/l charcoal rooted bulblets reached 70 % for a period of 2 months. Earlier trials to acclimatize plantlets started in January when they were transferred in pots with soil mixture, first in a mini cold-frame and after elongation of their leaves, in the experimental field plot. In September all survived (55 %) plantlets were transferred in trenches in open air conditions and 75 % of them survived their first wintering. Acclimatization was successful also in autumn when plantlets were transferred right in trenches in open air.

Key words: Leucojum aestivum, in vitro micropropagation, acclimatization

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P 047

INTRODUCTION OF NEW POLISH CULTIVARS OF SWEET BASIL (*OCIMUM BASILICUM* L.) TO CULTIVATION

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In 2002, after two years of examination two new Polish cultivars of sweet basil entered the Register of Cultivars Protected by Plant Breeders Right. The new cultivars named "Kasia" and "Wala" were bred in the Research Institute of Medicinal Plants. The economic value of these cultivars was evaluated in the comparative experiments. In the two-year experiments the following traits were evaluated: yield of herb, stem content in herb, seeds yield, weight of 1000 seeds, seed germination and essential oil content. The results showed that both cultivars of sweet basil gave good herb yield with low content of stems (34 – 35%) in the herb. Content of essential oil was high and oscillated from 1,12% (cv.Wala) to 1,50% (cv.Kasia). Both cultivars gave good yield of seeds, which varied from 380 kg/ha (cv.Kasia) to 430 kg/ha (cv.Wala). These cultivars could be easily propagate in Polish climate because of simple and certain seed reproduction. The farmers who produce herb of sweet basil are provided with seeds of both cultivars obtained in the maintenance breeding.

Table 1: Economic value of cultivars of sweet basil which were bred in RIMP

	cv. KASIA	cv. WALA
Yield of fresh herb [q/ha]	135	150
Yield of dried herb [q/ha]	21,5	23,5
Yield of herb without stems [q/ha]	14,2	15
Content of stems in herb [%]	34	35
Content of essential oil [%]	1,48	1,12
Yield of seeds [kg/ha]	380	430
Weight of 1000 kernels [g]	1,29	1,38

Key words: sweet basil, cultivar, raw material, essential oil content

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P 048

CULTIVATION EXPERIENCES WITH *ARNICA MONTANA* L. IN SOUTH FINLAND

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Three cultivation experiments were carried out with non-endemic *Arnica montana* L. during 1990 -2003 at Mikkeli, South Finland (61°44'N, 27°18'E). According to the experiences, arnica can

be cultivated successfully in this area, using organic cultivation methods. The estimated life cycle of cultivated plantations seems to be 6-8 years. The flowering period generally lasted three weeks, between 20th June and 10th of July. The dry flower drug yields picked by hand ranged 2,5-5,3 kg/100m². The total sesquiterpene lactone contents of the dry drug yields were 0.440-0.598 %. The germination of seeds was in average 89 %. Black plastic mulch seemed a suitable method for reducing the hand weeding. Since arnica populations are decreasing in its original habitats, its cultivation seems to be suitable for the small size farms at this Nordic latitude, as an alternative special new culture.

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P 049

SAGE (*SALVIA OFFICINALIS* L.), BORAGO (*BORAGO OFFICINALIS* L.) - BREEDING AND CULTIVATION

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The task was solved under support of the Ministry of Agriculture Czech Republic under Nr. QD 0129 – „Inovation of technologies selected medical, aromatic and spice plants, with the substances and their utilization“. The base for this work was variety Sage Krajová. On the area 200 m^2 we found a several plants with fertile pollen. Plants with the fertile pollen we will use for the next breeding. By sowing is good pre-emergence application of the linuron (Afalon 45 SC) and isoproturon (Tolkan FLO). After leaf creation is possibly to use herbicide on dicamba base (Banvel 480 S 0,1l/ha) and bentazone (Basagran Super 1,5 l/ha).Seed crop 2002- by hand without desication 313 $kg.ha^{-1}$, maschinery crop with desication Reglone 247 $kg.ha^{-1}$.Seed crop2003-maschinery crop after desication Reglonem 94 $kg.ha^{-1}$. Extreme draught decrease the seeds yealds. Price for the seeds is forcer higher.Borago cultivation in mixture with safflower or canari-grass. Pre-emergence application of the Burex or Afalon in reduce dose.

P 050

THE RESEARCH ON THE BIOLOGY OF GERMINATION AND CULTIVATION OF *SALVIA MILTIORRHIZA* BUNGE

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Salvia miltiorrhiza Bunge (*Lamiaceae*) is an important Chinese medicinal plants. The root of this species contains mainly diterpenes of abietane type and phenolic acids. It affects cardiovascular system.
Research was carried out in the Research Institute of Medicinal Plants from 1996 to 2004.
Germination of diaspores of *Salvia miltiorrhiza* in laboratory analysis does not depend on the influence of light and temperature within the range 18 – 35 ^{0}C. It depends on the atmospheric conditions during blossoming and maturation of seeds. The best germination of seeds was observed in

autumn (October, November) and in spring (April, May). After 10 years of storage in temperature 5 ^{0}C the diaspores of *Salvia miltiorrhiza* germinated in about 20 %.
In the field cultivation the potassic fertilization has the strongest effect on the content of phenolic acids and on the mass of roots of *Salvia miltiorrhiza*. The effect of atmospheric conditions in particular years is also essential. The optimal doses of fertilizers (pure component content in kg · ha^{-1}) were following: N – 100, P_2O_5 – 30, K_2O – 180. Having used this fertilizing combination the average content of phenolic acids was 4,62 % and the average aerial dry mass of roots was 13,21 g per 1 plant after one year of cultivation.

The work was supported with a grant by the Polish Committee for Scientific Research No PBZ-KBN-092/P05/2003

Key words: biology of germination, mineral fertilization, Salvia miltiorrhiza Bunge

P 051

EFFECT OF VARIETY ON CHLOROPHYLL CONTENT IN SPINACH LEAVES

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Spinach was grown under field conditions in the vicinity of Belgrade (Serbia). A two-year trial was conducted (2001 and 2002). The following varieties (hybrids) were used: Matador, Viroflay, Norvak, Marisca F1, Polka F1. A greater chlorophyll amount was found in the hybrids compared with the varieties. In 100 g of leaves the amount of chlorphyll registered was 11.78 mg and 11.96 mg. The variety Matador was similar to the hybrids yielding 10.95 mg chlorophyll. Its lagging behind amounted to 0.84 and 1.02 mg. The varieties Viroflay and Norvak were found to be considerably poorer in chlorophyll and the differences ranged from 1.09 mg to 2.28 mg. Spring spinach was investigated (March-May).
In 2002 chlorophyll amount in spinach was greater by 0.61 mg. The greatest differences were noted in the variety Viroflay (1.03 mg) followed by Matador (1.01 mg), etc. On average hybrids were found to be more resistant.
The benefit of chlorophyll is well known being similar to haemoglobin contained in blood. Thus its regular intake with plant originating products is recommendable.The major sources of chlorophyll are vegetable crops including spinach. From the medical standpoint it is highly recommendable enhancing liver and heart function and improving blood count.

Key words: spinach, varietis, hybrids, vegetable, chlorophyll

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P 052

VARIATION OF ELECAMPANE ROOT YIELD REGARDING THE GENOTYPE

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Intensively researching different genotypes of elecampane we are able to present the results of yield achieved during three years lasting microexpiriments (2001, 2002, 2003), realized in The South Banat area. Comparing the results of yield it was concluded that all five genotypes had higher yield, both fresh and dry elecampane root mass. The highest yield of 28.617 kg ha^{-1} of fresh, and 8.301 kg ha^{-1} of dry root had the genotype ″NŠ″, and it was more than a standard cultivar called ″Domaća″.
Considerable results were achieved by genotypes ″SŽ″ (77% higher yield of fresh mass, and 70% of dry mass), also the genotype ″BM″ (62% higher yield of fresh mass, and 84% of dry mass). The worst yield had the genotype ″SG″ and standard cultivar ″Domaća″, mostly because of late planting, in the second year of the research.

Key words: e*lecampane, yield, dry root, g*enotype, standard cultivar.

P 053

GROWING RESULTS OF SOME MAP SPECIES AT MOUNTAINEOUS REGION OF SERBIA

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The aim of this investigation was to test possibilities of growing of following MAP species in mountainous region: *Origanum vulgare* L., *Gentiana lutea* L., *Mentha x piperita* L., *Angelica archangelica* L. *and Plantago lanceolata* L. Testing was conducted in ecological conditions of mountain Tara (1004 m a. s. l., g. latitude 43°$_N$, 53', 41" and g. longitude 19°$_E$, 33', 41") in order to introduce these MAP species into culture and to organize production of seeds and planting material in ecologically non-contaminated environment. The experiments started in autumn 2001, and lasted up to autumn 2003, and they were conducted at small scale, in order to select appropriate plants for given growing conditions. Special attention was devoted to solve the optmal mode of nursery plant production for Yellow gentian (without hormonal treatment). The yield of Angelica was 8778 kg/ha of fresh roots (dry 2090 kg) with 0,7% of essential oil. The yield of Peppermint herb (domesticated »Mitcham« cultivar) of superior quality (57% of leaves containing 3.38% of essential oil) was 1558 kg/ha. The yield of stolones was 7500 kg/ha. In the first growing year the yield of Oregano was 1057 kg/ha of dry aboveground part of plant (*herba*) and the yield of Ribwort plantain dry leaves was 2260 kg/ha.
All tested MAP species gave acceptable results regarding development of their aboveground plant parts as well as seed bearing. The obtained data points out that these cultures may be successfully grown in agro-ecological conditions that offers mountain Tara. In following period investigation on economical parameters in order to estimate growing thrift are going to be faced.

Key words: growing in mountainous region, Yellow Gentian, Angelica, Peppermint, Oregano, Ribwort plantain.

P 054

OCCURENCE OF DISEASES AND PESTS ON SAGE IN SOUTH-WESTERN SLOVAKIA (*SALVIA OFFICINALIS* L.)

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Production of medicinal plants is followed by many problems, so as production of sage (*Salvia officinalis*). One of the important factors is presence of insects and pathogens. In sage cropping is impossible to avoid certain diseases [1, 2]. During 2003 – 2004 pests and pathogens of sage were observed at location Kolíňany in south-western Slovakia. Following pathogens were found on mature sage plants: *Sclerotinia sclerotiorum*, *Alternaria s*pp., *Fusarium* spp., and *Erysiphe* spp. On aboveground parts of the plants was detected presence of different insect species. Total 30 insect species were determined. The species frequently associated with sage were *Eupteryx (Cicadella) atropunctata*, *Bibio marci*, aphids and mites (Acarina).

Key words: sage, salvia officinalis, pests, diseases

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P 055

BREEDING OF CARAWAY IN CZECH REPUBLIC

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Caraway is the oldest spice cultivated in the territory of the Czech Republic. In the 2nd half of 19. century, deciduous cultivars named Český („Czech“) and Moravský („Moravian“) were planted, later it was the deciduous variety Ekonom. In 1978, the non-deciduous variety Rekord was created by the means of ionizing radiation application, along with the Prochan and Kepron varieties.
In the varieties comparison, Kepron reached maximum values of yield attributes, but is also the most variable. The breeding comes out from the Kepron variety, which responded strongly to the weather course during three growing periods. The Kepron variety is not plastic, it is unbalanced and thus suitable for the breeding.
As ensued from the physiologic monitoring of registered varieties, the highest essential oils content has the variety Rekord; this variety has also the deepest dormancy, which can be used for the breeding.

The research is supported by National Agency for Agricultural Research – NAZVA QF 4156, 2004-2007: The use of current varieties of caraway (Carum carvi L.) and new methods of caraway breeding for the qualitative and quantitative parameters enhancement and the Ministry of Education, Youth

and Sports (MŠMT ČR) within the program "The reinforcement of research in universities", reg. No. VS97014 .

Key words: Caraway, breeding, non-deciduous, essential oil content

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P 056

EDIBLE WILD SPECIES OF MACROMYCETES RECORDED IN THE REPUBLIC OF MACEDONIA

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Fungi represent a very heterogeneous group of organisms. However, studies to date have been concerned mainly with Ascomycota and Basidiomycota. The other orders have been studied insufficiently. Thus far, in the Republic of Macedonia approximately 1,250 species of fungi have been recorded [2, 3]. The majority of them belong to the orders Ascomycota (130), Basidiomycota (1,050), Myxomycota (10), Oomycota (20) and Zygomycota (35). The Division of Mycology, within the Faculty of Natural Science (Institute of Biology), possesses a large collection of macroscopic fungi called Fungi Macedonici. The collection contains approximately 10,000 specimens as well as a fungi database named MACFUNGI. This plentiful gene fond of fungi might be a potential source of food. The content of edible species (about 200 species) has not been researched enough, and this is an important reason why in Macedonia these species have not been exploited as a source of food. In the course of the last ten years, nutritionally important components have been determined in 53 species of cultivated or wild edible mushrooms found in various parts of Macedonia [1]. In addition, in the Republic of Macedonia there is no tradition of consuming wild species of macromycetes. Nevertheless, in the last few years several incidents due to consumption of poisonous fungi have occurred, whose outcome was lethal. Therefore, in the present study an attempt has been made to present the edible wild species of macromycetes recorded in the Republic of Macedonia together with the literature data. Their large number facilitates the selection of those characterised by a significant nutritional quality.

Key words*:* edible macromycetes, R. Macedonia

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P 057

FIRST REPORT ON *PHYTOPLASMA DISEASE* IN PURPLE CONEFLOWER IN SERBIA

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Purple coneflowers (*Echinacea* spp.) are the new and important medicinal plants in our country. *Echinacea purpurea* and *E. angustifolia* are the two species grown commercially in Serbia since last five years. After second year of growing in the plantation in Pančevo and Indjija disease symptoms were noticed. Diseased plants show leaf "reddening" and have extremely numerous, small, branched, axillary shoots coming from the stem nodes, with abnormal flower development. Ray and disk flowers turned green and leaf-like. The flowers fail to produce seeds. Almost all infected plants were died. Up to 30% of all plants were infected.
Electron microscope examination of ultra-thin section of vessels' zone from leaves of diseased plants showed phytoplasma bodies. These bodies were oval to spherical, lacked cell walls and surrounded by single membranes. It indicates that phytoplasma might be considered as the causal agent of redness of coneflowers. This is the first report of phytoplasma disease of coneflower, as well as of medicinal plants at all.

Key words: Purple coneflower, Echinacea purpurea, Echinacea angustifolia, disease, phytoplasma

P 058

OCCURENCE OF DISEASES AND PESTS ON SWEET BALM (*MELISSA OFFICINALIS* L.) IN SOUTH-WESTERN SLOVAKIA

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During 2003 – 2004 pests and pathogens of sweet balm were observed at location Kolíňany in south-western Slovakia. On stem of mature sweet balm plants was isolated *Fusarium* spp. and on leaves was observed powdery mildew (*Erysiphe* spp.). On aboveground parts of plants were detected various insect species. Total about 20 insect species were determined. The species frequently associated with sweet balm belonged to family Cicadellidae (*Eupteryx atropunctata, Emelyanoviana mollicula, Empoasca decipiens*). These insects sucked on sweet balm leaves and caused whitish or brown spots. Species belonged to family Cicadellidae can transmit several important viruses [1, 2].

Key words: sweet balm, Melissa officinalis, pests, diseases

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P 059

MENTHAE LONGIFOLIAE FOLIUM UNDER TOPSIN M TREATMENT: NOTE I. THE CHEMICAL PROFILE OF ESSENTIAL OIL

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The hydrodistilled essential oils from the leaves of Mentha longifolia L. (Topsin M 0,1% and 0,4% treated plants) were analysed comparative to untreated plants (control) by means of GC-MS. The yield of the essential oils ranged about 3 % at control and 2,38%-2,22 % at treated plants. The major oil constituents in all samples were found to be aromatic compounds: carvacrol, thymol and oxygenated monoterpene 1,8-cineole. Althoug all investigated oils contain a similar array of constituents, the relative contribution of them varies at treated samples (both 0,1% and 0,4 %) comparative to control samples, resulting in characteristic chemical profiles for each volatile oil.

Key words: essential oil, Mentha longifolia, Topsin M

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P 060

MENTHAE LONGIFOLIAE FOLIUM UNDER TOPSIN M TREATMENT: NOTE II. THE ANTIMICROBIAL INVESTIGATIONS ON ESSENTIAL OIL

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In the present study there were examined the antimicrobial activity of essential oils from leaves of *Mentha longifolia* in relation to the phytosanitary treatments with Topsin M 0,1% and 0,4% applied in experimental cultures from 2003. Qualitative antimicrobial activity was evaluated against four gram negative bacteria, four gram positive bacteria and Candida albicans. Under identical conditions on the same microbial cultures pure components of essential oils (carvacrol, thymol and 1,8-cineole) were tested. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the volatile oils and their components against Staphylococcus aureus were also determined. Generally, all tested oils showed good antimicrobial activity against all microrganisms, with the exception of Ps. aeruginosa.The phenols carvacrol and thymol had the most potent antimicrobial activity against all tested strains; only Ps. aeruginosa was resistant. We noticed some qualitative differences in antimicrobial activity of examined essential oils (from treated plants comparative to untreated plants). On the other hand, the volatile oils from untreated plants were more active against S. aureus (CMI=25 μg/ml) than volatile oils of treated plants (CMI=800 μg/ml at 0,1% treatment and 160 μg/ml at 0,4% treatment).

Key words: essential oil, Mentha longifolia, Topsin M, antimicrobial activity

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P 061

EFFECTS OF *HYPERICUM PERFORATUM* ALCOHOLIC EXTRACT ON RESERPINE-INDUCED GASTRIC ULCERS IN RAT

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Male Wistar Bratislava rats weighting 90-120 g from Cluj-Napoca University of Medicine and Pharmacy Breading Center. Animals were kept in standard laboratory conditions, natural dark/light cycle. Five randomized groups were formed, 8 rats/group, and treated i.p. 5 mg/kg reserpine to induce gastric ulcer.
Group I received water through gavage, 15 minutes before, and 4 hours after reserpine; group II received ethanol 30%; group III, IV and V received alcoholic extract of Hypericum perforatum in three different dilutions: 1:8, 1:4, 1:2 respectively. Treatment was performed after the same schedule as group I. Animals were sacrificed after 8 hours from reserpine. Number and severity of gastric ulcers was observed. Student's t test was used for statistical significance, at $p < 0.05$.
Results no difference was observed between groups treated with water and ethanol; Hypericum extract of 1:8 concentration increased number and severity of gastric ulcers; on the contrary, Hypericum extract in 1:4 and 1:2 concentration reduced number and severity of gastric lesions.
Conclusion: Effects of Hypericum perforatum alcoholic extract administered by gavage are dose-dependent: small doses aggravates gastric ulcers in rats, increased doses has a gastro-protective effect.

Key words: Hypericum extract, ulcers, reserpine

P 062

BIOLOGICAL ACTIVITY OF LINALOOL

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Essential oils of different medicinal and aromatic plants expresses broad spectrum of biological activities against different bioagents (microbial, insects etc.). Except essential oils, we tested pure terpenoids (linalool, camphor, α and β-thujone, 1,8-cineole, myrcene). The results of the antibacterial activity assays of linalool on different Gram-positive and Gram-negative bacterial strains indicated that this terpenic alcohol possess very strong activity. In antifungal investigations we used the macro- and micro dilution method in order to determine the MIC (minimal inhibitory concentration) and MFC (minimal fungicidal concentration). MIC in microdilution assay were 2.0 - 6.0 μl/ml (2.0-5.5 μl/ml) same as in macrodilution assay 3.0-7.0 μl/ml (2.0-6.5 μl/ml), so we can say that linalool possess strong antifungal activity. Antigenotoxic potential of linalool was performed with short-term bacterial tests in *Escherichia coli* K12, repair proficient SY252 strain, and *Salmonella typhimurium* TA100 strain. Antimutagenic activity was detected by inhibition of UV-induced mutations. The results indicate the significant antimutagenic effect of linalool (60%). Literature data shows that linalool exhibits

antioxidative properties with no cytotoxic effect on mammalian cells (1,2). In our investigation we included examination of insecticidal, antifeeding, contact and digestive activity of linalool on caterpillars (L_2) of *Limantria dispar* L. in the laboratory conditions. It was found that this terpenenic alcohol expressed very low toxicity (contact and digestive activity) but significant antifeeding activity on caterpillars of *Limantria dispar*. High antimicrobial, antimutagenic and antifeeding for insects activity enables linalool to be a source of a new lead compound with natural origin for control of bioagents.

Ministry for Science and Environment of the Republic of Serbia supports this study, through the project BTR.0519 (Control of bioagents in medicinal plants)

Key words: linalool, antimicrobial activity, antifeeding activity, bioagents

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P 063

COMPARATIVE INVESTIGATION OF *HYPERICUM PERFORATUM* L. POPULATIONS IN SERBIA

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In two-year period, three pre-selected *Hypericum perforatum* populations (V2, V3and D4) were tested on yield, hypericin yield and content and intensity of wilting disease, at two localities in Serbia. Testing was conducted at lowland locality of South Banat (Pančevo, 80 m. a. s. l., g. latitude 44°$_N$, 52', 20" and g. longitude 20°$_E$, 42', 25") and at locality in Western Serbia (Tara mountain, 1004 m a. s. l., g. latitude 43°$_N$, 53', 41" and g. longitude 19°$_E$, 33', 41").
H. perforatum cultivation was established in spring (winter production of nursery plants). In climatic conditions of South Banat, in the first year, it gave two harvests and yielded 1070-2440 kg/ha while in climatic conditions of Tara mountain it gave one harvest and yielded 220 - 720 kg/ha of dry herb.
In general, best yields of dry herb in the second year achieved population D4 (ca. 2000 kg/ha of dry herb) above all due to a better tolerance on unfavourable climatic conditions and better plant density preservation in comparison to other two tested populations (V2 and V3). The lowest hypericin yield was recorded in the first harvest of the first vegetation (0,3 - 1,3 kg/ha), and the highest one in the first harvest of the second vegetation (1,7 - 3,8 kg/ha). Due to the best yield of dry herb and satisfactory hypericin content, population D4 stands out for a best source of hypericin among all tested populations. In comparison to locality Pančevo, intensity of wilting disease (caused by *Colletotrichum gloeosporoides*) recorded in the second year of growing, was lower at Tara locality. This observation points out to significance of ecological factors in development of this disease, meaning that the mountainous climatic conditions impose themselves as a better solution for growing of *H. perforatum* in terms of avoidance of wilting disease then the lowland growing conditions.

Key words: *Hypericum perforatum*, populations, locality, yield, hypericin, wilting disease.

P 064

CYTOGENETICS AND MORPHO-ANATOMY IN *STEVIA REBAUDIANA* BERTONI

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Stevia rebaudiana is an herb from the *Asteraceae* family, indigenous from the higher elevations of Northern Paraguay near the Brazilian borders (Soejarto et al, 1983). Due to non-caloric sweeteners extracted from its leaves, mainly stevioside, this plant has gained importance as a crop for the pharmaceutical and food industries.
Chromosome's number in *Stevia rebaudiana* is identified as 2n=22. There are four morphological types – M, m, sm and st. Chromosomes arms ratio is found from 1.00 to 3.5. The chromosomes length is comprised between 1.68 and 4.22μm. We consider *Stevia rebaudiana* karyotype as asymmetric.
The anatomical structure of *Stevia rebaudiana* vegetative organs frame to the general plan structure of the *Asteraceae* in general. The opposite leaves disposition is considerate like a peculiarity, because the most *Composite* species has alternate leaves. In *S. rebaudiana*, fully differentiated functional glandular trichomes are already present at early stages of leaf development; in addition these glands are formed only on leaf primordia and very young leaves at various stages of differentiation.

Key word: Stevia rebaudiana, caryotype, alternate leaves, glandular trichomes

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P 065

COMPARATIVE KARYOTYPE ANALYSIS IN MEMBERS OF *UMBELIFERAE* FAMILY

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The aim of our work is to establish the karyotype for three member of *Umbeliferae* family, three aromatic species: *Petroselinum sativum* Hoff., *Anethum graveolens* L. and *Coriandrum sativum* L. Chromosome's number in *Petroselinum sativum* is identified as 2n=22, the same number as in *Anethum graveolens* and *Coriandrum sativum*. The similarity stops here because in *P. sativum* we found M and m chromosomes, M and m chromosomes in *A. graveolens* and m, sm st and T chromosomes in *C. sativum*. Chromosomes arms ratio is from 1.00 to 1.22 in *P. sativum*, from 1.0 to 1.68 in *A. graveolens* and from 1.33 to 9.06 in *C. sativum*. The chromosomes length is comprised between 2.24 and 3.33μm in *P. sativum*, between 1.66 and 3.03μm in *A. graveolens* and from 4.26 to 5.15μm in *C. sativum*.

Key words: Petroselinum sativum, Anethum graveolens, Coriandrum sativum, karyotype, Umbeliferae

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P 066

THE STRUCTURE OF SOME *ERICACEAE* SPECIES

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In this paper some anatomical features of 3 *Vaccinium* species (*V. vitis-idaea* L., *V. myrtillus* L., *V. oxycoccos* L.) and one species of *Andromeda* (*A. polifolia* L.) are presented. From the genus *Vaccinium* (blueberries) have a long history of use for medicinal purposes. As an edible fruit, blueberries are low in calories and sodium, contain no cholesterol, and are an excellent source of fiber. The pectin content within the fiber has been proven to lower blood cholesterol.
The structural features are related wit the live conditions (peat-bog). At the young stems of *V. myrtillus* and *V. vitis-idaea* the cortex consist in a network of large and small cells; in the mature stem the large cells of the cortex are disorganized, resulting aeriferous cavities. In *V. oxycoccos* the cortex is thin, with 1-2 sclerenchyma layer under the epidermis. The vascular tissue acquire early a secondary structure. The xylem has numerousness vessels, with thin walls. The lamina has bifacial structure in all analyzed species; the palisade parenchyma is bi-layered in *V. myrtillus* and *A. polifolia*, uni-layered in *V. oxicoccos.* In *V. vitis-idaea* (with sempervirent leaves) the structure differs in relations with the age of the leaf: at the 1 year leaves the palisade parenchyma has 2-3 layers of isodiametrical cells, without starch deposits – cuticle is thin; the 2 years leaves has 3-layered palisade, with long cells, with a lot of starch grains (identifiable by histochemical methods) – cuticle is very thick, especially above the upper epidermis.

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P 067

COMPARATIVE ANATOMY OF SOME SPECIES FROM *EQUISETUM* GENUS

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In this paper different aspects of histo-anatomical structure of 4 species of Equisetum (*E. arvense* L., *E. telmateia* Ehrh., *E. fluviatile* L. and *E. ramosissimum* Desf.) was studied. *Equisetum* aerial parts are traditionally used in poor digestion, to improve bile flow and as a liver protective. It is also a diuretic used in water retention and arthritis.
The structural features are correlated with the environmental conditions and functionality of the vegetative organs. The epidermis has cells with thick walls at the internodal level and with thin walls at the nodal one. The external cortex contain palisade parenchyma in *E. arvense, E. ramosissimum* and

in superior internodes of *E. fluviatile*. In *E. telmateia* principal axis the palisade tissue is missing; it is present in lateral branches. The aeriferous canals from middle cortex are a commune feature of this genus. They missing only in median and superior internodes of *E. fluviatile*; on the other hand, at this species the central aeriferous cavity is very large. The central cylinder is usually delimited by a casparyan endodermis. In *E. fluviatile* this endodermis is missing, but a layers of orderly cells (with thick external and lateral walls) surrounded each vascular bundles (possible with value of endodermis, with the role to control the water flux in the stem). The vascular bundles are formed by phloem (sieve cells), well developed in all analyzed species and few xylem vessels (tracheids) localized near the aeriferous lacuna from the bundle or from a part and another of the phloem (*E. ramosissimum*).

Key words: *Equisetum*, palisade parenchyma, aeriferous cavity, endodermis

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P 068

EFFECT OF BASTARD BALM (*M. MELISSOPHYLLUM* L.) ETHER OIL FULERENE AND CCL_4 ON LIPID PEROXIDATION OF LIPOSOMES PRODUCTION OF OXYGE RADICALS

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Bastard balm leaves (Melittis melissophyllum L.) contain no more than 0.1% of essential oil which is of complex and variable composition. Among the more than 50 aroma compounds yet identified, citronellal (dominantly the (R) enantiomer), β-caryophyllene, nereal, geranial, citronellol and geraniol amount to about 70% of the oil. The composition is similar to that of lemon grass, but balm oil can be identified by its typical pattern in chiral compounds; for example, enantiomerically almost pure (R)-(+)-methyl citronellate is a good indicator of true balm oil. Beside that, leaves contain flavonoids, triterpenes, monoterpene and phenolpropanoid glycosides, phenolic acids, sterols and salts. Ether oil exhibit spasmolitic action. Ether oil acts as a muscle relaxant, sedative and narcotic, antibacterial and antifugal.

In this study we investigated the effect of bastard balm oil on lipid peroxidation in liposomes (LPx) and production of $OH^{\bullet}$ radicals . Beside that, we exhamined synergic effects of this ether oil and fuleren, as well as synergic effect of ether oil of bastard balm with CCl_4. Intensity of lipid peroxidation was determined from the amount of malonildialdehide (MDA) produced during the process of lipid peroxidation of membrane lipid components using the method of Buege-Aust. The effects of these extracts on production of $OH^{\bullet}$ radicals was determined by monitoring the chemical degradation of deoxyribose. Pure preparations of pyralene and ascarel were used in different amounts.

Results: Significant reduction of LPx intensity was achieved with addition of 30 μl ether oil of bastard balm. Fullerene derivative alone reduced LPx intensity, especially with 20 μl of fullerene. In combination with fullerene derivative, ether oil of bastard balm, containing 20 μl of ether oil and 30 μl

fullerene, and 30µl etheroil and 30 µl fullerene induced decrease in LPx intensity. Similar results are obtained in investigation of $OH^{\bullet}$ radical production. Fullerene, as well as its combination with ether oil decrease the production of $OH^{\bullet}$ radicals, and the best protective effect was achieved using the combination of 30 µl ether oil and 30 µl fullerene.
CCl_4 alone induced statistically significant increase of LPx intensity, and in combination with bastard balm ether oil, significant decrease was achieved only using 30 µl of ether oil and 30 µl CCl_4. Also CCl_4 increased the production of $OH^{\bullet}$ radicals. All added volumes (10, 20 and 30µl of ether oil) in combination with CCl_4 caused reduction of $OH^{\bullet}$ radical production, and the most efficient was combination of 30 µl of ether oil with all added volumes of CCl_4 (10, 20 and 30µl).
In conclusion, it can be pointed out that ether oil of the investigated plants contain different compounds which exhibit antioxidative properties. Those substances are included in the processes of antioxidative protection using different mechanisms or synergic effects of the reduction of LPx intensity.

P 069

EFFECTS OF LAUREL (*LAURUS NOBILIS* L.) LEAVES AND BERRIES EXTRACTS AND PCBS ON PRODUCTION OF OXYGEN RADICALS

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Laurel *(Laurus nobilis L.)* is one of the most well known medicinal plants used in history. In traditional medicine, ether oil from laurel leaves is used as carminative, excito-aromatic and in perfumes industry. The fruits were used as spice, and today they are the source of oil. Oil is usually used in the mixture with other medicaments. The laurel plant contains several classes of secondary plant products. The fruit consists of 30% fat and 1% ether oil. Ether oil mostly contains cineol and □-pinen. The aim of this research was to investigate effects of the laurel leaves and poppies and their combination with PCBs, on the intensity of lipid peroxidation of liposomes.
Crude methanol extracts of macerated laurel leaves and poppies were obtained in extraction of poppies with 70% methanol. After evaporation to dryness, dry matter was dissolved in water and extracted with ether, chloroform, ethyl-acetate, and n-butanol, thus leaving water solution as well. 10% (v/v)solutions of extracts in 50% ethanol were prepared. The effects of these extracts on lipid peroxidation of liposomes was investigated according to Fukuzawa. Also, the effects of these extracts on production of $OH^{\bullet}$ radicals was determined by monitoring the chemical degradation of deoxyribose. Reaction is initiated by hydroxyl radicals obtained in Fenton's reaction, which yields products that react with thyobarbituric acid (TBA test). Obtained products, among which malonyl-dialdehyde is the most important, are determined by spectrophotometric metod according to Buege-Aust. Pure preparations of CCl_4 was used in different amounts.
All extracts of laurel leaves reduced the OH radical production, except n-BuOH extract. In combination with Adcarel, the best results were obtained with EtOAc extract, as well as H_2O extract. In combination with Pyralene all laurel leaf extracts exhibited protective properties. The best protective effect was again achieved using EtOAc extracts, with all added amounts of Pyralene.
In case of the extracts of laurel poppies all extracts exhibited significant effect to the decrease in production of OH radicals. In combination with Ascarel, all extracts had protective effect, of which the best was proven to be H_2O extract. Combination of extracts of laurel poppies with solution of Pyralene induced no significant changes compared to the control.
According to the given results, it can be concluded that the most efficient extract is the ethyl-acetate extract, and it probably contains substances which affect the metabolism of polychlorinated biphenyls,

thus eliminating their prooxidative activity. Different results with different extracts indicate various contents of secondary biomolecules and total flavonoids in the investigated extracts.

P 070

ALTERNATION OF ESSENTIAL OIL – *SALVIA SCLAREA* L.

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Samples of essential oil Salvia sclarea L. obtained in 1989, and analysed by gas chromatography, were kept in a refrigerator, at $+4^0$C. They were analysed again after 15 years. The content of linalool, linalylacetate and geraniol, has been determined. The decrease of linalool content and linalylacetate content increase has been noticed.

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P 071

QUALITY COMPOUNDS OF THE ESSENTIAL OILS OF SOME ALTERNATIVE AROMATIC FOOD PLANTS

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Researches on introduction of useful aromatic plants have been carried out in NBG NAS of Ukraine throughout 1999-2003 years. The purpose of the researches is enrichment of the cultivated flora of Ukraine with new species, varieties, cultivars and hybrids of aromatic plants. *Ocimum basilicum L., O. Gratissimum L., O. sanctum L., Dracocephalum moldavica L.* and *Nepeta cataria var.citriodora Beck.*) of Lamiaceae family are an aromatic plants that contains natural essential oils.
Essential oil was obtained by water distillation procedure for 2 h using Clevendger-type apparatus, and identification of components by high effective gas chromatography in a "Chrom 41", using a capillary column, and flame ionization detector.
The dried leaves of *O. basilicum* contain to 1,73% essential oil, *O.gratissimum* – 2,37, *O.sanctum* – 0,70 %, *D.moldavica* – 0,65%, *N.cataria* - 2,57% essential oil. The major compounds in the oils are linalool (*O. basilicum*), eugenol (*O.gratissimum*), 1,8-cineole, eugenol, methylchaviol (*O.sanctum*), citral (*D.moldavica*), geraniol (*N.cataria var.citriodora*). 9 main components were characterized in the essential oil of *O.basilicum*, representing 83,82% of the total oil. The most abundant component was linalool which constituted 45,99% of the total oil. 10 main components were characterized in the essential oil of *O.gratissimum*, representing 85,21% of the total oil. The most abundant component was eugenol which constituted 72,08% of the total oil. 8 main components were characterized in the essential oil of *O.sanctum*, representing 65,04% of the total oil and 2 unknown components 23,38% of the total oil. 7 main components were characterized in the essential oil of *D.moldavica*, representing 93,66% of the total oil. The most abundant component was citral which constituted 34,78% of the total oil. 6 main components were characterized in the essential oil of *N.cataria*, representing 79,91% of the total oil. The most abundant component was geraniol which constituted 23,26% of the total oil.

All this plants can be cultivated in the Ukraine for culinary and medicinal purposes as a fresh herb and as a dried spice.

Key words: food aromatic plants, biochemical compounds

P 072

NATIVE AND DERIVATED PRENYLISOFLAVONES

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Naturally occuring prenylflavonoids, distributed in plants, are very popular for their various biological activities. Two prenylisoflavones osajin (I) and pomiferin (II) were isolated from the fruits of *Maclura pomifera* (Moraceae) and identified by spectral analysis [1]. Iso-osajin (III) and iso-pomiferin (IV) were prepared from native isoflavones by reaction of native compounds with formic acid. Cyclization of prenyl chain in position 6 with hydroxyl group in position 5 leads to the new dimethylpyrane ring. Antioxidant activity of all these compounds was evaluated using methods with 1,1-difenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzthiazolin)-6-sulfonic acid ($ABTS^{+}$). Compounds III and IV exhibit decrease of antioxidant activity. From I and II were prepared two bromderivates by halogenation and separation using flash chromatography. These compounds are tested for their antifungal properties.

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Key words: Maclura pomifera, Pomiferin, Osajin, DPPH

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P 073

VOLATILE CONSTITUENTS OF *SALVIA* SPECIES NATIVE TO CONTINENTAL PARTS OF SERBIA AND MONTENEGRO

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The essential oils of *Salvia reflexa* Hornem., *S. nemorosa* L. and *S. glutinosa* L. from Pannonian plane (northern Serbia) and *S. verticillata* L. from Tara mountain (western Serbia) have been studied. The oil content ranged from 0.06-0.42%, and therefore, investigated species could be assigned to the

group of oil-poor members of *Lamiaceae*. By the means of GC-MS, 27 constituents were identified in the oil of *S. reflexa*, 23 in *S. nemorosa* and 13 in *S. glutinosa*. Oil specimen of *S. verticillata* comprised of 26 constituents. In contrast to many other *Salvia* species whose essential oils are characterized with high percentage of monoterpenes, our samples had high content of sesquiterpenes. The major constituents in *S. reflexa* oil were palmitic acid (28.02%) and sesquiterpene alcohol spathulenol (22.55%), while in the oil of *S. nemorosa* and *S. glutinosa* oxygenated sesquiterpene caryophyllene oxide was the main compound, averaged 23.36% and 33.34%, respectively. In the oil of *S. verticillata* sesquiterpene hydrocarbons *trans*-caryophyllene (10.2%) and β-cubebene (8.6%) were dominant compounds. Results obtained agree with previous reports that oil-poor species of *Lamiaceae* possess oil rich in sesquiterpene compounds [1]. In contrast to *S. officinalis* from Adriatic coast (i.e. Dalmatia and Montenegro), wild growing *Salvia* species from continental areas are particularly rich in oxygenated sesquiterpenes. In order to clarify species relationship of *Salvia* genus considering the effect of various environmental factors, further studies of wild growing *Salvia* species are necessary.

Key words*: Salvia* spp, essential oils, oxygenated sesquiterpenes

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P 074

EFFECTS OF DIFFERENT MISTLETOE (*VISCUM ALBUM* L.) EXTRACTS ON CANCER PREVENTION AND INHIBITION OF CARCINOGENESIS OF ERLICH CARCINOMA

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Mistletoe extracts are nowadays widely used in therapy of hypertension and have been shown to possess significant antitumour activity, *in vivo*. Main groups of compounds found in mistletoe are lectins, viscotoxins, flavonoids, phenylcarbonic acids, polysaccharides, alcaloids, biogenamines (feretylamine, tyramine, hystamine, acetylholine), tannins, terpenoids, saponnins, phytosterols, aminoacids, vitamins, etc. Some of those compounds are proved to have cytotoxic effects and immunomodelling potential.

In this paper effects of different aqueous extracts obtained from mistletoe leaves from different host trees on, *in vivo*, cancer prevention and inhibition of carcinogenesis of Erlich breast carcinoma were examined. Animals used in experiments (MNRI mice) were devided into groups. The first was control group (mice with transplanted Erlich carcinoma), the other groups received standardized aqueous extracts of mistletoe leaves collected from pear and plum host trees as a late therapy, prevention and early therapy. We have observed significant reduction of cancer incidence in all groups that received mistletoe extracts in comparison to control. Carcinogenesis of Erlich breast carcinoma was inhibited almost 100% in male animals that received mistletoe extracts as a prevention, and approx. 85% in female animals. We also observed significantly reduced number of carcinoma cells in animals with developed carcinoma and high citotoxicity of mistletoe extracts expressed through high percentage of damaged carcinoma cells. In addition, biochemical markers of oxidative stress, analysed in blood and liver, were also significantly reduced.

Key words: Viscum album, anticancer, antioxidant

P 075

BENZOPHENONE DERIVATIVES FROM *HYPERICUM ANNULATUM* AND THEIR *IN VITRO* ANTINEOPLASTIC AND CYTOPROTECTIVE ACTIVITIES

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Four known benzophenones, annulatophenone (1), annulatophenonoside (2), acetylannulatophenonoside (3), hypericophenonoside (4) [1, 2] and a new benzophenone O-glucoside neoannulatophenonoside (5) were isolated from aerial parts of *Hypericum annulatum.* The new structure was identified as 3',5',6-trihydroxy-4-methoxybenzophenone-2-O-β-D-glucopyranoside by means of chemical, physical and spectral evidence. The benzophenones and gentisein (6), a trioxygenated xantone, which was produced by hydrolysis of hypericophenonoside were tested for cytotoxic activity against the human chronic myeloid leukemia-derived K-562 cells and the acute myeloid leukemia-derived HL-60 cells, using the standard MTT-dye reduction assay for cell viability. In addition their ability to modulate the effects of the antineoplastic agent cisplatin, when applied concomitantly in non-cytotoxic concentrations were evaluated against the same cell lines. The results from the in vitro screening program revealed that the previously described compound 1 was the most active cytotoxic agent, whose efficacy was comparable to that of 6. The other compounds 2, 3 and 4 were less active, whereas the newly described compound 5 was least cytotoxic, causing marginal effects against K-562, but not against HL-60. The evaluation of the combinational effects of the tested compounds and cisplatin, revealed that with the only exception of gentisein they significantly ameliorated the cytotoxic effects of the antineoplastic agent. The results from the glutathion (GSH) levels determination revealed that the observed cytoprotection was at least in part modulated via GSH elevation, following treatment with 2, 3 and 5.

Key words: Hypericum annulatum, benzophenone-O-glycosides, spectral analysis,antineoplastic, cytoprotective activities

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P 076

COMPARATIVE PHYTOCHEMICAL STUDIES OF *MENTHA PIPERITA* GENOTYPES MULTIPLIED *IN VITRO*

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The *in vitro* multiplication of *Mentha piperita* aimed to obtain a uniform vegetal material, with raised vigour and bioproductive characteristics similar to those of the original plant – original genotype. By means of the comparative phytochemical study, we quantitatively and qualitatively

analyzed the content of volatile oils, polyphenols, flavons and sterolic compounds. The analyses were performed by TLC and gas – chromatography on 4 mint genotypes. We analyzed both inflorescences and *herba* used in the phytopharmaceutical practice due to the phytocomplex containing polyphenols, flavons and triterpens.
The highest content of essential oils was registered by variants 3V (C-371/200), with a content of 1.210 ml% and by 1V (Cristal) with a content of 1.070 ml%; the qualitative determination showed the fact that there are no major differences between the number of the volatile fractions; menthol is domineering as well as a fraction similar to thymol. In *herba* we identified about 8 sterolic fractions (cholesterine, ursolic and oleanolic acid) and a biosynthetic spectrum diversified for polyphenols (caffeic, chlorogenic, ferulic and cumaric acids) and flavons (luteolin and apigenin). In biosynthetical spectrum of the flavonic and polyphenolic compounds from the herba is almost identical in the analyzed variants (5-8 spots), with few exceptions that are in the normal variation limits.
The data obtained confirm that, by means of the micropropagation technique, the plants preserve the bioproductivity characteristics, the manifested phenotypical expression being determined by the genotype.

Key words: genotypes, tissue culture, volatile oils, flavons, polyphenols

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P 077

COMPARATIVE PHYTOCHEMICAL INVESTIGATIONS IN *MELISSA OFFICINALIS* SPECIES OF DIFFERENT PROVENIENCES

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Melissa officinalis contains volatile oils, flavonoids and bitter substances and is used to treat heart neurosis, to prevent sleeplessness. It is also as diaphoretic, carminative, digestive tonic, febrifuge and its antiviral and astringent affect recommends balm in the treatment of the affections of the oral and pharyngeal mucous membrane (1, 2).
Balm was cultivated in different habitats to evaluate the influence of the environmental factors and of the biosynthetic potential. We made ethanolic extracts in different concentrations (40, 50 and 70%) and dosed the content of flavons (rutin, cvercetol) and polyphenols (caffeic, ferulic and cumaric acids) by means of chromatography. The chromatographic technique was done by HPLC, by isocratic Jasco coupled with a UV-VIS detector.
The analyses showed the variation of the rutin and cvercetol content in the ethanolic extracts, the highest quantities are in the 70% extract (1194.857µg/mL) compared to 40% (754.428µg/mL) and 157.81µg/mL (70%) and 100.003µg/mL (40%) respectively. The caffeic, ferulic and cumaric acids revealed in the extracts observe the same biosynthetic model – the higher concentrations belonging to the 70% extracts.

Key words: different habitats, ethanolic extracts, HPLC, flavons, polyphenols

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P 078

ANTIMICROBIAL PROPERTIES OF ESSENTIAL OILS AGAINST SPORE FORMING BACTERIA

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The spores are the critical target for decontamination regimens since they are more resistant to many harsh treatments than their growing-cell counterparts. In addition many spore forming bacteria are the causative agents of serious diseases. Therefore the search for agents able not only to suppress the growth of vegetative cells but also to inactivate spores is of great importance. Since the essential oils (EO) exhibit antimicrobial properties we investigated their potential activity against vegetative cells and spores of eight spore forming bacteria belonging to genus *Bacillus* (*B.thuringiensis*, *B. sphaericus*, and *B. subtilis*). The EOs used in this study were obtained from *Origanum vulgare* ssp. hirtum, *Origanum dictamnus, Mentha pulegium, Mentha spicata* and *Salvia fruticosa*. All EOs tested and their main components exhibited a variable degree of antimicrobial activity against vegetative cells of all bacterial strains tested, with exception of camphore, which appeared to be active only against *B. sphaericus* strains. The highest antimicrobial activity was observed with EOs derived from *Origanum* species. Additionally all EOs as well as their main components were able to kill spores of *B.thuringiensis* strain HD-1 at variable levels. The highest spore-killing activity was observed with the EO derived from *Origanum vulgare* ssp. hirtum and with carvacrol (one of the main component of the oil). Spore killing by carvacrol appears to render spores defective in germination.

Key words : essential oils, Origanum, carvacro, spores, antibacterial

P 079

ELICITATION OF CALLUS TISSUE OF *RHODIOLA KIRYLOWII* (REGL.) MAXIM

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Rhodiola Kirylowii (Regl.) Maxim (Crassulaceae family) grows in North Asia and China.
The active constituents were isolated from roots of *Rhodiola Kirylowii* and identified as: salidrosid, tyrosol, daucosterol, cyanogenic glucoside-lotaustralin [2, 4] and beta-sitosterol [2].
Rhodiola Kirylowii is treated as an adaptogenic plant. Results show that oral administration of *Rhodiola Kirylowii* can significantly reduce pathologic damages done to rat's viscera due to hypoxic environment of altitude and can efficiently protect from cardiopulmonary disorders caused by

changing the altitude [6]. The elicitation as a method to enhance the active compounds content in the callus culture of *Rhodiola sachalinensis* [5] and *R. rosea* [1, 3] was used.
In this investigations two kinds of elicitors were used: yeast extract and methyl jasmonate. Yeast extract was added to the growing callus in concentrations of 1-10 mg of elicitor per culture. Methyl jasmonate was used in four different concentrations. The elicited callus was collected and dried after 5-7 days. The fresh and the dried biomass was measured. The content of active compounds was determined. The obtained results are presented in the tables.

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Key words: *Rhodiola Kirylowii*, elicitation, methyl jasmonate, yeast extract.

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P 080

IN VITRO SPECIFIC ANTIMICROBIAL PROPERTIES OF BALKANIC *ARTEMISIA* EXTRACTS

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3 samples of methanolic leaves extracts of balkanic *Artemisia : A. absinthium* L., *A. annua* L., *A. vulgaris* L., have been tested against bacteria and yeasts representative of human and cats and dogs skin microflorae.
Among the three extracts two inhibited the growth of a lot of the tested strains. On the one hand, the only *Pseudomonas aeruginosa*, *Moraxella sp*. and *Alcaligenes* resisted to a concentration of 1000 ppm. On the other hand *A. annua* and *A. vulgaris* extracts showed a good activity against the strains responsible for dogs and cats externa and media otitis: *S. intermedius* and *Malassezia pachydermatis*. Those extracts showed also bacteriostatic activity on saprophytic Staphylococci and Micrococci generating exoenzymes responsible for bad odours of the skin surface (ie smelly feet). On the contrary, *Moraxella* and *Alcaligenes* without exoenzymes were spared, so able to colonize with benefit the human skin.

Key words: Artemisia absymthium, annua, vulgaris L., in vitro antimicrobial properties, human skin microflora, cats and dogs skin microflora.

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P 081

COMPARATIVE FATTY ACID ANALYSIS OF *TELEKIA SPECIOSA* (SCHREB.) BAUNG

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Fatty acids in petroleum-ether extracts prepared from the flower, stem, and leaf of *Telekia speciosa* (Schreb.) Baung. (syn. *Buphthalmum speciosum*) (Compositae) were studied by capillary gas chromatography-mass spectrometry (GC-MS). The flower and leaf extracts were found to contain similar fatty acids, namley palmitic, linoleic, and oleic acids, whereas the stem extract contained only caproic acid.

Key words: *Telekia speciosa*, Compositae, *Buphthalmum*, fatty acid, GC-MS

P 082

SPECIFIC ANTIMICROBIAL ACTIVITIES OF MEDITERRANEAN ESSENTIAL OILS

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Essential oils (E.O.) are well known as antimicrobial agents. Nevertheless a lot of them do not possess such properties. Among the very wide diversity of bacteria and fungi, many show an important resistance. Mediterranean and subtropical countries furnish many chemotypes with different properties. In this study, 21 E.O.(essentialy from the Lamiaceae, Apiacea and Lauraceae) were tested against bacteria and fungi representative of human and animal skin microflora. Among the 21 samples tested, only seven were selected. Their main constituents are already well known for their antimicrobial properties : eugenol for *Oscimum gratissimum* chemotype eugenol, thymol/carvacrol for *Origanum vulgare* and *Satureia hortensis*, geraniol for *Monarda fistulosa var. geraniolifera*, citral for *Litsea citrata*, satured and insatures aldehydes for *Coriandrum sativa* leaves.
The mains applications would be in cosmetology as deodorants as they inhibited bacteria generating bad odours, and also in veterinitary medicine for local treatment of externa and even media otitis.

Key words: Mediterranean essential oils, antimicrobial properties, human skin microflora, cats and dogs skin microflora

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P 083

CONSTITUENTS OF *IRESINE HERBSTII* –ISOLATION AND BIOLOGICAL CHARACTERISATION

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Substances with antioxidant activity are in the centre of interest because of their influence on various diseases. Ethanolic extract of *Iresine herbstii* Hook (Amaranthaceae) shows an antioxidant activity [1]. *Iresine herbstii* is erect or ascending perennial herb native to tropical South America, now spread to tropical and subtropical areas all over the world. For our study were used plants cultivated in faculty greenhouse.
Ethanolic extract of the aerial part was separated by colon chromatography on silicagel. After rechromatography were obtained two isoflavonoids – known 2´,5-dimethoxy-6,7-methylenedioxyisoflavone (tlatlancuayin) [2] and 2´,2,5-trimethoxy-6,7-methylenedioxyisoflavanone. Both substances were tested for antioxidant activity using methods with 1,1-difenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzthiazolin)-6-sulfonic acid ($ABTS^{+}$). Isolated isoflavonoids have lower antioxidant activity in comparison with catechin.

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Key words: Iresine herbstii, isoflavonoids, antioxidant activity

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P 084

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF PROPOLIS AND ITS QUALITY CONTROL

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Propolis is a natural resinous hive product with various biological activity. It is has been used sience ancient times in folk medicine in many regions of the world and is still widely used as a component in pharmaceutical and cosmetic products. Chemical composition of propolis depends on

the plant sources available to bees at different locations. In the Temperate zone, propolis has similar chemical composition and orginates from bud exudates of poplar trees. Its main compounds are flavonoids, phenolics acid and their esters. The most of them possess high antibacterial and antifungal activities. For this reason quantification of the active components is a good choice and one of the steps for quality control of the propolis.
Three spectrophotometric methods for quantification of the main groups bioactive compounds in poplar type propolis were developed. The spectrophotometric assay based on aluminium chloride complex formation was applied to quantification of total flavones and flavonols, using galangin as the standard. For quantification of total flavanones and dihydroflavonols, colorimetric method from DAB9 was used with pinocembrin as the standard. Total phenolics were measured by the Folin-Ciocalteu method and mixture pinocembrin:galangin 2:1 were used as the standard. The validation was performed using a model mixture of "poplar" compounds. 10 poplar type propolis samples from Bulgaria and 8 samples from Macedonia were analysed by above-mentioned methods.

Key words: propolis; phenolics; flavones; flavanones; spectrophotometric quantification

P 085

ANTIOXIDANTS IN *ALLIUM ROSEUM* L.

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Allium genus produces a large number of chemical substances, which can have value as medicinal agents. Therefore *Alliums* have been grown for many thousands of years for their therepeutic and prophylactic properties but also because religious significance flavor and taste. Being used for medical purposes in great number of countries, for centuries, *Allium* plants have been shown to prevent tumour promotion, cardiovascular diseases, and ageing processes that are associated with free radicals and lipid peroxidation. Bulbs and leaves of *Allium cepa* L. and *Allium psekemense* B. Fedtsch are proven brain and blood vessels protectors as well as antioxidants and free radical scavengers [1]. Antioxidants are important substances which possess the ability to protect the body from damage caused by free radical-induced oxidative stress. In this study antioxidant activity of leaf, bulb, stalk and bloom of *Allium roseum* L. was investigated. Next biochemical parameters were determined: activities of antioxidant enzymes (superoxide dismutase, catalase, peroxidase, glutathione peroxidase), quantities of malonildialdehyde, superoxide and hydrohyl radicals and reduced glutathione, contents of total flavonoids and soluble proteins and DPPH scavanger activity. Our results indicated that *Allium roseum* L. exhibit antioxidant ability in all investigated plant organs and therefore it could be used in human diet as the source of antioxidants. Furthermore, ESR signal of DMPO-OH radical adducts in the presence of bulb phosphate buffer (pH 7) extract was reduced for 84.61 %.

Key words: Allium roseum, antioxidant, ESR

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P 086

HYDROETHANOLIC EXTRACTS OF *HYPERICUM* SPECIES MODERATELY MODIFY *IN VITRO* SEROTONIN REUPTAKE

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In many countries today the preparations based on extracts of *Hypericum perforatum* L. (St. John's wort) are licensed for depression, anxiety and insomnia [1]. Although it has been shown that *H. perforatum* affects monoamine, glutamate and GABA neurotransmitter systems by different mechanisms, it is proposed that the inhibition of monoamine transmitter synaptic reuptake is the base of its therapeutic potential [2,3]. The serotonin (5-HT) reuptake inhibition, induced mainly by hyperphorin, is indicated to give the greatest contribution to the antidepressive effect of this plant, like it does for the most of new synthetic antidepressive drugs [3,4]. We investigated potential blockade of in *vitro* serotonin reuptake by the extracts (70% EtOH extracts of dry flowering aerial parts) of six other *Hypericum* species: *H. hirsutum* L., *H. richeri* Vill., *H. acutum* Mnch., *H. androsaemum* L., *H. maculatum* Crantz. (collected from natural populations in Serbia and Montenegro), and *H. barbatum* Jacq. (cultivated). Methods of isolation of rat cortical synaptosomes and ^{3}H-5-HT uptake *in vitro* are described elsewhere [5]. In comparison to the 5-HT reuptake blocking effect (IC_{50} = 6 μg/ml) of the raw commercial hydroethanolic extract (Institute "Josif Pančić" Belgrade), neither of other extracts were so effective. The only respectable activity exhibited *H. androsaemum* (IC_{50} = 60.5μg/ml), while all other extracts have IC_{50} values between 100 μg/ml and 1 mg/ml, except *H. hirsutum* (> 1mg/ml). We may conclude here, if any of explored *Hypericum* species could have an antidepressive potential, it would not be by means of the synaptosomal 5-HT reuptake inhibition.

Key words: Hypericum species, hydroethanolic extracts, serotonin uptake, antidepressive

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P 087

INVESTIGATION ON ANTIOXIDANT ACTIVITY OF *CARLINA ACANTHIFOLIA* ALL.

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C. acanthifolia All. (syn. *C. utzka* Hacq., Compositae), is a perennial species with leaf rosette, without stem (rarely with short stem) and with singular capitula, up to 10 cm in diameter. It is widespread in the hills and mountains of E Serbia [1]. From its leaves C-glycosylflavones (orientin, isoorientin, schaftoside and isoschaftoside) were isolated [2]. Since polyphenolic compounds have

high antioxidant potential, we studied the antioxidant activity of hydromethanolic (70% MeOH) extract of the flowering aerial parts of this species.
Using the FRAP assay [3] determined total antioxidant activity (TAA) of extract was 0.6 μmol/mg (*ca* 13 times lower than L-ascorbic acid). TAA was in correlation with total phenols content determined spectrophotometrically using Folin-Ciocalteu reagent [4]. Radical scavenging activity was tested against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) [5] and OH radicals [6]. Inhibition of DPPH radical was in concentration-dependent manner: 3.5, 5.8, 28.6, 54.8 and 94.7 at concentrations of 5, 10, 50, 100 and 250 μg/ml (IC_{50}=90 μg/ml). Influence on scavenging of OH radical was measured in Fe^{3+}-EDTA-H_2O_2-deoxyribose system, following degradation of 2-deoxyribose into TBA-reactive substances. At concentrations of 6.25-200 μg/ml, extract scavenged OH radical reaching maximum of inhibiton (*ca* 57%) at a range of concentrations of 12.5-50 μg/ml. TBA test was used in order to examine influence of extract on Fe^{2+}/ascorbate induced lipid peroxidation (LP) in liposomes [7]. At concentrations of 25.0, 62.5, 125.0 i 250.0 μg/ml extract exerted inhibitory effect of 32.0, 39.9, 47.3, and 42.6%, respectively.

Key words: *Carlina acanthifolia,* FRAP assay, DPPH radical, OH radical, TBA test

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P 088

ESSENTIAL OIL COMPOSITION OF *HYPERICUM PERFORATUM* L., *HYPERICUM TETRAPTERUM* FRIES AND *HYPERICUM OLYMPICUM* L. GROWING IN GREECE

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Hypericum L. is a large genus of herbaceous or shrubby plants, which occur widely in temperate regions of the world. *H. perforatum* L. and *H. tetrapterum* Fries belong to the section Hypericum. They are perennial herbs, glabrous, with black glands present on stems, leaves, anthers and sometimes on sepals and petals. *H. olympicum* L. belongs to the section Olympia (Spach) Endl. It is a perennial herb, often woody at the base, with black glands present on anthers and sometimes elsewhere [1].
Aerial parts of the plants were collected during the flowering stage from the following locations: *H. perforatum* was collected from prefecture Ioannina, *H. tetrapterum* from prefecture Trikala and *H. olympicum* from prefecture Fthiotida. The essential oils were obtained by hydrodistillation. The chemical analysis of the oils was carried out using GC and GC/MS method [2].
The oil content of the aerial parts of *H. perforatum* was 0.28%, of *H. tetrapterum* was 0.20% and of *H. olympicum* was 0.18%. The most abundant constituents of *H. perforatum* essential oil were α-pinene (21.0%) and 2-methyl-octane (12.6%), while in *H. tetrapterum* the dominating components were α-copaene (11.3%) and α-longipinene (9.7%). In the essential oil of *H. olympicum* the major

components were germacreneD (16.0%) and (*E*)-caryophyllene (7.4%). All oils were characterized by a high content of sesquiterpene hydrocarbons (35.2%, 43.6% and 47.9%, respectively).

Key words: Hypericum perforatum; Hypericum tetrapterum; Hypericum olympicum; essential oil composition

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P 089

ESSENTIAL OIL OF *ACINOS MAJORANIFOLIUS* (MILL.) SILIC (LAMIACEAE) FROM MONTENEGRO

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Acinos mayoranifolius (Mill.) Silic is endemic, spread across the western border area of Croatia and Herzegovina and the western part of Montenegro. It is a distinctly Mediterranean mountainous species. It inhabits open limestone rocky terrains, rims of karst forests, on heights between 20 and 1400m (Silic, 1979, 1984). *A. majoranifolius* is distinguished from the rest of the *Acinos* species by its aroma, and therefore the composition of its essential oil is presented in this work.
We have studied the essential oils of three populations of *A. majoranifolius* collected from different localities in Western Montenegro: mountain Orjen, Njegusi and Lijeva Rijeka. The samples were gathered in the flowering period. The essential oil was obtained by hydrodistillation from the dried aerial parts of the plants. The analyses of the oils were carried out using GC/MS. The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and mass spectra with those obtained from authentic samples and/or the MS library (Adams, 1995).
The yield of the essential oils was between 0.5% and 0.6%. Pulegone was the main component of all the oils (65.4%-81.3%). The Mt. Orjen and Njegusi populations had a high content of isomenthone (11.4% and 15.4%), while this compound was detected only in traces in the Lijeva Rijeka population. Isopulegone and caryophyllene oxide were found in similar concentrations in all populations.

Key words: Acinos mayoranifolius (Mill.) Silic, pulegone, isomenthone, isopulegone, caryophyllene oxide

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P 090

INTERACTION OF EXTRACTS FROM THREE *STACHYS* SPECIES WITH RAT LIVER MAO SYSTEMS

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Previous studies have shown that *Stachys* species (Labiatae) exert different pharmacological effects such as anxiolytic, antioxidant, and anti-inflammatory [1-3]. Aim of our work was to investigate possible effects on MAO activity of three endemic *Stachys* species: *S. plumosa* Griseb., *S. beckeana* Dörfler & Hayek and *S. anisochila* Vis. et Pančić. The flowering aerial parts of species were collected from natural populations in Serbia. Dry plant material was extracted with $CHCl_3$ and then with MeOH (distinguished as liposoluble and hidrosoluble fractions, respectively). All dry extracts were dissolved in DMSO (up to 5 %) and used in experiments in a range of concentrations (1 μg/ml – 3 mg/ml). Partially purified mitochondrial fraction from rat liver homogenates [4] was used as MAO source in competitive radioassays with ^{14}C-tyramine (55 mCi/mmol) [5]. Specific MAO inhibitors: 200 nM pargiline and 30 nM clorgiline were used to resolve partial MAO A and MAO B activity, respectively. All extracts show moderate to poor potential to inhibit MAO systems, and no one exhibits a somewhat acceptable value of IC_{50} < 100 μg/ml. The utmost potential to reduce MAO A activity, among the tested extracts, had a liposoluble fraction of *S. beckeana* (IC_{50} = 201 μg/ml), while hidrosoluble extract of *S. anisochila* showed the greatest inhibition of MAO B (IC_{50} = 112 μg/ml; 316 μg/ml for MAO A). All other extracts had IC_{50} values above 330 μg/ml and up to 3.2 mg/ml. The actual level of *in vitro* MAO blocking potentials that we found here for *Stachys* extracts hardly suggests their pharmaco-physiological effects by this mechanism.

Key words: Stachys plumosa, Stachys beckeana, Stachys anisochila, MAO inhibition.

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P 091

FLAVONOIDS AND FREE RADICAL SCAVENGING ACTIVITY OF EXTRACTS OF *GENISTA SERICEA* WULF.

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The genus *Genista* L. (Fabaceae) is represented by 13 species in the Serbian flora [1]. We studied flavonoid composition and free radical scavenging activity (RSC) of extracts of *Genista sericea* Wulf. The flowering aerial parts of this species were extracted by $CHCl_3$, and than by MeOH. Dried MeOH extract was dissolved in water and successively extracted by Et_2O, EtOAc and n-BuOH.

Using HPLC method in the tested extracts several flavonoids were determined. In the $CHCl_3$ extract isoflavon aglycones were dominant (genistein, formononetin, prunetin and daidzein were identified). In the Et_2O extract, besides mentioned aglycones, there were luteolin glycosides (luteolin 7-O-β-glucoside was identified) and isoflavon glycosides (ononin as the main component, genistin and daidzin were identified). EtOAc was selective for glycosides. In the n-BuOH extract the main component was an isoflavon glycoside.
RSC of examined extracts was investigated to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. DPPH scavenging effects was recorded spectrophotometrically, monitoring the transformation of DPPH stable radical into reduced form (DPPH-H) [2]. The strongest scavenger activity showed EtOAc extract (IC_{50}=2.7 μg/ml), than Et_2O extract (IC_{50}=6.5 μg/ml), n-BuOH extract (IC_{50}=12.2 μg/ml) and $CHCl_3$ extract (IC_{50}=29.3 μg/ml). The obtained results suggest a high antiradical property of *G. sericea*, which is mostly due to the presence of different flavonoids in investigated extracts.

Key words: *Genista sericea,* HPLC, flavonoids, DPPH radical

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P 092

ESSENTIAL OIL COMPOSITION OF *ROSMARINUS OFFICINALIS* FROM GREECE

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Rosemary (*Rosmarinus officinalis*) is one of the most important aromatic and medicinal species of the family of Labiatae. Being a perennial plant, its growth habit is as a shrub of great longevity, well adapted to the Mediterranean warm and dry climates (subhumid and arid regions) [1].
The plant is extensively used in the Mediterranean basin, where it is also present in the vegetable gardens because of its use as an aromatic plant.
In our continuing research on Greek aromatic plants we analysed the essential oil of a natural population of *R. officinalis* from Zakynthos island collected during full blossoming.
The essential oil was obtained from the dried aerial parts by hydrodistillation and analyzed by means of GC and GC/MS.
Thirty eight components were identified constituting the 97.3% of the total oil. The major components identified were α-pinene (24.1%), camphor (14.9%), 1,8-cineole (9.3%), camphene (8.9%), α-terpineol (8.8%) and borneol (8.0%).

Key words: Rosmarinus officinalis; essential oil composition; α-pinene; camphor; 1,8-cineole

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ANTIOXIDANT ACTIVITY OF NATIVE AND THERMAL TREATED *QUERCUS CERRIS SEMEN*

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We investigated antioxidant activity of kernels of Turkish Oak, *Quercus cerris* L. (Fagaceae). Dry MeOH extracts of native (sample 1) and thermal treated kernels (10 minutes at 200°C; sample 2) were used for investigations.
Total antioxidant activity (TAA) was determined using FRAP assay [1]. TAA of samples 1 and 2 was 1.38 and 2.77 μmol/mg, respectively. Comparing to L-ascorbic acid these values were *ca* 5.5 and 2.5 times lower, respectively. Both extracts scavenged stable DPPH radical [2] in concentration-dependent manner (concentration applied from 5-200 μg/ml), with IC_{50}=8.66 μg/ml (sample 1) and 11.77 μg/ml (sample 2). TBA test was used to measure inhibitory effect on Fe^{2+}/ascorbate induced lipid peroxidation (LP) in liposomes [3]. Inhibition was more prominent for sample 2 (IC_{50}=41.75 μg/ml) than for sample 1 (IC_{50}=54 μg/ml). Antioxidant effect on refined sunflower oil was determined under Schaal oven test conditions, following the change of peroxide value (PV) [4]. Higher PV of sample 1 indicated its lower antioxidant activity than sample 2.
Determined higher antioxidant activity of thermal treated kernels was correlated with polyphenols content. Total polyphenols content determined spectrophotometrically using Folin-Ciocalteu reagent [5] was 15% in sample 1 and 31% in sample 2 (calculated as catechin). Gallic acid and rutin contents determined by HPLC were 0.85% and 0.90%, respectively in sample 1, and 4.55% and 5.00%, respectively in sample 2 [6].

Key words: *Quercus cerris semen,* FRAP, DPPH radical, lipid peroxidation, polyphenols content

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P 094

ESSENTIAL OIL COMPOSITION OF *MALABAILA AUREA* BOISS.

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Malabaila is a small genus of the family Umbelliferae distributed in East Mediterranean to C. Asia and Iran, presented with 3 taxa in Europe [1]. Some species of *Malabaila* are locally edible [1]. *Malabaila aurea* is a biennial herb present in Balkan Peninsula [2]. Very little is known in the

literature about this slightly aromatic plant and this prompted us to analyse its essential oil composition.
The plant material was collected during flowering-fruiting stage in May 2003 from Arachova village, (Viotia Prefecture, Greece). The essential oil of the studied taxon was obtained by hydrodistillation for 3 h in a Clevenger-type apparatus from the air-dried aerial parts. The chemical analysis of the oil was carried out using GC-MS.
The major component of the oil was found to be octyl isobutyrate (40%), an aliphatic ester with a herbaceous and fruiting green odor, used as a flavoring agent.
The biostatic activity of the oil was evaluated using the broth dilution method [3]. The oil exhibited antibacterial activity against *Staphylococcus aureus* ATCC 25923 (MIC value 5 μl/ml; MBC value 10 μg/ml. *Candida albicans* was highly susceptible to the analysed oil, presenting a MIC value of 1.25 μl/ml as well as a good fungicidal activity in concentration of 2.5 μl/ml.

Keywords: Malabaila aurea; essential oil composition; octyl isobutyrate; biostatic activity

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ESSENTIAL OIL COMPOSITION OF *PHLOMIS CRETICA* C. PRESL

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The genus *Phlomis* (family Labiatae) comprises 12 species, which are naturalized in Europe. *Phlomis cretica* C. Presl is a dwarf shrub up to 45 cm high, with yellow flowers found in the East Mediterranean region [1]. Some species are mentioned by Dioskorid as herbal drugs and are used ethnopharmacologically in herbal medicine possessing antimicrobial, tonic and stimulant activity [2].
Phlomis cretica was collected during the flowering stage from Chania (Crete Island) in May 2003. The air-dried crashed verticillasters (sample A) and leaves (sample B) were separately subjected to hydrodistillation. The oils analyses were carried out using GC/MS and the chemical composition of the volatile metabolites was determined.
Thirty six compounds, representing 91-100% of the oils, were identified. The qualitative composition of the oils did not present any differences. The main components of *P. cretica* verticillasters oil (sample A) were: germacrene D (34.0%), germacrene B (11.0%) and (*E*)-caryophyllene (9.2%), whereas in the leaf oil (sample B) germacrene D was the dominating component (47.9%), followed by α-pinene (11,2%) and γ-curcumene (7.3). Both oils were rich in sesquiterpene hydrocarbons comprising 79.1% and 82.7% respectively, whereas oxygenated sesquiterpenes accounted an amount of 4.6% in verticillasters oil and only traces in leaf oil.

Key words: Phlomis cretica; germacrene D; α-pinene; germacrene B; essential oil

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P 096

ESSENTIAL OIL COMPOSITION OF FOUR *SALVIA* SPECIES GROWING WILD IN GREECE

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Salvia, the largest genus of the Labiatae family includes about 900 species widespread all over the world [1]. In our continuing research on the essential oils of Greek *Salvia* plants, we investigated the essential oils of *Salvia verticillata* (Sectio Hemisphace), *Salvia verbenaca* (Sectio Plethiosphace), *Salvia glutinosa* and *Salvia candidissima* (Sectio Aethiopis). To our knowledge the oil of the previous reported *Salvia* species from Greek origin have not been studied.
The essential oils of the examined *Salvia species* were obtained from the dried aerial parts by hydrodistillation and analyzed by means of GC and GC/MS.
The essential oil composition varied within the species. The major components identified in the examined oils of *Salvia* were:
S. verticillata- *β*-pinene (30.7%), *p*-cymene (23.0%), isopropyl ester of lauric acid (16.8%), α-pinene (7.6%) and nerolidol (5.2%). *S. verbenaca*- *β*-phellandrene (30.3%), (*E*)-caryophyllene (16.1%), methyl ester of 6-decanoic acid (15.0%), fenchone (9.4%), isopropyl ester of lauric acid (7.8%), camphor (7.0%) and (*Z*)-*β*-ocimene (6.6%).
S. candidissima- α-pinene (11.2%), 1,8-cineole (9.89%), *p*-cymene (7.4%), myrtenal (6.5%), pinocarvone (6.2%), camphene (5.7%).
S. glutinosa- butyl butyryl lactate (26.7%), sclareol (11.9%), caryophyllene oxide (10.7%), manool (6.8%), 13-epi-manool (5.6%).

Key words: Salvia verticillata; Salvia verbenaca; Salvia candidissima; Salvia glutinosa; essential oils

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P 097

PHENOLIC METABOLITES FROM GREEK LICHENS

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Lichens are symbiotic systems, an association of a fungus and an alga, which produce interesting metabolites in relatively high amounts [2]. Lichen biosynthesise a wide spectrum of unique aromatic derivatives, having a taxonomic significance, often linked together as esters known as depsides and depsidones. Several lichen extracts have been used for various remedies in folk medicine. Pharmacological evaluation of lichens has often indicated the presence of metabolites with antibiotic, antimycobacterial, antiviral, analgesic and antipyretic properties [4]. In the framework of our current investigations towards the isolation of bioactive metabolites from natural sources we were able to collect and chemically analyse two lichen species found in abundance in Greece. *Ramalina fraxinea* was collected from Prefecture Grevena and *Parmelia perlata* from Prefecture Hleias. To the best of our knowledge these organisms have not been chemically investigated in the past.

The shade-dried plant materials were macerated with CH_2Cl_2-MeOH (2:1) and the extracts were subsequently submitted to a series of chromatographic separations to eventually afford from the medium polarity fractions a number of phenolic metabolites. The extracts of both lichens were found to contain small amounts of atraric acid and yielded as main constituent the bioactive metabolite atranorin, previously reported as a significant taxonomic marker for various lichen species [1,3]. Besides the above mentioned metabolites from *Parmelia perlata* were also isolated the metabolites 3-formyl-2,4-dihydroxy-6-methyl-benzoic acid methyl ester, chloroatranorin and 3-chloro-5-formyl-4,6-dihydroxy-2-methyl benzoic acid methyl ester. The structures of the isolated compounds were determined on the basis of their spectral characteristics and with the aid of one and two-dimensional NMR experiments.

Key words: Ramalina fraxinea; Parmelia perlata; atranorin; chloroatranorin

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P 098

THE HERBICIDES RESIDUE MEASUREMENT METHOD BY THIN LAYER CROMATOGRAPHY (TLC), USING BIOCHEMICAL METHODS AT SOME MEDICINAL PLANTS

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The paper describes the thin layer cromatography technics and the most important biochemical reveable method used at herbicide residue measurement.
The thin layer cromatography is very useful tool in the identification of known and unknown compounds. It has been used in pesticide residue analysis owing to its applicability for solving the various separation problems and for gaining semiquantitative information on the pesticide level in the sample or for confirmation of pesticides identified by GLC or HPLC.
Some herbicide, especially photosinthesis inhibitors Afalon (linuron) and Gesagard (prometrin) have been used at coriander and chamomile crops. The reveable method for detection and quantification was Hill inhibition that is the most selective and sensitive, enable to determinate the herbicide residue at MDQ (minimum detectable quantity) values equal to 1 ng that correspond to lower LOD (limit of determination) 0,001 mg / kg, which respect the legislative requirments. The cleanup of extracts (one of the most important step in analysis) has been carry out by GPC (gel permeable cromatography), on SX-3 filled columns.
The specify of this TLC method is detecting the primarily herbicides inhibiting the photo synthesis, the reagent mixture for detection being:
borax buffer solution: mixture of 350 ml 0.05 mol borax solution
DCPIP reagent: 200 mg of 2.6 - dichlorphenol – indophenol Na-salt is dissolved in 500 ml borax buffer solution.
chloroplast suspension from wheat leaves
The conditions for analysis, after cleanup step are: adsorbent layer: silica gel G-60, thickness : 0,25 mm, on glass support ; activation time : 30 minutes at 105 ^{o} C, mobile phase : ethyl acetate ; concentration of herbicide into medicinal plant matrix: 1 mg / kg; spotulated volum:1-20 µl, Hamilton syringe; spotulated quantity: 1-20 ng; detection: chloroplast suspension reagent; visualisation: below a 60W Wolfram lamp for a few minutes; colour reaction: blue against a greenish background; the

detection and quantification are carried out by visual comparison of the spot of interest (standard and sample)- by comparing the Rf values and the spot size of the component to be determined to those of the standard- depends on the linear range. The chromatograms will be presented.

P 099

COMPARATIVE INVESTIGATION OF *HYPERICUM PERFORATUM* L. POPULATIONS IN SERBIA

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In two-year period, three pre-selected *Hypericum perforatum* populations (V2, V3and D4) were tested on yield, hypericin yield and content and intensity of wilting disease, at two localities in Serbia. Testing was conducted at lowland locality of South Banat (Pančevo, 80 m. a. s. l., g. latitude 44°$_N$, 52', 20" and g. longitude 20°$_E$, 42', 25") and at locality in Western Serbia (Tara mountain, 1004 m a. s. l., g. latitude 43°$_N$, 53', 41" and g. longitude 19°$_E$, 33', 41").
H. perforatum cultivation was established in spring (winter production of nursery plants). In climatic conditions of South Banat, in the first year, it gave two harvests and yielded 1070-2440 kg/ha while in climatic conditions of Tara mountain it gave one harvest and yielded 220 - 720 kg/ha of dry herb.
In general, best yields of dry herb in the second year achieved population D4 (ca. 2000 kg/ha of dry herb) above all due to a better tolerance on unfavourable climatic conditions and better plant density preservation in comparison to other two tested populations (V2 and V3). The lowest hypericin yield was recorded in the first harvest of the first vegetation (0,3 - 1,3 kg/ha), and the highest one in the first harvest of the second vegetation (1,7 - 3,8 kg/ha). Due to the best yield of dry herb and satisfactory hypericin content, population D4 stands out for a best source of hypericin among all tested populations. In comparison to locality Pančevo, intensity of wilting disease (caused by *Colletotrichum gloeosporoides*) recorded in the second year of growing, was lower at Tara locality. This observation points out to significance of ecological factors in development of this disease, meaning that the mountainous climatic conditions impose themselves as a better solution for growing of *H. perforatum* in terms of avoidance of wilting disease then the lowland growing conditions.

Key words: Hypericum perforatum, populations, locality, yield, hypericin, wilting disease

P 100

APPLE JUICE FORTIFICATED WITH HERB EXTRACTS

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Fruit e.g. apple is a rich source of biological active compounds. Antioxidants are very interesting group. They have role to protect a easy oxidize compounds in presence of oxygen. Presence of antioxidants is mostly in a skin of fruit. During technological process of fruit do not get to sufficient fortification of juice by antioxidant active compounds. Consequence is deterioration quality of the juice in the short time. This problem may be solved by fortification juices with natural or synthetic protective compounds. Commonly used ascorbic acid (vitamin C) is possible to substitute for

example with herb extracts. Fortificated apple juice with extract of lemom balm and oregano markedly improved quality of juice during storage time, and positively influenced taste and aroma of juice. Antioxidant activity of apple juice already was increase after addition 0,01 ml/L of etanolic lemon balm and oregano extracts (100 g of herb was extracted 48 h with 70% etanol). The DPPH method and method the formation of phosphomolybdenum complex were used to evaluation of antioxidant activity of apple juice.

Key words: antioxidants, apple juice, phosphomolybdenum complex

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P 101

ARE SECONDARY METABOLITES OF MEDICINAL PLANTS RESPONSIBLE FOR TOLERANCE TO TOXIC METALS?

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Primary metabolism refers to the processes producing the primary metabolites such as carbohydrates, proteins, nucleic acids, carboxylic acids and fats, all essential for the survival and well-being of the plants. All plants possess the metabolic pathway by which this compounds are synthesized and utilized. On the other hand, secondary metabolites are non-essential to life, but they contribute to the plant's fitness for survival. The division between primary and secondary metabolism is not entirely clear, however both types are linked together because of primary metabolism provides the organic compounds that are the starting materials for the secondary metabolic pathways. It is well known that the shikimate pathway links metabolism of carbohydrates to biosynthesis of aromatic amino acids and many aromatic secondary metabolites. The exclusive process producing carbohydrates is photosynthesis. Considering the fact that the photosynthesis of medicinal plants was not yet intensively studied, we focused our attention on *Hypericum perforatum* L. and *Matricaria recutita* (L.). We found that both species are not only cadmium hyperaccumulators but they are tolerant to cadmium treatment. Specific secondary metabolites of these plants such are hypericin, pseudohypericin (*H. perforatum*), (-)-α-bisabolol oxide B, apigenine, and some flavonoids, e.g. quercetin (*M. recutita*) could sequestrate cadmium ions what could be also resulted in higher tolerance of these medicinal plants to studied toxic metal.

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Key words*:* primary and secondary metabolism, medicinal plants, metal tolerance

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RESEARCHES ON JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS* L.). ITS UTILIZATION IN PHYTOPHARMACEUTICAL PRODUCTS FOR THE TREATMENT OF DIABETES AND ASSOCIATED DISTURBANCES

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The most common acute diabetic complications are metabolic problems (hyperosmolar hyperglycemic nonketotic syndrome or HHNS) and infections. The long term complications are macrovascular complications (hypertension, dyslipidemia, myocardial infarction, stroke), microvascular complications (retinopathy, nephropathy, diabetic neuropathy, diarrhea, neurogenic bladder, impaired cardiovascular reflexes, sexual dysfunction), and diabetic feet disorders. There is a metabolic link between insulin resistance and the associated risk factors for atherosclerotic, cardiovascular diseases, especially hypertriglyceridemia.
The effect of soluble fibers on lipid metabolism, blood glucose and lipid profiles are well known. Oligofructose, the soluble fiber from Jerusalem artichoke, decreases serum cholesterol and serum lipid levels and lowers the triglyceride levels in blood (inhibits hepatic lipogenesis).
The paper presents different isolation methods of polyphenolic and ructan phytocomplex from different organs of vegetable raw material. Mono and disaccharides and polyphenol complex were determined by a thin-layer chromatographic densitometric method. Some specific products with hypotrigliceridemic, hypocholesterolemic, immunomodulatory and antioxidative effects were elaborated in granule and tablet forms.
A specific granule- form product was elaborated to improve foot peripheral blood circulation. All this products are beneficial for human health, especially for the diabetics.

Key words: Jerusalem artichoke (Helianthus tuberosus), inulin, polyphenols, diabetes, natural products

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BIOLOGICAL ACTIVE ADDITIONS TO THE MEDICAL OINTMENTS AND COSMETIC CREAMS

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We investigated possibilities of using vegetable oils with iodinized fat acids as addition when medical ointment and cosmetic creams produced. Was established that scin condition was improved when used iodinezied vegetable oil as separately as in combination with animal fats, particulary with ostrich fat improve scin condition and quality of cosmetic remedies. Vegetable oils when iodinized becoming effective applicable remedy and carry out conduction biological active iodine into tissue. Such cosmetic and medical creams have high biological activity and speed up the treatment of specific and nonspecific inflamatory defect of scin and mucosal membrans when: infected wounds, Fissures, psoriases, chronic dermatitis, chronic arthritis.

Key words: iodinized oil, skin, cosmetic cream, medical oitment

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STANDARTIZATION OF *UNCARIA TOMENTOSA* DISPERG.

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By extraction of inner bark of *Uncaria tomentosa (Willd) D.C. Rubiaceae* and subsequent chromatographic procedures total alkaloid mixture was produced, which contains 8 substances of penta and tetracyclic oxindole alkaloid group. One alkaloid was isolated and identified as isopteropodine by means of spectral (UV, MS), GC and HPLC data. HPLC and densitometric HPTLC methods were developed for quantification of isopteropodine in crude drug, dipsperg and capsules of *Uncaria tomentosa.*

Key words: Uncaria tomentosa, isopteropodine, disperg, HPLC, densitometry

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DENSITOMETRIC HPTLC METHOD FOR DETERMINATION OF TOTAL CONTENT OF SILYBIN AND ISOSILYBIN IN SILYMARIN CONTAINING PRODUCTS

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A HPTLC densitometric method was developed for quantification of total content of silybin and isosilybin in silymarin and silymarin containing products. The two isomers were separated from accompanying compounds using mobile phase of chloroform-acetone-formic acid (9:2:1). The detection limit was found to be 25 ng silybin/isosilybin per spot. The linear regression from 50 to 200 ng silybin/isosilybin per spot was established. The method was successfully used for quantification of two isomers in different samples silymarin and silymarin containing products. The relative standard deviation was found to be 2.2-4.8 % (n=10).

Key words: densitometry, silybin, isosilybin, silymarin

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STUDY ON DIURETIC ACTIVITY OF *MAYDIS STIGMA* EXTRACTS IN RATS

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Maydis stigma (corn silk) is a herbal drug reputed for the treatment of urinary ailments in various traditional medicine systems [1-4]. To determine its influence on urinary volume and the excretion of sodium, potassium and chloride, 5 % and 10 % decoctions were administered daily to adult male Wistar rats for eight days. The concentration of electrolytes and urea in plasma, the influence of treatment on urinary pH value as well as creatinine clearance were also investigated. Daily oral administration of 5 % decoction at the dose of 10 ml/kg lead to a significant and acute diuresis in rats, reaching the peak value in the first 24 hours of treatment. Over a similar period, application of 10 % decoction did not affect urinary excretion of water, but significantly increased the pH value of excreted urine. A significant decrease in sodium and chloride plasma levels was observed in both treated groups. The creatinine clearance was markedly increased after the treatment with both extracts. Our findings indicate that diuretic effect of 5 % aqueous *Maydis stigma* extract is in accordance to the increase in glomerular filtration rate and inhibition of sodium and chloride tubular reabsorption, caused by still unidentified intrinsic factor, but not the salt-loading effect.

Key words: Maydis stigma; Diuretic effect; Electrolyte excretion; Inhibition of tubular reabsorbtion

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DIURNAL DYNAMICS OF NECTAR SECRETION IN *OCIMUM BASILICUM* L. GROWN IN DIFFERENT HABITAT CONDITIONS

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Ocimum basilicum was grown from seed in natural and experimental habitat conditions to determine influence of microclimatic parameters on nectar secretion. Evaluation of basil nectar production was performed by measuring the total nectar amount per flower during twenty four hours, as well as by determining diurnal dynamics of nectar production in two hour intervals. At the time of nectar sampling, microclimatic parameters were measured: air humidity, air temperature and evaporation.
Nectar volume was established by the microcapillary method according to Kuliev (1951), based upon measuring height of the nectar column in the microcapillary glass tube and converted into ml. The

nectar was withdrawn from the flowers with microcapillaries (diameter 0,5 or 0,6 mm) without destructing nectaries.
Comparative analyses of nectar secretion in *O. basilicum*, grown in dissimilar microclimatic conditions, revealed a close relationship between this physiological process and external factors. Nectar amount varied as a function of microclimatic habitat conditions (air temperature, humidity and evaporation). Higher daily nectar amount per flower was recorded in basil grown in experimental field (0.351ml), under increased air humidity, and decreased temperature and evaporation. Diurnal dynamics of nectar secretion were fairly different in these two habitats. The highest nectar production was recorded at 10.00 in natural (0.103 ml) and at 8.00 in second locality (0.163 ml). After that, secretion had more or less rapid decreasing tendency toward evening, and minimal nectar amount was sampled at 18.00 in both localities (0.006–0.016 ml). Air humidity and evaporation were positively and temperature negatively correlated with diurnal dynamics of nectar production.

Key words: nectar production, basil

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RHAPONTICUM CARTHAMOIDES – PERSPECTIVE CROP FOR MARGINAL AGRICULTURAL REGIONS

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The plant *Leuzea carthamoides* DC. [syn. *Rhaponticum carthamoides* (Willd.) ILJIN] from the *Asteraceae* family is one of the perspective alternative crops for cultivation in Czech marginal agricultural regions. It is useful as an adaptogenic medical plant containing important biologically effective compounds as phytoecdysteroids, flavonoids and triterpenoid glycosides. Extracts from *Rhaponticum carthamoides* are often used as neurostimulators, aphrodisiacs, antisclerotics, antioxidants, bacteriostatics and anabolics. It is also a special forage crop comparable to other fodder plants in the nutrients´ contents. Growing of the plant is very convenient from the economical scope of view. Calculated expenses reach the value of 87 000 Kc (~ 2740 Euro) per hectare for the three years period of growing, where the largest part consists of the costs for afterharvest processing. The incomes from growing reach the value of 300 000 Kc (~ 9450 Euro) per hectare. Thus it is necessary to have sufficient capital and technical equipment for growing this plant and especially to ensure contracting sale of the product.

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NURSERY PLANTS PRODUCTION OF FEVERFEW - *TANACETUM PARTHENIUM* (L.) SCHULTZ-BIP. (*ASTERACEAE*)

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Interest in Feverfew has been recently renewed since it is believed that its active compound parthenolide also has beneficial effects in prophylactic treatments of migraine. Increasing demand for standardized quality row material of Feverfew impose necessity to develop appropriate growing techniques and introduce this plant species into culture.
Four high parthenolide populations selected for further work on the basis of criteria important for cultivation came out as a result of preliminary investigations. In two-year experiments started in 2002, two models (winter and summer) of nursery plant production organized in multi-cell containers have been investigated. Experiments were conducted in following periods: winter production in green house conditions - from late January till end of March, and summer production in the open environment - from end of July till end of September. Nursery plants development was monitored up to the stage of 5 pairs of permanent leaves, when the plantlets were ready for transplantation into the open field.
Differences in tempo of development between tested populations ranged from 2-8 days, depending of stage of development; 3 of 4 populations expressed similar tempo (1-2 days difference), while the fourth one expressed a bit greater difference. In both models of nursery plants production stage of germination occurred 5-6 days and stage of seedlings 7-8 days following the date of sowing. The I pair and the III pair of permanent leaves are formed, depending on climatic conditions, in winter production 20-22 days and 39-40 days following the date of sowing and in summer production 15-16 days and 30-34 days, respectively. The nursery plants reached its appropriate stage for transplantation (five pairs of permanent leaves) in winter production 56-57 days and in summer production 47-51days following the date of sowing. The height of ready nursery plants and their overall appearance also showed differences between the examined models of production, the winter ones' leaf petiols being in general more longer then in summer ones, which also looked more compact. In general, summer production gave nursery plants of better quality, in shorter period of time and with lower production expenses.

Key words: Feverfew, nursery plants production

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ANTIMICROBIAL PROPERTIES OF ESSENTIAL OILS AGAINST SPORE FORMING BACTERIA

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The spores are the critical target for decontamination regimens since they are more resistant to many harsh treatments than their growing-cell counterparts. In addition many spore forming bacteria are the causative agents of serious diseases. Therefore the search for agents able not only to suppress the growth of vegetative cells but also to inactivate spores is of great importance. Since the essential

oils (EO) exhibit antimicrobial properties we investigated their potential activity against vegetative cells and spores of eight spore forming bacteria belonging to genus *Bacillus* (*B.thuringiensis, B. sphaericus*, and *B. subtilis*). The EOs used in this study were obtained from *Origanum vulgare* ssp. hirtum, *Origanum dictamnus, Mentha pulegium, Mentha spicata* and *Salvia fruticosa*. All EOs tested and their main components exhibited a variable degree of antimicrobial activity against vegetative cells of all bacterial strains tested, with exception of camphore, which appeared to be active only against *B. sphaericus* strains. The highest antimicrobial activity was observed with EOs derived from *Origanum* species. Additionally all EOs as well as their main components were able to kill spores of *B.thuringiensis* strain HD-1 at variable levels. The highest spore-killing activity was observed with the EO derived from *Origanum vulgare* ssp. hirtum and with carvacrol (one of the main component of the oil). Spore killing by carvacrol appears to render spores defective in germination.

Key words: essential oils, Origanum, carvacro, spores, antibacterial

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NEUROCHEMICAL SCREENING OF XANTHONES FROM *GENTIANA KOCHIANA*

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Gentiana kochiana Perr. et Song. (Gentianaceae) is plant used in traditional medicine as antihypertensive [1]. Its vasorelaxing property is based on the activity of 1,3,7,8-substituted xanthones [2]. These xanthones, gentiacaulein and gentiakochianin, were also reported for a strong inhibition of MAO enzymes *in vitro*[3]. We extend this research by evaluating the *in vitro* interaction of dimethyl-ether (Et_2O) extract of *G. kochiana* and its two purified xanthones with specific dopamine (DA) and serotonin (5-HT) receptors, and synaptosomal 5-HT reuptake, in the light of its possible antidepressive potential. Mitochondrial fraction from rat liver was MAO source in competitive radioassays with ^{14}C-tyramine (55 mCi/mmol) [4], rat cortical synaptosomes were applied for the ^{3}H-5-HT reuptake experiments, while the rat striatal and cortical membrane preparations, for the competitive radiologand binding assays at DA (D_1, D_2) and 5-HT ($5HT_{1A}$, $5HT_{2A}$, $5HT_{2C}$) receptors [5]. Neither the extract, nor the both xanthones, showed significant association with any of 5-HT and DA receptors (Ki >1mM). Also, they did not substantially modify synaptosomal 5-HT uptake in synaptosomes. However, the Et_2O extract of G. kochiana exhibit a high potency to block MAO-A (IC_{50}=0.22μg/ml), and to a lesser extent MAO-B enzyme (IC_{50}=17μg/ml). It seems that it depends mostly on gentiacaulein (IC_{50} for MAO-A and MAO-B are 0.49μM and 560μM, respectively), while gentiakochianin participate with similar affinity for both enzymes (IC_{50}, MAO-A: =164μM; MAO-B:132μM).

Key words: Gentiana kochiana, xanthones, MAO inhibition, radioligand binding assays, serotonin uptake

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VOLATILE METABOLITES OF *NEPETA NUDA* SSP. *NUDA* FROM GREECE

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Nepeta is a large genus of the family Labiatae, which comprises of more than 250 species, distributed in Europe, Asia, Middle East and a few areas of Africa [1]. Antibacterial, fungicidal and antiviral activities, has been attributed to nepetalactones, the characteristic metabolites of *Nepeta* oils. Several species of the genus are used in folk medicine particularly for their diuretic and bacteriostatic activity.
Nepeta nuda L. is a common species of this genus found throughout Europe [2]. In the frame of our research on Greek aromatic plants, we studied two natural populations of *Nepeta nuda* L. ssp. *nuda*, from Mt Parnassos (sample A) and from Mt Oiti (sample B).
Air-dried leaves and inflorescences of samples A and B were subjected separately to hydrodistillation and the obtained oils were analyzed by means of GC-MS method.
Even though the qualitative oil composition of the two different samples was rather similar, marked quantitative differences were observed. In the inflorescences oils 4aα,7α,7aβ-nepetalactone was the dominant constituent (75.7% in sample A; 63.1% in sample B). Quantities of 4aα,7α,7aα-nepetalactone were also found in both oils. The main metabolites of the leaves oils were 1,8-cineole (16.8%), 4aα,7α,7aβ-nepetalactone (24.7%) and caryophyllene oxide (16.3 %) for sample A, while in sample B β-pinene (8.8%), 1,8-cineole (43.7%) and 4aα,7α,7aβ-nepetalactone (12.2%) were the principle components.

Key words: Nepeta nuda subsp. nuda; essential oil composition; 4aα,7α,7aβ-nepetalactone

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ESSENTIAL OIL COMPOSITION OF GREEK *ECHINOPS* SPECIES

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Echinops genus (Compositae) includes c. 120 species, 12 of which are represented in Europe and distributed from Mediterranean to C. Asia and tropical African mountains. The root of *E. kebericho* (a member of this genus) is used to cure intestinal diseases in cattles, while its smoke is effective against typhus and fever, and as a snake repellent. No data has been published on the chemical analysis of *Echinops* oils. In continuing our research on the essential oils of Greek plants, we have investigated the slightly aromatic inflorescences of *E. graecus* Miller and *E. ritro* L.
E. graecus belongs to the section Oligolepis Bunge and is endemic to E. Greece, while *E. ritro* belongs to the section Echinops and occurs in S., S. E. and E. C. Europe.

The examined species were collected during the flowering stage; *E. graecus* in May 2003 from Prefecture Attiki (sample A) and *E. ritro* in June 2003 from Prefecture Viotia (sample B). The hydrodistilled essential oils, which were very low in yield, were analyzed by GC-MS.
The major component of sample A oil was methyl chavicol (42.5 %), an oxygenated monoterpene with insecticide activity. The main constituents of sample B oil were the bioactive compounds (*E*)-2-hexenal (21.4%), 1,8-cineole (16.3%) and *p*-cymene (12.2%).

Key words: Echinops graecus; Echinops ritro; essential oil composition; chavicol; (E)-2-hexenal

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SOME ASPECTS CONCERNING THE GAMMA RAY INDUCED EFFECTS IN SUCCESSIVE GENERATIONS OF *WITHANIA SOMNIFERA* DUN.

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In 1999 we founded a research program to bring the species *Withania somnifera* to *in vitro* and conventional culture and to amplify its variability by gamma ray treatments. The results of some of these investigations were presented in previous papers. The biological material for these tests came from the Botanical Garden of Chisinau.
Gamma ray treatment in M_1 and M_3 with doses ranging between 1 and 15 Krad enhanced plant variability concerning: height, branching, individual biomass, fruit number and biomass per plant. In M_4 generation (2003), under the soil and climatic conditions of Piatra Neamt, at the moment of plant harvesting, we noted that root length at irradiated plants ranged between 19.5 and 31.0 cm, compared to 22.6 cm in control (C) , stem length between 43.2 and 88.2 cm (C=71.4 cm), number of stem basal branches - from 2.11 to 2.66 (C=2.66%), individual plant biomass varied between 91.2 and 337 g (C=223.8 g), individual root mass from 14.5 to 46.3 g (C=30.0 g), fruit number per plant from 53.0 to 302.6 g (C=179.7 g), and fruit biomass per plant 15.4-78.6 g (C=42.4 g).
The highest values of the tested parameters were registered at the variants treated with 1 and 3 Krad. Flavon content in herb ranged between 0.991 and 2.439% d.m. (C=1.657%) and the polyphenols between 0.087 and 0.202 % (C=0.265%). Several individuals valuable for *Withania somnifera* melioration were selected and registered high values for all the tested parameters($V_{2\text{-}2}$, $V_{4\text{-}2}$, $V_{7\text{-}1}$, $V_{7\text{-}2}$ etc.). They will be analysed on the regard of the stable display of these characters during the next generations.

Key words: Withania somnifera**,** gamma rays, physiological indices, flavons, polyfenols

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P 115

INFLUENCE OF THE ENVIRONMENTAL FACTORS ON THE HEAVY METALS CONTENT IN SOME MEDICINAL PLANTS

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In 1997 – 2002 there was observed an accumulation of most potential toxic heavy metals (Cd, Pb, Cr, Ni) in 29 medicinal plants species in the region of Eastern Slovakia.
The aim of the work was concentrated on the evaluation of selected risk aspects of medicinal plants gathering/cultivation with respect to the environmental stress in the air – polluted areas of the region.
Contents of heavy metals were determined by the means of GFA – AAS method on Shimadzu 660. Obtained results showed the most accumulation of toxic heavy metals in following order: Cd – German Camomile anthodia (0,527 $mg.kg^{-1}$), St. John´s worth herba (0,850 $mg.kg^{-1}$), European Mistletoe – (0,479 $mg.kg^{-1}$); Pb – (13,06 $mg.kg^{-1}$), St. John´s worth – (2,87 $mg.kg^{-1}$), Nettle herba – (5,12 $mg.kg^{-1}$); Cr total: Dandelion radix – (3,26 $mg.kg^{-1}$), Burdock radix – (0,77 $mg.kg^{-1}$); Ni: Burdock radix – (8,6 $mg.kg^{-1}$), Dandelion radix – (9,2 $mg.kg^{-1}$), German Camomile anthodia – (8,7 $mg.kg^{-1}$).
Results confirmed that contents of heavy metals in medicinal plants are depended on the several factors: specific ability of some species to overaccumulate various toxic heavy metals, chemical and physical characteristics of soil, locality of plant cultivation/growth and contamination of region.

Key words: medical plants, heavy metal, pollution

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CULTIVATION OF CARAWAY IN CZECH REPUBLIC

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The coriander essential oil is used in the food, cosmetics and partly in pharmaceutical industry. Nowadays, new possibilities of use in plant preservation and paint matters stabilization emerged. The essential oil is gained by the steam distillation and contains mostly linalool, terpinene, thujene, geraniol, fenchene, cymene, limonene, camphor, borneol etc.
The essential oil content varies according to the locality and is also affected by weather course during the breeding year. The experiments with caraway in Czech Republic were established in sugar beet growing region and corn growing region in years 2002-2003. In the examined years, the weather course was different in both habitats (Huštěnovice, Dolní Újezd). During the ripening, spells of drought occured, especially in the year 2003 and were higher in the habitat Huštěnovice (corn growing region). The essential oil content was higher in the Dolní Újezd standpoint in both years and the higher essential oil content occured in the year 2003. The geraniol content didn't vary according to the habitat, but it did from one examined years to the other. The content of limonene varied according to both examined years and standpoints. The content of linalool didn't vary according to none of examined factors.

Keywords: Coriander, planting, essential oil content

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ELECTROMAGNETIC PROCESSING OF BASIL AND CARAWAY SEEDS TO IMPROVE INITIAL GROWTH

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Electromagnetic processing of seed of cultivated plants is increasingly used in Serbia and the world to improve the yields and quality of cultivated plants. The results of these efforts are encouraging and prompt further study.

Our trial with electromagnetic seed processing was carried out under controlled conditions in a nutrient solution. Basil and caraway seeds were treated with various frequencies up to 100 Hz with second decimal place accuracy. After processing, the seeds were germinated and grown in a nutrient solution for 26 days. Fresh and dry masses of young plantlets were measured. The experiment consisted of two series with four replicates each.

In basil, fresh seedling mass increased by 6.7-11.8% (1.2-3.19 mg/seedling), while the dry mass rose by 6.5-12.2% (0.06-0.2 mg/seedling). The best treatment in both cases was Frequency No. 11.

In caraway, the fresh mass of seedlings increased by 0.3-26.1% (0.06-4.71 mg/seedling), while the dry mass increased by 2.2-22.6% (0.06-0.42 mg/seedling). The best results were obtained by Treatment E and Treatment G, respectively.

Key words: Caraway, basil, frekvencies, RIES.

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STUDY OF BIOLOGICAL EFFICIENCY OF MEDICINAL PLANT EXTRACTS IN BARLEY GROWING PROCESS

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In the field small-plot experiments over the period of three years (1999–2001) the biological efficiency of medicinal plants, primary and secondary products of extraction, has been tested. Thirty primary products of extraction, one secondary product of extraction and two chemical fungicides were tested. Chosen fungicides has more effectivity in physiological impact. Variant without treatment (control) was used to compare and detect impact of extracts and fungicides. Model crop was spring barley.

Different impact of extraction products was confirmed on spring barley yield. During testing period we achieved spring barley yield variation from 75 % to 120 % in comparison to untreated control. Different year had a major impact on the yield. Different impact of tested preparations on production parameters signs the existence of eventual risks and demonstrates a need for the future research solution. Compared to the untreated control variant there was a fundamental increase of yield at the most part of tested preparations. The yield increase was above 5 % - in some cases we recorded spring barley yield increase above 10 %.

Generally, so-called physiological – harvest effect of extraction products was slight but comparable to both tested fungicidal variants.

Key words: biological efficiency, physiological effect, plant extracts, fungicides, spring barley

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THE EVALUATION OF ANTIOXIDANT AND ANTIMUTAGENIC ACTIVITY OF A POLYPHENOLIC COMPLEX ISOLATED FROM *VITIS VINIFERA*

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Antioxidants could prevent mutagenic effects induced by free radicals. We tested the antioxidant activity of a polyphenolic complex from seeds and skins of grapes and the protective effect on genotoxicity induced by cyclophosphamide (CP) in the mouse bone marrow polychromatic erythrocytes using in *vivo* micronucleus test.
The antioxidant activity was evaluated *in vitro* on an experimental model using liver homogenates from Wistar rats intoxicated with CCl_4.The polyphenolic complex from *Vitis vinifera* proved a high efficiency (78%) as protective agent against lipid peroxidation.
The micronucleated polychromatic erythrocytes (mPCE) frequency at 24 h and 48 h after an i.p. administration of CP (50 mg/kg) was significantly lower in polyphenolic complex – pre-treated mouse bone marrow than those observed in the group treated with CP only. Also, in CP – treated animals a significant reduction of the ratio PCE/NCE was observed suggesting that CP induce erythropoietic cells toxicity. The proportion of PCE at 24 h in polyphenolic complex – pre-treated animals was upper than those observed in the positive control group while at 48h, PCE/NCE overreach the negative control group rate. This demonstrates the protective effect of the polyphenolic complex against CP induced cytotoxicity and mutagenicity induced and this effect is attributed to ability of plant polyphenols to inhibit cytochrome P_{450} enzymes that activate chemical carcinogens and to suppress covalent binding of biologically reactive ultimate metabolites of CP to DNA.

Key words: polyphenolic complex, antioxidant activity, antimutagenic effect, micronucleus test

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USING OF IODINIZED VEGETABLE OILS FOR FLUORINE METABOLISM STIMULATION

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Iodinized vegetable oils in combination with sodium fluoride can be used for the stimulation of hard bone structure formation, including tooth enamel reduction with prophylactic action against osteomalacia as well as caries prophylaxis, above all in infants.
This two-component composition enables the effective capturing of fluorine by hard bone structures, the iodized fat acids of vegetable oil carrying the function of a conductor for fluorine, for the

formation of protein-mineral iodinazed, and subsequently due to the displacement of iodine by fluorine, the iodinized-fluorine protein-mineral complexes in hard bone structures with the formation of fluorine apatites that favors the bone tissue regeneration, and as to the tooth enamel, it gets acidproof.
The action of the give composition is directed to the reduction and hardening of the tooth enamel and gums, as well as caries prophylaxis and osteomalacia through the increase of the effectiveness by adding fluorine in bone tissues with retention of hardness and preventing from brittleness, it having antiparadontic and antiseptic action.

Key words: iodine, fluorine, vegetable oil, caries, antiseptic

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ANTIFUNGAL ACTIVITY OF TWO *ACHILLEA* SPECIES

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In continuation of our research of wild growing species from Serbia, the methanol extracts of *Achillea lingulata* and *A. alexandri-regis* have been screened for potential antifungal activity. The previous pharmacological studies have showed that herb extracts of both plants possess significant anti-inflammatory and antioxidant activity [1, 2], as well weak antibacterial activity [3]. Dried and grounded aerial parts was extracted on room temperature with light petroleum and methanol (75%, V/V), respectively. The solvents were evaporated under low pressure and activity of the methanol extract tested.
Antifungal activity was tested using microdilution technique, on microtitar plates, 96-system [4, 5] and macrodilution method on Petri dishes [6]. The following microorganisms are form Mycoteca of Mycological laboratory, Institute for Biological Research, Belgrade, Serbia and Montenegro: *Trichophyton mentagrophytes, T. rubrum, T. tonsurans, Microsporum canis, Epidermophyton flocosum* and *Candida albicans.* Minimum inhibitory (MICs) and minimum fungicidal concentrations (MFCs) were determined.
Both extracts showed antifungal activity against all tested fungi with MIC values range of 100-150 μg/ml and MFC values of 100-300 μg/ml. Antifungal potential of tested extracts was significant, but lower than reference antimicrobial agent chloramphenicol (MIC 30-50 μg/ml; MFC 50-100 μg/ml).

Key words: Achillea, antifungal activity

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USING OF VEGETABLE OILS AS MEANS FOR ACTIVATION AND TRANSPORTATION OF SOME MICROELEMENTS IN BIOLOGICAL SYSTEMS

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The possibilities of chemical binding together of iodine microelements and selenium with unsaturated fat acids of various vegetative oils and their usage as biologically active food additive for bioobject have been investigated.
The ability of chemical binding of marked iodine microelements with unsaturated fat acids of vegetable oils by organically binded iodine formation and the manifestation of its expressed biological activity lying in individual metabolic regulation as its normalizing factor in the chain of hypophysis-thyroid regulation has been established. The possibility of vegetable oil usage as the means for transportation and biological activation not only of iodine but by means of it also of selenium and their usage as biologically active food additive as metabolic regulator and effective antioxidant have been determined. This can be an effective normalizing corrector of the thyroid gland function as well as aminoacid-peptide metabolism in norm under stress reactions and pathologies.
The high effectiveness of these food additives under condition of the mentioned microelements insufficiency, hypothyroid and same hyperthyroid states as well as in pathologic processes, including genesis has been revealed.

Key words: iodine, selenium, vegetable oil, metabolism

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ALLIUM URSINUM L. – MEDICINAL PLANT OF FUTURE?

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Allium ursinum L. (Family Liliaceae) – is a native woodland plant growing about 30-40 cm tall.
A. ursinum prefers woodland conditions in a moist well-drained soil. It will succeed in most soils and prefers moist conditions, thought it will also succeed where the soil is very wet in the winter. It grows well in shady to sunny conditions. When given suitable conditions it will form a dense carpet of growth and can be invasive. The plant comes into growth in the middle to late winter, flowers in the spring and then dies down completely by the middle of summer.
The plant is anthelmintic, antiasthmatic, anticholesterolemic, antiseptic, antispasmodic, astringent, cholagogue, depuritive, diaphoretic, diuretic, expectorant, febrifuge, hypotensive, rubefacient, stimulant, stomachic, tonic and vasodilator. It ease stomach pain, is a tonic to the digestion, it can be used in the treatment of diarrhoea, colic, wind, indigestion and loss of appetite. All parts of this plant are edible. The leaves are delicious raw or cooked and we have harvested them as early as the middle of January in mild winters.The whole herb can be used in an infusion against threadworms, either ingested or given as an enema. The herb is also beneficial in the treatment of asthma, bronchitis and

emphysema. The juice is used as an aid to weight loss and also be applied externally to rheumatic and arthritic joints. Allium ursinum is disinfectant and repellent, too.
Possibility of cultivation in Slovakia: A.ursinum is rare and protected plant. Possibility to order of seeds or bulbs from abroad (Germany) and try of cultivation in moisture places.

Key words: Allium ursinum L., properties, cultivation conditions, Slovakia

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OCIMUM SANCTUM L. - AND ITS PROSPECTS IN SLOVAKIA

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Ocimum sanctum L.(Family Lamiaceae). is a well-known sacred plant of the Indian subcontinent. It is grown as a perennial plant in its native tropical environment but can also be grown as an annual in cooler regions. It is found growing naturally in moist soil nearly all over the globe. In India, Hindus grow it as a religious plant in their homes and temples and use its leaves in routine worship. This plant is also grown as a pot herd and in home gardens. It is not a water loving herb. Over irrigation make this herb weak and unhealthy. Ocimum sanctum leaves contain a kind of bright yellow volatile oil which is useful against insect and bacteria. The principal constituents of this oil are eugenol, eugenol methyl ether and carvacrol. The oil is reported to possess anti-bacterial properties and acts as an insecticide. It inhibits the in vitro growth of Mycobacterium tuberculosis and Micrococcus pyogenes var. aureus. It has marked insecticidal activity against mosquitoes. The juice of leaves, and or a concoction, a kind of tea, gives relief in common cold, fever, bronchitis, cough, digestive complaints, etc. When applied locally, it helps in eradicating ringworms and other skin diseases. Oil is also used as ear drops in case of pain. The seeds are also used in curing urinary problems.
Possibility of cultivation in Slovakia: Experience with cultivation are exist (north-east Slovakia). But conditions for cultivation are better in south Slovakia. Plants can not freeze.

Key words: Ocimum sanctum L., utilization, cultivation conditions, Slovakia

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OCCURENCE OF MEDICAL PLANT SPECIES ON THE PASTURES OF WESTERN CARPATHIAN MOUNTAINS

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During the years 1992-2002 the occurrence of medical and toxic plants was studied on the Western Carpathian Mountain pastures in the regions of Kysuce, Orava and Liptov. Results of 517 floristic analysis show, that from the overall number of 175 plant species there were 21 grass species, 7 leguminous species, and the rest was constituted by the other herbs, 45 species (25 %) from these were classified as medical herbs and from these 8 species belong to the valuable plants considering

their fodder value [1,2,3]. The highest share was constituted by *Taraxacum officinale* (3.57%), *Carum carvi* (2.79%), *Achillea millefolium* (2.59%), *Alchemilla vulgaris* (1.97%), *Trifolium pratense* (1.36%), *Plantago lanceolata* (0.75%), *Pimpinella saxifraga* (0.03%) a *Heracleum sphondylium* (0.01%), the others belonged to the less valuable species. 9 of toxic species were classified as medical herbs, the biggest share of them was constituted by *Hypericum perforatum* (1.13*%), Glechoma hederacea* (0.09%), *Euphrasia rostkoviana* (0.06%), *Odontites vulgaris* (0.05%), *Cardamine pratensis* (0.03%), *Equisetum arvense* (0.02%), *Tanacetum vulgare* (0.01%), *Colchicum autumnale* (0.01%), and *Carlina acaulis* (0.01%). The medical herbs can be used not only in human medicine, but also in veterinary medicine [4]. The share of studied species significantly influenced the fodder. When the mowed aboveground biomass was given to the manger, the animals did not have so wide choice, as they do directly on the pasture. The medical herbs helped preserve good state of health, and conversely toxic plants caused toxicoses, even long-term ones. On the pastures the toxic species were by the animals avoided.

Key words: grassland, predominance, medical herbs

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COMPARATIVE STUDY OF SOME SPECIES *ECHINACEA* (MOENCH) GENUS

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Medicinal plants are the main natural source of medicinal substances and natural drugs. The various species of Echinacea plants are often misidentified [1]. Since commercial products of Echinacea purpurea have been freguently adultarated with Partheniu and other species Echinacea. The analysis of Echinacea roots has become of great importance.
The present work deals with the root structure of selected Echinacea species, their anatomical structure and content and quality of the essentional oil [2], in relation to the individual plants organs, and their ontogenetic phasis.
Since the roots of therapeutically used Echinacea species can be differentiated structurally (shape, anatomy), the roots of some species are very similar in their ultramicroscopic structure.

Key words: Echinacea, anatomical structure, quality of essentional oil

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QUALITATIVE PROPERTIES AND CONTENTS OF ESSENTIONAL OIL OF SOME SPECIES OF *SALVIA* GENUS

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The content and chemical composition of the volatile oils from three species *Salvia genus* studied (GC). The plants were cultivated in three various localities and the ones were collected in the period of their optimal stage of growth and development. The quality of volatile oil from *Salvia lavandulaefolia* Vahl. is characteristic with high content camphor and 1,8-cineole and little content thujone. *Salvia sclarea* L. which in essentional oil contain when main component linalool and linalyl-acetate, have big growth and higher yield biomass like Salvia officinalis L.

Key word: Salvia, volatile oils, limonen, thujone

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QUALITATIVE PROPERTIES AND BIOLOGICAL ACTIVITY FROM THREE SELECTED SORTS OF *ECHINACEA* MOENCH. GENUS

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The some genus species of the Echinacea Moench is very interesting for their biological activity. In these times, a world wide renaissance of the use of natural substances and medicinal plants takes place. Not replaceable position occupy the plant sorts which exert so-called immuno modulation activity. Significance of an immuno stimulation, meant as proflaxy and therapy of wide palette of diseases connected with the immunity system, strongly increases in the recent years. A row of phyto pharmaceuticals containing extracts from plants of the Echinacea Moench genus is nowadays exploited for the enhancement of defense mechanism. Until now, however, we do not know all the constituents and biological effects of the most known sorts, i.e. Echinacea atrorubens and Echinacea purpurea.
In present work we have searched for a plant cultivar of A. atrorubens that would demonstrate the cytotoxic activity after appropriate isolation of the active compounds. Ethanol-water soluble extratc from fresh radix (EREx) and plants (EP) as well as the essential oils (ERSl) were under study. Their cytotoxic activity on HeLa cells was compared with that of five compounds of plant origin: caffeic acid (CA), quercetin (Qe), chlorogenic acid (CHA), quercitrin (Qi), and mixture of flavonoids (F).

The cytotoxic efficiency of the extracts and essential oils decreased in the order EREx, ERS1, ERS2, EP, and antioxidants CA, Qe, CHA. Quercitrin and flavonoids showed no toxic effect in the concentration rande 4 – 500 μg/ml. The extract from fresh radix (EREx) inhibited the most cytotoxic effect when compared with all compounds tested ($1D_{50}$ = 19.6 – 33.7 μg/ml). Furthermore, the EREx and ERS1 prevented the treated cells to form multilayer focuses and thus probably exhibited their antineoplastoic transformation capacity.

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YIELD OF *SATUREJA HORTENSIS* AS INFLUENCED BY TERM OF SOWING DAY IN CONDITION OF DANUBIAN LOWLAND IN SLOVAKIA

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During years 1999-2001 there was based field trial with *Satureja hortensis* in Botanical garden of Slovak agricultural University in Nitra. The trial was established on heavy clay soils with average humus content of pH 7,05. Climatic conditions were classified as very warm and dry with moderate winters. Leaf biomass creation as influenced by term of sowing day was evaluated. Sowing dates were repeat after 10 days (3 times) in each year when the first date of sowing was in year 1999 the April 16th, in 2000 the April 14th and in 2001 the April 17th.
In 1999 for the first time plants were harvested on July 16th and for the second time on September 9th, in 2000 on July 21st and September 22nd, in 2001 on July 22nd and September 22nd. The highest yields – 1150 $g.m^{-2}$ were generally found in the first term of sowing day during period of years 1999-2001. Delay by 10 days in the second term caused decrease of average yields on level 1030 $g.m^{-2}$ or on level 911 $g.m^{-2}$ respectively in the third term of sowing day.
Presented results documents that technology of direct sowing can replace growing of *Satureja hortensis* from seedlings in cases when proper climatic conditions during spring period are utilized as soon as possible.

Key words: Satureja hortensis, sowing day, climatic conditions

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ANALYSIS OF CULTIVATED SAGE (*SALVIA OFFICINALIS* L.) IN THE WARM AGRO-CLIMATICAL CONDITIONS

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Different cultivars of sage - ´Krajova´, ´Comune´, ´Primorska´ (Slovak, Italian, Yugoslav proveniences) were introduces into the cultivation in the warm agro-climatical conditions, in the Kolinany [1] . The productive characteristics of dry sage herb and qualitative-quantitative analysis of

its essential oils were observed. Our results confirmed high productive potential and required quality of mentioned sage cultivars. An advantagenes of intorduced cultivars can be defined on the ground of high dry herb production, compositon of essential oil and resistence agaist sage diseases [2, 3].
Result of the research are presented thanks to the financial support of the scientific project solution of the Scientific Grant Agency at the Ministry of Education of Slovak republik and the Slovak Academy of Sciences 1/9091/02: Optimisation of the intensification factors of Salvia sp. and Melissa sp. cultivation technologies and their effect on qualitative parameters.

Key words: sage (*Salvia officinalis* L.), cultivar, cultivation, quality of essential oil

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SERVICE TREE (*SORBUS DOMESTICA* L.) – MEDICINAL BUT ALSO ECONOMICALLY VALUABLE NEGLECTED FRUIT TREE

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In the history was service tree very popular, at present is its occurrence rare and is visibly disappearing. Attention is focused not only for endangered species, but also as perspective, economically valuable tree.
From the point of fruit production has many important properties. Beside consuming of fresh fruits, it is possible to process fruits in households on tasty and healthy products, for example dried fruits, preparation of so-called "service tree salami", canned fruits, from unrotted fruits thick pap or jam [1]. We can use them on preparation of marmalades, jams, juices, jelly and candied fruit. In some Balkan countries are fruits used on filling of roasted ducks or chicken [4]. In some Russian countries is very high quality vinegar produced from fruits [2].
In the history were service tree fruits used in public medicine for cure of problems in digestion of humans and animals. Old nations in Mediterranean region used service tree as food and drug [3]. Fialová [2] found in in fruits phytoncides, which in low concentrations have antibacterial and antimycotic effect. In the flowers were detected estrogenic compounds, in seeds oils and amygdaline.

Key words: Service tree (Sorbus domestica L.), utilization, fruits, food products

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P 132

GENUS *CRATAEGUS* L. – HAWTHORN IN SLOVAKIA: A POTENTIAL PLANTS FOR PHARMACEUTICAL USE

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Hawthorn is an welknown medicinal plant from ancient to present days. First reference is known from Theophrastos around 330 years BC., means as crataigos = crataegus hardwood., commonly used name for species *C. laevigata* and *C. monogyna*. Traditionally these two species were collected in Central and W. Europe for common and farmaceutically commercial uses too. Phytochemistry of both species is similar, but some compounds (flavonoids) shown differences. In the Central Europe 16 species are native in hedges, pastures and forests, occurrence of many and various interspecific hybrids were estimate. In the territory of Slovakia 14 species and 5 interspecific hybrids was find Results of study autochthonous populations of hawthorn on territory of Slovakia has brought new opinions of their taxonomical structure. Much more of analysed populations are conspecific, consist more than 2 species. Populations with 1 species are frequently occurs in south parts of Slovakia, ussualy as species *C. monogyna* or *C. laevigata*, northern populations are cospecific. One of them *C. lindmanii* is threatened species with very rare occurrence Recommended taxonomical determination of the shrubs before harwesting of desiderative portions.

Key words: Crataegus species, pharmaceutical importance, Slovakia

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EX SITU COLLECTIONS OF MEDICINAL PLANTS IN BOTANICAL GARDEN OF SLOVAK UNIVERSITY OF AGRICULTURE

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Botanical garden of Slovak University of Agriculture in Nitra is a typical small botanical garden (only 30 years old history) with a few activities as for example: care for indoor and outdoor collections, delivering of Index seminum, education of university students and some research activities (investigation of autochthonous threathened plants and seeds collecting).
One of the bigest special living collection is the collection of medicinal and aromatic plants, founded more than 30 years before. Some of them, *Mentha x piperita* L., *Althea officinalis* L., *Salvia officinalis* L., *Thymus vulgaris* L., *Hyssopus officinalis* L., *Agrimonia eupatoria* L., *Peganum harmala* L. and *Aloë vera* L., *Belamcanda chinensis* (L.) Leman, *Eucalyptus globulus* Labill as tropical plants were planted with a special attention.
Other threatened species, *Tribulus terrestris* L., *Drosera rotundifolia* L., *Daphne cneorum* L., are planted as a core collection from the native population in Slovakia. Samples of seeds are stored in National genebank in Research institute of crop production in Piešťany. Herbarium specimens of each seed samples are stored in Botanical garden and in Herbarium of Department of Botany, Faculty of agrobiology and Food Resources, SUA.

Key words: botanical garden collections, medicinal plants, genepool protection ex situ, seedgenebank

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THE OCCURENCE OF PUNCTURE VINE (*TRIBULUS TERRESTRIS* L.) AND POSSIBILITIES OF GROWING IN SLOVAKIA

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Tribulus terrestris L. is a plant which grows in moderate climates. This plant species is native in the South and East Europe, for example in Bulgaria, Romania, Hungary etc. In Slovakia is endangered species [1] .
The research work was divided into the following parts: monitoring, reconnoitring and collesction of an genepool of puncture vine. Its identification and evaulation as well as use during 1999 – 2002.
The yield of dry haulm was taken from two experimental fields Nitra (400,5 g.m $^{-2}$) and Streda nad Bodrogom (374,25 g.m $^{-2}$). The content of furostanol saponins analyse HPLC method was find out in limits from 0,48 ± 0,03 % (seeds, locality Nesvady) to 3,71 ± 0,03 % (whole plants, locality Streda nad Bodrogom). The quantity of furostanol saponins are various in different plant part and was modified by the growing conditions. The optimum conditions for growing puncture vine is in warm agroclimatical conditions.

Key words: Tribulus terrestris L., occurence, growing, furostanol saponins, Slovakia

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PERSISTENCE OF DDVP IN STORED MEDICINAL PLANTS

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Nuvan 500-EC was used in combating moving forms of stored product pests in ready made herbal drugs, set for further processing and packing, aiming to resolve whether these products could be treated in such a way but keeping level of DDVP residues within acceptable boundaries. Treatment was performed employing ULV-technique by the use of 10 ml of formulated product per 100 m^3. Samples of selected herbal drugs from three uncovered boxes, hawthorn, senna, and mint, were taken before and 3, 7 and 14 days after treatment, and subjected to determination of DDVP residues. Extraction of herbal drugs samples (5 g) was performed with dichloromethane (100 ml) at laboratory temperature during 24 hours. After filtration and rinsing to collect 100 ml of corresponding dichloromethane extracts, these extracts were evaporated to approximately 2 ml and then under the nitrogen to dryness. Resulting residues were transferred to narrow medium size vials, dissolved in n-

hexane to 5 ml and analysed by GC/FPD. On the basis of preliminary GC-testing (GC/FID, GC/MS and GC/FPD), contents of DDVP residues in processed samples were determined from the calibration curve (GC/FPD), covering range between 0.4-80 ng/g of DDVP. Residues of DDVP in hawthorn, senna and mint, ranging between 3.1-4.8, 0.1-0.2 and 4.9-28.2 ppb, respectively, were recorded in treated samples (recovery: >98%). Since upper level of residues, after 14 days, was lower then 5 ppb in all cases, performed treatment could be marked as admissible and quite acceptable for common practice.

Keywords: DDVP, pest control, persistence, residues, medicinal plants

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DETERMINATION OF MALATHION IN CHAMOMILE (*CHAMOMILLAE FLORES*)

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The samples of chamomile are analyzed by the gas chromatography using two different columns and two different detectors, flame photometric detector (FPD) and alkali flame ionization detector (AFID) what is valid confirmation of the malathion presence (or the absence).
Content of malathion in samples of chamomile, bought in the supermarket, was less then 0.01 mg/kg, which is under the limit of detection for this method.
Sample of chamomile blossoms, raw material for a production of tea for the market, contained 0.112 mg/kg of malathion, which represents potential hazard for consuming.
We believe that increased concentration of malathion is result of improper usage of formulations with malathion as active ingredient for plant protection in excessive doses, e.g. that recommended directions for usage were not obeyed. Also, it is possible that the found amount of malathion is the consequence of the usage of the malathion-based formulations in combating mosquito.

Key words: malathion, gas chromatography, residue determination, chamomile

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CALENDULA OFFICINALIS AS AN OILSEED CROP FOR INDUSTRIAL USE

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Calendula officinalis is used as a traditional medicinal plant - floral drug. Recently it has also been tested as an alternative oilseed crop for industrial use [1]. For this purpose Calendula officinalis has been monitored during experiments at the Czech Agriculture University in Prague from 1997 to 2000 in marginal areas. Two cultivars have been used in experiments, Czech cultivar Plamen and German cultivar Regina. In addition to agrotechnical data, oil content and oil composition have also

been evaluated during sequential sampling (August) starting when oil production in seed begun up to well-ripened seeds from harvest. Oil content in seeds was determined by Soxhlet extraction. Obtained values from sequential sampling corresponded with oil producion laws in other oilseed crops according to which with increasing seeds maturity oil content also increases. Seeds in vegetation have been unequally ripe during harvest and those more mature came out. Oil quality was determined according to fatty acids composition [2]. Good quality oil has low content of palmitic acid (2-3 %) and high content of calendic acid (41-52 %). Juvenile seeds have higher content of palmitic acid. During the three years of experiments the oil content varied and the most favourable year was in 1999 when the oil content was the highest (between 17-21 % for both cultivars). Fatty acids composition corresponded with standard. Based on total evaluation of Calendula officinalis as the oilseed crop in marginal regions cultivar Plamen was at the lower limit of standard, oil content was between 11-13 %, palmitic acid content was between 4-8 % and calendic acid content was around 42 %. Cultivar Regina had higher oil content (14 %) and oil composition corresponded with standard. Low oil content (9-12 %) makes problem for further processing. Predominant cause of low oil yield is a thick seed coat, which could be removed only by breeding cultivars with thin seed coat.

Key words: Calendula officinalis L., oil content, acids composition, cultivar Plamen and Regina

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BIOACTIVE PHENOLICS FROM *SARCOLOBUS GLOBOSUS*, A BANGLADESHI MEDICINAL PLANT

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The bark of Sarcolobus globosus is used in traditional medicine in Bangladesh but its chemical constituents are not known. A hydromethanolic extract of Sarcolobus globosus bark was suspended in distilled water and extracted successively with diethyl ether, ethyl acetate and n-buthanol. The n-buthanol extract was fractioned by column chromatography on Diaion HP-20. There have been collected twelve main fractions which were further subjected to antioxidant assays (15-lipoxygenase inhibition and diphenylpicrylhydrazyl scavenging activity). 1H and 13C NMR spectra were also recorded for all these fractions. Fractions 4 and 5 were subjected to centrifugally accelerated thin-layer chromatography followed by preparative high-pressure liquid chromatography. There have been isolated four compounds: compound 1 – vanillic acid 4-O-beta-D-glucoside, compound 2 – glucosyringic acid, compound 3 – tachioside, compound 4 – isotachioside. Their structures were determined by means of 1H and 13C NMR spectroscopy, mass spectrometry and infrared spectroscopy. Compounds 1 – 4 were assayed for diphenylpicrylhydrazyl scavenging activity and 15-lipoxygenase inhibition. IC_{50} values for diphenylpicrylhydrazyl scavenging activity: tachioside: 84 ± 2μM, isotachioside: 130 ± 2μM. IC_{50} values for 15-lipoxygenase inhibition: vanillic acid 4-O-beta-D-glucoside: 161 ± 8 μM, glucosyringic acid, tachioside, isotachioside: > 167 μM.

Key words: Sarcolobus globosus, vanillic acid 4-O-beta-D-glucoside, glucosyringic acid, tachioside,isotachiosid

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ECOLOGICAL CULTIVATION OF MEDICINAL PLANTS AND THEIR ANALYSIS

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State research solutions and development tasks in the first year of the experiment were aimed to the possibilities of selected medicinal plant species using ecologically certified cultivation: Peppermint (*Mentha x piperita* L.), Calendula [Pot Marigold] (*Calendula officinalis* L.), Ribwort (*Plantago lanceolata* L.), Yarrow (*Achillea millefolium* L.) in agro-ecological conditions of the testing area Rudnik, district of Myjava (Slovak Republic). In the year 2003 characteristics and specification of agro-ecological conditions of the testing area were worked out, culls of soil samples and biological material were realized. Analyses of potential contamination content in the soil samples did not exceed allowed limits for the content of heterogeneous substances in the soil.
The yields of the fresh overgrown phytomass were as follows: *Mentha x piperita* L. ranged from 910 up to 1 050 $g.m^{-2}$ (i.e. 187 – 238 $g.m^{-2}$ of air-dried drug), *Calendula officinalis* L. from 253 to 472 $g.m^{-2}$ (51 – 198 $g.m^{-2}$), *Plantago lanceolata* L. from 1 490 to 1 600 $g.m^{-2}$ (278 – 298 $g.m^{-2}$) and *Achillea millefolium* L. from 1 490 to 1 600 $g.m^{-2}$ (580 – 630 $g.m^{-2}$) in correlation with variants of the experiment.
Expected results of the research, which will be realized during the years 2003 – 2005, contribute to the project of cultivation of the tested medicinal plants species in ecological production. These results of the research are presented with thanks to the financial support of the State research and development tasks: "Ecological and economic rationalization of the primary plant production", CU 01 VE 08 SE 03: "Ecological cultivation and quality of selected medicinal plants" solved under the coordination of the Research Institute of Plant Production in Piešťany, Slovak Republic.

Key words: *Mentha x piperita* L., *Calendula officinalis* L., *Plantago lanceolata* L., *Achillea millefolium* L., ecological cultivation

Leonardo da Vinci project at the Slovak University of Agriculture in Nitra:

"Developing Plant Protection Vocational Training Material for SMEs farmers"
(No.: HU/02/B/F/PP-136012)

Information society, globalization, the challenges of the European Union manifest themselves also in the area of agriculture. Agriculture gets new roles in the society: must be sustainable, ecological and create quality of living society. In the area of agriculture such changes have taken place in Central – Eastern Europe, which entirely changed the structure of the agricultural farms with family enterprises coming to the fore.

An International Consortium has submitted a project proposal to the European Commission under Leonardo da Vinci Program. The aim of the project is to launch modern adult professional training programme with production technology approach and to collectively handle plant pathology, parasites and weeds.

The partners of the Transnational project are: Trebag Property- and Project management Ltd, Hungary, Czech University of Agriculture Prague, ***Slovak University of Agriculture in Nitra***, Municipality of Malaga, Bács-Kiskun County Agricultural Chamber, Cserháti Sándor Technical and Agricultural Secondary School, Agricultural Technological Center of Research and Vocational Training "C.V.T. Georgiki Anaptixi", Global Interface, Spain.

We develop the teaching material in a module system, which is based on 14 + 2 modules. Fourteen modules include the most important grown gardening and field plants, while the +2 modules olive and citrus species. The structure of the modules is as follows: morphology, life cycling, pathology, pests, weeds, short guidance, and short test.
The final product of the project is a teaching material, which is based on CD-Rom. The project also contains a pilot test training held by each partner countries.

Project coordinator in the Slovak Republic:

prof. Ing. Magdaléna Lacko-Bartošová, PhD.,
Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra.

The project website:
http://www.plantprotection.hu/
http://www.fapz.uniag.sk/PlantPro/ENG/

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