38th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

FINAL PROGRAMME
BOOK of ABSTRACTS

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38th International Symposium on Essential Oils
ISEO2007

Graz, Austria
September 9-12, 2007

Program

Book of Abstracts

Organisation:

Institute for Applied Botany and Pharmacognosy, University of Veterinary Medicine, Vienna

Joanneum Research Graz

Institute of Pharmaceutical Sciences, Karl-Franzens-University Graz

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General Information

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Elisabeth Stahl-Biskup, University of Hamburg, Germany

Symposium Venue

The Karl-Franzens-University Graz, Austria, hosts the Symposium. All lectures except the lectures of the Young Scientists Workshop will be presented in the Aula of the University. The Young Scientists Workshop is located at HS 1.15 and posters will be presented in rooms HS 1.11 to HS 1.14.

Instructions for oral contributions

The duration of a plenary lecture is 30 min, of keynote lectures 25 min plus 5 min for discussion, and of short lectures 10 min plus 5 min for discussion. For oral presentations the following equipment is available: PC, beamer, overhead. Please contact the Organizing Secretariat if you need a slide projector. To avoid technical problems, personal laptops cannot be utilized; a disc, CD-ROM or memory stick should be provided at least one hour prior to the beginning of the session. Precautions will be taken to avoid any unauthorized copying of data. Speakers should meet the chairperson and provide her/him with a short CV 20 minutes prior to the beginning of their session.

Instructions for poster contributions

Participants are responsible for mounting their posters and removing them. The organizers will provide the equipment needed. The size of the posters should be 84 cm x 119 cm (DIN A0) or 33 inch x 47 inch, respectively (width x height). All posters will be presented during the whole symposium.

Registration

The registration desk will be located on the first floor, in the foyer of the aula of the Karl-Franzens-University. On Monday and Tuesday, the registration desk will be open from 8.00 to 17.00 and on Wednesday from 8.30 to 14.00. Participants are kindly requested to wear their name badges at all times.

Incoming messages and message board

Messages received by the desk will be posted on the message board located at the registration desk. Participants may also use this board to leave messages to other delegates.

Symposium website

The symposium homepage will be constantly kept up-to date until December 2007: http://www.iseo2007.org.

Language

The official language of the congress will be English.

Insurance / Liability

The congress organisers cannot be held responsible for any loss, theft, damage or injury to any person or property during the congress, whatever the cause may be. The liability of persons and enterprises providing means of transportation or other services, however, remains unaffected. The customer takes part in all tours and trips at his own risk.

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Scientific Programme

			Monday, September 10, 2007	
09:00	09:30		Opening ceremony	Aula
			Molecular Biology and Biosynthesis of Terpenes	Aula
			Chair: Robert P. Adams	
09:30	10:00	PL-01	Degenhart J: "The Origin of Terpene Diversity in Plants"	
10:00	10:30	KL-01	Kampranis S: "Engineering Substrate and Product Specificity in a Terpene Synthase"	
10:30	11:00		Coffee Break	
			Chair: Czeslaw Wawrzeñczyk and Jan Demyttenaere	
11:00	11:30	KL-02	Castilho P: "Valorisation of Essential Oils by Chemical and Biotechnological Modification of Key Components"	
11:30	11:45	SL-01	Demirci F: „Biotransformation of p-Cymene to Thymoquinone“	
11:45	12:00	SL-02	Noma Y: „Biotransformation of Limonene and Related Compounds by Newly Isolated Low Temperature Grown Citrus Pathogenic Fungi and Red Yeast“	
12:00	12:15	SL-03	Zope D: „Synthetic Substitutes for the High Class Natural Materials“	
12:15	12:30	SL-04	Asakawa Y: „Biologically Active Compounds from Malagasy *Cinnamosma* Species and Liverworts“	
12:30	12:45	SL-05	Joulain D: „The Essential Oils of the Heartwoods of Two Closely Related Endemic Species from New Caledonia: *Neocallitropsis pancheri* and *Callitris neocaledonicum*“	
12:45	14:15		Lunch Break	
			Body(Odour) - Fragrance – Interactions	Aula
			Chair: Elisabeth Stahl-Biskup and Gerhard Buchbauer	
14:15	14:45	PL-02	Preti G: "Human Body Odors and their Interactions with Fragrances"	
14:45	15:15	KL-03	Heuberger E: "Fragrance Effects in Humans - Is It All in Our Heads?"	
15:15	15:30	SL-06	Dürbeck K: "The Aromatherapy Educational Standard @ Forum Essenzia e.V."	
15:30	15:45	SL-07	Van Zyl R: „Pharmacological Interactions of Nature Identical Essential Oil Constituents“	
15:45	16:15		Coffee Break	
			Chair: Ana-Cristina Figueiredo and Karl-Heinz Kubeczka	
16:15	16:45	KL-04	Elisabetsky E : "Behavioural Effects of Inhaled Linalool in Mice: Implications for Aromatherapy"	
16:45	17:00	SL-08	Orhan I: „In vitro Anticholinesterase Upshots of Selected Essential Oils from Lamiaceae and Their Components“	
17:00	17:15	SL-09	Mastelic J: „Free and Bound Volatile Compounds from Rocket, *Eruca sativa* (L.)“	
17:15	17:30	SL-10	Nemeth-Zambori E: „Influence of the Plant Organ on the Compounds of the Essential Oil in Some Labiatae Species“	
17:30	17:45	SL-11	Ibrahim H: „Chemical Distribution in the Essential Oils of Two Closely Related Species of *Alpinia* Endemic to Borneo“	
17:45	18:00	SL-12	Baser KHC: „Essential Oils of *Ferula* Species of Turkey“	

			Tuesday, September 11, 2007	
			Developments in Quantitative Essential Oil Analysis	Aula
			Chair: Luigi Mondello and Carlo Bicchi	
08:30	09:00	PL-03	Grob K: "Quantitative GC – an Overview of the Problems, the Solutions and the Limitations"	
09:00	09:15	SL-13	Soulier C: „Characterisation of Essential Oils Using GC Exact Mass Time-of-Flight Mass Spectrometry: Application of Chemometrics for the Identification of Potential Markers“	
09:15	09:45	KL-05	Rubiolo P: "Quantitative Analysis in Essential Oils: is it a Difficult Task?"	
09:45	10:15	KL-06	Chaintreau A: "A Suitable Sample Preparation: a Prerequisite for Quantitative Analysis"	
10:15	10:45		Coffee Break	
			Chair: Chantal Menut and Fatih Demirci	
10:45	11:00	SL-14	Mondello L: "Innovative Approaches for the Analysis of Essential Oils (Fast-GC, MDGC, GCxGC, LCxLC)"	
11:00	11:15	SL-15	Ravid U: „Distribution of Some Chiral Monoterpene Ketones and Alcohols in Aromatic Plants“	
11:15	11:30	SL-16	Van Vuuren SF: „A Review of the Methods Used to Study the Antimicrobial Activity of Aromatic Plants“	
11:30	11:45	SL-17	Khalid H: „Antifungal Activity of Essential Oils of Some Sudanese Plants“	
11:45	12:00	SL-18	Zrira S: „Chemical Composition and Biological Properties of Moroccan *Ammi-visnaga* extracts“	
12:00	12:15	SL-19	Eloff JN: „A Comparison of Work on Volatile Versus Non-Volatile Antimicrobial Plant Compounds“	
12:15	13:45		Lunch Break	
			Young Scientists Workshop (HS 1.15)	HS 1.15
			Chair: Uwe J. Meierhenrich	
13:45	14:15	KL-07	Viljoen A: "Maximizing Opportunity – the Value of Having a Multidisciplinary Approach to Researching the Aromatic Flora of South Africa"	
14:15	14:30	SL-20	Bozin B: „Stabilization of Corn Oil by the Essential Oil of Oregano“	
14:30	14:45	SL-21	Ryabchenko B: „In vitro Antiviral and Anticancer Properties of Selected Aroma Samples“	
14:45	15:00	SL-22	Perriot R: „Chemical Composition of Mimosa Absolute“	
15:00	15:30		Coffee Break	
15:30	15:45	SL-23	Breme K: „Natural Extracts Analysis: Identification of Sulphur and Nitrogen Containing Compounds with Organoleptic Interests“	
15:45	16:00	SL-24	Radulovics N: „Volatile Glucosinolate Hydrolysis Products of Three Serbian Crucifers“	
16:00	16:15	SL-25	Novák I: „Investigation of Different *Thymus* Species with GC and "Electronic Nose"“	
16:15	16:30	SL-26	Wagner S: "Investigations in Distillation of Different Herbs in Pilot Plant Scale Regarding Oil Yield, Oil Composition and Development Stage"	

			Workshop Recent Developments in Industry	Aula
			Chair: Herbert Böchzelt	
13:45	14:00	KL-08	Böchzelt H: „Extraction Technologies: An Overview“	
14:00	14:15	SL-27	Ferhat M: „Comparison of Different Extraction Methods: Cold Pressing, Hydrodistillation, and Solvent Free Microwave Extraction, Used for the Isolation of Essential Oil“	
14:15	14:30	SL-28	Serafini L: „Design of a Microwave-based Pilot Module for Extraction of Essential Oils“	
14:30	14:45	SL-29	Kineci S: „Production of Essential Oil Using Adsorbent Resins from Waste Water That Remained While Rose Oil Production“	
14:45	15:00	SL-30	Albitar N: „The Instantaneous Controlled Pressure Drop (DIC) Technology for the Extraction of Essential Oils from Oregano and Jasmine“	
15:00	15:30		Coffee Break	
15:30	15:45	SL-31	Wolff AC: „Pressurized Liquid Extraction (PLE) for the Extraction of Essential Oil Compounds of Aromatic Herbs“	
15:45	16:00	SL-32	Lack E: „Supercritical Fluid Technology for the Production of Flavours and Fragrances and for Particle Generation“	
16:00	16:15	SL-33	Gamse T: „Extraction of Spanish Hyssop with Supercritical CO_2“	
16:15	16:30	SL-34	Venskutonis R: „Application of Liquid Carbon Dioxide for the Isolation of Essential Oils from Various Botanical Species“	
16:30	16:45	SL-35	Ramachandra YR: „Subcritical CO_2 Fractionation of Some Floral Concretes“	
16:45	17:00	SL-36	Brown B: „Stimulating Black Economic Empowerment in the Essential Oils industry in South Africa –Experiences and Lessons Learnt“	
17:00	18:30		Poster session	
19:00			Departure Symposium Dinner	
			Wednesday, September 12, 2007	
			Essential Oils in Animal Health and Nutrition	Aula
			Chair: Eva Nemeth-Zambori and Hassan Khalid	
08:30	09:00	PL-04	Windisch WM: "Essential Oils in Agricultural Lifestock Feeding"	
09:00	09:15	SL-37	Steiner T: „Efficacy of Phytogenics in Comparison to Antibiotics in Weaned Piglets“	
09:15	09:30	SL-38	Michiels J: „Fast Intestinal Absorption of Free Carvacrol and Thymol Compromises Antibacterial Effects Against the Pig Gut Flora“	
09:30	09:45	SL-39	Kaushik P: „Fumigation Toxicity of Spice Oils Against *Callosobruchus maculatus* in Grain Storage Systems“	
09:45	10:00	SL-40	Özkan G: „The Influence of Harvest Time on Essential Oil Composition, Phenolic Constituents and Antioxidant Properties of Turkish Oregano (*Origanum onites* L.)“	
10:00	10:30	KL-09	Reichling J: “External Application of Essential Oils in Animals”	
10:30	11:00		Coffee Break	
			Regulatory Affairs – Overregulation?	Aula
			Chair: Peter Liddle and Daniel Joulain	
11:00	11:30	PL-05	Demyttenaere J: “Recent EU-Legislation on Flavours and Fragrances and its Impact on Essential Oils”	
11:30	11:45	SL-41	Southwell I: „Regulatory Control of Essential Oils. Tea Tree Oil: A Case Study“	
11:45	12:15	KL-10	Burfield T: “Overregulation is Destroying Natural Aromatics”	
12:15	12:45		Brian Lawrence: Summary	Aula
12:45			Closing Ceremony	Aula

Abbreviations: PL....Plenary Lecture; KL….Keynote Lecture; SL….Short Lecture

LECTURES

Molecular Biology and Biosynthesis of Terpenes

PL- 1 The Origin of Terpene Diversity in Plants

Degenhardt J
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With over 30.000 different structures, terpenes form the largest group of plant natural compounds. This enormous structural diversity is attributable in large part to the actions of terpene synthases. These enzymes convert the linear C10, C15 and C20 terpenyl diphosphates to the parent skeletons of monoterpenes, sesquiterpenes and diterpenes, respectively. Perhaps the most extraordinary property of terpene synthases is their ability to produce complex mixtures of products, in some cases up to 50. It has long been appreciated that this catalytic virtuosity arises from a carbocationic mechanism in which, after displacement of the diphosphate residue, the resulting carbocationic species undergo a very broad range of of possible metabolic fates, including sucessive ring formation, hydride shifts and other rearrangements to generate a plethora of products.
To study the biosynthesis and composition of *Origanum vulgare* essential oils, we have identified six terpene synthases from EST libraries of leaf glandular trichomes. These terpene synthases produce almost all terpenes of oregano essential oil. Each of the enzymes form multiple terpenes in fixed, stoichiometric ratios. Variations in the essential oil compositions of two oregano lines were due to variation in terpene synthase expression levels rather than changes of the reaction mechanisms of the terpene synthases themselves. These analyses also suggested a catalytic pathway for the biosynthesis of the aromatic, hydroxylated terpenes thymol and carvacrol which are the products of secondary modification of monterpene hydrocarbons. Taken together, the presence of large terpene synthase gene families, multiproduct enzymes and secondary modifications of the terpene products provide the plant with efficient mechanisms to generate a large structural diversity of terpenes.

KL- 1 Engineering Substrate and Product Specificity in a Terpene Synthase

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Mediterranean Agronomic Institute of Chania, Alsyllio Agrokepiou, PO Box 85, Chania 73100, Crete, Greece

Elucidation of the structure of 1,8-cineole synthase from *Salvia fruticosa* (SfCinS1) combined with analysis of functional and phylogenetic relationships of enzymes within *Salvia* species enabled the identification of active site residues responsible for product specificity. Thus, SfCinS1 was successfully converted to a sabinene synthase with a minimum number of rationally predicted substitutions, while identification of the asparagine sidechain essential for water activation enabled the introduction of 1,8 cineole and α-terpineol activity to *S. pomifera* sabinene synthase. Moreover, a single amino-acid substitution was sufficient to enlarge the active site cavity enough to accommodate the larger farnesyl pyrophosphate substrate and lead to the efficient synthesis of sesquiterpenes, while alternate single substitutions of this critical amino-acid yielded five additional terpene synthases. The identification of such high plasticity residues in the terpene synthase active site provides structural insights into the evolution of terpene synthases. The combination of specificity and promiscuity observed in terpene synthases may be a snapshot of an evolutionary mechanism that maintains a dynamic state between desirable specific activities and a capacity for rapid change.

KL- 2 Valorisation of Essential Oils by Chemical and Biotechnological Modification of Key Components

Castilho P
Centro de Química da Madeira, Departamento de Química,Universidade da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, PORTUGAL

Essential oils are valuable materials themselves, with a large range of applications. Their added value can vary substantially depending on usefulness, composition or availability of plant material. Abundant, cheap components of essential oils can be converted on other compounds, not so easily accessible by means of chemical modification, either after isolation of key components or on the oil as obtained. The same can be performed on oils containing large percentages of toxic substances that rend them unacceptable for the usual applications of an essential oil.

Our group has been working on the valorisation of pine gum turpentine, of which Portugal is a large producer, obtained separately from the country two main pine species, *Pinus pinaster* Ait and *Pinus pinea* L.: the former is α-pinene rich, while the last has a (-)-limonene content over 80%.

Heterogeneous catalysis was used to convert selectively α-pinene into its ethers via alkoxydation with short linear chain alcohols. The same transformations were performed on *P. pinea* essential oil, obtaining several limonene oxides with application in the perfumery industry. *P. pinea* EO and pure limonene was also subject of solid acid catalysed aromatization yielding p-cymene. This method has the potential to compete with cumene disproportionation in the industrial formation of p-cymene as an intermediate in p-cresol production.

Heterogeneous catalysts of natural and synthetic origin have been fine tuned for the modification of other key components of essential oils: we used citronellal to produce isopulegols, with high stereo and enantiomeric selectivity. Recently, we have been reducing pulegone, a toxic component of the essential oils of several Labiatae, into pulegols using conventional chemical reducing agents, baker's yeast and grated carrot to find out the time of reaction increases while the enantiomeric purity increases in this order.

SL- 1 Biotransformation of p-Cymene to Thymoquinone

Demirci F[1], Berber H[2], Başer KHC[1]
1 Deparment of Pharmacognosy, Faculty of Pharmacy; 2 Department of Chemistry, Faculty of Science, Anadolu University, 26470 Eskişehir, Turkey.

Monoterpenes are important natural product resources which are consumed in chemicals, cosmetics, foods and pharmaceuticals sectors. A number of pure monoterpenes are obtained from essential oils [1]. Such compounds can be derivatised via biological techniques such as biotransformation, enzymatic biocatalysis to obtain value added speciality chemicals.
The monoterpene hydrocarbon p-cymene was used as a precursor for biochemical transformation by various microorganisms to produce thymoquinone and analogues. Furthermore, biomimetic substituted metalloporphyrin derivatives of chlorophyll were also utilized for the efficient synthesis of hydroxylated monoterpenes. Chromatospectral techniques were utilized for structure elucidation. Biological activity e.g. free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and antimicrobial activity of the metabolites were investigated using microdilution assay against a battery of human pathogenic bacteria and fungi.

Acknowledgements: The authors would like to thank TÜBİTAK 106T117 for financial support and Pharm. E. Civisov for his assistance.

References: 1. Baser KHC. and Demirci F. (2007) Chemistry of Essential oils. In: Flavours and Fragrances, Chemistry, Bioprocessing and Sustainability. Springer Verlag, Berlin.

SL- 2 Biotransformation of Limonene and Related Compounds by Newly Isolated Low Temperature Grown Citrus Pathogenic Fungi and Red Yeast

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1 Faculty of Life Sciences, Tokushima Bunri University; 2 Faculty of Pharmaceutical, Sciences, Tokushima Bunri University,Yamashiro-cho, Tokushima 770-8514,Japan, E-mail: ynoma@tokushima.bunri-u. ac.jp

In the continuing studies on microbial transformation and the searching of useful compounds of terpenoids [1-2], the biotransformation of (+)-(1) & (-)-limonene, (+)-(2) & (-)-α-terpineol, (-)-terpinen-4-ol (3) and (+)- & (-)- limonene epoxides by newly isolated low temperature grown kinds of several citrus pathogenic fungi and red yeast was carried out .

Cultivation and biotransformation was carried out according to the previous report [1]. The stereostructure of the metabolites were established by a combination of high resolution NMR spectroscopy, x-ray crystallographic analysis and the chemical reaction.

Penicillium sp. KCPYN converted 1 to (+)-isopiperitenone(4), (+)-2α-hydroxy- 1,8-cineole(5), (+)-limonene-1,2-trans-diol(6), (+)-p-menthane-1,2, 8-triol(7) and (+)-trans-sobrerol(8) as main products. Compound 5, 7 and 8 were formed from 2. *Penicillium* sp. YuzuYN converted 1 to (+)-trans-carveol(9), (+)-carvone, (-)-dihydro carvone, 4 and 6. *Aspergillus* sp.CBAYN also converted 1 to 4 and 6. Red yeast biotransformed 1 to afford 4, 6, 9 and (+)-cis-carveol(10) as main products. The predominat formation of carveols and limonene-1,2-diols from limonene is a very interesting phenomenon from the view point of the production of bottrospicatols as plant seed germination inhibitor [3] and carvone as useful perfume.

The formation of 5 and 7 from 2 led out attention to the biotransformation of 3. Compound 3 was also biotransformed to give (+)-2α-hydroxy-1,4-cineole and (+)-p-menthane-1,2,4-triol.

We will discuss the microbial and chemical transformation of (-)-limonene and limonene epoxides.

References: 1 Noma Y. and Asakawa, Y.,1995. Biotechnology in Agriculture and Forestry, Springer-Verlag Berlin Heidelberg,33,62-96; 2 Noma Y. and Asakawa, Y.,2006.37th ISEO, Abstract paper, L-22 and P-91(Grasse); 3 Noma Y and Nishimura H. 1987.Agric.Biol.Chem.,51, 1845-1849,.

SL- 3 Synthetic Substitutes for the High Class Natural Materials

Zope DD, Patnekar SG, Kanetkar VR
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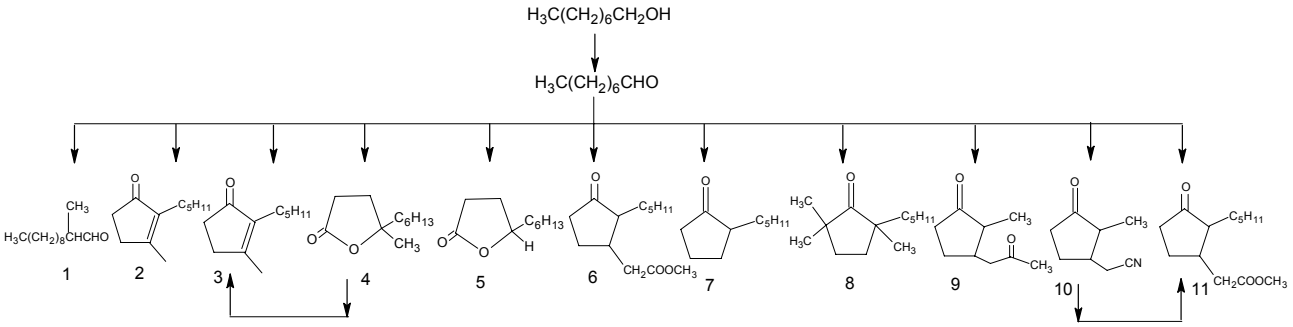
Jasmone and methyl jasmonate, found in jasmine flowers (*Jasminium grandiflorum*), are important perfume ingredients. These compounds have focused continuous attention and being convenient testing targets for many new synthetic methodologies, because of their economic values. Inevitably, synthetic chemists turned their attention not only to the naturally occurring substances themselves, but also to analogues and in particular those which are more easy to prepare or which are having practical advantage over the natural prototypes such as dihydrojasmone, methyl dihydrojasmonate (hedione), 2-pentyl cyclopentanone (delphone), 2,2,5-trimethyl-5-pentyl cyclopentanone (veloutone), magnolione etc.[1,2,3]. The present paper reports the synthesis of such cyclopentenones and its derivatives starting from n-octanol which is chiefly, easily and abundantly available commercially. n-Octanol has functionality suitable for the synthesis of cyclopentenones and its analogues. Oxidation of n-octanol furnished n-octanal, which on Knovenagel condensation with malonic acid gives 3-decenoic acid. Cyclization of this 3-decenoic acid using polyphosphoric acid yields 2-pentylcyclopent-2-ene-1-one. The synthetic utility of n-octanol for the synthesis of speciality aroma molecules has been discussed in this paper as:



Note: In the above mentioned scheme the compound No. 2 & 3 (Dihydrojasmone) and compound No. 6 & 11 (Hedione) are mentioned twice as they have prepared by two different novel routes.

Acknowledgements : D. D. Z. acknowledges CSIR, Govt. of India, for the SRF award.
References: 1.Dhananjay D Zope, et al. (2006), Flavour & Fragrance Journal, 21, 395-399.2.Dhananjay D Zope, et al. Flavour & Fragrance Journal, in press.3. Charles S Sell, Dorman L A, (1983), Perfumer & Flavorist, 8 (2), 68-74.

SL- 4 Biologically Active Compounds from Malagasy *Cinnamosma* Species and Liverworts

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In Madagascar, there are a number of endemic medicinal plants which have not yet been investigated pharmacologically. We focus on the chemical constituents of Malagasy pungent medicinal plants and several liverworts. *Cannela*, *Cinnamosma*, *Cinnamodendron* (=*Capsicodendron*), *Pleodendron* and *Warburgia* species belonging to the Canellaceae produce hot-tasting substances. *Cinnamosma fragrans*, *C. macrocarpa* and C*. madagascariensis* are used for ailments especially malaria symptoms, rheumatism, muscle aches and cough etc. From *C. fragrans* we isolated drimane sesquiterpenes, such as cinnamodial, cinnamolide, bemadienolides, cinnamosmolide, bermarivolide, fragrolide, capsicodendrin cinnafragrin A and ugandensolide, cinnafragrin together wit a large amount of mannitol and δ-tocotrienol [1].
From *C. macrocarpa*, bemadienolide, cinnamacrins A-C together with three related drimanes were isolated. Capsicodendrin, cinnamodial and cinnafragrin B showed α-glucosidase inhibitory activity. Cinnamodial and capsicodendrin indicated inhibitory activity against murine leukemia cells (IC 50: 0.18, 0,75 μg/ml) and human T-lymphocyte cells (0.45-0.59 μg/ml). Both compounds also demonstrated antiviral activity in vesicular stomatitis, Coxsackie B4 virus or respiratory syncytial virus at MIC 0.60-60 μg/ml as well as anti-herpes simplex virus-1 and 2, vaccnina virus at MIC 0.64μg/ml [2].
The volatile components of the Malagasy liverworts, *Bazzania decrescens*, *B. madagassa*, *B. nitida*, *Frullania* species, *Isotachis aubertii*, *Marchantia pappeana*, *Mastigophora diclados*, *Plagiochia barteri*, *P. heterospipa*, *P. terebrans*, *Radula appressa* and *Thysananthus spathulistipus* were chemically investigated and a number of terpenoids and lipophilic aromatic compounds were isolated and their structures and biological activity studied [3].

References: 1 Harinantenaina, L., Takaoka, S. (2006) J. Nat. Prod. 69, 1193-1197. ; 2 Harinantenaina, L., Asakawa, Y., De Clercq E. (2007) J. Nat. Prod. 70, 277-282; 3 Harianantenaina, L. , Asakawa, Y. Nat. Prod. Commun. 2007, 2, 1-9.

SL- 5 The Essential Oil of the Heartwood of *Neocallitropsis pancheri* from New Caledonia

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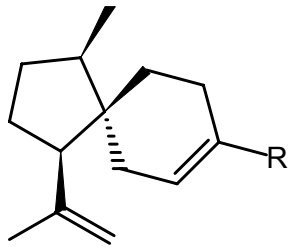
The essential oil obtained by steam distillation of the heartwood of *Neocallitropsis pancheri* was analyzed by conventional methods (physical fractionations, GC, GC-FTIR and GC-MS). To ensure univoqual identification of terpenoids, separation and isolation of individual compounds were achieved by silicagel column chromatography, preparative GC etc. followed by extensive NMR studies. A new sesquiterpene hydrocarbon: α-neocallitropsene **1**, and the corresponding primary alcool, α-neocallitropsol **2**, were identified. The stereochemistry and relative configuration of **1** and **2** were subsequently confirmed after completion of the total synthesis of **1** [1]. The determination of the absolute configuration of **1** and **2** is now disscussed with regard to recently published data [1,2], and a comprehensive analysis of the essential oil is presented.



1 R = CH_3 : α-neocallitropsene

2 R = CH_2OH : α-neocallitropsol

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Body(Odour) – Fragrance - Interaction

PL- 2 Human Body Odors and their Interactions with Fragrances

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The human axillae (underarms) are a unique source of human odor as well as primer and modulator pheromones. Ameliorating underarm odors is a focal point for a multi-billion dollar consumer product industry. These factors have motivated research aimed at identifying the nature, abundance and biogenesis of axillary odorants. A high density of eccrine, sebaceous and apocrine glands is found in the axillae. Moisture from the eccrine glands and sebum from sebaceous glands help (leave as help) support a dense microflora; apocrine secretions contain the odorless precursors to the characteristic axillary odor. Interactions between secretions from apocrine glands and the axillary microflora lead to a complex mix of odorants. The compounds that constitute the characteristic axillary odor have been elucidated. Emotional stress or fear increases apocrine and eccrine gland output and should result in greater odor production. We created a stress-related axillary odor using apocrine secretions and employed the technique of cross adaptation to screen dozens of fragrance materials to determine which most effectively blocked the male or female stress-related odors (SROs). Fragrance chemicals commonly employed in deodorant preparations and the SROs were found to interact in a gender-specific way, based upon both the gender of the odor producer and the "sniffer." More fragrance chemicals blocked female SRO to female sniffers than blocked male SRO to female sniffers; a large number of fragrance chemicals blocked female SRO in male sniffers. This study was unique in demonstrating a gender difference in the ability of a malodor to "break-through" the cross-adapting odorant. Additional studies also demonstrated that differences between male and female SRO's were hedonic and not based upon intensity. These results demonstrate that it is possible to design gender-specific products using the correct blends of fragrance materials.

KL- 3 Fragrance Effects in Humans - Is it all in Our Heads?

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During the past decade, aromatherapy research has greatly advanced our knowledge of the effects of essential oils and their constituents on the human body and mind. However, the mechanisms involved in such effects are still poorly understood. Several mechanisms based on odor valence, odor semantics and expectations of odors as well as on pharmacological principles have been proposed to mediate the effects of fragrances in humans [1]. In this presentation, an overview is given about these mechanisms and their efficacy in the modulation of human autonomic, emotional and cognitive arousal. The proposed mechanisms will be discussed in relation to a number of recent [2-7] and ongoing investigations by our group. In particular, psychological and pharmacological effects of essential oils and their constituents will be compared and the impact of the route of administration, such as inhalation and dermal application, will be evaluated.

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SL- 6 The Aromatherapy educational standard @ Forum Essenzia e.V.

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The legal framework as established in the context of Forum Essenzia includes three aspects of an extended definition of aromatherapy, including the application of genuine and authentic essential oils and vegetable oils through therapeutics (AromaTherapy), nursing staff (AromaCare) and the family use as AromaCulture. Based on the legal framework conditions in the EU there is need for an international standardized curriculum of aromatherapy education. Working groups, like the Aromatherapy Councils in UK and USA, work with a Core Curriculum.
At Forum Essenzia the criteria for the curriculum of Aromatherapy courses are the genuine interest in the application of essential and vegetable oils, and respect for the legal framework conditions. The essential criteria evolve around questions like: what constitutes the common body of knowledge within the Forum Essenzia community? Is it evidence based enough to create the exam along with grading guidelines? Can a Forum Essenzia standard guide the educators and schools in the German speaking world? Can it be organised under the ECTS –European Credit Transfer in facilitating student mobility and international curriculum development?
The Forum Essenzia introductory seminar course provides students with the basics of aromatherapy, explaining biology, chemistry, processing and application of essential and vegetable oils. The Forum Essenzia core educational programme offers 4 technical 2-day seminars on Biology, Chemistry, Processing and Application of essential and vegetable oils. As advanced vocational training options Forum Essenzia organises technical seminars and conferences.

SL- 7 Pharmacological Interactions of Nature Identical Essential Oil Constituents

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The pharmacological properties of essential oils have been widely studied. However the interaction and contribution of the individual constituents to the overall activity remains virtually unexplored. In this study, thirteen essential oil constituents (EOC′s) from six structural groups were tested for their antimalarial, antimicrobial, and toxicity properties, using the tritiated hypoxanthine incorporation, the minimum inhibitory concentration (MIC) microplate and the tetrazolium-based cellular viability assays, respectively. The EOC´s were found to inhibit the growth of chloroquine-resistant Plasmodium falciparum with IC50 values ranging between 0.9 to 1528.8µM, with *E*- and *Z*-(±)-nerolidol, (-)-pulegone, (+)-α-pinene and linalyl acetate being the most active. In combination, the two least active EOC's, ρ-cymene and carvacrol interacted synergistically (∑FIC = 0.09), which was comparable to the interaction between *E*- and *Z*-(±)-nerolidol and quinine (∑FIC = 0.01). Eugenol was the least toxic and (+)-α-pinene had the most favourable safety index. In combination, *E*- and *Z*-(±)-nerolidol with either (-)-pulegone or quinine potentiated each other′s toxicity. The EOC´s inhibited the microbial growth to varying degrees in millimolar concentrations, with carvacrol being the most active against *Staphylococcus aureus* and *Candida albicans*, while eugenol was the most active against *Escherichia coli* and *Bacillus cereus*. When tested against *C. albicans*, (+)-β-pinene interacted antagonistically with (-)-menthone (∑FIC = 9.80), but synergistically with 1,8-cineole (∑FIC = 0.35). Against *E. coli*, the combination of *E*- and *Z*-(±)-nerolidol and geranyl acetate displayed an additive interaction, while an indifferent interaction was observed between *E*- and *Z*-(±)-nerolidol and eugenol. The combination of an EOC (carvacrol or eugenol) and a standard antimicrobial (ciprofloxacin or amphotericin B) resulted in synergistic interactions against all four microorganisms. These results show that the pharmacological activities of EOC′s can vary greatly when used in combination. Therapeutically, they have the potential to be used as adjuncts in the combat against drug resistance.

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KL- 4 Behavioral effects of inhaled linalool in mice: implications for aromatherapy.

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Introduction: Linalool is a monoterpene, often the major component of plant essential oils. Numerous linalool producing plants have been used in traditional systems of medicine, commonly for purposes that remind sedative effects as definied by western pharmacology. Psychopharmacological evaluations of linalool (ip or icv) revealed marked sedative and anticonvulsant effects in diverse mice models. Neurochemistry identified an interesting and complex mechanism of action, especially as a modulator of the glutamate transmission. Considering this psychopharmacological profile, reports of sedative and/or relaxing effects of inhaled essential oils in animals and in humans, and that aromatic species rich in linalool (e.g., lavender) are commonly used in aromatherapy, the purpose of this study was to examine the sedative effects of inhaled linalool in mice. For this purpose anxiolytic, hypnotic, cognitive and motor effects were examined. Furthermore we studied the effects of inhaled linalool in the anxiety produced by stress, aggressiveness and social interaction in mice. Methods: Diazepan 1mg/kg ip was used as positive control. Mice inhaled (60 min) tween 1%, linalool 1% or 3% and were immediately submitted to: light/dark, locomotion, barbiture sleep, or step-down avoidance tests. Other groups were submitted to immobilization stress after inhalation, followed by light-dark, aggression and social interaction tests Experiments were video recorded, and analyzed with Noldus software. ANOVA or Kruskal-Wallis/Mann-Whitney was used for statistic. Discussion: Data suggest that inhaled linalool 3% (but not 1%) is anxiolytic in doses that induce little sedation ($p<0.01$). Moreover it reverses the effects of immobilizations stress, decrease aggression, and increase social interaction in mice. These findings are in agreement with aromatherapy claims of linalool rich odors used for sedative purposes in baths or devices for enclosed spaces, such as candles, incenses, and the like.

Acknowledgements: CNPq e FAPESP

SL- 8 In Vitro Anticholinesterase Upshots of Selected Essential Oils from Lamiaceae and Their Components

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Alzheimer's disease (AD) is a degenerative neurological disorder characterized by senile plaques containing amyloid β protein and loss of cholinergic neuromediators in the brain. The most remarkable biochemical change in AD patients is reduction of acetylcholine (ACh) level in hippocampus and cortex in the brain [1,2]. Therefore, inhibition of acetylcholinesterase (AChE), the enzyme responsible for hydrolysis of ACh at the cholinergic synapse, is currently the most established approach to treating AD.
Although several studies have been thus far carried out on anticholinesterase upshot of essential oils, a few reports have mentioned about anticholinesterase activity of essential oil components. As well-known, essential oils are complex mixtures of components that show usually higher activities than their isolated components, and their final activities are generally concluded to be due to the combined effects of several minor components.
In this study, we have tested acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of sixteen essential oils from Lamiaceae plants; two from *Melissa officinalis* L. (organic and chemical fertilization), two from *Mentha piperita* L. and *M. spicata* L. (organic fertilization), two from *Lavandula officinalis* Chaix ex Villars (organic and chemical fertilization), two from *Ocimum basilicum* L. (green and purple-leaf varieties only organic fertilization), four from *Origanum onites* L., *O. vulgare* L. , *O. minutiflorum* Hausskn., and *O. majorana* L. cultivated using organic fertilizer, two from *Salvia sclarea* L. (organic and chemical fertilization), *S. officinalis* L. (organic fertilization), and *Satureja cuneifolia* Ten. (organic fertilization) by a spectrophotometric method of Ellman using ELISA microplate-reader at 1 mg/ml concentration. In addition, a number of single components widely encountered in most of the essential oils (γ-terpinene, 4-allyl-anisole, (-)-carvone, dihydrocarvone, (-)-phencone, cuminyl alcohol, cumol, 4-isopropylbenzaldehyde, trans-anethole, camphene, iso-borneol, (-)-borneol, L-bornyl acetate, 2-decanol, 2- heptanol, methyl-heptanol, farnesol, nerol, iso-pulegol, eucalyptol, citral, citronellal, citronellol, geraniol, linalool, α- pinene, β-pinene, piperitone, iso-menthone, linalyl oxide, linalyl ester, geranyl ester, carvacrol, thymol, menthol, vanilline, and eugenol were also screened for the same activity in the same manner. The results showed that almost all of the essential oils showed a very high inhibitory activity (over 80%) against both enzymes, whereas the single components were not as active as the essential oils, indicating synergistic interaction among those components.

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SL- 9 Free and Bound Volatile Compounds from Rocket, *Eruca sativa* (L.)

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Rocket, *Eruca sativa* (L.) belongs to Brassicaceae family. It contains glucosinolates and O-glycosides. The glucosinolates and their aglucones are biological active compounds. Composition of the obtained aglucones depends on the structure of glucosinolates and degradation conditions such as pH-values, temperatures, Fe2+ ions and specific protein [1]. Further, chemical analysis of aglucones depends on the mode of isolation and concentration.

The volatiles were isolated from fresh plant material of rocket (flowers, leaves and green beans) prior and after the autolysis [2] by two methods: simultaneous hydrodistillation-extraction and simultaneous hydrodistillation-adsorption, proposed method [3] . All isolates of volatiles were analyzed by GC and GC/MS. Isothiocyanates, nitriles, aliphatic alcohols, carbonyls, fatty acids, hydrocarbons, phenylpropane derivatives were identified. The major component in all isolates was 4-methylthiobuthylisothiocyanate. The volatiles which were isolated after the autolysis had larger number of compounds than the volatiles obtained without autolysis.

The main advantages of hydrodistillation-adsorption method over hydrodistillation-extraction method are: the isolation is particular, higher yield, simultaneous separation of volatile isolate in three fractions (polar, non polar and light volatile compounds).

O-glycosides of volatile compounds were isolated and purified by selective extraction and column chromatography from the same plant material. After hydrolysis of O-glycosides, using β-glucosidase, liberated volatile O-aglycones were also analyzed by GC and GC/MS. Compounds with sulphur and nitrogen (which are characteristic for glucosinolates) were not identified among these compounds. The main volatile O-aglycones were: 2-phenylethanol, benzyl alcohol, eugenol, (*Z*)-3-hexen-1-ol. Some of them were identified in volatile isolates together with the sulphur and nitrogen containing compounds, such as (*Z*)-3-hexene-1-ol, benzyl alcohol and 2-phenylethanol. The chemical composition of O-aglycones from rocket has not been reported, so far as we know.

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SL- 10 Influence of the Plant Organ on the Compounds of the Essential Oil in Some Labiatae Species

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Several ones of the most important medicinal and spice plants in the temperate area belong to the Labiatae family. It is generally known, that both the level of essential oil and its composition are influenced by ontogeny and organic composition [1].
In our recent study we wanted to describe the role of the plant organ on the quantity and quality of the oil, furthermore, comparing the characteristics of seven related species (*Hyssopus officinalis*, *Lavandula hybrida*, *Origanum vulgare*, *Salvia officinalis*, *Salvia sclarea*, *Satureja montana*, *Thymus vulgaris*). In each case the whole shoots of the cultivated populations were harvested at full flowering stage and separated into stem, leaf and flower samples. The essential oil was distilled and analysed by GC-MS method.
The results showed that in each species the level of the oil was highest in the flowers. The leaves displayed ½-1/3 of it in thyme, sage, savory and hyssop, while they had much lower levels (1/10) in lavender, oregano and muscat sage. The stem contained measurable amounts of oil only in *S. officinalis*.
The composition of the different organs was almost similar in case of hyssop, thyme and savory. Quantitative differences among them could be detected in the samples of oregano (e.g. β-phellandrene, terpinolene, charyophyllene) and sage (e.g. pinenes, α-thujone, borneol), while considerable qualitative differences were described in muscat sage and lavender. In the latter case the main compounds linalool, linalyl-acetate of the flowers are missing from the vegetative parts. In five of the seven species, a higher proportion of sesquiterpenes in the vegetative organs seems to be characteristic compared to the flowers. Similar was found already for Asteraceae species [2].

Acknowledgements: 1. Project nr. GVOP-3.2.1.-2004-04-0134/3.0, 2. Klara Demeter

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SL- 11 Chemical Distribution in the Essential Oils of two Closely Related Species of *Alpinia* Endemic to Borneo

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Alpinia ligulata and *Alpinia nieuwenhuizii* are two wild and morphologically very closely related species endemic to Borneo. Both species are recognised by their strongly paniculate inflorescence and singly borne flowers. *A. ligulata* is mainly distinguished from *A. nieuwenhuizii* by its large ligule and less floriferous inflorescence. The aim is to investigate whether the two morphologically allied species are also closely related in their chemical composition. In this paper, we wish to report for the first time, the chemical constituents detected in essential oils of *A. ligulata* and *A. nieuwenhuizii*. Hydrodistilled essential oils from the rhizomes of *A. ligulata* and *A. nieuwenhuizii* were analyzed by GC and GC/MS. Essential oil yield of *A. ligulata* was 0.8%(w/w) by dry weight while that for *A. nieuwenhuizii* was 0.9%(w/w) by dry weight. Remarkably the oils were found to possess much compositional similarity but are quantitatively different in the concentration of each component. Twenty compounds were detected in the essential oil of *A. ligulata* and twenty-one were detected in the essential oil of *A. nieuwenhuizii*. The major compound identified in both species was methyl cinnamate, which was present at 48.2% in *A. ligulata* and 66.7% in *A. nieuwenhuizii*. Previously the same compound was also detected as the major component in *Alpinia latilabris* by us [1] and other workers [2].

Acknowledgements: Universiti Malaysia Pahang, University of Malaya, Institute for Medical Research

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SL- 12 Essential Oils of *Ferula* Species of Turkey

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Ferula L. (Apiaceae) is represented in Turkey by 21 species of which 11 are endemic to Turkey (1-3). Dried fruits of 16 *Ferula* species were hydrodistilled and the oils were analysed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The *Ferula* species investigated are as follows: *F. anatolica* Boiss., *F. brevipedicellata* M.Sagiroglu & H.Duman, *F. communis* L., *F. coskunii* H.Duman & M.Sagiroglu, *F. duranii* M.Sagiroglu & H.Duman, *F. drudeana* Korovin, *F. elaeochytris* Korovin, *F. halophila* Pesmen, *F. haussknechtii* Wolff ex Rech., *F. hermonis* Boiss., *F. lycia* Boiss, *F. mervynii* M.Sagiroglu & H.Duman, *F. parva* Freyn & Bornm., *F. rigidula* DC., *F. szowitsiana* DC, *F. tingitana* L. Oil yields varied from trace to 3.7%. Monoterpene hydrocarbons (10 oils), naphthalene (4 oils), germacrene D (2 oils) and shyobunone derivatives (1 oil) were the main components.
This is the first concise study of the *Ferula* oils of Turkey.

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No		*Ferula* sp.	Yield	Main components (%)
1	E	*F. lycia*	tr	Naphthalene (16.6), α-pinene (14.7), caryophyllene oxide (10.6)
2		*F. communis*	tr	Naphthalene (50.0), nonacosane (18.2)
3	E	*F. duranii* yeni tür		Germacrene-D (24.8), naphthalene (10.3),
4	E	*F. anatolica*	tr	Germacrene-D (30.0), naphthalene (21.7)
5		F. hermonis		α-Pinene (71.7)
6		*F. elaeochytris*		α-Pinene (72.8), β-pinene (15.4)
7	E	*F. brevipedicellata* yeni tür		α-Pinene (64.7), β-phellandere (6.8), β-pinene (6.7)
8	E	*F. mervynii* yeni tür	tr	α-Pinene (79.9), sabinene (12.2), β-pinene (8.8)
9	E	*F. parva*		α-Pinene (34.0), eremophilene (8.9)
10		*F. haussknechtii*		α-Pinene (32.0), camphene (30.7), myrcene (7.3), bornyl acetate (6.6)
11	E	*F. coskunii* yeni tür		Sabinene (37.5), α-pinene (37.4), β-pinene (11.4)
12		*F. rigidula*		Camphene (14.6), α-pinene (13.0), δ–cadinene (12.5), α-cadinol (10.3), germacrene-D-4-ol (10.0)
13	E	*F. halophila*		β-Phellandere (14.2), eremophilene (8.6)
14	E	*F. drudeana*		epi-Isoshyobunone (37.5), shyobunone (25.4)

Developments in Quantitative Essential Oil Analysis

PL- 3 Quantitative analysis by GC: recent technical progress

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Gaschromatographic technology slowly improves in two directions: improved techniques and improved quality management, i.e. control of quality. The lecture reports improvements relevant for our laboratory.

1. Techniques for large volume injection became simplified in so far as from the many options available, 3 techniques were selected:
(i) on-column injection by the retention gap technique (with or without early vapour exit),
(ii) on-column injection with concurrent solvent evaporation and
(iii) splitless injection with concurrent solvent recondensation (CSR).
2. Injector-internal thermal desorption from lipids, a kind of headspace at high temperature in the injector, is a promising technique also for the analysis of essential oils.
3. The use of the sample matrix for deactivating the injector liner or the (pre-)column is an important tool for some analyses.
4. Quantitative analysis primarily suffers from failure of critical steps rather than a deviation somewhat beyond the anticipated uncertainty range. Such failures must be recognized. Method performance also tends to drift and it must be detected when critical thresholds are exceeded. For this reason, validation may be useful at the stage of method development, but quality of the data should been sured by verification, i.e. tools built into the method to enable checking for every analysis whether the critical steps were performed adequately. Verification tools must be designed for the purpose.

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SL- 13 Characterisation of Essential Oils using GC Exact Mass time-of-flight Mass Spectrometry: Application of Chemometrics for the Identification of Potential Markers

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Among the several essential oil families, citrus family is the most difficult to work with as its collection of essential oils exhibits very similar fragrances. Essential oils from the citrus family, originating from several countries and suppliers, were studied using gas chromatography exact mass time-of-flight mass spectrometry (GC-TOF-MS). The GC method developed to differentiate these oils was fast and general, and it can be applied equally well to analysis of various other complex essential oils. The elevated resolution of TOF-MS with exact mass capabilities facilitates the deconvolution of the data without the need for complete chromatographic separation.
A chemometric approach based on principal component analysis (PCA), using MarkerLynx application manager with SIMCA-P, was used to design the "fingerprint" \f „n" of forty individual essential oils and three mixtures composed of natural essential oils and synthetic components. Successful identification of potential markers of the essential oils allowed characterisation of three mixtures, using MarkerLynx with SIMCA-P and ChromaLynx to identify relevant similarities. The PCA model separated successfully all the essential oils, showing tight sample clustering of each individual oil variety.
These analytical tools are also important considering the authenticity and quality control of essential oils based products.

KL- 5 Quantitative Analysis of Essential Oils: Is it a Difficult Task?

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The implemented legislation in fields strictly involved with essential oils (EOs), such as food and cosmetics together with the evolution of method validation has required to adopt a different approach for EOs quantitative analysis. The aim of this presentation is to show some of the problems related to the quantitation of EO components. EO quantitative analysis requires a careful control of their sample preparation and analysis:

- the first critical point for a correct quantitation is sample preparation (e.g hydrodistillation or mechanical processes); because of the variability of the vegetable matrix, internal standards must always be used;
- the second one concerns the correct quantitation of characteristic EO components in the analytical step (e.g. GC/FID and/or GC/SIM-MS analysis of an EO) versus external and/or internal standards, although this task is not easy in particular with highly complex EOs.

On the other hand an absolute quantitation is not always necessary in particular in quality control when a reference material is available. These approaches will be illustrated through some examples concerning:

- the parameters necessary for a correct EO quantitative analysis
- the standardization of the sampling and analysis procedures
- the setting-up of marker components
- the setting-up of a chromatographic profile as a reference material

KL- 6 Suitable Sample Preparation: A Prerequisite for Quantitative Analysis

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In the domain of flavors and fragrances (F&F), a reliable quantification of volatile constituents is a crucial requirement for chemotaxonomic studies, or to assess the production quality of essential oils, compounded perfumes and aroma, etc. In addition, recent legislation requires the determination of an increasing number of restricted chemicals in the finished products. In almost all cases, the isolation of the flavor or the fragrance from its matrix (plant, food, cosmetics, detergents, etc) is a key step. It should provide the analyst with an extract whose composition is representative of target analytes in the starting material. Numerous publications suggest that authors are not always aware of methods compatible with the quantitative requirements of this first step.
In the present lecture, the main sample preparation techniques that are applicable to F&F will be reviewed, with a focus on their suitability in quantitative analysis. In most instances, their performances can be modeled using physical chemistry, which allows the prediction of the resulting analyte recoveries. Experimental examples illustrate these predictions for widely used techniques, such as simultaneous distillation-extraction, solid-phase microextraction, etc. The performances of less common techniques will also be reported, when applicable to F&F (e.g. Static-and-trapped headspace).

SL- 14 Innovative Approaches for the Analysis of Essential Oils (Fast-GC, MDGC, GCxGC, LCxLC)

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Essential oils may be considered medium to highly complex samples, which are formed of a volatile and non-volatile fraction. Gas chromatography (GC) is the analytical technique that has given the major contribution towards the determination of volatile profiles in essential oils. The study of the non-volatile fraction is, on the other hand, generally achieved through high performance liquid chromatography (HPLC).
The development of a series of methods has allowed not only the characterization of several essential oils but also, as a consequence, accurate judgements on genuineness, geographic origin, possible contamination and adulteration. A series of limitations, though, must be considered:

- single column chromatography often lacks the necessary resolving power to isolate all matrix components in an acceptable analytical time. Furthermore, extensive peak overlapping is a hinderance towards reliable MS structural elucidation.
- conventional chromatographic methods sometimes ensuring satisfactory separations on essential oils samples, are also characterized by a substantial disadvantage: the cost in analytical time. This becomes a limitating factor especially for laboratories with a high sample throughput and/or where there is a need for quick and correct results.

These aspects regard not only essential oil analysis but also a vast amount of matrices in different fields. As such, in the past years, there has been an increasing interest within the chromatographic community towards the development of more effective separation methods.
The present contribution is focussed on the most advanced monodimensional (micro-bore column GC) and multidimensional chromatographic techniques (comprehensive GC, comprehensive LC, heart-cutting multidimensional GC) today employed in essential oil analysis. A series of applications on different essential oil samples will be described in order to demonstrate the effectiveness of these approaches.

SL- 15 Distribution of Some Chiral Monoterpene Ketones and Alcohols in Aromatic Plants

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The isolation of chiral building blocks with high enantiomeric or diastereomeric purity from aromatic plants is important for the syntheses of more complex chiral bioactive molecules. The chiral building blocks are incorporated into the target molecules in such a way that the configuration of the stereo centers remains unchanged. Since the relative configuration of newly produced centers of chirality can be controlled, virtually any enantiomerically or diastereomerically pure product can be built around the chiral starting molecule.

Chiral GC-MS analyses of natural and synthetic *trans*- and *cis*-piperitone oxide were performed on a chiral capillary column in order to clarify the stereochemistry of their enantiomeric forms.

The two diastereomers of *cis*- and *trans*-piperitone oxide were detected in plants belonging to the Lamiaceae family, especially in *Mentha* and *Calamintha*. Only enantiomerically pure levorotatory piperitone oxides, (1S,2S,4S)-*trans*-piperitone oxide and (1S,2S,4R)-*cis*-piperitone oxide, were detected by chiral GC-MS analyses of *Micromeria fruticosa* and *Mentha longifolia*.

Relatively large quantities of *cis*- and *trans*-sabinene hydrate were detected in the genus *Origanum*. The enantiomers of *trans*- and *cis*-sabinene hydrate and their acetates were detected in the extracts of *O. dayi*, *O. majorana*, *O. vulgare* ssp. *vulgare* and *O. syriacum* ssp. *syriacum*.

We examined the content of the two diastereomers of thujone in eight aromatic plants. It was found that plant samples taken during the period of extensive renewed vegetative growth contained the highest level of thujones. Of the examined species, those rich in α-thujone were *Achillea fragrantissima*, *Artemisia maritima* and *Salvia officinalis*. The species rich in β-thujone were *Ac. fragrantissima*, *Ar. herba alba*, *Ar. maritima* and *Ar. arborescens*.

The differences in the enantiomeric or diastereomeric distribution of the chiral oxygenated monoterpenes enable distinction among the species.

References: 1. Larkov, O. et al., Flavour Fragr. J., 2007, 22, 328-333.

SL- 16 A Review of the Methods used to Study the Antimicrobial Activity of Aromatic Plants.

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Many studies reporting the antimicrobial activity of essential oils have been undertaken, however, there are still several disparities in the techniques where there is a need to standardize the various methods. Assays used to determine the antimicrobiological activity of aromatic plants should be carefully considered and carried out in a manner so that results yield accurate and reproducible results. It is of utmost importance that parameters such as plant collection, extraction, essential oil analysis and various details of the microbiological techniques are carefully defined. The various microbiological techniques such as disc diffusion, minimum inhibitory concentration, time-kill and synergy assays commonly used when assessing the antimicrobial activity of essential oils will be presented with reference to their application in studies on South African aromatic medicinal plants. These methods will be critically assessed with recommendations based on both literature reviews and practical experience. Some examples discussed will be the importance of method validation demonstrated with *Osmitopsis asteriscoides*. Variation of results between different methods will be illustrated for *Eriocephalus* spp. Reference to literature will demonstrate how results differ as noted in the investigation of 1,8-cineole. The antimicrobial efficacy of essential oil constituents will be demonstrated with reference to their interactions with other constituents and within the plant as a whole.

SL- 17 Antifungal Activity of Essential Oils of Some Sudanese Plants

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The antifungal activity of essential oils of eight plants namely; *Cyperus rotandus* L. (Cyperaceae), *Boswellia papyrifera* (Del. ex Caill.) Hochst. (Burseraceae), *Eucalyptus camaldulensis* Dehnhardt,(Mytraceaee) *Cymbopogon schoenanthus* (L.) Spreng.(Poaceae), *Citrus paradisi* Macfad.(Rutaceae), *Foeniculum vulgare* P (Apiaceae). Mill., *Ocimum basilicum* L.(Lamiaceae)& *Cymbopogon nervatus* (Hochst.) Chiov. (Poaceae), were screened for their antifungal activity against *Aspergillus: niger* and *Candida albicans* using Sabouraud Dextrose Agar for Agar diffusion Assay. The three former plant extracts showed best activity. Czapek Dox liquid medium and Sabouraud Dextrose Broth were used for a Dilution Assay. Minimum inhibition concentration (MIC) for *Aspergillus niger*, *A. flavus* and *Candida albicans* cultures were determined. *Cymbopogon nervatus* (Hochst.) Chiov. extract showed notable antifungal activity. Quantitative Analyses (Turbidimetry & Dry weight) were studied; the measurements verified antifungal activity of the essential oil.

SL- 18 Chemical Composition and Biological Properties of Moroccan *Ammi visnaga* Extracts

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Ammi visnaga, known as Khella, is an annual plant from the umbelliferae family which grows spontaneously in Southernmost Europe, Occidental Asia, Egypt, Algeria, Madeira and Acores. It is very wild spread in Morocco.
Its fruits, seeds and extracts have very interesting therapeutic actions and clinical indications: hypertension, chronic asthma, angina pectoris, vitiligo,…
In order to examine the potential uses of *Ammi visnaga*, we have examined the chemical composition of its different extracts and the antimicrobial activity of its essential oil.The essential oils were obtained by hydrodistillation using a Clevenger-type apparatus and by steam distillation using an industrial distiller. The CO_2 extract was obtained by supercritical fluid extraction. The essential oils were analyzed by gas chromatography and gas chromatography-mass spectrometry.
The major compounds of the *Ammi visnaga* oil obtained by hydrodistillation were 2-methylbutyl butyrate (41.8%), linalool (23.5%) and 2-methylbutyl 2-methylbutyrate (10%), while 2-methylbutyl 2-methylbutyrate (27.7%), linalool (22.7%) and isobutyl valerate (16.6%) were the main constituents of the steam distillation oil. The supercritical CO_2 extract was rich in geranyl linalool (22.7%), linalool (11%), visnagin (6.1%) and khellin (6.1%). The essential oil of *Ammi visnaga* showed a very interesting antibacterial activity against *Mycobacterium aurum*.
The Moroccan *Ammi visnaga* essential oil is a new oil which could find many other uses because of its flowered notes, green of galbanum type and Roman chamomile. It could also be used in aromatherapy.

SL- 19 A Comparison of Work on Volatile Versus Non-volatile Antimicrobial Plant Compounds

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Generally antimicrobial activity of volatile compounds obtained from plants is an order of magnitude lower than that of extracts of other plant secondary compounds. Many scientists work in this area. There are several advantages with working on volatile compounds. The techniques have been developed well. With GC-MS an impressive quantitative list of compounds present in the plant can be obtained. It is easy to determine diurnal changes in concentrations and between different populations of the same species.
There are also disadvantages. Steam distillation may lead to changes in the composition of volatile compounds. If a new compound is present it is difficult to identify. Bio-assay guided fractionation is complicated by the volatility. It is difficult to obtain enough of a single compound for in vitro or especially in vivo studies. Concentrations of volatile compounds are much more variable than that of non-volatile plant secondary compounds. Changes may take place between collection and analysis of plant material. Losses of volatile compounds can take place easily if precautions are not taken during e.g. determination of MIC or analysis by bioautography. Some compounds may even be lost from a sample holder during storage if the correct stopper is not used [1]. It is also difficult to determine the mechanism of activity without sufficient material. Based on the relatively high MIC values activity may be related to cell membrane damage rather than to a specific metabolic interaction.
The higher activity of organic plant extracts may be due to mixtures containing volatiles and non-volatiles.

Acknowledgements: The NRF provided funding for this work

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Young Scientists Workshop

KL- 7 Maximizing opportunity – the value of having a multidisciplinary approach to researching the aromatic flora of South Africa

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South Africa has offered the world two indigenous aromatic plants which have both been developed into a commercial success. Geranium oil (obtained from various cultivars of *Pelargonium graveolens*) and Buchu oil (from *Agathosma betulina*) are only two species out of the near 700 aromatic plants indigenous to South Africa. It remains ironic that most of the other aromatic species have until recently been neglected, with little research being carried out to explore their possible future use and application in consumer products. Most often, aromatic plants are studied either to: 1) determine the chemical composition of the oil; 2) search for interesting chemotypes, 3) investigate the biological activity (especially for plants used in African traditional healing), 4) to resolve or confirm a taxonomic placement. Generally these aspects are undertaken independently. In 1999 an ambitious project was initiated to record the essential oil composition and biological activities of South Africa's extra-special botanical resources. Using a classical multidisciplinary approach results obtained for some of the most abundant indigenous aromatic genera (e.g. *Salvia*, *Lippa*, *Eriocephalus*, *Agathosma*, *Pelargonium*) will be discussed in terms of results obtained for aspects 1-4 mentioned above. The paper is a brief reflection of past and present research and will unequivocally confirm the value (and privilege) in exploring one of the most biodiverse areas in the world.

Acknowledgements: All collaborators, past and present postgraduate students who have contributed to this project are greatly acknowledged.

SL- 20 Stabilization of Corn Oil by the Essential Oil of Oregano

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Evaluation of antioxidant activities of plant secondary biomolecules are intensified recently. Among large number of different biologicaly active constituents of higher plants, most powerful antioxidant effects are expressed by phenolic compounds and essential oils. However, only the investigations related to the practical use of these substances in different commercial preparations, together with in vivo assays could lead to the confirmation of their applicability. With respect to this, in the present study the results of effects of the oregano essential oil, as a strong natural antioxidant confirmed in in vitro examinations [1], on the peroxidation of the lipids in the corn oil (in the following concentrations: 9.6, 18.1, 21.3 i 31.9μl of essential oil per ml of corn oil).
Chemical composition of the corn oil and oregano essential oil were evaluated by GC-MS. Quality of the investigated corn oil (CO) and the mixture (ECO) is evaluated by free fatty acid (FFA) content, saponification (SN) and esterification number (EN) [2]. Antioxidant activity of the oregano essential oil is measured as the % of the lipid peroxidation inhibition (LP) in the corn oil, induced by two systems (Fe^{2+}/ascorbate and Fe^{2+}/H_2O_2) [1]. From the obtained results for FFA (CO-5.33, ECO up to 2.64mgKOH/g), SN (CO-140.3, ECO up to 171.2mgKOH/g) and EN (CO-135.0, ECO up to 168.5mgKOH/g), it is obvious that essential oil improves the quality of the corn oil, by esterification of free fatty acids. Also, the essential oil exhibited significant inhibitory effects on lipid peroxidation (LP) in corn oil, reaching the 50% of inhibition of LP in both systems of induction (in Fe^{2+}/ascorbate IC50=0.17μl/ml, and in Fe^{2+}/H_2O_2 system of induction IC50=0.07μl/ml).
Obtained results pointing on a great potential of the oregano essential oil as a natural additive, especially in the pharmaceutical and food industry.

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SL- 21 In Vitro Antiviral and Anticancer Properties of Selected Aroma Samples

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The anti-tumor, antiviral and cytotoxic activities of cinnamic acid, β-caryophyllene, caryophyllene oxide, natural and synthetic nerolidol, longifolene, geranic acid, farnesol (different isomers), and a farnesol-rich ylang-ylang-fraction were studied.
The anti-tumor activity of the compounds was performed in HeLa and Jurkat cell lines. Using a CytoTox-96®-Assay we found a strong anti-tumor effect of natural and synthetic nerolidol in concentrations <5 µM. Moreover, the concentration of the synthetic nerolidol required to reduce the number of HeLa or Jurkat cells by 50% (CC50) was even ten times lower than the effective dose required achieving 50% of cytotoxicity for cells (ED50). The antiviral properties of all compounds were investigated in an in vitro model by testing their inhibitory effect of plaque formation in 3T6 cells by mouse polyomavirus. Natural and synthetic nerolidol showed the highest inhibitory activity (CC50: 1,2±0,4µM and 3,2±1,4µM, respectively), followed by longifolene (CC50: 36±8µM). Noticeable, the corresponding ED50-values were up to ten fold higher.

SL- 22 Chemical Composition of Mimosa absolute

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Absolutes are natural extracts produced from aromatic plants that are widely used in perfume compositions. The producers now have to respect the European REACH protocol concerning the safety of chemical products, which implies the description of the chemical composition of marketed natural extracts. Up to now, absolutes have been mainly studied by GC, so there is a considerable lack of knowledge on non-volatile compounds in concretes and absolutes. The present work reports on the analytical study of mimosa absolutes. The volatile components of these extracts have already been studied [1-3] but the non volatile fraction is still scarcely known. At first, the samples were subjected to fractionation by column chromatography and/or preparative RP-HPLC, distillation, and chemical treatments, and then analysed by GC-MS, HPLC-MS, and NMR. Mimosa absolute proved to be a complex matrix with a large proportion of semi- and non-volatile compounds. The main volatile compounds are fatty acids derivatives (alkanes, alkenes, aldehydes, ketones, acetals, esters and alcohols) with even or odd number of carbon atoms. The semi-volatile fraction (eluting on an apolar GC column) is composed of pentacyclic triterpenic alcohols or ketones [4] like lupeol and lupenone. The non volatile part is mainly constituted of fatty and coumaroyl esters of triterpenic alcohols. This work is a starting study in a more general project devoted to the analysis of others absolutes used in perfumery, in the frame of the European REACH protocol.

Acknowledgements: Albert Vieille S.A., Region PACA.

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SL- 23 Natural Extracts Analysis: Identification of Sulphur and Nitrogen Containing Compounds with Organoleptic Interests

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In perfume and flavour chemistry, the study of natural extracts still is an efficient and challenging path towards the discovery of new odorant molecules. Interestingly, the human nose is to date the most sensitive detector for odours and GC-Olfactometry (GC-O), a technique using the human nose as detection device, is nowadays an important tool for flavour chemistry. The combination of conventional (GC-MS, GC/FID) and GC-O analyses of flavouring raw materials provides precious information on the composition and organoleptic impact of the constituents of the matrix. The impact of nitrogen and sulphur trace compounds can in some cases be revealed by GC-O studies because these molecules often have low perception thresholds and trace quantities are sometimes not detected by physical detection [1-4].
Here we report on the analytical pathway developed for natural extracts used in perfume and flavour chemistry [5]. In a first step, GC studies of Brassicales and Asterales extracts using classical detection devices such as MS for structural identification and FPD for sulphur specific detection were accomplished. In order to obtain physiological information on the organoleptic properties and olfactory impact of the constituents, Aroma Extract Dilution Analysis (GC-O-AEDA) was realized and calculation of Flavour Dilution (FD) factors revealed several components with strong influence on the matrix's odour.

Acknowledgements: Robertet S.A.

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SL- 24 Volatile Glucosinolate Hydrolysis Products of Three Serbian Crucifers

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In the past few decades the importance of glucosinolates, once known as mustard oil glucosides, has increased further following discovery of their potential as cancer-preventing agents, crop-protection compounds, and biofumigants in agriculture [1]. From this reason a detailed phytochemical profiling of plant species containing these secondary metabolites deserves attention. This work presents the first report on the glucosinolates present, inferred from the volatile products of natural autolysis (endogenous myrosinase), in three members of the family Brassicaceae from Serbia-*Draba lasiocarpa* Rochel, *Thlaspi praecox* Wulfen and *Conringia orientalis* (L.) Dumort. (*D. lasiocarpa* and *T. praecox* have never been investigated previously and *C. orientalis* for the first time from Serbia). A single goitrogenic volatile glucosinolate product, 5,5-dimethyl-2-oxazolidinethione, was detected by GC and GC-MS, suggesting the presence of only one glucosinolate, glucoconringiin (2-hydroxy-2-methylpropyl glucosinolate). All plant organs (flowers, leaves and roots) of *D. lasiocarpa* and *C. orientalis* contained this glucosinolate, but it was present only in the roots of *T. praecox*. Besides previously reported from *C. orientalis* (and isolated for the first time) [2], glucoconringiin seems to have a restricted occurrence in the family Brassicacae (previously identified only in *Draba aizoides* [3]), however also reported in other taxa of the order Capparales (*Reseda alba* [4] (Resedaceae), *Moringa stenopetala* [5] and *Moringa peregrina* [6] (Moringaceae)). Such a distribution may be of chemotaxonomic significance.

Acknowledgements: The authors acknowledge the Ministry of Science and Environmental Protection of Serbia for financial support (Project 142042).

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SL- 25 Investigation of Different Thymus Species with GC and „Electronic Nose"

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In the Hungarian wild flora five species represent the genus *Thymus*: *T. pannonicus*, *T. serpyllum*, *T. pulegioides*, *T. glabrescens* and *T. praecox*. The aim of our study was to evaluate some chemotaxa of the first 4 species both with analytical methods and with a chemosensor array, so-called „electronic nose".
The analytical measurements were carried out with a Shimadzu GC-14B type gas-chromatograph equipped with FID and an SE-30 type coloumn (0.25 µm film thickness), the temperature program was the following: 110 °C/3 min; 110-220 °C (8°C/min); 220 °C/5 min. The essential oil - hydrodistilled from each sample - was measured by chemosensor array (NST 3320 type electronic nose with 22 MOSFET sensors). The results were evaluated with PCA built in the NST Senstool software.
Among the 6 samples of *T. pannonicus* - collected from 3 different living sites in Hungary - two chemotypes were found: four samples are belonging to the thymol chemotype and two samples are geraniol chemotype. The samples with high thymol content in the essential oil could be distinguished with electronic nose. The *T. glabrescens* sample was found as carvacrole-rich type, *T. pulegioides* has two different chemovariety: a thymol- and a γ-terpinene-type, while among the *T. serpyllum* samples thymol- and geraniol-type were found. All of these samples could be distinguished in several steps from each other with the help of the chemosensor array too. Accroding to our results a quite huge chemical diversity could be found among *Thymus* species, which is considered to be a valuable basis for breeding activities. Electronic nose proved to be an appropriate tool to perform a quick distinction among the samples belonging to different chemotypes.

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SL- 26 Investigations in Distillation of Different Herbs in Pilot Plant Scale Regarding Oil Yield, Oil Composition and Development Stage

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Distillation of herbs to produce essential oils in pilot plant scale is influenced by many different parameters e.g. climatic and weather conditions of the years examined, genetic conditions or development stage of the plants at harvest. To carry out the total harvest of the herbs at an appropriate moment from an economical point of view (highest oil yield), a proper code for the expression of development stages of the plants has to be established.
The plant development of four different herbs (oregano, yarrow, clary sage and lemon balm) all grown by organic farming in one or more habitats in the northeast of Styria (Austria) was examined by using a development code for the plants adapted to the international codification of the development stages of mono- and dicotyledonous plants (1). Oil contents and oil yields were determined and calculated and the main compounds of the different oils were analysed by gas chromatography (GC/MS and GC/FID). The essential oils were obtained using a hundred litres distillation pilot plant, which in average processes about 10 to 15 kilograms of fresh plant material per batch and an apparatus in lab scale regarding the European Pharmacopoeia.
The results provide the possibility to determine the development stages of the harvested plants expressed as code, in combination to highest oil contents and yields and highest content of specific compounds in the oils examined.

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Workshop
Recent Developments in Industry – Extraction Technology

KL- 8 Extraction Technologies: An Overview

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Plant extracts produced through various extraction methods are important raw materials for the cosmetic, pharmaceutical, functional food, fodder and beverage industries. Within recent years, different "new" extraction methods have been developed and/or improved concerning the techniques for obtaining crude plant extracts, essential oils and fragrances. The major attempt of all these methods was the improvement of yields, reduction of extraction-time and the overall lowering of expenses compared to the traditional hydro-distillation (HD), cold-pressing (CP) or solvent extraction (SE). Furthermore, some of these new methods seem to have the potential to gain an additional overall economic value and therefore may have a huge impact on the future production of essential oils and fragrances. Examples for such technologies might be:

- Controlled pressure drop technology (CPD)
- Supercritical and liquid CO_2
- Solvent-free microwave extraction (SFME)

In addition the recovery of high value essential-oils from wastewater distillate might add significant economic value to few of the processes. Overall, some of these applications offer important advantages over traditional methods, e.g.: shorter extraction times and better yields. Concerning the possible impact of these new applications in the production of essential oils and fragrances, the technical improvement of these methods should be further observed intensively within the next years.

SL- 27 Comparison of Different Extraction Methods: Cold Pressing, Hydrodistillation and Solvent Free Microwave Extraction, Used for the Isolation of Essential Oil from Citrus Fruits.

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Traditional hydro-distillation (HD), cold pressing (CP), and innovative solvent-free microwave extraction (SFME) [1] methods have been compared and evaluated for their effectiveness in the isolation of essential oil from fresh Citrus peels. The microwave method offers important advantages over traditional alternatives, namely; shorter extraction times (30 min. against 3 hours for hydro-distillation and 1 hour for cold pressing); better yields (0.24% against 0.21% for HD and 0.05% for CP); environmental impact (energy cost is fairly higher to perform HD and for mechanical motor (CP) than that required for rapid SFME extraction); cleaner features (as no residue generation and no water or solvent used); increases antimicrobial activities; and provides a more valuable essential oil (with high amounts of oxygenated compounds). It offers also the possibility for a better reproduction of natural aroma of the essential oil from Citrus fruit comparable to CP but more than the HD essential oil. More, microwave procedure yielded essential oils that could be analysed or used directly without any clean-up, solvent exchange or centrifugation steps. Scanning electron microscopy provides more evidence of the clean microwave extraction, in contrast to the huge perforation on the external surface of the Citrus fruit peel in the case of conventional hydro-distillation. Finally, a mechanism of microwave extraction is proposed and discussed.

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SL- 28 Design of a Microwave-based Pilot Module for Extraction of Essential Oils

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Under a governmental funding programme in the estate of Rio Grande do Sul-Brazil, we have designed a microwave-based pilot module for extraction of essential oils in batches of 5 to 10 Kg of plant leaves. The leaf-mass is initially deposited in a glass container with circa 30L volume and then exposed to radiation from eletronic valves of 2.45 GHz and 800 to 3.000W. All the process variables were monitored and computer controlled by using a Windows XP plataform. In this microwave-based process, the electromagnectic energy is employed to disrupt rigid structures, facilitating the extraction process. This high-tech procedure has enumerous applications in the extraction of natural products from plants, such as essential oils, fragrances, dyes, pharmaceutical products and biological pesticides. The significant feature of this novel method is its efficiency regarding the absorbtion of radiation by the sample, which is much faster and this speeds up the whole process. As a result, the completion of extraction in reached in a much shorter time, at a lower temperature, without any addition of water, and these are important factors to stop any hydrolytic reactions from occuring in the sample.

Acknowledgements: Universidade de Caxias do Sul, Secretaria de Estado da Ciência e Tecnologia do Rio Grande do Sul.

SL- 29 Production of Essential Oil Using Adsorbent Resins from Waste Water that Remained While Rose Oil Production.

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Rose oil from *Rosa damascena* Mill. is well known and very important ingredient in parfumery. It is a reality that there is a lot of yield losses while distillation of rose oil. This work aimed to apply the adsorption method to the production of raw materials from waste water that remained while rose oil production by hydro distillation. A column was prepared with Amberlite XAD4 adsorbent resins. After regeneration of the resins with ethanol (96%) filtered waste water passed through the column. The alcohol phase was evaporated, and then some oil was obtained. The yields varied between 0.02% and 0.03%. The oil obtained from the waste water was analysed by GC/MS and compared with other materials obtained by other traditional technics. It was interesting the oil obtained from waste water had been contained very low methyl eugenol (0.002%) and very high phenylethyl alcohol (95%).

SL- 30 The Instantaneous Controlled Pressure Drop (DIC) Technology for the Extraction of Essential Oils from: Oregano and Jasmine

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The Instantaneous Controlled Pressure Drop (DIC) technology has been employed for the extraction of essential oils from oregano and jasmine. Some reference fragrance components separated from the extracts using GC/MS analyzer was examined. The effect of DIC operating conditions including: steam pressure, processing time and number of DIC cycles was studied and the optimum conditions were determined using experimental design based on Response Surface Methodology.
The DIC process is based on the thermo-mechanical effects induced by subjecting the raw material to a steam pressure (about 1.105 to 6 .105 Pa depending on the product) for a short time, followed by an abrupt pressure drop towards vacuum (about 0.50 kPa.) (1). The abrupt pressure drop may promote auto-vaporization of volatile components, coupled to an instant cooling of the products, avoiding thermal degradation, modifying the internal structure and causing the rupture of cell walls, which enhances the internal diffusion.
The steam pressure, the processing time and the number of pressure drop cycles seemed to depend on the product nature. For oregano, the best rate of extraction is obtained at 6.105 Pa in 5 minutes with 10 cycles. For jasmine, the best rate of extraction is obtained using: 2.5.105 Pa, 2 cycles, 30 sec and 3.4.105 Pa, 3 cycles, 24 sec.
By comparing the DIC with steam distillation, as conventional technique, we are getting quicker operation: a few seconds or minutes versus several hours. Finally, DIC doesn't induce thermal degradation and preserves initial composition of the essential oils.

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SL- 31 Pressurized Liquid Extraction (PLE) for the Extraction of Essential Oil Compounds of Aromatic Herbs

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The aim of this research was to find a fast and efficient extraction technique for essential oil compounds particularly with regard to the sensory characterisation of different provenances of *Origanum vulgare* using gaschromatography/ olfactometry (GC/O). In this context pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction) as a sample preparation technique that combines elevated temperature and pressures to achieve fast and efficient extraction of analytes was investigated and optimized [1].
In this connection air dried plant material was obtained from the Dr. Junghanns GmbH, Groß Schierstedt, Germany. The volatile compounds were extracted with PLE and analysed with gaschromatography/ massspectrometry.
To investigate the suitability of the new developed PLE method the precision of the experimental procedure was evaluated by calculating a series of six analyses. Variation coefficient of total area was 5.72%, which is acceptable. Furthermore the recovery of the method was studied applying a herb sample spiked with defined quantities of volatile standards under analysis conditions. The recoveries for each standard using the developed ASE method were satisfactory and ranged from 88.18% to 91.49%. To confirm the suitability the new developed ASE method needed to be compared with commonly used extraction techniques. With regard to that the ASE method has higher recoveries compared to hydrodistillation with recoveries between 72.71% and 99.65%. Another important advantage is the level of automation which results in saving time and labour. Concluding to the above said PLE is beside hydrodistillation, supercritical fluid extraction and solvent extraction yet another reproducible technique to extract essential oil compounds from plant matrices.

Acknowledgement: Center of Life Sciences, Institute of Bioanalytical Sciences (IBAS), Anhalt University of Applied Sciences; Federal Centre for Breeding Research on Cultivated Plants, Institute of Plant Analysis (IPA)

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SL- 32 Extraction of Spanish Hyssop with Supercritical CO_2

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In the present study, the natural product that is been processed is the perennial lamiaceous herb (*Hyssopus officinalis* L.) originating from Spain, because is an important medicinal plant used for antispasmodic, stomachic, antifungal and cough treatment. The extract of Spanish Hyssop was obtained from two different ways, using a traditional technique of extraction like Soxhlet extraction and SFE with CO_2 as supercritical fluid.
Soxhlet extraction was made by using three different solvents: n-hexane, 2-propanol and ethyl acetate, each one with different polarity, to obtain different kind of extracts. Each experiment lasted four hours and the extract was 9%, 24% and 14% respectively from n-hexane, 2-propanol and ethyl acetate. The volatile components of the extracts were identified GC/MS and FID techniques. For all different solvents 1,8-Cineol (Eucalyptol) was the main compound. The content of other compounds like Pinocamphone, Isophinocamphone and Pinocarvone, very common in other kinds of Hyssop from other places, was very low.
Supercritical extraction was made using CO2 as supercritical fluid. The experiments were performed at isothermal (25, 40, 60°C) and isobaric conditions (100, 200, 300 bar). Each experiment lasted four hours at constant CO2 flow. The chemical composition of the extracts was analyzed using GC/MS and FID techniques.

SL- 33 Supercritical Fluid Technology for the Production of Flavours and Fragrances and for Particle Generation

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Extraction of ingredients with supercritical fluid technology is an alternative to conventional methods like steam distillation and solvent extraction. A comparison of these technologies with high pressure CO_2 extraction is presented for a range of products, e.g. mace, jasmine, lavender, marigold, sandalwood, vetiver, clove, cinnamon, cardamom, rosemary, sage, ginger and hop. Two different kinds of supercritical fluid extraction plants can be used for the extraction of flavours and fragrances. Multipurpose plants have a two step separation system for the separation of oleoresins and essential oils. They also allow the use of co-solvents. On the other hand specialized plants for essential oils production are available, which operate at lower pressures (design pressure 150 – 200 bar).
Another technology involving supercritical CO_2 is particle generation. Two processes available on the market are CPF (Concentrated Powder Form) and PGSS (Particles from Gas Saturated Solutions). CPF is used for loading of liquids (e.g. also viscous extracts) on powder carriers with loads up to 80 %. PGSS allows particle generation from melts with tailor-made morphologies and encapsulation of ingredients. Both processes can be applied after the extraction of essential oils or other ingredients in order to create final products for the customers.

SL- 34 Application of Liquid Carbon Dioxide for the iiolation of Essential Oils from Various Botanical Species

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Extractions of various botanical species by using liquid carbon dioxide ($\tilde{L}CO_2$) were performed with the main aim to increase the content of essential oil in the extracts. Pilot plant scale extractions were performed by using 46 raw materials at 5$\tilde{5}$60 bar and ambient temperature. Different extraction cycles were applied to increase the yield. As a rule, the highest yields were obtained from the seeds, containing remarkable amounts of low polarity lipophylic substances, particularly essential and fixed oils. For instance, extraction of black cumin seeds during 5 hrs yielded 18. of yellow-reddish color, oil consistency and pleasant aroma liquid extract. High yields were also obtained from hops and some fruits of *Capsicum* spp. (cayenne, red bell pepper). The yields from herbs varied from 0.2 to 5.2. . Extraction of German chamomile, rosemary, St. John's wort, marigold, sweet marjoram and basil resulted in the highest yields, while the yields from such popular culinary herbs as savory, oregano and thyme were considerably lower, usually less than 1.5. $\tilde{L}CO_2$ extracts contained remarkably higher concentrations of the essential oils compared to the initial raw material: the content of volatile oil in the extracts was from traces to 47 %. The differences in the composition of the essential oils isolated by hydrodistillation and $\tilde{L}CO_2$ extraction were assessed by gas chromatography mass spectrometry. $\tilde{L}CO_2$ extraction enabled to isolate reasonable amounts of volatile fraction from some botanical species which usually do not yield essential oil by using traditional distillation techniques. $\tilde{L}CO_2$ extracts were also tested in the reaction with a free DPPH• radical and ABTS•+ radical cation; however, their radical scavenging capacity (RSC) was weak comparing with the extracts isolated with organic solvents. Herbal extracts possessed higher RSC comparing with seed, fruit and root extracts. Preliminary calculations indicate that production costs of $\tilde{L}CO_2$ extracts depend mainly on the price of raw materials and extract yield. Thus, the costs of some inexpensive and highly yielding seeds, such as coriander, caraway, fennel and black cumin were approximately $ 1$\tilde{0}$30 per kg, while the costs of the extracts with a very low yield went up to unacceptable levels of more than $ 1000 per kg.

SL- 35 Subcritical CO_2 Fractionation of Some Floral Concretes

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Essential oils still remain absolutely necessary for the manufacture of fragrances from the most sophisticated to cosmetic and even to household products. With application in the production of specialized fragrances and in aromatherapy, essential oils from floral materials have attained considerable importance. The so called concretes and absolutes from *Jasminum grandiflorum*, *Rosa damascena*, *Polyanthus tuberosa* and several other flowers produced through solvent extraction are traded in large quantities. However, the absolutes produced from the concretes by precipitation of waxes with ethyl alcohol still contain waxes and about 20% of fatty acid methyl esters. Removal of alcoholic solvents from the extracts not only leaves solvent residues but allows highly volatile constituents to escape with the solvent. Direct extraction of floral materials with supercritical CO_2 poses several problems. Due to the very low bulk densities of flowers and occurrence of the fragrance in low concentration, very large capacities of the plant are warranted. On the other hand fractionation of floral concrete with CO_2 to get absolute is an attractive alternative. We have recently prepared absolutes from the pentane extracts of a few floral materials viz. *Jasminum sambac* Aiton, *Quisqualis indica*, *Michelia champaka* Linn and *Pandanus fascucularis* Lam. We wish to report here improvements in yields and the comparative composition of the absolutes with those obtained in the conventional way.

SL- 36 Stimulating Black Economic Empowerment in the Essential Oils Industry in South Africa –Experiences and Lessons learnt

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In an effort to stimulate equitable economic growth, the South African Government created several legal instruments aimed at redressing inequality. These include the Black Economic Empowerment (BEE) Act, and BEE codes. These instruments are aimed at increasing economic participation and wealth creation amongst Black people.
The Technology Transfer for Social Impact (TTSI) office of the Council for Scientific and Industrial Research (CSIR) has a successful track record in developing enterprises (especially community-based enterprises) and stimulating economic participation especially for Black people in SA. This is done through the use of a structured Enterprise Development method, which is backed by computer-based tools and include feasibility studies, financial modelling, business plan development and implementation of business plans. Funding is obtained mostly from Government Departments.
TTSI is currently managing a Government funded programme aimed at developing a number of sustainable Essential Oils farming and value addition businesses across South Africa. These initiatives were used as case studies to assess whether the approach used was successful in creating sustainable businesses and stimulating BEE. A number of businesses were unsuccessful and had to be closed, and several were deemed to be successful. The results of the study confirmed that in order to be sustainable, these enterprises must operate according to formal business principles.

Acknowledgement: CSIR, Department of Science and Technology.

Essential Oils in Animal Health and Nutrition

PL- 4 Essential Oils in Agricultural Lifestock Feeding

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Essential oils (EO) have recently gained significant relevance for use as feed additives to agricultural livestock. The major reason was ban of antibiotic feed additives in the EC in 2006. Nowadays, EO compete with organic acids and probiotics for use as a natural feed additives to stabilize health and hence to improve productivity of agricultural livestock. This refers in particular to young monogastric species (piglets, poultry for fattening). But EO are used also as feed additives to ruminants, mainly for purpose of stabilizing and improving ruminal functions.
A variety of recent studies clearly demonstrate overall efficacy of feed additives based on EO to improve productivity of agricultural livestock. However, the modes of action and their individual contribution to the overall efficacy are still under discussion. Several studies have recently investigated more in detail the effects of EO (partially in comparison with antibiotic feed additives or organic acids) on gut microbiology, physiology, morphology, incl. molecular biology. The studies suggest that EO applied to monogastrics exert their beneficial efficacy by combination of several modes of action, such as direct antimicrobial and antioxidative properties, stabilization of passage rate of digesta, rise in digestive enzyme activities, but also stimulation of mucus production (which impairs adhesion of pathogens). In ruminants, the effects of EO on the animal's productivity arise mainly from beneficial modulations of ruminal microbiota leading to changes in fermentation pattern and production of volatile fatty acids.
When applied as feed additives to monogastrics, EO seem to be absorbed, metabolized and eliminated via urine quite efficiently. Metabolites of EO in edible tissues could not be detected except for blood and kidney. Obviously, EO used as feed additives do not pose a relevant safety risk to the consumer of products derived from those animals.

SL- 37 Efficacy of Phytogenics in Comparison to Antibiotics in Weaned Piglets

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In total 144 weanling pigs (BW 5.9 kg) were used to evaluate the efficacy of phytogenics in comparison to Antibiotic Growth Promoters (AGPs) in a performance study conducted at Kansas State University, U.S.A. Pigs were randomly assigned to one of the following treatments with eight replications (pens) per treatment and six pigs per pen: (1) Negative Control (NC) without phytogenics or AGPs; (2) NC + phytogenics (Biomin® P.E.P., 125 g/t); (3) PC (AGPs: neomycin sulfate, 140 g/t + oxytetracycline HCl, 140 g/t). Phase 1 and 2 diets, based on corn and soybean meal, were fed ad libitum from d 0 to 14 and d 14 to 42, respectively. Compared to the NC, average daily weight gain was increased by 6.2 and 10.8% through supplementation with phytogenics (P=0.02) and AGPs (P<0.001) (453 vs. 481 vs. 502 g) after 42 d. Feed:gain ratio was reduced (P<0.001) in pigs fed phytogenics as compared to the NC and PC (1.37 vs. 1.42 vs. 1.40). There was no difference (P=0.26) in feed:gain ratio between the NC and PC. Finally, feed intake was higher (P<0.001) in pigs fed the PC diet as compared to pigs offered the NC diet or phytogenics (705 vs. 642 vs. 658 g). The efficacy of the phytogenic feed additive under evaluation is partly based on a modification of the gut microflora [1]. In conclusion, phytogenics improved growth performance in weaned piglets fed diets without AGPs.

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SL- 38 Fast Intestinal Absorption of Free Carvacrol and Thymol Compromises Antibacterial Effects Against the Pig Gut Flora

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The effect of carvacrol and thymol on the gut flora and bacterial metabolites of piglets was investigated. Twenty five weaned piglets (castrates BLxLW; age 28 days; live weight 6.6 kg) were homogenously distributed to five treatments: control (C; wheat-pea based diet), C+500 mg.kg-1 carvacrol, C+2000 mg.kg-1 carvacrol, C+500 mg.kg-1 thymol and C+2000 mg.kg-1 thymol. The phytochemicals were dissolved in the soybean oil fraction and then mixed with the other feed components. All diets were given ad libitum. At 11/12 days post-weaning piglets were euthanized, digesta from stomach, proximal and distal small intestine (SI) were sampled for bacteriological, chemical and phytochemical analysis. Selective media were used for counting the following bacterial groups: total anaerobic bacteria, coliforms, streptococci and lactobacilli (viable counts; CFU log10/ml). The phytochemicals in the digesta were extracted twice with ethyl ethanoate and quantified by GC. No significant differences between treatments ($P>0.05$) were found for pH, dry matter, lactate and acetate content of digesta, neither for the bacteriological counts. However, thymol at 2000 mg.kg-1 feed showed a trend ($P<0.1$) for higher numbers of lactobacilli in the proximal SI and streptococci in the distal SI. The concentrations of carvacrol and thymol (2000 mg.kg-1 feed) were on average 517 and 544 mg.kg-1 digesta in stomach and 5 and 16 mg.kg-1 digesta in proximal SI. These concentrations were previously found to be too low to reveal a bactericidal effect [1]. Using 4M HCl insoluble ash as a marker, it was found that between 30 and 40% and between 50 and 63% of the phytochemicals had disappeared from gastric and proximal small intestinal digesta respectively. Carvacrol and thymol were not metabolized in in vitro simulations of the gastric and small intestinal fermentation [2], so it is concluded that the disappearance in this trial is due to fast intestinal absorption.

Acknowledgements: Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT), Brussels, Belgium

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SL- 39 Fumigation Toxicity of Spice Oils of Against *Callosobruchus maculatus* in Grain Storage Systems.

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Synthetic pesticides are used in gaseous form to control insects during stored conditions. Therefore, fumigation is considered as one of the best and most popular technique for stored grains protection due to insect infestation [1]. Presently various fumigants like methyl bromide, ethyl bromide being used as fumigant to protect grains, have to be phased out before this decade due to their deleterious environmental impacts and health hazards [2]. Hence there is an upcoming thrust by shifting the focus from synthetic pesticides to botanical pesticides indicating the need of development of new fumigant formulations based on plant products [3]. India being the land of spices has various potential spices which show promising bioactivity. Essential oils which are the active constituent of the spices contain volatile chemical which can be used as fumigant, repellent, antifeedant etc. against stored grain insects [4, 5]. *Callosobruchus maculatus* is one of the most devastating, serious, worldwide pest of legumes causing substantial economical losses under storage conditions. In the present study essential oils of spices and medicinal plants of Indian origin have been used for fumigation bioassay experiments. 10 essential oils viz. *Origanum majorana*, *Coriandrum sativum*, *Mentha piperita*, *Chimaphila maculata*, *Ylang ylang*, *Cymbopogon martini*, *Pimpinella anisum*, *Citrus bergamia*, *Melaleuca* spp. are selected for the present study. In the pure form, 20 µl/L concentration was found sufficient to produce mortality greater than 95 %. These essential oils having high volatility can be successfully employed for fumigation in stored legumes for preventing them from infestations of *Callosobruchus maculatus* for a considerable period of time.

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SL- 40 The Botanical Origins of Cedarwood Oils

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Commercial Cedarwood oils consist of Texas Cedarwood oil (*Juniperus ashei*), Virginia cedarwood oil (*J. virginiana*) and Chinese cedarwood oil (putatively *Cupressus funebris*). The first two oils have well defined botanical sources, however, the botanical source of Chinese cedarwood oil is debatable. *Cupressus funebris* is generally regarded as the botanical source of Chinese cedarwood oil. However, due the limited amount of mature forest trees of *C. funebris* in China, other species in the Cupressaceae that have wood oils high in α-cedrene, β-cedrene, thujopsene and cedrol might be utilized for cedarwood oil production. Wood samples of putative *C. funebris* were extracted and the extracts were analyzed and compared with several lots of Chinese cedarwood oil. Wood oils were also extracted from *Juniperus chinensis* and J. c. cv. torrulosa and analyzed. Considerable variation was found among the wood oils of putative *C. funebris*. The various lots of commercial Chinese cedarwood oils were very variable: α-cedrene (3.6 - 44.2%), β-cedrene (3.5 - 11.5%), *cis*-thujopsene (1.9 - 37.4%), cedrol (1.7 - 23.4%). The presence of β-biotol and β-biotone in several Chinese cedarwood oils seems to indicate that wood of *Platycladus orientalis* (*Biota orientalis*) was utilized in their production. It appears that Chinese cedarwood oil is derived from a mixture of woods from several Cupressaceae species. Compositional analyses of Texas and Virginia cedarwood oils are compared with commercial Chinese cedarwood oils.

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KL- 9 External Application of Essential Oils in Animals

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Evidence-based veterinary herbal medicine is still in its infancy. Most available data on medicinal and aromatic plants in veterinary phytotherapy arise from their ethnobotanical and ethnomedicinal use. For topical application essential oils should be diluted in a carrier oil, such as sweet almond oil, at not greater concentrations than 3 to 5%. Therapeutically essential oils can be used in different ways. For example, lavender oil can be inhaled to promote relaxation and sleep in dogs. A blend of lavender oil, geranium oil and lemon oil may be useful to control pet odor. Othor essential oils like tee tree oil (TTO), eucalyptus oil or lavender oil are recommended to combat fleas, ticks, mites, bacteria or fungi.
In a clinical trial a 10% TTO-containing cream was investigated for topical symptomatic treatment of pruritic and localised dermatitis in dogs. Its superior efficacy after 10 days of treatment was confirmed by significant improvements and resolution of the common clinical signs of pruritus and alopecia, which are typical for canine acute and chronic dermatitis.
When using essential oils, veterinarians must consider the differences between species and between herbivores and carnivores, along with other factors, like size and sex. Terpenes are metabolized by the liver to glucuronid, glycine and sulfate conjugates. Glucuronidation is an important detoxification mechanism present in most animals except in cats. Cats lacking in glucuronyl transferase activity are therefore highly sensitive to essential oils.

Regulatory Affairs – Overregulation?

PL- 5 Recent EU-legislation on Flavours and Fragrances and its Impact on Essential Oils

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In the last years several new European Regulations and Directives have been adopted or announced in relation to flavours and fragrances. As essential oils are very important ingredients for flavouring and fragrance applications, these new regulations will have a major impact on the trade and use in commerce of these essential oils.
The presentation will focus on some pieces of legislation that are of major importance for the Flavour and Fragrance Industry, such as REACH [1], the new Flavouring Regulation (part of the so-called "Food Improvement Agents Package") that will replace the current Flavouring Directive 88/388/EEC and that is currently under discussion at EU Parliament and Council level [2], ongoing actions at Commission level regarding the Cosmetic Directive (Public Consultation on Perfumery Materials) and the first amendment of the Detergent Regulation [3] (June 2006) which makes the labelling of 26 specific fragrance ingredients (so-called 26 allergens) mandatory. In line with the 7th Amendment of the Cosmetic Directive 76/768/EEC [4] the presence of these materials above the given threshold has to be declared irrespective of the way they are added (as such or as being part of 'complex ingredients' such as essential oils).
Also the issue on Hazard Classification and Labelling of dangerous substances and preparations, and essential oils containing hazardous components will be addressed and some examples will be given. This relates to the recent publication of the Commission Directive 2006/8/EC [5] amending the Dangerous Preparation Directive 1999/45/EC.

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SL- 41 Regulatory Control of Essential Oils. Tea Tree Oil: A Case Study

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Tea tree (*Melaleuca alternifolia*) oil is widely used in cosmetic products and has been shown to be a most effective antibacterial agent. Questions have been raised about various aspects of the current regulatory documentation presently available including safety, stability and the extent to which various components present within tea tree oil are absorbed through the skin.

Our evaluation of the available literature and data acquired from our own investigations suggest that, if used appropriately, tea tree oil is a most useful cosmetic with minimal safety concerns. It has minimal acute toxicity as evidenced in oral, dermal and inhalation toxicity studies where, for instance, ingestion of half a cup of oil (human, neat) did not cause death with symptoms arising, abating in 12 hours. Irritation and sensitisation can arise with its application but, in general, these are mild and include: gum irritation (nil, humans, 2.5%), eye irritation (slight, rabbits, 1-5% to severe HET-CAM >5%), skin irritation (weak, human, neat) and sensitisation (weak, mouse, LLNA; 2%, human, patch). Our own human percutaneous absorption studies suggest that absorption is mainly limited to one component (terpinen-4-ol) and is low (< 8 %).

Degradation of tea tree oil to sensitizing components is promoted under extreme storage conditions, hence it is recommended that products should always be kept away from light and below 25oC. We suggest p-cymene is an excellent marker for the likely formation of sensitizing components and recommend that it be below 2500ppm in any 2% cosmetic tea tree oil product applied to human skin.

KL- 10 Over-regulation is Destroying Natural Aromatics.

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Influential career toxicologists, using the precautionary principle as a regulatory ramrod, have been influential in forcing the aromatics trade to go through a critical period of excessive 'safety' regulation, resulting in depletion in both the range & volumes of essential oils, absolutes & resinoids actively deployed in retailed cosmetics. This has been achieved within the EU Cosmetics Sector via the Brussels Hyper bureaucratic Machine, & affects areas such as sensitizers, REACH, & dangerous preparations', as well as the ever more technocratic edicts from aroma-connected organizations e.g. EFFA Codes of Practice & IFRA Standards. Incredible demands have been resultantly heaped on aroma traders & producers, as well as cosmetic manufacturers, who are completely hamstrung by red tape & many of whom are unable to function without complex regulatory software programs. As a result, many aroma ingredient manufacturers have moved out of this hostile European regulatory environment to relocate in more industry-friendly places such as India or China, which have the additional advantage of lower investment & operating costs. Within the aroma concerns themselves, the rise of newly important posts, like that of the 'Regulatory Affairs Manager', has further devolved focus and power away from perfumery excellence, as company executives worry more about anti-fragrance campaigners, regulatory complicity & possible litigation, than they do about the integrity of the perfumery art. . Those natural aromatic ingredient producers adversely affected by over-regulation, either inside & outside the EU, are scarcely acknowledged & certainly not compensated or helped financially to produce regulatory complaint materials. This results in social hardship for local communities dependent on income from these ingredient producers, and the loss of sustainability for some threatened species; however nobody in authority responsible for this state of affairs, seems to express either the slightest interest, let alone regret.


POSTERS

P- 1 Chemical Polymorphism and Genetic Diversity of *Thymus caespititius*: Is there a Correlation?

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Thymus caespititius is an endemic species of the NW Iberian Peninsula and of the Madeiran and Azorean archipelagos. Previous studies on essential oils isolated from collective samples of *Th. caespititius* from populations grown on Azorean islands showed a remarkable chemical polymorphism (1-3). Since no correlation was found between the chemical composition of the oils and environmental factors, a new approach was started in order to evaluate of the possible relationship between chemical polymorphism and genetic variability.

Twenty six individuals were collected on the islands of S. Jorge and Terceira (Azores) during the flowering period. The volatiles, isolated by distillation-extraction, were analyzed by GC and GC-MS. Molecular analyses were performed using 17 RAPD (random amplified polymorphic DNA) primers. For the cluster analyses of the data obtained in both studies the NTSYS software was used.

The individual volatile composition confirmed the previous results obtained from the respective populations. However, molecular analysis of the same individuals did not show the same clustering as with the essential oil profiles and no straight correlation between chemical and molecular assessments could be found. In view of these preliminary results, other molecular methodologies should be explored in order to fully determine the influence of both environmental and genetic factors on volatiles composition.

Acknowledgements: Study funded by the Fundação para a Ciência e a Tecnologia, Lisbon, under research contract PTDC/AGR-AAM/70136/2006. The authors would like to thank Prof. Margarida Oliveira and Dr. Madalena Martins for their valuable help.

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P- 2 Inheritance of Selected Volatile Oil Constituents in Basil (*Ocimum basilicum* L.)

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Inheritance of natural plant products and aromatic volatile oil compounds is poorly understood. To test the hypothesis that methyl-*E*-cinnamate and methylchavicol in basil are inherited as single independent genes, controlled crosses were made between chemotypes rich in each of these constituents. Stable *Ocimum basilicum* populations selected for high methyl-*E*-cinnamate (79%), methylchavicol (95%), and linalool (82%) respectively, served as parents. Crosses were made using all these chemotypes: methylchavicol x methylcinnamate; linalool x methylchavicol; linalool x methylcinnamate. True hybrids were selfed in isolation and F_2 plants were analyzed for their aromatic volatiles. The parents, F_1 hybrids and the F_2 populations were evaluated under both greenhouse and field conditions. Aromatic volatile oils were extracted by hydrodistillation and the identification of essential oil constituents were confirmed by GC/MS. The F_2 segregation data was analyzed by Chi-Square test. Results show a single and independent inheritance for both methyl-*E*-cinnamate and methylchavicol.

P- 3 Large Scale Microbial Transformation of Cedrol

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In the continuing studies on the microbial transformation of terpenoids (Noma and Asakawa, 1995, 2005, 2006), the large scale microbial transformation of cedrol was carried out by several kinds of microorganisms.
Microorganism was cultivated ratatory (100 rpm) at 30ºC for 3-5 days in the 500ml Erlenmyer flask containing 200ml of Czapek pepton medium(1.5% glucose, 1.5% sucrose, 0.5% polypepton, 0.1% K_2HPO_4, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$; pH 7.0). After the full growth of microorganisms, a spoon or two spoons of cedrol (about 1.5-3.0g) was added to the cultured broth and biotransformed for 7 days under the same condition. After filtration of the cultured broth the aqueous layer was extracted with ether. The ether extract was applied to silica gel CC and isolated the metabolites. The stereostructure of the metabolites were established by a combination of high resolution NMR spectrum, x-ray crystallographic analysis and the chemical reaction.
We will discuss about the large scale cultivation by using several kinds of microorganisms for the effective production of cedrol metabolites.

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P- 4 Synthesis of New Jasmone Type Structural Moieties

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Cis-Jasmone the main perfumery compound found in jasmine flowers (*Jasminium grandiflorum*) has a great economical value because of its organoleptic properties. High cost and low availability of *cis*-jasmone moved the attention of the scientist to find the substituted compounds for it [1,2,3,4].
Certain variations in the structure and position of alkyl groups attached to the 2,3 position of the cyclopentenones have been made. The substitution at the 2 and 3 position of the 2,3-alkyl cyclopentenone can radically alter the olfactory characteristics of the material. We studied the organoleptic properties of the compounds as well as the intermediates synthesized. It was found that instead of jasmine floral note, the final substituted cyclopentenone gives the minty, earthy, woody note having vetiver as under note. Some of the intermediates also show interesting odour characteristics. Odour of 2,3-alkyl cyclopentenones have been compared with the other related compounds having same number of carbon atoms but different structures. We have converted the intermediate ketone in to the respective oxime which gives the green note. Volatilities of these chemicals have also been studied on the blotting paper.

Acknowledgements: The authors thanks Mr. S. R. Iyer and Dr. Charles S. Sell, Quest International Ltd, U.K.; Dr. S. D. Mestri, Symrise India Ltd. for olfactory evaluation and important comments. One of the author D. D. Z. acknowledges CSIR, Govt. of India, for the SRF award.

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P- 5 Synthesis of Chiral Odoriferous Oxy-derivatives of Gem-dimethylcyclohexene.

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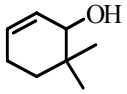
Compounds with gem-dimethylcyclohexene ring are often used in fragrance compositions [1]. Dynascone®, which is one of the components of the overall popular perfume Cool Water (Davidoff, 1988), possesses such a structural element. This fragrance compound possesses a green, galbanum–type odour with pineapple–like, hyacinth and metallic facets [2, 3]. Another known compound with this structural element is Artemone®, which has a herbal–grestic, thujone–like odour.
In the course of our syntheses of new biologically active bicyclic lactones with gem-dimethylcyclohexane ring, we have observed that many oxy–derivatives of cyclohexene possess very interesting odors. This observation pushed us to synthesize odoriferous oxy–derivatives of gem-dimethylcyclohexene.



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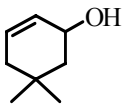

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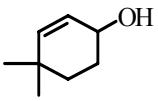

3

Here we present the synthesis of esters of racemic and enantiomerically enriched three alcohols 1, 2 and 3. The enantiomers of these alcohols were obtained via enzymatic esterification of racemic alcohols with vinyl acetate in the presence of lipase from *Candida cylindracea* and lipase from *Burkholderia cepacia*. The acetates and propionates of racemic and enantiomerically pure or enriched alcohols were synthesized. The comparison of odors of enantiomeric pairs confirmed the significant influence of configuration of chiral center present in the molecule on odoriferous properties of compounds studied.

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P- 6 Synthesis of a Polysantol® analogue.

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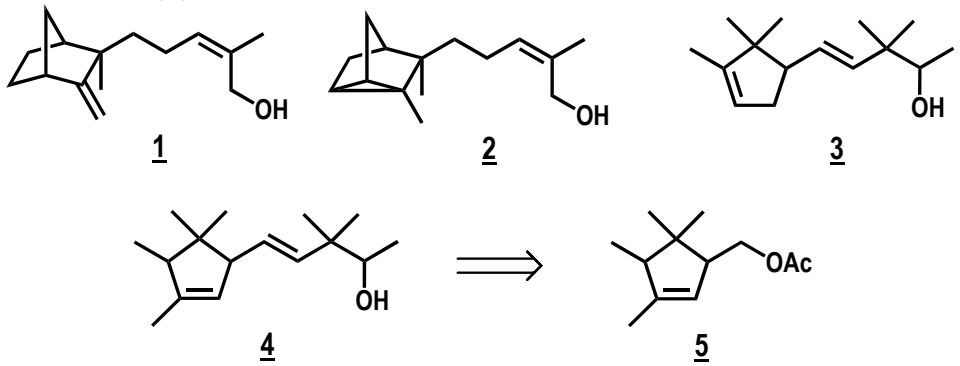
Sandalwood essential oil is an important ingredient in many perfume formulations. Its main odoriferous constituents are β- and α-santalols (1-2). Among the synthetic sandalwood odorants, Polysantol® (3) is an interesting material showing slight musky and cedar notes, in addition to the dominant sandalwood tonality. On the basis of previous structure-activity relationship studies [1-3] and in order to get more information on the sandalwood olfactophore, we synthesised a Polysantol® analogue (4), from natural trans-α-necrodyl acetate (5), the main component of Lavandula luisieri essential oil [4]. In this work, we present the synthetic process and the olfactive description of compound (4).



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Acknowledgements: Sophie Lavoine, Charabot SA.

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P- 7 Valorization of *Coleus tenuicaulis* Essential Oil by Chemical Transformations of 6,7-Epoxyocimene

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Coleus tenuicaulis (Lamiaceae) (syn. *Plectranthus tenuicaulis*, *Plectranthus minimus*) is a small erect, branched herb with pubescent stems and variegated leaves with purple dominant over green [1].
In the context of our chemical investigations on essential oils from tropical aromatic plants, the species growing in Gabon had been examined: hydrodistillation of its fresh leaves gave essential oils with about 1.5% yield; their analyses by GC and GC/MS showed that the volatile extracts were always dominated by a single component, (E)-epoxyocimene which represented more than 75% of the total composition [2].
In this study, we present the results of our further chemical investigations on this species. (E)-epoxyocimene was isolated from the essential oil by liquid solid chromatography on silica gel column and characterized by the classical spectrometric methods. Evaluation of its specific optical rotation $[\alpha]_{25}$ = + 3.2 indicated an enantiomeric excess of the (3R) epoxide which had been identified as a potential pheromone of the male fruit-spotting bug Amblypelta nitida [3, 4].
Chemical transformations of this component by different synthesis methods gave the hydroxylated and thiirane derivatives which were purified and characterized; their spectroscopic data are presented.
Considering the nice and intense odours of these molecules, the interest of their preparation from the natural epoxide is commented.

Acknowledgements: The authors would like to thank Y. Issembe and Lema for their helpful in identifying and collecting the plant material. This work is a part of a scientific collaboration program supported by the "Service de Coopération et d'Action Culturelle" française in Libreville (Gabon).

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P- 8 Synthesis and Odor of Cyclopropano–β–santalol

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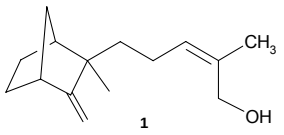
East Indian Sandalwood Oil, the essential oil of the wood of *Santalum album* (L.), is widely used in perfume industry. The bicyclic sesquiterpene alcohol β–santalol (1) is one of the most important compounds responsible for the sweet woody character of the essential oil. Therefore, it was and still is the target compound for many structure–odor–relationship studies [e.g. 1-3]. There it already could be shown that modification of the side chain of β–santalol (1) can lead to weakening or loss of the typical odor, the ring system also plays an important role in the odor properties of 1. On account of the ring strain cyclopropanes in many cases show olefinic behavior [4], thus, a cyclopropane ring could be a suitable substitute of the exocyclic double bond in 1 and therefore cyclopropano–β–santalol (2) should be an alluring target molecule whose synthesis and olfactory properties are reported.



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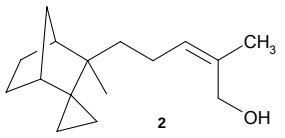

2

The synthetic route to the desired compound 2 was started from norcampher, which was α–alkylated in a two-step process followed by reduction of the ketone to the exycyclic double bond which then could be converted to a cyclopropane ring by Simmons-Smith reaction [5]. Acetal cleavage and successful conversion of the aldehyde to target compound 2 was affected by treatment with the corresponding Wittig-reagent and reduction of the ester after separation of the Z/E-isomeric mixture via TLC. Interestingly, none of the isomers of 2 exhibited the typical sandalwood odor.

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P- 9 Enzyme-mediated Reaction of Terpene Secondary Allylic Alcohols Towards New Chiral Esters with Olfactory Properties

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Biocatalysis offers a clean and ecological way to perform chemical processes, in mild reaction conditions and with high degree of selectivity. The use of enzymes, especially lipases, in organic solvents proves an excellent methodology for the preparation of single-isomer chiral odorants [1]. Terpene esters are found in many essential oils and are commonly used as flavor and fragrance compounds in a variety of foods and beverages for creating fruity aromas. Conventionally, the terpene esters are synthesized using chemical reagents. However, owing to a number of drawbacks in the chemical synthesis, the recent trend is more towards the use of biocatalysts, especially lipases, which can be used in organic solvents, to synthesize terpene esters [2]. In our previous papers we presented bioconversion to the acetates from mixture of diasteroisomers secondary bicycle[3.1.0]hexane alcohols obtained from monoterpene bicyclic hydrocarbon, (+)-3-carene, one the major constituent of resinous extract from Scotch Pine (*Pinus sylvestris* L.) [3]. Searching for the new chiral derivatives with olfactory properties we present herein chemoenzymatic reaction to obtain fragrance derivative of gem-dimethylbicyclo[3.1.0]hexane with new propionate and butyrate ester's moiety. All procedures and synthetic details with emphasis of stereo aspects and olfactory properties of new derivatives will be presented.

Acknowledgements: Supported by Ministry of Science and Higher Education, Grant No 3 T09B 092 28
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P- 10 Effects of Thymoquinone and Thymohydroquinone on Tumors: In Vitro and In Vivo Studies

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The aim of the study was to investigate the antitumor activity of the putative pharmacologically active constituents of *Nigella sativa* L. volatile oil thymoquinone (TQ) and thymohydroquinone (THQ). In the in vitro experiments, L929 mouse fibroblasts normal cells lines were used as a control and two tumor cell lines (squamous cell carcinoma (SCC VII) and fibrosarcoma FsaR)) were used. The cells were cultured with 0.1 and 0.01 mg/ml TQ and THQ for 24 h, and cytotoxicity assay was performed using crystal violet staining technique. In the in vivo experiments two murine tumor models (fibrosarcoma (FsaR) and squamous cell carcinoma (SCC VII)) were used. The used dose was equal for both substances. Antitumor affect of 4 intratumoral injections of TQ and THQ at the dose of 5 mg/kg was evaluated by comparison of tumor growth kinetics between treated and control animals. In vitro study showed that TQ and THQ exhibit statistically significant antitumor activity ($p>0.01$). The antitumor activity was dose dependent and more expressed against tumor cells than against L929 fibroblasts. The results of antitumor activity of TQ and THQ in vivo reached TGI of 52% and it was statistically significant ($p < 0.05$). The results indicate that THQ antitumor activity may be improved with a further increase in dose.

P- 11 Anticholinesterase Effects of *Foeniculum vulgare* Mill. and *Anethum graveolens* L. Essential Oils and Their Main Constituents

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The most common form of dementia among older people is Alzheimer's Disease (AD), which initially involves the parts of the brain that control thought, memory, and language. The oldest, on which most currently available drug therapies are based, is known as the "cholinergic hypothesis" and suggests that AD is due to reduced biosynthesis of the neurotransmitter called acetylcholine. Consequently, acetylcholinesterase (AChE) inhibition is thought to be rather important because of a notable reduction in activity of the cholinergic neurons.

On the other hand, some essential oils have been reported to display remarkable inhibition on AChE and butyrylcholinesterase (BChE), which are the key enzymes used in treatment of AD. Therefore, we herein investigated in vitro anticholinesterase effects of three essential oils of Apiaceae family, two of which belong to *Foeniculum vulgare* Mill. (fennel) collected at fully mature and flowering stages as well as *Anethum graveolens* L. (dill) by the spectrophotometric method of Ellman at 1 mg/ml concentration employing enzyme linked-immunosorbent assay (ELISA)-microplate reader.

Our data showed that all these oils had an extremely significant anticholinesterase activity. The essential oil of *A. graveolens* exerted 100±0.88% inhibition on AChE and 90.9±0.56% inhibition on BChE, while the essential oils of *F. vulgare* obtained at the fully-mature and flowering stages showed 94.3±0.12% and 80.8±0.64% inhibition toward AChE. Besides, several main components of abundantly found in both of the oils including trans-anethole, (-)-carvone, dihydrocarvone, (-)-phencone, α-pinene, thymol, and eugenol were also tested in the same manner and none of these components exerted significant anticholinesterase activity, which led to the suggestion that the exceedingly high anticholinesterase effect of these essential oils may possibly result from a synergistic interaction of all minor and major components, although -pinene was highly active against AChE (76.3±1.27%).

P- 12 The Impact of Natural Odors on Affective States in Humans

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The aim of the current study is to measure the emotional impact, odor intensity, and odor hedonics of complex fragrant compounds in two Vienna gardens and the "Fragrant Garden" at the University of Natural Resources and Applied Life Sciences in Vienna. A working hypothesis for the ongoing study is that olfactory stimuli, either solely or in combination, are able to evoke distinct emotional states in humans [1-3]. Laboratory studies have already shown significant correlations between certain fragrances and affective, as well as cognitive states in human beings [4-7]. Here we are interested in the relationship between complex natural odors and emotional states in the field - precisely in two Vienna gardens and the "Fragrant Garden" at the University of Natural Resources and Applied Life Sciences in Vienna. Affective reactions are measured with the MDBF questionnaire [8]. Additionally intensity ratings as well as hedonic evaluations (both on visual analogue scales) are given by the subjects. In total, 20 to 25 healthy non-smoking persons are tested in each garden at several time points according to the bloom of interesting fragrant plants. Preliminary data evaluations have shown significant effects of the natural odors in two of the three gardens on several measures of human affective states, precisely calmness, alertness and mood.

Acknowledgements: Vienna Science and Technology Fund (WWTF)

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P- 13 Compositional Characteristics and Toxicity of the Essential Oil of *Artemisia campestris* Grown in Lithuania

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Artemisia campestris is a perennial or biennial plant, which ranges temperate regions throughout the northern hemisphere, is common in most parts of Europe. The herb possesses anthelmintic, antiseptic, cholagogue, digestive, tonic and other bioactivities. The aim of the work was to evaluate chemical variability and bioactivity of essential oils of *A. campestris* grown in Lithuania.

Essential oil qualitative and quantitative analyses were performed for aerial parts of *A. campestris* collected from various habitats in Lithuania, in 2002-2007. Volatile components of the plants were extracted by simultaneous hydro distillation-extraction and analysed by gas chromatography (GC equipped with FID and a polar capillary column HP-FFAP, 30 m $\times$ 0.25 mm i.d.) and gas chromatography-mass spectrometry (GC/MS equipped with a nonpolar capillary column CB-5, 50 m $\times$ 0.32 mm i.d.). Qualitative analysis was based on a comparison of retention times and indexes and mass spectra with corresponding data in the literature [1] and computer mass spectra libraries.

Over 80 components of the essential oils were identified. The predominant constituents were germacrene D, (E)-caryophyllene and caryophyllene oxide in the most investigated samples. Among monoterpenes appreciable amounts of α- and β-pinene, myrcene, limonene and ocimene were determined. Some of the constituents were found only in minor quantities.

Morphological variability, histological characteristics and quantity of pigments (a and b chlorophylls) were investigated in the plant material.

Toxicity test was performed with *A. campestris* essential oils. The shrimp (*Artemia sera*) lethality test [2] in vivo was done and LC50 (95 % conf. interval) values were estimated.

Acknowledgements: Lithuanian Science and Studies Foundation, project "Biomarks" (V-05/2007, reg. V-07025).

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P- 14 Are Odorants Pharmaceuticals? - Pharmacodynamic Aspects of Fragrance Effects on Human Attention

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Several studies have used pharmacological magnetic resonance imaging (phMRI) paradigms to investigate the effects of psychopharmacological agents on neural activity in the human brain. Kleinschmidt et al. [1] studied the effects of sedative (Diazepam) and stimulant (Methamphetamine) drugs. Activation after intravenous administration of the psycho stimulant was found especially in subcortical gray matter structures and the cerebellum, while the sedative abolished this activation. Previous investigations in which fragrances were applied to the skin have shown physiological as well as behavioral activation for 1,8-cineole while (-)-linalool proved sedative [2]. In this study we aim to demonstrate that these effects are mediated by central nervous system processes. The fragrances dissolved in peanut oil (20% v/v) were applied to the skin of the lower abdomen. Peanut oil was used as a placebo. In order to exclude any olfactory stimulation odorless air was supplied via breathing masks. The phMRI experiment was a combination of block- and event-related design features over a length of 100 minutes. During each imaging session subjects had to perform a visual vigilance task (VT) and a sensorimotor control task (CT). phMRI data were analyzed by means of statistical parametric mapping using the general linear model (GLM). Functional activation within predefined regions of interest (ROIs) associated with sustained attention, such as the ACC, the lateral prefrontal and right frontal cortices, and the superior temporal / inferior parietal cortices of the right hemisphere, were derived by a subtraction analysis (VT – CT).

Acknowledgements: Jubiläumsfonds der Österreichischen Nationalbank Grant No. 11362

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P- 15 Effects of Bergamot Essential Oil on Anxiety-related Behavior in Rats

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Bergamot essential oil (BEO), extracted from peel of a *Citrus bergamia*, Risso, has been widely used in aromatherapy to reduce stress and anxiety (1), although there are only a few scientific findings concerning these effects. A previous study (2) showed that BEO significantly increased γ-aminobutyric acid (GABA) in rat hippocampus, suggesting its potential for anxiolytic properties. Therefore the aim of this study was to investigate the effect of BEO (1%, 2.5% and 5% w/w) on anxiety-related behaviour compared to that of diazepam, a typical anxiolytic drug, using an elevated plus-maze (EPM), a widely used animal model of aversion. Inhalation of BEO (1% and 2.5% w/w) and intraperitoneal administration of DZP (1 mg/kg, i.p.) significantly increased percent of entry in the open arms. The percent of time spent in the open arms was significantly enhanced in rats treated with either inhalation of BEO (2.5% and 5% w/w) or DZP (1 mg/kg, i.p.). Total arm entries tended to increase after BEO inhalation in a dose-dependent manner but the effect was only significant at the highest dose (5% BEO w/w), suggesting an alteration in locomotor activity at this dose. All three doses of bergamot oil (1.0%, 2.5% and 5% w/w) trended to increase rearing but the effects were not significant. In conclusion, the present study indicates that BEO exhibits an anxiolytic-like profile in the EPM test similar to DZP-treated rats. Future studies need to investigate the precise mechanism of action of BEO involved in this anxiolytic effect.

Acknowledgements: Faculty of Medicine, Srinakharinwirot University, Dr. Reinaldo Nóbrega de Almeida, Assistant Professor Dr Tapanee Hongratanaworakit.

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P- 16 The Effect of Therapy for Aged Patient with severe Alzheimer's disease

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The effects of art and aroma therapy for aged patient with severe Alzheimer's disease were studied. Since the first experiment in 1999, we have been observing and recording positive results in the form of a reduction in problem behavioures among demented senior citizen's consistence with art game program. This result suggests that art therapy could change the behavior property and that the change might differ according to the personality of the patient.
We are proposing the art as the series of pilot experiments. In the preparing the research report we have asked the consent of the patients and family members we have changed some details of identity to respect the privacy and human right of the patients.
Amongst the senior citizens with dementia at the welfare institution, Mrs. A had the most severe symptoms. The staff at first judged that there would be too many difficulties if participated in the art therapy sessions. However, as the sessions advanced Mrs. A began to express human emotions which she never showed in her daily life. She seemed to a make great effort to transcend the limits of her dementia by projecting her inner world onto her artwork.
Even the professional staffs at welfare institutes tend to give up on senior citizens with heavy dementia and treat them in a perfunctory manner. However as all of us will eventually become old, it's natural to hope for a human like existence up until the end of life.
Art therapy is an especially effective treatment method for senior citizens with dementia because art activities provide them with a time in which they can gain a feeling of self-respect.
Surely art therapy is a valuable method for allowing senior citizens with dementia to live in a, more human like manner. Even thought Mrs. A dementia symptoms were especially severe, the drawing activities she participated in activated her latent abilities, so as a result, she momentary returned to her original self. As a future research topic, the writer plans to conduct a long-range statistical study of the effect of art therapy. The effect of art and aromatherapy for aged patient with severe Alzheimer's disease will be studied.

P- 17 Medicinal Plants as Sources of Resveratrol and its Derivatives

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Stilbene polyphenols are subject of numerous researches as antioxidant, cardioprotective and antitumour properties of resveratrol have already been proved. The aim of this study was to determine the polyphenolic composition and antioxidant properties of ten medicinal plants traditionally used in Croatian folk medicine. Antioxidant capacity of ethanolic plant extracts was determined as percentage of inhibition of stable α,α-diphenyl-β-picrylhydrazyl radical (DPPH). Total phenols (TP) were determined by Folin-Ciocalteu method. Stilbene polyphenols were analyzed by HPLC-RP. Significant differences in TP concentrations and antioxidant properties of analyzed plant extracts were confirmed, as expected. The total phenolic contents ranged from 508 to 1646 mg gallic acid equivalents (GAE)/100 g of dry plant material (Vitis idaeae folium > Mirtilli folium >Fragariae folium > Helichrysi flos >Teucrii montani herba > Menthae folium > Salviae folium > Poligonii herba >Symphity folium >Urticae folium). All plant extracts exhibited excellent capability of free radical scavenging. When DPPH was compared at equal concentration value of TP (100 mg GAE/L of plant extract), it ranged from 49.24 to 91.03 %. Vitis idaeae folium, Mirtilli folium, Fragariae folium and Salviae folium extracts showed the best DPPH results. HPLC separation indicated the differences in the polyphenolic composition of plant extracts. While Helichrysi flos and Menthae folium extracts were rich in flavonoid quercetin, stilbene glucosides (isorhapontin, piceid and/or astringin) were found in Salviae folium, Fragariae folium and Teucrii montani herba extracts. As these plants are well-known in phytomedicine their medicinal properties may be related to the presence of these resveratrol derivatives.

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P- 18 Comparative Study of Composition and Toxicity of *Lippia alba* Essential Oils from 15 Different Regions of Colombia

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Essential oils from *Lippia alba* (Miller) N.E Brown (Verbenaceae) plants, grown in 15 different regions of Colombia, were obtained by hydrodistillation and by microwave-radiation assisted hydrodistillation and analyzed by GC-MS [1]. Their cytotoxicity against *Artemia franciscana*, expressed as the mean lethal concentration (LC50), was determined by counting the numbers of dead nauplii alter 24 and 48 h incubation periods. LC50 values between 6.95 and 21.05 µg/mL were obtained for the 15 *L. alba* essential oils studied. The chromatographic analysis of the oils evaluated in this study revealed the presence of 125 different compounds. GC-MS analysis positively identified 83 of these compounds, which represented over 93% of the total composition. The comparison of the essential oil compositions, based on Principal Component Analysis, revealed the existence of three different chemotypes. One chemotype was characterized by its high carvone content (ca. 41%); another chemotype was rich in citral (neral + geranial, ca. 55%). The third one was an intermediate ("hybrid") chemotype, not previously reported in the scientific literature, with carvone (25%), limonene (22%) and citral (21%), as its main components. Variation of the hydrodistillation time showed that the highest carvone content in the oil obtained from the first chemotype plant material was obtained after 75 min.

Acknowledgements: Colciencias, Grant 432-2004.

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P- 19 Variability of *Artemisia vulgaris* Essential Oil Composition and Toxicity

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Artemisia vulgaris (mugwort) is a common weed in the most parts of Europe. The herb has been known not only as an edible plant (mostly like a spice), but also as a folk medicine resource. Mugwort essential oils are used for their insecticidal, antimicrobial, hepatoprotective and analgesic activities. The aim of the work was to investigate chemical variability and bioactivity of the essential oils of *A. vulgaris* from Lithuania.
Aerial parts of mugworts were collected from various habitats in Lithuania (2002-2007). Volatile components of the plants were obtained by hydrodistillation-extraction and analysed by gas chromatography (GC, detection-FID, a polar capillary column HP-FFAP, 30 m $\times$ 0.25 mm i.d.) and gas chromatography-mass spectrometry (GC/MS, a nonpolar capillary column CB-5, 50 m $\times$ 0.32 mm i.d.). Qualitative analysis was based on a comparison of retention times, indices and mass spectra with corresponding data in the literature [1] and computer mass spectra libraries.
Among major constituents were sabinene, β-pinene, 1,8-cineole, thujones, artemisia ketone, chrysanthenyl acetate, germacrene D and caryophyllene. Principal Component Analysis was performed on the basis of the oils quantitative content and according to the main constituents the oils were distributed among chemotypes by cluster analysis. The data obtained in this work showed a remarkable chemical variation of the mugwort essential oils.
The shrimp (*Artemia sera*) lethality test [2] in vivo was done and LC50 (95 % conf. interval) values were estimated.
The obtained results indicated existence of chemical polymorphism of *A. vulgaris* essential oils and large differences of their toxicity.

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P- 20 In Vitro Metabolism of (-)-Camphor using Human Liver Microsomes and CYP2A6

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The in vitro metabolism of (-)-camphor was examined in human liver microsomes and recombinant enzymes. Biotransformation of (-)-camphor was investigated by gas-chromatography-mass spectrometry (GC-MS). (-)-Camphor was oxidized to 5-exo-hydroxyfenchone by human liver microsomal cytochrome (P 450) enzymes. The formation of metabolites of (-)-camphor was detected by the relative abundance of mass fragments and retention time on gas chromatography (GC). CYP2A6 was the major enzyme involved in the hydroxylation of (-)-camphor by human liver microsomes, based on the following lines of evidence. First, of eleven recombinant human P 450 enzymes tested, CYP2A6 catalyzed the oxidation of (-)-camphor. Second, oxidation of (-)-camphor was inhibited by (+)-menthofuran and anti-CYP2A6 antibody. Finally, there was a good correlation between CYP2A6 contents and (-)-camphor hydroxylation activities in liver microsomes of 9 human samples.

P- 21 In Vitro Antiprotozoal Activity of Several Essential Oils Against *Cryptosporidium parvum*

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Cryptosporidium parvum is an intestinal protozoan parasite (Apicomplexa) that infects a wide range of host animals including humans. Especially young or immunocompromised animals are susceptible to infection and mass propagation of the parasite which is often accompanied by loss of weight and water, retarded development and increased susceptibility to other diseases. This results in reduced animal health and economical losses for the animal production industry, for example in calf rearing. Although essential oils were tested in field trials against related parasites of the genus *Eimeria*, reports on activity against *Cryptosporidia* are scarce. In the present study the in vitro activity of several essential oils, among them a mixture used in a commercial product, and some of their predominant compounds were assessed. A cell culture assay with HCT-8 host cells was chosen for determination of anti-cryptosporidial effects of test substances. Intracellular parasite development was evaluated microscopically after labelling by indirect fluorescent antibody technique (IFAT). Cell vitality assays were conducted simultaneously to assess eventual deleterious effects on the host cells. Some of the essential oil samples inhibited *C. parvum* development while being tolerated by the host cells at the effective concentrations. Monensin as a positive control substance inhibited parasite development in a highly reproducible way. These findings attribute to evaluate the role of essential oils in combating or alleviating cryptosporidia- or coccidia-related diseases.

P- 22 Rapid Extraction of Volatile Compounds Using a New Simultaneous Microwave Distillation- Solvent Extraction Device

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Simultaneous distillation–extraction (SDE) is routinely used by analysts for sample preparation prior to gas chromatography analysis. In this work, a new process design and operation for microwave assisted simultaneous distillation – solvent extraction (MW-SDE) of volatile compounds was developed. Using the proposed method, isolation, extraction and concentration of volatile compounds can be carried out in a single step. To demonstrate its feasibility, MW-SDE was compared with the conventional technique, Simultaneous distillation–extraction (SDE), for gas chromatography-mass spectrometry (GC-MS) analysis of volatile compounds in a fresh aromatic herb, *Zygophyllum album* L., a wild salty desert herb belonging to the family Zygophyllaceae. SDE method required long time (3 h) to isolate the volatile compounds, and large amount of organic solvent (200 mL of hexane) for further extraction, while MW-SDE needed little time (only 30 min) to prepare sample, and less amount of organic solvent (10 mL of hexane). These results show that MW-SDE–GC-MS is a simple, rapid and solvent-less method for determination of volatile compounds from aromatic plants.

P- 23 Application of Rapid and Microscale Isolation Techniques for the Analysis of Volatiles from Two *Heracleum* Species

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The genus *Heracleum* (Apiaceae) encompasses about 70 species world-wide. In Turkey, *Heracleum* is represented by 14 species and 7 of them are endemic [1]. The fruit volatiles of *Heracleum trachyloma* Fisch. & Mey. and *Heracleum antasiaticum* Manden. were obtained by three rapid and microscale isolation techniques, namely, microdistillation (MD), microsteam distillation − solid-phase microextraction (MSD-SPME) and head space - solid phase microextraction (HS-SPME) with subsequent GC/FID and GC/MS analysis. The microdistillation is a capillary technique which offers fast (55 min) and efficient distillation technique with good qualitative and quantitative results, making it valuable in comparison to conventional methods. HS-SPME and MSD-SPME techniques are rapid, simple, inexpensive, solventless and highly sensitive extraction techniques which allow isolation of the volatiles for a quite short time (3 min) from a small amount of plant material (0.5g). MSD-SPME procedure involved of concurrent solid-phase microextraction combined with continuous hydrodistillation of the oil. The compositions of volatiles obtained by three microscale techniques were very close to those hydrodistilled in a Clevenger-type apparatus. Hydrocarbon esters were found in high abundance in all the oils. Octyl acetate was the major constituent of *H. antasiaticum* fruit volatiles, while in the oils of *H. trachyloma* octyl acetate, hexyl isobutyrate and hexyl 2-methylbutyrate were the major compounds.

Acknowledgements: Authors are grateful to AUBIBAM for the use of microdistillation instrument.

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P- 24 Gas Chromatographic–Mass Spectrometric Analysis of Volatiles Obtained by Four Techniques from *Salvia rosifolia* Sm. and Evaluation of Antimicrobial Activity

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Salvia rosifolia Sm. (Labiatae) is an endemic species belonging to one of the most diversified genus *Salvia* in Turkey with 52% endemism ratio [1]. The volatile constituents from the aerial parts of *S. rosifolia* were obtained by four different isolation techniques and then analyzed by GC/FID and GC/MS methods [2,3]. Conventional hydrodistillation (HD), microwave assisted hydrodistillation (MWHD), microdistillation (MD) and microsteam distillation – solid phase microextraction (MSD-SPME) techniques performed for 180 min, 45 min, 55 min and 0.5 min, respectively, yielded volatiles of similar composition. MWHD procedure produced the oil in similar yield (0.39%) to the conventional HD (0.4%). α-Pinene (15.0-35.0%), 1,8-cineole (13.7-25.8%), β-pinene (5.9-13.6%), β-caryophyllene (1.4-5.2%), and caryophyllene oxide (1.3-4.6%) were identified as major constituents in the oils. Antibacterial and anticandidal activities of the oil obtained by HD and the main constituents (α-pinene and 1,8-cineole) were evaluated using the micro-dilution broth method against selected six Gram(+) and Gram(-) bacteria and fungal strain *Candida albicans*. The oil was found active against *S. aureus* (MR) with a MIC of 125 µg/ml. *E. aerogenes* and *S. epidermidis* strains were the less sensitive to the oil. In addition, the oil indicated a strong antifungal activity against *C. albicans* with MIC of 500 µg/ml.

Acknowledgements: Authors are grateful to NAPRALERT for the use of database, and to AUBIBAM for the use of microdistillation instrument.

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P- 25 Direct Thermal Extraction of Fragrance Compounds from Cosmetic Matrices Using Thermal Desorption (TD/GCMS)

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Volatiles present in flavour and fragrance products can be determined by extracting those compounds thermally from the matrix. The benefit of this technique is that end products like shower gel or hand soap can be placed directly onto a glass wool plug in the desorption tube without any sample preparation. In this experiment a Shimadzu TD-20 thermal desorber was coupled with a GCMS-QP2010S.
For the experiments described here, the cold trap temperature was set to minus 20 °C in this mode. The analytes are then thermally extracted from the samples placed on a plug of deactivated glass wool in the sample tubes and refocused in the cold trap. The latter is afterwards heated to 300 °C using the fastest heating rate, i.e. 30°C/min in order to transfer the refocussed analytes as a sharp band to the column (RTX-5, 10m, 0.18 mm, 0.4 μm). The inner diameter of the column was selected to be smaller compared to standard application in order to get faster analysis time with no loss of resolution [1].
As fragrance related end products like shower gel or shampoo contain lots of matrix compounds with higher boiling points, the temperature for desorption was optimized. Therefore the desorption temperature selected was set to 80 °C. For this temperature clean chromatograms were recorded almost free from matrix interference. The split ratio was set to 200:1. The mass spectrometer was operated in full scan mode. More than 60 peaks including up to 10 potential allergens were detected in the chromatograms recorded with shower gel and hand soap within less than 11 minutes retention time. For identification, the Shimadzu FFNSC 1.2 library and the NIST 05 library were used with linear retention index filter applied. The method can be used as a routine tool to screen volatiles from cosmetic end products. In order to quantify the potential allergens, an external calibration was performed by injecting the standard allergen solution directly into a glass wool plug in the sample tube. The concentrations of the detected potential allergens in shower gel and hand soaps cover a range between 0.5 and 1100 ppm. The combination of columns with reduced inner diameter (and shorter length in comparison with standard GCMS) with thermal desorption was proven to be very successful in routine analysis.

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P- 26 Extraction and Chemical Characterization of Essential Oils from Plants of Genus *Schinus* from South Brazil

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The aim of this study was the extraction and chemical characterization of essential oils obtained from two species of plants of the Anacardiaceae family, *Schinus terebinthifolius* and *Schinus molle*, which were collected in the south Brazil. Two distinct processes were used to extract the essential oils from the plant leaves the first was hydro-distillation, and the other method was extraction with supercritical CO_2. The experiments using supercritical fluid were conducted at various combinations of temperatures of 40 and 60°C and pressures of 90 and 120 bar. The essential oils yields for *Schinus terebinthifolius* using the hydro-distillation process was 1.0% (dry solid basis), and 2.2% for *Schinus molle*. For the extraction with supercritical CO_2, the highest yields were 0.54% (*Schinus terebinthifolius*) and 0.89% (*Schinus molle*) at the following conditions: 40°C and 120 bar. The analytical method used for the chemical characterization was the gas chromatography (GC) with hexadecane as the internal standard. While the essential oils obtained by hydro-distillation were richer in non-oxygenated monoterpenes, the oils obtained by the extraction with supercritical CO_2 presented higher quantities of sesquiterpenes.

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P- 27 The Influence of Distillation Fractions on Essential Oil Composition of *Salvia officinalis* L.

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Sage (*Salvia officinalis* L., Lamiaceae) is well known as common medicinal plant. This work aimed to determine the essential oil composition of *Salvia officinalis* in separate fractions with sequential intervals during the hydro-distillation. The essential oils were extracted from the dry leaves in Clevenger apparatus. The sage oils in leaves were collected from sequential intervals of 0-30, 31-60, 61-90, 91-120, 121-150, and 151-180 min during the hydro-distillation, and analyzed on a gas chromatography-mass spectrometry (GC-MS). The total oil yield was 1.80%, and 86.6% of the oils was released in the first 30 min. The sage oil was characterized by high percentage of monoterpene alcohols representing in particular 1,8-cineole, α-thujone, β-thujone and camphor, and hydrocarbons representing in particular viridiflorol/ledol, agathadiol and manool. Essential oil composition was significantly different in the separate fractions. The amount of monoterpene alcohols decreased, whereas the hydrocarbons steadily increased up to late fractions, especially from 30 min. During the first 30 min the fractions consisted for the greatest part of 1,8-cineole (19.58%) and α-thujone (19.74%), β-thujone (9.22%), camphor (24.82%) and the fractions after 151 min contained only small amounts of 1,8-cineole (1.41%) and α-thujone (0.83%), β-thujone (0.38%), camphor (0.63%). On the other hand, during the first 30 min the fractions contained small amounts of viridiflorol/ledol and agathadiol, manool, and the fractions after 151 min consist for the greatest part of viridiflorol/ledol (20.58%) and agathadiol (15.81%), manool (44.11%). The other essential oil components showed the least amount of change during the distillation process.

P- 28 Volatile Compounds of Lavender Intact Flowers (*Lavandula angustifolia* L.) and Extracted by Supercritical Carbon Dioxide and Sonication from North-East Italy

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In view of more extensive cultivation of aromatic plants in north Italy, three samples of *Lavandula angustifolia* L. flowers, from different parts of Friuli Venezia-Giulia, were studied in order to evaluate their quality.
Headspace solid-phase micro extraction (HS-SPME) coupled to gas chromatography and mass spectrometry (GC-MS) analysis of fragrance from lavender intact flowers was carried out. Sensory analysis of the lavender flowers was performed too. The same lavender flowers were extracted by supercritical carbon dioxide and by sonication and analysed by GC-MS. All major components for the essential oil of *L. angustifolia* defined by ISO and AFNOR standards were detected in the samples.
The volatile composition of the supercritical fluid extracts resulted very close with the natural pleasant aroma of flowers evaluated by HS-SPME. The volatile composition of lavender extracts obtained by supercritical carbon dioxide confirmed the effectiveness of this extraction method to produce fragrance to be not distinguishable from that of the starting materials. Sonication improved the yields of lavender fragrance and shortened the extraction time in comparison to steam-distillation method. Ultrasonic probe was operated on different ultrasonic power using ethanol 70% as extraction solvent .The effect of ultrasonic power on the extraction of volatile compounds showed differences in efficiency when the probe was operated at different amplitude. At 100% amplitude for 3 min the extract was similar to that of hydrodistillation performed for 3 h. The Middle-Friuli lavender was evaluated as the highest quality as it was known that good lavender contains a high proportion of linalyl acetate and linalool and a low proportion of camphor.

P- 29 How Many Fennel Seeds are Necessary to get Accurate Results?

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Essential fennel oil is not distributed equally in fennel seeds. Only 1, 2 or 3 seeds cannot represent a whole plant. So the question was, for instance, how large a sample must be in order to obtain a deviation of less than 3 % in parallel determinations. For the solution to the problem 99 fennel seeds of the cultivar "Berfena" were extracted separately in 1 mL isooctane with internal standard. After shaking in a vibrating mill the extracts contained the essential oils of the single seeds. All 99 extracts were examined by GC. The essential oil values were composed of the sum of the individual components. Fig.1 shows the inhomogeneous distribution of the essential oils contents in the 99 fennel seeds. Fig. 2 shows, the more seeds are contained in the sample the smaller is the deviation from the average value.

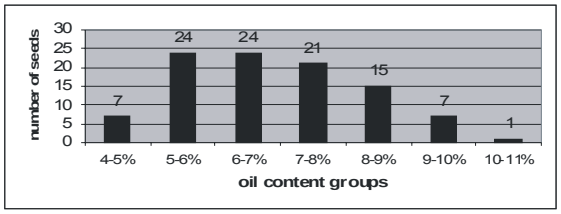

Fig.1: Oil content groups of 99 fennel seeds

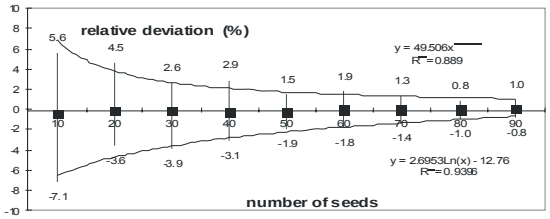

Fig.2: The relative deviation of essential oil values from the average in function of the seed number

Since there are no identical fennel seeds, 100 random sample groups with 10, 20, 30 etc. were formed and the relative deviations from the average values were calculated. The result supports our experience that the samples must have at least 200 mg (approx. 40 seeds) in order to keep a standard deviation for the essential oil value less than 3%.

P- 30 Test of the reliability of SPME-method for Essential Oil Analysis

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Recent analysis on several species of Lamiaceae revealed that the non-equilibrium solid-phase micro-extraction (SPME) method is a simple and useful tool to investigate the content of single oil glands (Kubeczka, 1997; Grassi et al., 2004; Johnson et al., 2004; Novak et al., 2006). Using this method, the content of a single oil gland can be directly sampled with an SPME fibre and injected into a gas chromatograph (GC), without any further extraction procedure.
To confirm the reproducibility and reliability of this non-equilibrium SPME method three test arrangements were compared on the same test item, essential oil of *Salvia sclarea*: 1. essential oil diluted in dichloromethane and injected by GC-autosampler. 2. equilibrium headspace-SPME analysis of the essential oil. 3. essential oil drops, simulating essential oil glands, were directly sampled by non-equilibrium SPME with three different SPME-fibre coatings.
The results of the method comparison confirmed good reproducibility and reliability of the non-equilibrium SPME method, giving standard deviations only slightly higher than the essential oil diluted in solvent and injected by the autosampler. The three different fibre-coatings and the essential oil directly injected showed relatively similar results. The headspace analysis, however, had a distinct terpene pattern compared to the other treatments.

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P- 31 Linear Retention Indices in Enantioselective GC Coupled with Mass Spectrometry (ES-GC-MS) as a Tool to Identify Enantiomers in the Flavour and Fragrance Fields.

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In flavour and fragrance field the absolute and/or relative configuration of the optical isomers may induce marked differences in the organoleptic properties. Enantiomer recognition and enantiomeric excess (EE) and/or ratio (ER) determination are therefore extremely important in this field, so as to define a correlation between chemical composition and organoleptic properties, to implement quality control, to characterise a sample or to determine its origin, to detect fraud or adulteration of "natural" samples and to study biosynthetic pathway of a compound.

Mass spectrometry is well known to be unable to discriminate between optical isomers giving indistinguishable spectra, not being a selective probe, As a consequence GC-MS cannot be used alone neither to determine which enantiomer is present in a sample, nor to establish the predominant one through its EE and/or ER. Linear Retention Indices (LRIs) obtained by Enantioselective(ES)-GC-MS on a column coated with a chiral selector is therefore necessary to identify a given optical isomer in a complex mixture unequivocally. In the chiral recognition of optically-active isomers, LRIs and MS spectra have to be combined but, unlike conventional GC-MS analysis, mass spectra (or single ion monitoring) are used to locate the two enantiomers in the chromatogram, and LRIs to identify them.

This study deals with the building-up of a MS library of optically-active compounds in the field of flavours and fragrances combining active LRIs in parallel to MS spectra as an additional tool to identify a chiral compound.. Some applications showing the effectiveness of this approach in chiral recognitions of optically-active isomers in a complex mixture will be also presented.

P- 32 Solid-phase Microextraction of Volatile Compounds from *Laurobasidium lauri* from Madeira Archipelago.

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Laurobasidium lauri (Geyler) Julich is a fungus parasite of *Laurus novocanariensis* and *Laurus nobilis*, used in Madeira Island folk medicine as anti-inflammatory, anti-rheumatic, vulverary and blood depurative in the form of alcoholic tincture. It is known locally as madre de louro (laurel's mother).

In previous studies on both madre de louro and its host *Laurus novocanariensis*, we found two lipophilic sesquiterpenes lactones, costunolide and dehydrocostulactone, as the biological active components. These two lactones were present, albeit in small amounts, in the essential oils obtained by hydrodistillation of both species.

In the present study, headspace solid phase microextraction (SPME) coupled to fast gas chromatography-mass spectrometry (GC-MS) was used to analyze the volatile compounds of *Laurobasidium lauri* from Madeira Archipelago, Portugal.

Sampling sensitivity was optimised by evaluation of sample matrix, sampling size, salt addition, extraction temperature, and extraction time and fibre type. The best response was obtained for an extraction temperature was 40ºC, extraction time of 20 min and desorption time of 5 min.

Five different fibres were evaluated: polydimethylsiolxane (PDMS), carbowax/divinylbenzene (CW/DVB), polydimethylsiloxane/divinylbenzene (PDMS/DVB), polydimethylsiloxane/carboxen (PDMS/CAR) polyacrylate (PA), and polydimethylsiloxane/ divinylbenzene/carboxen 1006 (PDMS/DVB/CAR). The best results were obtained with PA, where monoterpenes, sesquiterpenes and oxygenated compounds are all well resolved.

The volatile fraction of *Laurobasidium lauri* is composed mainly of non-oxygenated compounds, with predominance of sesquiterpenes and germacrene D as the main constituent. Costunolide and dehydrocostulactone were not detected in any chromatogram, not being adsorbed in any of the fibres, in the present experimental conditions.

Acknowledgements: This work was partially supported by Biopolis project, INTERREG programme. S. Gouveia is grateful to FCT and FEDER for a PhD grant SFRH/BD/24227/2005.

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P- 33 Extraction and Fractionation of Lovage (*Levisticum officinale* Koch) Essential Oil Components by Supercritical Carbon Dioxide

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Sub- and supercritical CO_2 extractions of lovage (*Levisticum officinale* Koch.) roots were performed to determine the effect of CO_2 parameters on the extract yield and the composition of volatile essential oil compounds. First series extractions were performed to establish the effect of CO_2 pressure and temperature; second, to determine the dynamics of the extraction at constant temperature. It was found that by varying the pressure from 80 to 300 bar and the temperature from +10 °C to +55 °C the best result was obtained at 50 °C and 200 bar. During the second series the temperature was kept at 50 °C and the extracts were collected at various stages of extraction process at the pressures from 80 to 350 bar. The extract yield considerably increased by raising the pressure from 80 to 200 bar, however, further pressure increase to 350 bar was not so efficient. The composition of volatile compounds was examined by gas chromatography and mass spectrometry. Particular attention was focused on the content of phthalides, which are considered as key flavor compounds of lovage. Among 8 identified phthalides, cis-ligustilide was most abundant in all extracts; its content depending on extraction parameters and process time varied in a very wide range, from 2 to 65 %. Model systems, containing important lovage essential oil components, α-terpinyl acetate and 3-n-propylidene phthalide, as well as lovage leaves, stems and seeds were used for the second task aiming at the enrichment of lower quality leaf and stem essential oil with valuable root compounds, phthalides. The percentage content of the main constituents in the extracts varied in a wide range depending on their ratio in the initial mixture. By using solvent circulating system with two separators at different parameters it was possible to obtain phthalide enriched fraction both from the model matrix and plant material.

P- 34 Reliable Identification of Enantiomers Domprised in Flavour and Fragrance Matrices by Means of GC-Enantio/MS/O

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The analysis and resolution of optically active compounds in flavour and fragrance matrices, still represents to be a trend in analytical research. Many natural flavour materials occur as a specific chiral isomers, and their odour can be very distinctive and characteristic [1]. In general, enantiomers may differ either in odour quality, intensity or activity [2].
The great demand for enantiopure substances imposed the development of appropriate and accurate analytical techniques and methods, e.g. enantioselective gas chromatography hyphenated to mass spectrometry (GC-Enantio/MS) Even though it shall be outlined that in the latter technique the similarity in the enantiomers fragmentation pattern may result in unreliable peak assignment. To support MS data, linear retention index (LRI) values of resolved enantiomers were determined in four chiral stationary phases. However, when closely eluting enantiomers are considered, with LRIs separated by few index units, this value looses reliability. To cope with this limit, the differences in sensory quality of enantiomers were considered, and olfactometric analyses were performed enabling in several cases a sustainable characterization of enantiomers.
The present research is focussed on the application of enantioselective gas chromatography/ mass spectrometry/ olfactometry (GC-Enantio/MS/O) for the correct determination of sensory properties and differentiation of individual enantiomers comprised in essential oils.

Acknowledgements: Shimadzu Corporation.
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P- 35 Revisited Enantioselective Investigation of Citrus Oils by Means of GC-Enantio/MS

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Citrus oils are characterised by a volatile fraction which comprises 90-95% of their composition. These oils, in particular the Italian oils, exert an important economical [1], as also olfactive impact, and are widely used for flavour, beverage, food, cosmetics, pharmaceutical, and chemical purposes [2]. As well recognised a wide variety of components are comprised in these essential oils belonging mainly to the class of monoterpenes and sesquiterpenes hydrocarbons and their oxygenated derivatives. Compounds known to frequently present chiral centres.
The enantiomeric distribution of the components of essential oils, usually achieved through conventional gas chromatographic (GC) methods using chiral stationary phases, can provide useful information on the determination of authenticity, quality, applied extraction technique, geographic origin and biogenesis of the oils [3].
The present research is focussed on the enantioselective analysis of several citrus essential oils aiming an accurate, and revisited, determination of their enantiomeric composition. The latter was established for lemon, bitter and sweet orange, bergamot, mandarin, citron, neroli, petitgrain citronier, petitgrain bigarade, and petitgrain mandarin oils by means of GC hyphenated to mass spectrometry (MS)- GC-Enantio/MS, using distinct stationary phases immobilized with different chiral selectors. To contour the limit caused by the similarity in MS fragmentation pattern expected for enantiomers, linear retention indices (LRI) were determined and applied as an additional tool for their differentiation.

Acknowledgements: Shimadzu Corporation.

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P- 36 High Speed Gas Chromatography – Full Scan Mass Spectrometry for the Determination of Fragrance Allergens

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In the 7th Amendment to the European Cosmetics Directive, the Scientific Committee for Cosmetics & Non-Food Products established twenty-six fragrance components, widely used in cosmetic products, as being responsible for a series of skin allergies [1]. The regulation foresees that any allergen, present in excess of 100 mg/kg in rinse-off and of 10 mg/kg in leave-on formulations, must be reported on the product label. A fast GC-full scan quadrupole mass spectrometric method for the determination of allergens in perfumes is presented. Reliable peak identification was achieved through a twin-filtered MS library matching procedure, considering a minimum degree of spectral similarity (90%) and retention data (a linear retention index window was applied). Peak quantification was carried out by using an extracted ion. The fast GC-MS method was validated in terms of intra-day retention time and peak area precision, limits of detection and quantification and method linearity.

Acknowledgements: Shimadzu Corporation.

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P- 37 Comparison of the Chromatographic Fingerprints of Horseradish and Mustard Oils (Brassicaceae) with the Support of an Innovative GC-MS Database.

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Horseradish oil is obtained from the root of *Cochlearia armoracia*, that is a perennial, herbaceous plant easily found in Europe and Asia. The oil is characterized by high diffusivity, pungency and hot flavour, as well as the freshly grated root [1]. For these qualities, it is very common the culinary use of horseradish in sauces and dressings, as an alternative to mustard oil. The latter is obtained from the dried ripe seeds of *Brassica nigra* or B. *juncea* [2]. Both the oils are used not only in food, but also in traditional medicine as rubefacient, decongestant and antibacterial. In this study, the volatile fingerprints of the two oils have been compared, after their quali-quantitative determination by means of GC-FID and GC-MS. Attention is claimed on the use of a laboratory-constructed database (FFNSC library) containing spectra related to fragrance and flavour field; each spectrum is provided with the experimental Retention Index, used by the software as a filter to reduce the list of possible candidates for an unknown compound [3]. For quantitative analysis, accurate determination of response factors has been carried out and data have been reported as g/100 g.

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P- 38 The Impact of Drying on the Composition of Volatile Constituents of Bosnian Pine (*Pinus heldreichii* Christ. var. *leucodermis*)

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Bosnian pine (*Pinus heldreichii* Christ. var. *leucodermis*) is a plant endemic to the Balkan Peninsula and Southern Italy. The free volatile constituents of this plant have been studied in the context of biosystematic investigations related to the environment [1, 2]. Essential oils are dominated by monoterpene hydrocarbons such as limonene, pinenes and terpinenes, followed by sesquiterpenes germacrene, Δ-cadinene, α-humulene, and caryophyllene. The aim of this study was to determine the impact of drying on the chemical composition of Bosnian pine essential oil. Samples of plant material (pine needles) were collected in central Herzegovina near Mostar (Bosnia and Herzegovina) in January 2007. Each sample was collected in triplicate, pooled and then divided in two parts: one was investigated as fresh plant material (100 g) and other (500 g) was dried in shaded place at room temperature and submitted consecutively to five investigations within air-drying period for 30 days. The essential oil was isolated by hydrodistillation in Clevenger type apparatus for 2 h. The yields of essential oils were determined by gravimetric method and expressed as a mean value. Volatile constituents were analyzed using a Hewlett-Packard GC-MS system (GC 5890 series II, MSD 5971A). The comparative analysis of oils isolated from fresh and dried plant material showed a minor increase in essential oil yield and no significant qualitative differences in oil composition after drying. On the other hand, some quantitative differences were found, especially in major components limonene and germacrene. The oil obtained form dried plant material showed remarkable increase in abundance of limonene (22.7% - 42.8%), α-pinene (1.0%-14.2%) and β-pinene (0.6%-5.26%). However, a substantial decrease was noted for germacrene (52.5% - 21.9%).

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P- 39 Influence of Drying Parameters on the Composition of Volatile Compounds of *Helichrysum italicum* G. Don ssp. *microphyllum* (Wild) Grown in Sardinia

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The shelf life of spices is traditionally extended by drying. Drying, however, may result in a series of physical and chemical alterations, which negatively affect the quality. This paper deals with the effect of different drying temperatures and air fluxes on the volatiles in *Helichrysum italicum* G. Don ssp. *microphyllum* (Willd) of Asteraceae family grown in Sardinia. The plant produces an essential oil from its blossoms which is used for medicinal purposes. It is anti inflammatory, fungicidal, and astringent. It soothes burns and raw chapped skin; it is used as a fixative in perfumes. It has an intense fragrance.
Fresh aerial parts of cultivated *Helichrysum italicum* G. Don ssp. *microphyllum* (Willd) were collected and soon transported at the laboratory, divided into two batches. Both batches were subjected to the extraction of the volatiles compounds and subsequent GC-MS analysis, the first sample in the fresh form, the second one after drying, which was done with a laboratory pilot dryer equipped. Three drying temperatures were used, 30, 38 and 45°C, while two volumetric flow rates were set for each temperature.
The fresh and conserved plant material was submitted for 3 hours to hydro distillation using a Clevenger-type apparatus according to Italian Official Pharmacopoeia X. The oils were stored at –20°C until they were analyzed. From the first analyses we observed that, in Sardinia two different ecotypes are present, where the principal constituent is neryl acetate and 5-eudesmen-11-ol, respectively. Considering the analyzed samples 48 constituents accounting around 96.5% of total volatile components of essential oils have been identified and quantified. Among the identified constituents those present in highest percentage were neryl acetate (or 5-eudesmen-11-ol), linalool, neryl propionate and limonene. From the first analyses we observed that using traditional drying conditions no substantial decreasing of the interesting compounds is noted between fresh and dried plants. For this reason we will examine also other different way of conservation of this plant material with the aim to preserve all the active constituents of Sardinian *Helichrysum* to increase the shelf life of the vegetal material and consequently the commercial value of this plant.

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P- 40 The Effect of Sodium Chloride on the Content and Composition of Tarragon Essential Oil

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The genus *Artemisia* (compositae) involves 34 perennial and annual species in Iran, and two of them are endemic [1]. The present report is a part of a project aimed at the enhancement and development of research activities on medicinal and aromatic plants in Iran. Previous investigations have been carried out on chemical composition [2-4,8,9], biological activity of essential oil [2,6,8], anticonvulsant activity [3], antioxidant activity [4], Loss of essential oil due to drying [5] and effect of NAA on the accumulation of volatile oil components in cell-cultures of tarragon (*Artemisia dracunculus*) [7].
The present paper deals with the influence of sodium chloride on the essential oil of aerial parts of Tarragon plants. The plants were treated under field condition and were irrigated with different levels of NaCl (100,150,225 mM) twice in a week. After a week aerial parts of plants were harvested and dried in a dark place so that the continuous air stream flows through. The essential oil of dried aerial parts was obtained by hydrodistillation using a Clevenger apparatus. The essential oil of one of treatments (225 mM) and its control (with water without NaCl irrigated) were analyzed by GC/MS(Hewlett-Packard 6890/5973) equipped with a fused silica capillary HP-5MS column. The main compounds of essential oil were cis-β-ocimene (5.2%), trans-β-ocimene (4.0%), methyl chavicol (82.3%), for control plants. The yield of essential oil decreased in all treatments. The percentage of methyl chavicol in tarragon essential oil showed a decrease by NaCl while the other main compounds showed an increase.

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P- 41 The Effect of Indol Acetic Acid and Naphthalene Acetic Acid on Composition the Essential Oil of *Artemisia dracunculus* L.

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The genus *Artemisia* (compositae) involves 34 perennial and annual species in Iran, and two of them are endemic [1]. The present report is a part of a project aimed at the enhancement and development of research activities on medicinal and aromatic plants in Iran. Previous investigations have been carried out on chemical composition [2-4,8,9], biological activity of essential oil [2,6,8], anticonvulsant activity [3], antioxidant activity [4], Loss of essential oil due to drying [5] and effect of NAA on the accumulation of volatile oil components in cell-cultures of tarragon (*Artemisia dracunculus*) [7].
In the present work aerial parts of *Artemisia dracunculus* (tarragon) were treated with indol acetic acid (IAA) and naphthalene acetic acid (NAA) under field conditions for a week. Then, the aerial parts were harvested and dried in a dark place so that the continuous air stream flows through. The essential oil of dried aerial parts was obtained by hydrodistillation. Foliar spray of indol acetic acid (IAA) and naphthalene acetic acid (NAA) increased oil yield of tarragon. The essential oil of two treatments and their control (water sprayed) were analyzed by GC/MS. The main compounds of essential oil of control plants were methyl chavicol (82.31%), *cis*-β-ocimene (5.15%), *trans*-β-ocimene (4.01%). It was concluded that IAA and NAA decrease methyl chavicol content of tarragon oil and promote the amounts of other main constituent components.

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P- 42 Authenticity Assessment of Essential Oils – the Key for Product Safety and Traceability in the Field of Animal Feeding

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For several years, feed additives have been offered on the market for ecological as well as conventional animal feeding. Based on secondary plant components they do not involve any risk of antibiotic efficiency promoter and partly contribute their advantages.
Compounds like carvacrol in oregano and savory oil, thymol in thyme oil or anethol in anise and fennel oil correlate with an improved feed conversion which was the result of latest research work. On this basis, various product groups were established on the market.
The aim of the project is to develop an analytical method using multielement/ multicomponent isotope ratio mass spectrometry (GC-IRMS) to discriminate the source (naturally or synthetic) of such essential oils, which are increasingly used in animal feeding. This method can be used to confirm the natural origin and adulterations can be identified reliably.
The isotope ratios of the components carvacrol, thymol and trans-anethol are evaluated primarily. The corresponding authentic oils are analysed and compared with commercially available oils as well as with synthetic products of the single components from different synthetic processes.
The trials show good results for discrimination between natural and synthetic substances, which are demonstrated on the poster.

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P- 43 Variation of the Composition of the Essential Oil of *Asarum canadensis* Cultivated under Forest Canopy

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Asarum canadensis, Wild Ginger or Canada Snakeroot, are a common plant of the maple grove of the center and south-east of Canada whose rhizome has a strong flavour of ginger and contains a very aromatic essential oil, is used in aromatherapy or medicinal and perfumery. The analyses of oils were carried out by gas chromatography (GC) and were confirmed by gas chromatography coupled with mass spectrometry. The chemical composition of the essential oil of the seedlings of asaret which were transplanted in maple grove of La Pocatière, Cape-St-Ignace, Pohénégamook and Grondines was followed during three years. The harvest of certain seedlings was carried out to make it possible to estimate the growth of the seedlings during each season as well as the composition of the essential oil of the rhizome. In the rhizomes, one mainly finds monoterpenes such as methyl eugenol and linalol which characterize this essential oil as well as a very aromatic notes conferred by geranyl, bornyl, neryl and linalyl acetate. Methyl eugenol varies from 53.8% to 73.1% and the linalool varies from 3.9% to 17.4% according to the age of the plant, its site and the rate of light received.

P- 44 Effects of Biofertilizers Application on Seed Yield and Active Substance in Fennel (*Foeniculum vulgare* mill.)

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Biofertilizers are used in sustainable agriculture with aim of remove or reduce of chemical inputs in order to reach to quality increasing and sustainability of yield. Also quantity and quality of active substance is important in medicinal plant production.
The main aim of this study was to determine the effects of biofertilizers on seed yield and active substance in Fennel. The experiment was carried out at Hoomand Research station, Damavand/ Iran in 2005. The factors were mycorrhizal inoculation (inoculated and non-inoculated), biophosphate fertilizer (0, 30 and 60 kg/ha) and vermicompost (0, 5 and 10 ton/ha) to gether a fertilizer control treatment (N= 90, P= 60 and K= 90 kg/ha). Used vesicular arbuscular mycorrhizal fungus was *Glomus intraradices* and applied boiphosphate fertilizer consisted bacteria of *Pseudomonas striata* and rock phosphate. Also, consumed vermicompost in this study, was prepared by using from animal manure and beneficial earth worms (*Eisenia foetida*).
The design was a factorial experiment in the base of randomized complete blocks design with 19 treatments and 3 replications. The measured characteristics were seed yield, essential oil content in seed and anethole content in essential oil. Mean comparison was carried out using the Duncan multiple range test (at 5% level).
Results showed that the highest seed yield, essential oil content in seed and anethole content in essential oil were obtained with mycorrhization. Biophosphate fertilizer also showed significant effect on essential oil content in seed only. The maximum essential oil content in seed were obtained from biophosphate fertilizer (60 kg/ha).
The highest seed yield, essential oil content in seed and anethole content in essential oil were obtained with application of 10 ton/ha from vermicompost. Three factors intraction also indicated significant effects on essential oil content in seed. Comparison of control versus biofertilizer treatments was significant so that seed yield in treatment of include mycorrhization, consumption of 30 kg/ha from biophosphate fertilizer and 10 ton/ha from vermicompost and essential oil content in seed in treatment of contain mycorrhization, application of 60 kg/ha from biophosphate fertilizer and 10 ton/ha from vermicompost were higher than control.

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P- 45 Evaluation of Drug Quality and Polymorphism of *Thymus pannonicus* All.

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Thymus pannonicus All. (Eurasian/Hungarian thyme) is the most frequent indigenous thyme species in Hungary. Regarding the 26 model areas surveyed (2001-2006), many different base rocks and soil types have been found as a substrate of its populations. It could be established that *T. pannonicus* prefers the carbonate containing base rocks to volcanic ones or to acidic sandstone. Concerning soil types, the species is most widely occurs on rendzina, bare soils, humic sand or on chernozem. Our results support the generalist character of Hungarian thyme, however, show wider distribution of values than it was described previously (Mártonfi et al., 1996) concerning soil preferences.
Almost all populations surveyed met the requirements of the Pharmacopoeia Hungarica Ed. VIII. (2004) (min. 0.3 ml/100 g essential oil) concerning dried flowering parts (Serpylli herba). The volatile oil content varied between 0.140 ml/100 g (min.) and 1.750 ml/100 g (max.) values with the average of 0,690 ml/100 g. Essential oil analyses were performed by capillary GC (GC: Agilent Technologies 6890 N, column: HP-5, detection: FID/MS). The monoterpene phenolic chemotypes, known from previous publications (Sur et al., 1988; Mechtler et al., 1994), were the most frequently detected in the Hungarian wild growing populations, too: thymol (67.5 %); thymol/p-cymene (36.5-63.7/ 11.5-27.3 %) and thymol/p-cymene/γ-terpinene (26.8-60.0/ 6.9-21.5/ 5.9-20.9 %). According to our results, nine new chemovarieties have been described as follows: 1. thymol/γ-terpinene/p-cymene (40.0/ 20.2/ 14.7 %); 2. thymol/p-cymene/borneol (41.9/ 20.2/ 10.3 %); 3. thymol/linalyl-acetate/γ-terpinene (27.7/ 18.8/ 18.6 %); 4. p-cymene/geraniol (53.7/ 15.8 %); 5. geraniol/γ-terpinene (25.3/ 25.4 %); 6. linalyl acetate/geranyl acetate (36.2/ 20.2 %); 7. germacrene D/β-caryophyllene (43.4/15.0 %); 8. caryophyllene-oxid/β-cubebene/linalool (45.1/15.7/13.8 %); 9. β-cubebene/linalool/linalyl acetate (25.5/ 7.6/ 7.4 %).

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P- 46 Usage of Eugenol in Coating of Pakchoi (*Brassica chinensis* L.) Seed for Fungal Inhibition

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Seed coating with chemical pesticide has been continuously improved especially the last 10 years in European and North America countries. At thus become interest and a challenge to explore whether the western know-how of seed coating techniques and the local bio-substance for fungus control can be combined to produce an efficiency bio-pesticide coated seed to answer the environmental awareness.
The objective of this study was to constitute the biofungicide from essential oil for coating Pakchoi (*Brassica chinensis* L.) seed. Twenty one isolates of pathogenic molds were found from Pakchoi seed by agar method (ISTA) and 20 isolates were found from soil by pour plate method. Four isolates from the seeds were identified as seed-borne fungi i.e. *Aspergillus* sp., *Rhizopus* sp. and *Alternaria* sp. , and one isolate from soil was identified as a soil-borne fungus, *Fusarium* sp. .
At 1% concentration of eugenol, it could completely inhibit mycelial growth and spore germination of all the seed-borne and soil-borne fungi tested. The amount of 1-5% eugenol combined with fixed coating material, i.e. 2% chitosan, 1% acetic acid, 0.1% lignosulfonic acid, 0.7% food color and distilled water, in seed coating technique was investigated. The optimized ratio of seed to coating material 1:1 was also used in the experiments.
The efficiency of Pakchoi seeds coated with 1-2% eugenol of coating film showed that the seeds gave higher germination percentage than those mixed with captan but the inhibitory effect of the seeds against phytopathogenic molds was less. The efficiency of coated seeds growing in green house was also performed by planting the test seeds in pots with appropriate ratios of phytopathogenic molds to soil. The results showed that Pakchoi seeds coated with 1-2% eugenol of the coating film could germinate and produce the healthy seedlings better than those mixed with captan.

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P- 47 Anti-chemotactic Properties of the Essential Oils of Four Species of *Baccharis*

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Migration from peripheral circulation to injury sites is one of the main steps of the inflammatory response undertaken by leukocytes, particularly polymorphonuclear cells, with the aim of eliminating the aggressor and repairing the damaged area. In this study, the effects of the essential oils (EO) extracted from the aerial parts of *Baccharis articulata* (Ba), *B. crispa* (Bc), *B. dracunculifolia* (Bd), and *B. gaudichaudiana* (Bg) in interfering with the human leukocytes migration induced by casein were investigated using the Boyden's chamber method. Peripheral blood leukocytes harvested from volunteers were treated with increasing EO concentrations (10-4 -10-2 µl/ml) for 30 min at 37°C, and induced to migrate towards 0.5% casein gradient. Dexamethasone (Dexa, 10-5 M) was used as an inhibitory agent, while DMSO, the diluent used to incorporate the EO into the aqueous medium, was used for monitoring the cell spontaneous migration. From the species tested, only *B. articulata* and *B. dracunculifolia* inhibited significantly the casein-induced granulocyte chemotaxis at 10-2 ul/ml when compared with DMSO (95.2±3.0%), with respectively 41.7±10.2% [n=5; $F(14.47)=6.56$; $p<0.01$], and 53.9±12.5% [n=5; $F(14.47)=6.56$; $p<0.05$] of the cells recovered from the lower chamber compartment. Moreover, these effects were even better than those observed for Dexa (59.0±7.2%) [n=8; $F(14,47)=6,56$; $p<0,01$], which is a drug often used in similar in vitro studies. The data herein presented supports the usefulness of *Baccharis* EO for treating inflammatory diseases and also suggest these effects may be mediated through opioid receptors as casein binds to several opioid receptors with different affinities expressed on human leukocytes surface.

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P- 48 Effect of the Year on the Production of Chamomile Strains (*Matricaria recutita* L.) of Different Origin

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Changes in morphological and chemical characteristics of chamomile (*Matricaria recutita* L.) have been studied effected by the vegetation year. The experiments were carried out in 2005-2006 at the research field of the Faculty. In 2005 and 2006, characteristic differences were registered in the quantity and distribution of the precipitation. In open-field plots we studied 28 populations originated from Hungarian natural habitats, comparing them also with 4 registered cultivars.
Concerning plant-height and flower-diameter in each strain we got significantly higher values in 2006 than in 2005. We found, that a larger flower-diameter was achieved mainly due to longer ray-flowers.
We established a strong effect of the year on the essential oil level, however, increase of decrease was dependent on the strain. On the other side, the main compounds of the oil (chemotype) proved to be more stable during the years. Beside the stable qualitative profile of the oil, we registered some quantitative differences between years. The proportion of chamazulene decreased in 2006 by 1.3 %, while the ratio of α-bisabolol and bisabolol-oxide A and B increased by 44 %, 57 % and 17 % respectively. Nevertheless, only the variability of the level of α-bisabolol proved to be significant.
In the contents of flavonoid components we also observed great variability comparing the two years. While the amount of isoquercitrin and quercitrin increased in 2006 (by 216 % and 240 % respectively), that of rutin and hyperoside decreased significantly (by 50 % and 37 % respectively). Also in the case of apigenin the change was confirmed statistically, while the content of chlorogenic acid did not changed significantly.
According to our results, we summarised the effects of the year (weather conditions) on the morphological and chemical performance of chamomile. They should be taken into consideration during evaluation of accessions, breeding and maintenance of varieties.

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P- 49 *Valeriana officinalis* L. – Comparative Evaluation of Different Selected Lines and Populations

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Due to the increasing demand on Valerian (the roots and rhizome of *Valeriana officinalis* L. - Valerianae rhizoma et radix) the lack of a morphologically homogeneous cultivar adapted to the Hungarian ecological circumstances and characterised by standard chemical composition and good drug-yield has become a serious problem, since today an officially registered Valerian cultivar does not exist in Hungary.
The accumulation of the chemical constituents is highly affected by different ecological and cultivation conditions. Another problem is the great morphological and production-biological diversity of the plants.
During the research aiming to produce a new cultivar, selected lines – I/5, II/2, III/3, IM – and populations – Pannon, Wien, Lengyel, Német – were compared with relevance to their morphological and production-biological properties in the experimental field of the Department of Medicinal and Aromatic Plants (Corvinus University of Budapest), Soroksár.
According to the morphological characteristics (length of the leaves, length and width of the leaflets, length of the roots, number of root collars, thickness of the lateral roots etc.) the samples were significantly different from each other. Lines I/5 having wide and lobed leaves, and II/2 bearing slashed leaves as well as the very heterogeneous population of Wien were characterised by the strongest plant vigour and the biggest drug yields.
In the case of the chemical constituents the essential-oil content of the examined samples was varied between 0.518 – 1.038 ml/100g dry material. Significant differences were also detected in valerianic acid (0.193 – 0.427 %) content as well as in the amount of valtrats (0.534 – 0.916 %).
Consequently it can be ascertained that based on the results of several years, selected line of I/5 – owing to its advantageous production-biological and chemical characteristics – seems to be a good plant material for further selection-breeding work of Valerian in the future.

P- 50 Effect of Garlic- and Rosemary Oil on Performance of Broiler Chicken

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As effect of the endemics during the last years (e.g. BSE, avian flu) and changes of the consumers' needs (e.g. ban of growth promoters), regulations of animal nutrition became stricter than ever. To maintain livestock performance it is necessary to search alternative growth promoters. Based on the intensive research in that field one of the perspective group of novel feed additives would be the plant extracts and oils. In the present study effects of garlic- and rosemary-oil were studied.
Four groups of day old Hubbard hybrid chickens were included in the experiment (n=200) for six weeks. Control group was fed on commercial compound feed free of added antioxidant and antibiotics. This feed was supplemented with garlic oil (0.05% - group G), or rosemary oil (0.15% - group R) or their combination (0.05% garlic and 0.15% rosemary oil – group GR) in the three treated groups, respectively. Weight and feed consumption were measured weekly. In virtue of these data, daily weight gain and feed conversion ratio were calculated.
Average feed consumption of group GR was the lowest in the starter and highest in the grower period. Slaughter weight of the birds in the R group was significantly ($P<0.01$) higher than that of the control ones, while the others were smaller. Daily weight gain in each treated groups exceeded that of the control group, especially in the growing period. Considering feed conversion, rosemary oil supplementation has shown the best results.
Altogether, the most promising results were obtained with rosemary oil supplementation. Garlic oil has shown practically no effect, while in the combined treatment it even has deteriorated the positive effect of the rosemary oil.

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P- 51 Changes in the Volatile Profile of *Artemisia alba* Turra Associated with Plant Material Storage – Monoterpene Fraction

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Monoterpenes are frequently credited as carriers of the specific and desirable aroma of many essential oil containing plant species. Unfortunately, these volatiles readily undergo different temperature and light driven transformations [1]. The aim of this study was set to investigate (by GC and GC-MS) the relative compositional changes in the monoterpene fractions of seven different hydro-distilled essential oils, from the same *Artemisia alba* Turra plant material, isolated during one year long storage period. Although the dominant compounds of the monoterpene fraction of the oil obtained from fresh plant material (artemisia alcohol (18.3%), yomogi alcohol (11.1%), camphor (9.3%) and artemisia ketone (8.0%)) were also found in the other investigated oils (longer storage period), their relative amounts varied significantly. The main component of the oils isolated 5 and 15 days after the plant material harvest was artemisia ketone (23.54 and 20.38%, respectively), however, in the oils obtained after 1, 3, 6 and 12 months of storage the most prevailing constituent was camphor (up to 27.2 %). The observed changes in the relative amounts of monoterpenes, their identity and number of detected components, strongly suggest that the main processes occurring during plant material storage, were the unequal evaporation of compounds from the plant material, and their chemical transformations (e.g. oxidation of artemisia alcohol to artemisia ketone).

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P- 52 Variability of Essential Oil Composition of *Santolina insularis* (Genn Ex Fiori) Arrig. Sardinian Endemic Plant

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Santolina insularis (Genn. ex Flowers) Arrig (Asteraceae) is an endemic species present in the Gennargentu area, in the center-oriental Sardinia and in the Iglesiente calcareous ground. The whole aerial plant or its essence, has been used in the past (1) as anthelmintic in children; moreover was used by the shepherds to curative purpose as vermifuge for the horses. Some literature references have been found on essential oil from *Santolina insularis* growing wild in Sardinia (2-4) but no studies were performed on variability of *S. insularis* essential oil growing wild in the different areas. This first screening was carried out collecting in the four main geographical areas where *Santolina* is growing wild (Marganai, Buggerru, Bruncu Spina and Urzulei). The fresh plant material was submitted to hydro distillation for 3 hours using a Clevenger-type apparatus according with Italian Official Pharmacopeas X. The oils were stored at –20°C until they were analyzed. For the identification of essential oil constituents GC/Mass analyses were carried out with a Hewlett Packard G1800B-GCD System using the same conditions and column used for GC analyses. Particularly the three constituents myrcene, β-phellandrene and artemisia ketone are the most representative compounds and are present in high concentration.
Considering the characterizing constituents we observe that myrcene can be useful to identify Buggerru essential oil where β-phellandrene is in very small amount, on the contrary, artemisia ketone allowed us to recognize Buncu Spina essential oil. This information can be used to identify the origin of the essence with a simple gaschromatographic analysis.

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P- 53 Yield and Quality of the Essential Oil from Greek Oregano (*Origanum vulgare* ssp. *hirtum*) Selected Populations Cultivated in Serbia and Greece

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Four Greek populations of *Origanum vulgare* ssp. *hirtum* were cultivated in two different agroecological conditions, in Pancevo (Serbia) and Macedonia (Greece). Experimental plots were established in both countries, in the year 2005 and in 2006, with nursery plants from selected homogeneous populations, in three replications. Cultivation measures applied on experimental plots were identical in both countries. The aim of testing was to estimate suitability of local agroecological conditions for large-scale cultivation of Greek Oregano, whose major essential oil characteristics have to be high yield and high content of carvacrol. Such a quality herb of Greek Oregano is highly appreciated as raw material for the production of phytomedicines. The yields of the essential oils were determined in a Clevenger-type apparatus, from air dried plant material harvested in the stage of full flowering. The quality of the distilled essential oils was determined by cross-correlation of results obtained by GC/FID and GC/MS analysis. Leading results of conducted testing are presented in the table.

Parameters registered	Essential oil content (%)		Carvacrol content (%)		Sum of thymol and carvacrol (%)	
Cultivation details	Greece	Serbia	Greece	Serbia	Greece	Serbia
Population 1	6.2	3.3	80.9	80.7	85.7	82.9
Population 2	7.0	4.5	80.9	79.2	83.9	81.0
Population 3	5.4	3.3	88.2	78.3	88.5	79.6
Population 4	6.4	5.0	82.1	77.0	86.0	77.2

Greek Oregano cultivated in local (Greek) agro ecological conditions gave higher yield of herbal drug (Origani herba), with significantly higher content and more appreciated composition of the oil in all the investigated cases.

P- 54 Effect of Essential Oils and Temperature on Storage Improvement of Sour Cherry

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Sour cherry (*Prunus cerasus* L.) is one of the important fruit in temperate zone of Iran. Hard storability of the fruit limits fresh consumption and therefore the main portion of sour cherry used in processsing industries. There are a few reports on the storage of sour cherry therfore the research was conducted to increase the storage periods of the fruit by the natural compound such as Clove and Black Zira essential oils and grape seed extract in two temperature regims (10 and 25°C). The treatments were 500 and 1000 ppm of each essential oils or grape seed extract as emulsion and essential oils fumigation as 1ml in each package with and without covered by plastic film. Before treatment, physical and biochemical properties such as fruit weight, TSS, pH and titrable acity (TA) of the fruits were tested. After treatment, each package was considered at 48 hour intervals for weight loss, fruit quality, stem browning, fungal spoilage and fruit surface shriveling. At the end of the experiment TSS, TA, and pH of treated fruit juice also were analysed. Our results shown that sour cherry fruits had good condition in ambient temperature (25°C) and in refrigerator temperature (10°C) after 2 and 4 weeks respectively. Our data also shown that low temperature (10°C) especially in covered boxes with plastic film in comparison with open boxes had positive effects on storability of the fruits. All natural compounds had significant effects on the measured factors during the experiment. Clove and Black Zira essential oils (500ppm) had the best results in all measured factors. Fumigation methods beacuse of the high concentration decrease fruit quality.

P- 55 Chemical Composition of the Essential Oil of Leaves of Avocado Tree Cultivated in Iran.

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Avocado (*Persea americana* mill.) (Lauraceae) is a tree. It is chiefly grown in temperate regions and sparsely grown in tropical regions of the world, native to the Central American region, is appreciated worldwide because of its special organoleptic characteristics and nutritional value (1-2). It is recommended for anemia, exhaustion, hypercholesterolemia, hypertension, gastritis, and gastroduodenal ulcer (3).The leaves have been reported as an effective antitussive, antidiabetic, and relief for arthritis pain by traditional medicine practitioners of Ibibio tribe in South Nigeria. These trees are cultivated in some regions of Iran. The leaves of avocado (*Persea americana*) were collected in February 2006 from Ramsar, Province of Mazandran, and North of Iran where the Avocado trees were cultivated. The essential oil isolated by hydrodistillation from the leaves of *Persea americana* tree was analyzed by means of GC and GC/MS. Thirty-five components representing 97.7% of the oil were characterized. Methyl eugenol (31.2%), β-caryophyllene (16.9%), estragol (9.0%), δ-cadinene (4.8%), β-pinene (4.2%) and α-pinene (3.2%) were found as the major components in the oil.

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P- 56 Caraway Essential Oil Influences Gut Flora Under In Vitro and In Vivo Conditions

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Preparations based on caraway (*Carum carvi* L., Apiaceae) and its essential oil (Carvi aetheroleum; EO) are very common alternative therapy for gastrointestinal disorders in our region and worldwide. The aim of this work was to investigate the effect of EO of caraway both on *Enterococcus* sp. and *E. coli*, as usual components of mammalian gut flora, presenting in vitro conditions, and on mice gut flora, as in vivo conditions. The chemical profile of EO was determined by gas-chromatography mass-spectrometry method (GC-MS) [1]. Dilutions of EO in n-hexane (20% and 50%) were tested in in vitro survey by hole-plate agar diffusion method [1]. In in vivo testing single dose of EO emulsion for p.o. application, adapted and calculated from recommended daily dose for man [2], was applied 4 h before sacrifying of animals and collecting the content of caecum, proximal and distal colon and faeces. The number of colony-forming-units (CFU) was estimated after the inoculation of 0.1 mL of appropriate dilution in plates and incubation (72 h, at 37°C) under aerobic and anaerobic conditions. According to the GC-MS, the most probably responsible components for observed effects of EO of caraway were carvone (78.8%) and limonene (10.1%). The 50% solution of EO inhibits growth of *Enterococcus* sp. and *E. coli* 30.67±9.23 mm and 17.4±2.79 mm, respectively, while the 20% solution of EO inhibits growth of *Enterococcus* sp. 20.75±15.43 mm and of *E. coli* 12.4±3.78 mm. Therapeutic dose of EO of caraway produced slight rise in CFU of mice gut samples under aerobic conditions (table; results are expressed as log10 CFU/g of sample).

	aerobic conditions				anaerobic conditions			
	caecum	colon		faeces	caecum	colon		faeces
		proximal	distal			proximal	distal	
control	10.4±0.9	10.5±1.0	10.6±1.1	10.9±1.0	10.2±0.4	10.5±0.6	10.8±0.8	10.9±0.5
essential oil	11.4±0.3	11.2±0.2	11.1±0.8	11.3±0.7	10.0±0.2	10.2±1.1	10.1±1.1	10.3±0.7

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P- 57 Research into Effects of Harvest Hour and Wait Times in *Rosa damascena* Mill. in the Quality of Rose Oil

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Several factors affect the yield and quality in rose farming. One of the significant factors affecting oil quality is harvesting hours and postharvest wait times. In this study, the effect of the harvest hours (05.00, 07.00, 09.00, 11.00, 13.00, 15.00, 17.00, 19.00, 23.00) and postharvest wait times (1, 7 and 14 hours) on the quality of *Rosa damascena* Mill. used in rose oil production were investigated. In order to determine the quality of oil, the rates of ethanol, citronellol, nerol, geraniol, phenyl ethyl alcohol, nonadecane+nonadecene, methyl eugenol, heneicosane and citronellol/geraniol were studied. Oil prolonged wilting periods (0, 12, 24 and 36 h), were shown to influence the oil composition (*Rosa damascena* Mill.) (1). In this study, the essential oils were hydrodistilled from fresh roses using a Clevenger apparatus. The oils were analysed using a gas chromatograph (GC). The citronellol, geraniol, nerol, phenyl ethyl alcohol and methyl eugenol content of oils were found to range between 10.2-48.8%, 1.6-29.3%, 0.3-9.7%, 0.2-1.5% and 0.6-4.8% respectively. In a previous study, it was proven that upon fermentation the citronellol to geraniol ratio increased (2). It was established that the rates of citronellol/geraniol, an important criterion in the determination of the quality of rose oil, changed between 0.6 and 15.3. Changes in the rates of citronellol/geraniol correlate well with harvest hours and wait times. The study clearly indicated that in order to obtain quality oil with higher yield it is necessary to pick flowers in early hours of the day and distillation should be affected without much delay.

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P- 58 Growth Performance and Carcass Composition of Pigs Fed on a Mixture of Essential Oils of *Thymus vulgaris*, *Rosmarinus officinalis* and *Allium cepa* L.

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A total of 160 crossbred pigs (PI x Hybrid) were assigned into two treatment groups according to bodyweight and sex. Pigs were housed in pens of 10 animals and fed on a basal diet (negative control; NC) or the basal diet supplemented with 100 ppm of the phytogenic feed additive AROMEX ME Plus (mixture of essential oils from thyme, rosemary and onion plus saponins from Quillaja; AME+). Grower feed was given from 20.7 to 47.5 kg and finisher feed was fed from 47.5 to 114.8 kg. The dietary supplementation of the phytogenic product significantly improved daily weight gain during the grower period (NC: 572 g, AME+: 635 g) and over the whole experiment (NC: 706 g, AME+: 733 g), while weight gain during finisher period was numerically increased (NC: 770 g, AME+: 784 g). Daily feed intake was slightly higher (NC: 1.91 kg, AME+: 1.96 kg) and feed conversion slightly lower (NC: 2.66 kg/kg, AME+: 2.63 kg/kg) in the phytogenic group compared with the control. Mortality was numerically reduced in the AME+ group (NC: 11 pigs lost during experiment, AME+: 4 pigs lost during experiment). Carcass composition (lean meat ratio, backfat thickness) was not affected by the dietary treatment. It is concluded that the phytogenic AROMEX ME Plus can be used for improvement of performance of fattening pigs. Positive effects on growth performance might be explained by improved digestibility of nutrient due to the stimulatory effects of the essential oils on digestive processes.

P- 59 Lavandin: Honey Volatiles in Comparison with Essential Oil

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Honey aroma is very complex, involving many tens of volatile compounds [1]. The analysis of volatile fraction of unifloral honey provides useful information for the botanical and geographical origin determination. Croatian Lavandin honey is generated mostly from the nectar of *Lavandula hybrida* Reverchon. Volatile compounds from three samples of unifloral Lavandin honey (pollen content > 20%) were investigated. The volatiles were isolated by ultrasonic solvent extraction (USE) and hydrodistillation (HD) and analysed by gas chromatography - mass spectrometry (GC-MS) on HP-20M column. The obtained results vary greatly upon the isolation method employed. USE is more suitable for the isolation of low and high molecular weight compounds providing potential biomarkers for unifloral honey origin determination [2,3]. Aliphatic and aromatic hydrocarbons, terpenes, phenylpropane derivatives and carboxylic acids were identified. The content of ubiquitous benzoic acid and phenylacetic acid was low that could be important for comparison with other unifloral honeys. Major qualitative differences were observed in comparison with the volatile profiles of *Lavandula stoechas*, *Lavandula angustifolia* and *Lavandula angustifolia* x *latifolia* honeys from Portugal and France [5]. Coumarin was also identified and it can be used as an indicator of honey freshness [5]. HD has main drawbacks because of the drastic isolation conditions (boiling water) that lead to the formation of artefacts (Strecker degradation and Maillard reactions). The isolated honey volatiles were compared with three samples of Lavandin essential oil obtained by steam distillation. Common monoterpene compounds among the honey volatiles and the essential oil were 1,8-cineole, *cis*- and *trans*-linalool oxide, camphor, linalool, terpinen-4-ol, borneol and geranyl acetate.

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P- 60 Volatile Constituents from Selected Liverworts

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Liverworts (Hepaticae) are estimated with ~8000 species in 380 genera and 74 families and traditionally separated two morphologically defined classes, Jungermanniopsida and Marchantiopsida [1]. They are considered to be oldest terrestrial plants although no strong scientific evidences have appeared in the literature [2]. Liverworts contain a wide variety of terpenoids and aromatic compounds which constitute the characteristic oil bodies, and occasionally produce their own peculiar dimeric compounds such as bisbenzyls, dimeric isocuparens, having interesting biological activity [3-5]. This current study is consisted of GC-mass spectrometry analysis of some selected liverworts such as *Porella platyphylla* (L.) Dum. (Jungermanniaceae, Porellaceae), *Scapania nemorosa* L. Dum. (Scapaniaceae), *Bazzania japonica* (Lac.) Lindb. (Lepidoziaceae), *Dumortiera hirsuta* (Sw.) Nees (Marchantiaceae), *Radula perrottetii* Gott. (Radulaceae), *Pellia endiviifolia* (Dicks.) Dum. (Dilaenaceae), *Wiesnerella denudata* (Mitt.) Steph. (Conocephalaceae) and *Conocephalum conicum* (L.) Corda. (Conocephaceae), growing in different locations. *Scapania* species contain many ent-sesquiterpenoids, labdane diterpenoids and phenolic compounds [5]. In addition, *Porella* species are a rich source of drimane, pinguisane, guaiane, pseudoguaiane, germacrane, aromadendrane and striatane sesquiterpenoids. *Porella platyphylla* is a nonpungent type of *Porella* species that produces a wide range of terpenoids [6]. So, this study also concerns with the identification of volatile components of the Japanese Liverworts as well as the some heavy compounds isolated from American *Scapania nemorosa* and French *Porella platyphylla*, Ecuadorian *Noteroclada*, *Symphyogyna*, Mexican *Asterella*, Kretan *Fossombronia*, and German *Pellia endiviifolia*. Structural elucidation of these compounds were elucidated by 1H and 13C-NMR, DEPT, 2D-NMR, and MS experiments besides the comparison of spectral data in literature.

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P- 61 Volatile Constituents of *Allium sphaerocephalon* L. Flower Heads

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Although formally classified in the family Liliaceae, represented by 280 separate genera and 4000 species, recent taxonomic revisions have seen members of the genus *Allium* placed in the family Alliaceae. Distributed throughout most regions of the temperate world including Europe, Asia, America and Africa, *Allium* species have a long history in common folklore and as sources of therapeutic principles. Volatile chemicals are produced throughout enzymatic hydrolysis of non-volatile sulfur storage compounds, termed S-alkenyl-L-cysteine sulfoxides [1] that can be additionally recombined into approximately 50 sulfur compounds. Given such chemical diversity, members of the genus *Allium* deserves much attention as a possible new source of biologically active compounds. The subject of this study was set to analyze the hydro-distilled essential oil of *Allium sphaerocephalon* L. flower heads (by GC and GC-MS analysis). The essential oil of foul odor obtained in 0.0074 % (w/w) yield was characterized by the presence of terpenoids (68.9%), sulfur containing compounds (21%) and fatty acid derived compounds (3.0%). The terpenoid fraction mainly consisted of sesquiterpenoids (hydrocarbons 26.1%, and oxygenated derivatives 37.3%), while the monoterpenoids were represented by 5.5% (hydrocarbon monoterpenes were detected in trace amounts only). The most abundant constituents of terpenoid origin were 6-α-hydroxy-germacra-1(10),4-diene (12.3%) and α-cadinol (9.3%). 3,5-Diethyl-1,2,4-trithiolane (7.2%), the most dominant sulfur containing compound, could be responsible for the specific unpleasant olfactory sensation.

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P- 62 Head-space Volatiles of Marula (*Scelerocarya birrea*, subspecies *caffera*)

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Of all fruit trees indigenous to South Africa, the marula has received most attention in terms of domestication and commercialization [1]. Several products such as beer, juice, jam and jelly have been developed and successfully marketed, the most recent being a marula liqueur [2]. Despite the traditional and commercial uses the flavour constituents remain poorly studied. Headspace volatiles of the fruit pulp and the whole fruits were investigated using solid phase micro-extraction (SPME) and GC-MS. The two major compounds in the fruit pulp are β-caryophyllene (91.3%) and α-humulene (8.3%). Twenty eight volatiles representing 86.9% of the total composition were identified in the headspace of the intact fruits. Heptadecene (16.1%); benzyl 4-methylpentanoate (9.3%), cyclo-pentendecane (6.3%) and (*Z*)-13-octadecenal (5.6%) were present in levels higher than 5%. (*Z*)-3-Nonen-1-ol (8.6%) described as “fresh mushroom waxy green melon” was the major alcohol detected in the headspace of the whole fruits.

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P- 63 Seed Oil Content and Composition of Different Iranian Accessions of *Anethum graveolens* L.

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Dill (*Anethum graveolens* L.) is an aromatic, spice plant that belongs to the Apiaceae family. It is a cool temperate plant native to Europe and cooler regions of Asia but extremely adaptable to different environmental conditions (3). Dill is grown in home gardens in every part of Iran (2). Seeds and herbs are used as culinary spices. The object of this work was to study the essential oil composition of seeds in plants growing in different parts of Iran. Thirteen accessions were kindly prepared from local gardeners. The oils were extracted by hydro distillation and their components were identified using GC and GC-MS (1). There was considerable variation in essential oil content and composition. Oil yield was varied between 0.16-1.8 % w/w. Major and most variable components of oils were; carvone (37.0-55.6%), limonene (21.5-45.8%), dihydrocarvone (5.2-14.9%) and dill-apiole (0.1-19.2 %).

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P- 64 Essential Oil Content and Chemical Composition of *Astrodaucus persicus* Boiss Cultivated in Iran

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Astrodaucus persicus Boiss (Syn. *Daucus persicus*) is an annual herbaceous plant belonging to the family Apiaceae [1]. It is native to Mediterranean region and wildly grown on temperate region of Iran such as north, northeast and northwest of Iran. Its height is 40-120cm depending on the place of cultivation [2]. Content and composition of the essential oil of *Astrodaucus persicus* cultivated in Iran have been studied. Oil was prepared by hydro-distillation and analyzed by GC and GC/MS. The oil content was 0.8% w/w. Eighteen components were identified from the oil. The major components were geranyl acetate (39.0%) a-pinene (13.2%), sabinene (12.9%), trans methyl isoeugenol (7.9%), myrcene (7.2%), carotol (4.4%) and limonene (3.8%).

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P- 65 Composition and Antimicrobial Activity of the Rhizome Essential Oils of Two *Athamanta Turbith* Subspecies

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In this study, the rhizomes essential oils of *Athamanta turbith* ssp. *hungarica* (Borbás) Tutin and *A. turbith* ssp. *haynaldii* (Borbás & Uechtr.) Tutin (Umbelliferae) [1] were analysed by GC-FID and GC-MS. Twenty-one compound were identified in the oil from *A. turbith* ssp. *hungarica* (sample 1), and thirty-three components in the oil from *A. turbith* ssp. *haynaldii* (sample 2). Both oils were characterized by the high amount of phenylpropanes (88.0% and 72.6%, in sample 1 and 2, respectively), with myristicin (54.2 and 22.3%, respectively) and apiole (32.6 and 48.5%, respectively) being the main components. The content of monoterpenes in sample 2 was 15.7% (oxygenated 11.9%), while this class of compounds was present only in traces in sample 1. In the both oils sesquiterpene hydrocarbones were present in higher amounts (ca. 11.0%) compared to oxygenated once (0.1-0.7%). Besides the dominant phenylpropane components, germacrene B (3.6%) and γ-muurolene (2.9%) in sample 1, and terpinolene (11.9%) and δ-amorphene (4.1%) in sample 2, represented the most abundant compounds. The studied essential oils exerted similar antimicrobial activity, with best effect detected against *Micrococcus luteus* and *Klebsiella pneumoniae*.

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P- 66 Chemical Composition and Antibacterial Activity of the Essential Oil of *Carum montanum* from Algeria.

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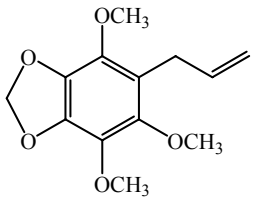
Carum montanum (Coss. et Dur.) Benth. et Hook. Syn. *Selinopsis montana* Coss. et Dur. (Apiaceae) is a species endemic to North Africa. In this study, the aerial parts of *Carum montanum* growing wild in Algeria were hydrodistilled, and the resulting essential oil was analysed by GC and GC-MS. The composition of the oil was dominated by nothoapiole (68% GC), a rare, highly oxygenated phenylpropanoid which has been already identified in few essential oils [1-4], but never in such a high amount. This compound was isolated by column chromatography, and its NMR spectral data were fully described, with 1H and 13C signals attribution by NOESY and 2D NMR. Additionally, the antibacterial activities of the total oil and pure nothoapiole were evaluated against several strains of Gram + and Gram – bacteria. An interesting selectivity was evidenced as both proved to be totally inactive against all the strains tested except on some bacteria from *Staphyloccus* genus.

Nothoapiole :



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P- 67 Essential Oil Analysis and Antimicrobial Investigation of *Cymbocarpum erythraeum* (D.C.) Boiss.

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This study aimed to analyse the essential oil of *Cymbocarpum erythraeum* (D.C.) Boiss. (Umbelliferae) and to evaluate its antibacterial and antifungal activity. Aerial parts of this plant were collected from west Azarbaijan of Iran in August 2004. Air dried parts of the plant were ground and subjected to hydrodistillation by using a Clevenger type apparatus [1] to give a yellow oil in 1.1% v/w yield. 24 components were identified in this essential oil, through combination of GC and GC/MS. The major components in essential oil of *C. erythraeum* were: 2 –decanal (56%), l-decanal (14%), n-decanol (8.1%), n–octanal (2%), 3-dodecan-l-al (4.9%), dodecanal (2.4%). Cup plate method [2] was used for the antimicrobial test. MIC (minimum inhibitory concentration) values were shown 4.8mg/ml to 38.04mg/ml. The MIC values were determined to be: 4.8mg/ml against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and 38.04mg/ml against *Bacillus subtilis*. The MIC value for *Candida albicans* determined to be 4.8mg/ml. The essential oil showed no significant activity against *Pseudomonas aeruginosa* and *Aspergillus niger*.

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P- 68 Essential Oil from *Daucus carota* ssp. *carota* Grown in the Wild in Vienna

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Wild carrot (*Daucus carota* ssp. *carota*, Apiaceae) is widely distributed in Europe and occurs on dry meadows, along roadsides and on slopes. It is present throughout the municipal area of Vienna [1]. The present study reports the composition of the essential oil from different plant parts of the wild carrot collected at two locations in the urban area. At one site the plants could be collected during two consecutive years. The essential oils were obtained by hydrodistillation in a Clevenger type apparatus and were analysed by GC/MS. At harvest time the plants had fully developed fruits and gave oil yields of 0.2%, 0.1-0.3% and 0.8-1.6% (v/w) for roots, leaves and fruits, respectively. The root oil was dominated by terpinolene (26% and 56% at one site on two consecutive years) and contained beside α-pinene, β-pinene and myrcene also the phenylpropanoid myristicin. Leaves had α-pinene and sabinene as main compounds, followed by myrcene and limonene. Also in the fruits α-pinene and sabinene dominated, but in one year also an appreciable amount of geranyl-acetate could be recorded. The results confirm the great variability of essential oils in carrots [2-4].

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P- 69 Antimycobacterial Activity of Essential Oil of *Daucus littoralis* Sibth. & Sm. (Apiaceae), from Turkey

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WHO estimates that between 2000 and 2020, nearly one billion people will be newly infected, 200 million people will become sick, another 35 million people will die from TB if it can not be taken under control (1-3). The problem is arising due to the fact that Multidrug-Resistant Tuberculosis (MDR) patients are resistant to isoniazid, rifampicin and other antituberculosis drugs (4, 5). Thus new therapeutic agents are required urgently (6). In the course of screening for antimycobacterial activity, we have detected significant activity in the essential oil of *Daucus littoralis* Sibth. & Sm. Composition of the oil was characterized by gas chromatographic (GC) and gas chromatographic/mass spectrometric (GC/MS) analyses. The genus *Daucus* (Apiaceae) is represented in Turkey by 6 species, one being endemic. *Daucus carota* L. which is a well known species whose roots are used as food and its fruit oil is used in perfumery [7, 8]. Air dried aerial parts of *D. littoralis* were subjected to water distillation using a Clevenger type apparatus. The essential oil yield was 0.2% on dry weight basis. The main compound in the oil was found as cis-chrysanthenyl acetate (46.8%). The aim of this study has been to determine new potent antimycobacterial compounds for the treatment of tuberculosis. This is the first report on the chemistry and antimycobacterial activity of *D. littoralis*. In vitro evaluation of antimycobacterial activity was carried out using the MGIT Mycobacteria Growth Indicator Tube, 7H9 Broth Base (Becton Dickinson). Essential oil of *D. littoralis* and a standard drug, streptomycin, were assayed against *M. tuberculosis* ATCC 25177 (H37Ra) and the growth of *M. tuberculosis* was observed using a 365 nm UV reader. It showed significant activity (MIC 196 µg/ml) against *M. tuberculosis*.

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P- 70 Essential Oil Composition of the Different Parts of *Eryngium corniculatum* Lam. from Spain

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The *Eryngium* L. genus belongs to the Apiaceae family and, with about 250 species, has a cosmopolitan distribution. On the Iberian Peninsula grow 14 of the 26 species described in Flora Europaea. *Eryngium corniculatum* Lam. is an annual or biennial species endemic to this region, which grow in the margins of seasonal lakes and riverbeds. The essential oil from the different parts (stems + leaves, inflorescences and roots) of this species, gathered in Guadalajara (Spain) has been extracted by steam distillation and analysed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MS).
Although quantitative and qualitative differences have been found between the analysed parts, all of them contained the same principal compound, 2,4,6-trimethyl benzaldehyde, with a percentage composition of 50.0%, 50.8% and 29.8% for stems + leaves, inflorescences and roots respectively. Besides, other representative constituents of the oil were similar in the different fractions: the stems + leaves oil showed 2,4,5-trimethyl benzaldehyde (3.8%), α-pinene (3.4%), (E)-nerolidol (2.4%) and (2Z,6E)-farnesol (2.1%), while in the inflorescences fraction where found to be chrysanthenyl acetate (4.0%), α-pinene (4.0%), 2,4,5-trimethyl benzaldehyde (3.3%), (2Z,6E)-farnesol (2.0%), (E)-nerolidol (2.1%) and (Z)-β-ocimene (2.1%), and in the roots oil phyllocladene (13.0%), (E)-nerolidol (9.4%), β-eudesmol (4.1%) and (2Z,6E)-farnesol (2.1%). As far as we know this is the first report about the chemical composition of this Iberian Peninsula endemic species. It is worth mentioning the presence of monoterpenes as main fraction for an *Eryngium* species.

P- 71 A Comparison Between the Content and Chemical Constituents of the Essential Oil, Solvent Extract and Hydro-distillation Extract of *Ferula gumosa* gum

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Persian galbanum (*Ferula gumosa* Boiss) belongs to the Apiaceae Family. The gum, which is accumulated in the roots, occurs as a soft, sometimes almost semi liquid mass [1]. It is used for manufacturing of transparent liquid glues. Its ingredients are used as stabilizer in perfumes and also applied in hygienic and cosmetic industries [2]. Comparison between hydro-distillation and chemical extraction by hexane on the gum of Persian galbanum (*Ferula gumosa* Boiss) was investigated. According to the results, methods of essential oil extraction of *Ferula gumosa* gum significantly affected on its essential oil content and constituents. The essential oil content of gum extracted by hydro-distillation method was 33.0% and thirty-four constituents were identified by GC and GC/MS. The major components were β-pinene (76.3%), 1-8-cineol (4.0%) and β-phellandrene (3.3%). The essential oil content of gum extracted by hexane was 36.0% and twelve compositions have been analyzed by GC and GC/MS in the oil and the main components were β-pinene (64.3%), bulnesol (5.9%), guaiol (3.7%0), α-bisabolol (3.3%), myrcene (3.3%) and β-phellandrene (3.1%).

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P- 72 Chemical Composition of Essential Oil of *Ferula orientalis* L. from Iran

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The genus *Ferula*, which belongs to the Umbelliferae family, is widespread in the Mediterranean area and Central Asia [1]. The flora of Iran comprises 30 species of *Ferula* of which 15 are endemic [2,3]. The resins of the plants have been used in china as a remedy for malaria and dysentery and also as an insecticide [4]. Other medical uses are also reported such as sedative, antispasmodic, against teeth pain, asthma, cough, epilepsy, fever, irritable colon, as an antihysterie, for feminine sterility and against rheumatism [5-7].

Air-dried aerial parts of the *F. orientalis* were ground and subjected to hydrodistillation, for 4 hours, using a Clevenger-type apparatus to produce yellowish oil in 0.35% (w/w) yield based on dry weight. The composition of the essential oil from aerial parts of *Ferula orientalis* has been analyzed by GC and GC/MS. The constituents of the essential oil were identified by comparison of theire mass spectra and retention indices (RI) with those given in the literature and those authentic samples [8]. Sixty-four components were identified in the essential oil of *F.orientalis*, representing 86.5% of the oil, of which the 2-ethyl-1-octen-3-one (13.9%), cis- verbenol (9.0%), caryophyllene oxide (5.0%) , p-mentha-1,5-dien-8-ol (2.4%), myrtenol (2.4%), were found to be the main constituents.

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P- 73 Composition and Antibacterial Activity of the Essential Oil of *Ferulago confusa* Velen.

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Water-distilled essential oil from crushed fruits of *Ferulago confusa* Velen , collected from Bursa: Keles- Orhaneli in Turkey, was analyzed by GC and GC/MS. Ninety one components were identified representing 99.5 % of the oil. Cis-chrysanthenyl acetate (37.7 %) and α-pinene (36.7 %) were characterized as main constituents.
The antibacterial effects of the *Ferulago confusa* oil were evaluated by using microdilution broth method. The oil showed moderate inhibitory effects on the selected human pathogenic bacteria having MIC values 0.6 to 2.5 mg/mL.

P- 74 Antioxidant Activity of the Essential Oils Isolated from Seeds and Aerial Parts of *Foeniculum vulgare*

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Foeniculum vulgare essential oils were isolated, by hydrodistillation, from the dried aerial parts and seeds of commercial source, with different distillation times: 30min, 1h, 2h and 3h. The essential oils were analysed by GC and GC-MS. The antioxidant ability was followed by three distinct ways: thiobarbituric acid reactive species (TBARS) method, reduction of the stable radical DPPH (2,2-diphenyl-1-picryl-hydrazyl) method, and deoxyribose assay method for scavenging of hydroxyl radical.
Trans-Anethole (31-36%), α-pinene (14-20%) and limonene (11-13%) were the main components of the essentials oil isolated from commercial *F. vulgare* aerials parts, whereas methyl chavicol (79-88%) was dominant in the seeds oils.
Considering the concentrations tested (100 – 2000mg/L), the essential oils obtained after 1h of hydrodistillation showed the best antioxidant activity: 73% at 750mg/L for aerial parts and 80% at 1000mg/L for seeds.
With the DPPH method and using concentrations between 1400 and 24600mg/L, the plant oils had better activity than the seed oils. The plant oils isolated for 30min and 1h showed higher anti-radicalar efficiency (AE) than the respective seed oils. In this case, the best activity was found with the oils isolated for 2h and 3h.
Generally the ability of the oils for scavenging OH radicals increased until 750-1000mg/L (100 – 2000mg/L). The maximal activity (48%) was obtained with the 30min distillation seed oils. For plant oils, the best activity (44%) was obtained with the oils isolated for 2h.

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P- 75 Chemical Composition of the Essential Oil from Flowers, Stems and Leaves of *Malabaila secacul* (Miller) Boiss. from Iran

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Malabaila secacul, which grows in Armenia, Iraq and Syria, besides Iran is one of five perennial species of the genus *Malabaila* (family Umbelliferae) are found in Iran among these three are endemic [1]. Only one investigation has been carried out on the chemical composition of the essential oils of the genus *Malabaila*, and this deals only with the fruits of *Malabaila suaveolens* Coss. [2]. To the best of our knowledge this is the first report on the essential oils from different parts of *Malabaila secacul*. The aim of our study is to compare the yields and to identify the constituents of the essential oils of flowers, stems and leaves of *Malabaila secacul* (Miller) Boiss. growing wild at the flowering stage in Northeast of Iran. The colorless oils were obtained by 3-hours' hydrodistillation, using a Clevenger-type apparatus, of the flowers, stems and leaves in yields of 0.073%, 0.036% and 0.022% (w/w), respectively. GC and GC-MS revealed 20 compounds, representing 97.7% of the flower oil. The major constituents were hexyl-3-methyl butyrate (26.3%), β-elemene (15.8%), hexyl-2-methyl butyrate (14.9%) and hexyl butyrate (10.6%). The stem oil of the plant was characterized by higher amounts of β-elemene (18.5%), β-selinene (17.5%), germacrene (16.0%), germacrene B (9.2%) among the fourteen detected components, which together comprised 87.9% of the total oil. Nineteen compounds, representing 68.9% of the leaf oil, of the plant were identified. Among these, β-elemene (24.9%), caryophyllene oxide (10.8%) and humulene epoxide II (5.0%) were the major ones.

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P- 76 Chemical Composition of the Essential Oil from Flowers, Stems and Leaves of *Peucedanum aucheri* Boiss. from Iran

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Peucedanum aucheri is one of the 21 species of the genus *Peucedanum* (family Umbelliferae) family found in mountains and forests of Iran, and one of the 11 species that are endemic [1]. Although there have been some investigations of the chemical compositions of essential oils from species *Peucedanum* [2-4], this, to the best of our knowledge, this is the first report on the essential oils from different parts of *Peucedanum aucheri*. The aim of our study is to compare the essential oil yields and to identify their constituents in the flowers, stems and leaves of *P. aucheri* Boiss. growing wild at flowering stage in Northeast Iran. The pale yellowish colored oils were obtained by 3-hours' hydrodistillation, using a Clevenger-type apparatus for 3 hours, of the flowers, stems and leaves, in yields of 0.49%, 0.64% and 0.27% yield (w/w), respectively. GC and GC-MS analysis revealed 18 compounds, representing 98.2% of oil, in the flower. Of these, α-phellandrene (27.2%), β-phellandrene (16.6%), α-pinene (12.2%), p-cymene (10.3%) and δ-3-carene (7.8%) were the major constituents. Eighteen compounds representing 98.1% of the stem oil of the plant were identified. Of these α-pinene(21.2%), α-phellandrene (18.1%), p-cymene (17.7%) and β-phellandrene (15.8%) were present in largest amounts. The leaf oil of the plant was characterized by higher amounts of α-phellandrene (20.7%) , α-pinene (16.8%), β-phellandrene (16.2%) and p-cymene (14.6%), among the seventeen detected components that comprised 97.2% of the total oil. In the oils of all parts of the plant, monoterpenes and monoterpene hydrocarbons predominated over oxygenated monoterpenes.

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P- 77 GC-MS Analysis of the Composition of the Essential Oil and Headspace from Aerial Parts of *Peucedanum tauricum* during Development

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The aim of this work was the analysis of the essential oil and headspace of fresh aerial parts (leaves, flowers and fruits) of *Peucedanum tauricum* (Apiaceae) obtained by hydrodistillation and headspace solid-phase microextraction (SH-SPME). Analyses were performed by GC-MS FID (70 eV). Changes in the composition of the essential oil and the headspace were found related to the time of development and the part of the plant [1]. The presence of some sesquiterpenoids: α-guaiene, guaia-6,9-diene, guaia-1(10), 11-diene and guaia-9,11-diene, previously detected and isolated from fruit of the *P. tauricum* [2, 3] can chemotaxonomically characterize the investigated plant oil with respect to those from other plants from the genus *Peucedanum* L..

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P- 78 Composition of the Essential Oils of *Pimpinella anisactis* Rech.f. and *Pimpinella saxifraga* L. From Iran

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The Iranian flora comprises of *Pimpinella*, 6 of them are endemic. Some *Pimpinella* species are used as flavouring agents and for medical purposes, e.g., Anise (*Pimpinella anisum* L.) is an annual, herbaceous aromatic plant, which is native to the eastern coast of Mediterranean to Asia Minor region. The essential oil from *Pimpinella anisum* fruit has antiseptic, antispasmodic, carminative, digestive and fungicide effects. It is also used in perfumes, toothpaste and liquor industry.

The essential oils obtained by hydrodistillation of the aerial parts of *Pimpinella anisactis* Rech .f. and *Pimpinella saxifrage* L., were analyzed by GC and GC/MS. The oil of *P. anisactis* was characterized by higher amounts of decanal (34.5%) and decanol (14.1%), which were among the thirty components comprising 96.3% of the total oil detected. Twenty four compounds representing 90.0% of the oil of *P. saxifraga* were identified, being trans-α-bergamotene (20.1%), β-sesquiphellandrene (10.8%) and β-bisabolene (10.1%) the major ones. The composition of both oils differs quantitatively and qualitatively.

P- 79 Quantitative and Qualitative Study of Components in Essential Oil *Pimpinella anisum* L.

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Anis is the very important medicinal plants which use in different food and medicinal industries. Dry fruit on anis (*Pimpinella anisum* L.) was collected from Tabriz in summer and its oil was obtained by steam distillation in 2.7% w/w yield. Chemical composition of essential oil was identified by GC and GC/MS. Among 11 compounds identified, the major components were trans-anethol (more than 92%). Other constituents were involved 8% of the oil that the most important of them are: - zingiberene (0.94%), eugenil acetate (3%), γ-gurjunene (1.75%) and estragol (1.2%).

P- 80 Study on the Chemical Compositions of the Essential Oils of Stems, Leaves and Flowers of *Prangos acaulis* (DC) Bornm

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The chemical composition of the essential oils obtained of stems, leaves, and flowers of the *Prangos acaulis* at flowering stage were isolated by hydrodistillation and investigated by GC/MS. A total of ten compounds constituting 97.6% of stems oil, seventeen compounds constituting 99.5 % of leaves oil, and twenty one compounds constituting 98.1% of flowers oil have been identified and quantified. The major components of stems oil were 3-ethylidene-2-methyl-1-hexen-4-yne (56.8%) and α-pinene (34.2%) The major components of leaves oil were α-pinene (39.5%), 3-ethylidene-2-methyl-1-hexen-4-yne (37.9%) and α-terpinene (10.9%) and the major components of flowers oil were α-pinene (25.1 %), 3-ethylidene-2-methyl-1-hexen-4-yne (23.5 %), α-terpinene (17.2 %) and limonene (13.6 %).

P- 81 Chemical Composition and Antibacterial Activity of the Essential Oil of *Prangos ferulacea* (L.) Lindl.

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The genus *Prangos* (Umbelliferae) involves about 30 species [1]. Various species of this plant have been widely distributed in the brackish regions of Iran. The common applications of *Prangos* species are as emulient, carminative [2], tonic antiflatulent, anthelmintic, and antibacterial agents [3-4]. A literature survey reveals that steam distilled from aerial parts and seeds [5] and hydrodistilled fruits [6] of *Prangos ferulacea* have been the subjects of some reports. However, present work showed different constituent types and percentages for the aerial parts components of *Prangos ferulacea* in comparison with previous reports.

The homogeneous coarse powder of air-dried aerial parts (150 g) of *Prangos feulacea* was subjected to hydrodistillation by using a Clevenger-type apparatus for 3 h. The obtained volatile oil was dried over anhydrous sodium sulfate and kept under nitrogen in a sealed vial at -10 ˚C. Afterwards, injection a 0.2 microliter portion of the hydrodistilled essential oil to a GC/MS instrument led to a total of 27 compounds among which 21 ones were identified comprising 96.23% of the oil composition. The major compounds were β-phellandrene (20.4%), α-terpinolene (15.3%), α–pinene (11.6%), δ-3-carene (11.1%), -phellandrene (9.1%) and trans-β-ocimene (9.7%).

In addition, determination of the antibacterial activity of the oil was carried out according to the agar well diffusion method [7]. The essential oil of the plant showed antibacterial activity against *E. coli* (PTCC1330) and S. saprophytacus (PTCC1113) with minimum inhibitory concentration (MIC) values of 20.48 and 8.19 g/ml, respectively. On the other hand, the oil did not show any activity against *B. cereus*.

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P- 82 Volatile Constituents of Different Parts (aerial parts, fruits and roots) of *Scandix australis*

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The genus *Scandix* L. (Umbelliferae) comprises 9 species and subspecies in Europe [1]. Six of these taxa i.e. *S. australis* subsp. *australis*, *S. australis* subsp. *grandiflora*, *S. pecten-veneris* subsp. *pecten-veneris*, *S. pecten-veneris* subsp. *brachycarpa*, *S. pecten-veneris* subsp. *macroryncha* and *S. stellata* are distributed in Greece. *Scandix* species are known under the vernacular name myronia [2] and young plants are used in Greek traditional cooking. In the present study the essential oil composition of different parts of *S. australis* was investigated. The plant material was collected from Kalymnos island (Greece) in April 2007. *S. australis* s.l. is an annual herb with 1 to 3-pinnate leaves, 1-3 rays, bracteoles ovate to narrowly oblong-ovate, often membranous margined, flowers with white petals and fruits somewhat compressed laterally with a beak at least twice as long as the seed-bearing part [1]. The essential oils of the fresh aerial parts, the ripe fruits and the roots were analyzed separately by means of GC-FID and GC-MS. All oils were characterized by the abundance of phenylpropanoids. Methyl chavicol (66.5%), methyl eugenol (10.3%) and (Z)-β-ocimene (10.2%) were the main components in the oil from the aerial parts. Methyl chavicol (89.9%) was also the primary constituent in fruit oil while in root oil methyl eugenol (63.0%) was the dominant component.

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P- 83 Composition of *Tordylium aegyptiacum* (L.) Lam. Essential Oil

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Umbelliferae is one of the most important families of flowering plants with aromatic herbage. The genus *Tordylium* L. (Umbelliferae) is represented by 16 species including 6 endemic species in Turkey [1-3]. *Tordylium aegyptiacum* (L.) Lam. (Syn.: *Hasselquistia aegyptiaca* L.) grows in roadsides, fields, waste places as an annual plant in 25-60 cm height with very attractive fruits. It was collected in southern Turkey during the fruiting period in Antakya. Crushed fruits were subjected to hydro-distillation to yield oil in <0.1 yield. The essential oil was analyzed by GC and GC/MS. In total, sixty compounds were characterized, representing 95.6 % of the oil. The main constituents were determined as hexadecanoic acid (40.1 %), β-caryophyllene (10.6 %), octyl octanoate (8.8 %) and caryophyllene oxide (8.5 %). Phytochemical and biological activity studies are quite limited on these species. The essential oils of *T. apulum*, *T. pestalozzae*, *T. pustulosum*, *T. lanatum*, *T. trachycarpum*, *T. hasselquistiae*, *T. ketenoglui* growing in Turkey have been analyzed by our group [4-7]. The compositions of essential oils obtained from *Tordylium* L. species of Turkish origin studied up to now comprised octyl esters, octanol and hexadecanoic acid as predominant compounds similar to those in *T. aegyptiacum* oil. However, *T. aegyptiacum* oil contains high amount of sesquiterpene hydrocarbons such as β-caryophyllene and caryophyllene oxide as different from the other Tordylium essential oils. This is the first report on the essential oil of *T. aegyptiacum*.

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P- 84 Essential Oil Composition of *Trachymene incisa* subsp. *incisa* Rudge from Australia

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The Apiaceae is a large cosmopolitan family with many well-know crops species, such as edible carrot *Daucus carota* L., celery *Apium graveolens* L., or parsnip *Pastinaca sativa* L. [1]. In Australia this family is widely represented with a total of 42 genera which contain a large number of species and subspecies, most of them endemic to this huge continent [2]. Within the genus *Trachymene* around 55 species have been described being endemic to Australia 37 of them. The diversity of this genus has been recently studied in detail by Hart and Henwood [3]. *Trachymene incisa* is a perennial herb widespread in forests and woodlands in eastern Australia. Nowadays *T. incisa* contains two subspecies that can be confused although they differ in their fruits and distribution. While subspecies *incisa* shows mericarps dull, smooth or tuberculate to papillate, subspecies *corrugate* has mericarps shining, smooth or tuberculate [3]. The subspecies *incisa* is particularly variable in terms of the size, shape and indumentum of the leaves and it is likely that the subspecies contains more than one taxon.

Trying to clarify this variability the leaves oil composition of three populations of typical *T. incisa* subsp. *incisa* and two populations of a particularly large, hairy variant from the Northern Tablelands on New South Wales have been extracted by hydrodistillation and analysed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MS). Preliminary results show sesquiterpenes as main compounds of their essential oil. The principal compound in the different samples analysed have been identified as β-selinene and bicyclogermacrene. An exhaustive study should be done to test if the chemical and morphological variations could be caused for environmental factors.

With this research we continue with our previous studies about the chemical composition of the Apiaceae family in Australia (4-6) and contribute to the best knowledge of *Trachymene incisa* subsp. *incisa*.

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P- 85 Essential Oil Composition and Antimicrobial Activities of Three *Achillea* Species

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Achillea L. (Asteraceae) is presented for a wide circumscription with 140 species throughout the world [1]. According to recent studies on the Turkish *Achillea* genus, that is represented by 44 species (49 taxa) of which 22 are endemic to Turkey [2-4]. For therapeutic means especially *A. millefolium* L. (Yarrow) has been used as anthelmintic, anti-inflammatory, astringent, choleretic, antispasmodic, antiviral, diuretic, analgesic and antihaemorrhagic [5, 6].
Flowering aerial parts of *Achillea magnifica* Hub.-Mor., *A. sipikorensis* Hausskn. and *A. membranacea* (Labill.) DC. (endemic species for Turkey except *A. membranacea*) were hydrodistilled to obtain essential oils that were then analysed by GC and GC/MS. The main components of the oils are shown in the table below.
The antimicrobial activities of the oils were evaluated by using micro-dilution broth method. Achillea magnifica oil showed a strong inhibitory effect on *Streptococcus epidermidis* with a MIC value 0.0652 mg/ml.

Essential Oil	Main Components %
A. magnifica	myrtenol; 9.5, myrtenal; 7.9, hexadecanoic acid; 7.1, β-pinene; 5.4
A. sipikorensis	camphor; 16.0, 1,8-cineole; 15.9, caryophyllene oxide; 6.7
A. membranacea	hexadecanoic acid; 33.9, nonacosane; 11.1, tetradecanoic acid; 7.8, heptacosane; 6.9

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P- 86 Composition and Antimicrobial Activity of *Achillea coarctata* Essential Oils

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Achillea coarctata Poir. is a perennial herb with yellow ligules growing in dry hilsides and sandy oils, distributed from S.E.Europe eastwards to S. W. Ukraine [1]. Aerial parts of *A. coarctata* were collected from Greece during the flowering stage, in July 2001. The plant material was separated in flowerheads (sample 1) and leaves (sample 2); the two samples were subjected to hydrodistillation and analyzed by GC and GC-MS. The main compounds in flowerheads and leaf oils were 1,8-cineole (26.9% and 29.1% respectively), camphor (22.1%, 9.2%) and borneol (5.0%, 6.8%). Both oils were characterized by the abundance of monoterpenes (69.4% and 65.5%, respectively). The microbial growth inhibitory properties of the isolated essential oils were determined against the microorganisms *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12288), *Micrococcus luteus* (ATCC 10240), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (NCIMB9111), *Enterococcus faecalis* (ATCC 29212), and the yeast *Candida albicans* (ATCC 10259 and ATCC 24433).

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P- 87 Quantitative and Qualitative Analysis of Wild *Achillea eriophora* Essentials Oil from Iran

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Achillea is one of the most important genera of the Asteraceae family. There are 19 species of this genus in Iran. *A. eriophora* is a rare species of *Achillea* that is endemic to Iran and grows exclusively in Southern provinces of Iran (especially in “Fars” South East of Iran). It contains a high level of essential oils in comparison to other *Achillea* species. In this study, in order to identify the essential oils content and components of the species, top flowering part of the plants collected from “Jahrom” (South of “Fars” province) and after drying its essential oil was extracted by „ \f „n“ Clevenger” apparatus. Then its chemical compounds were identified by GC-MS. The amount of essential oil in this species was 2% (v/w dry weight basis) and more than 80% of constituents were identified successfully. The main components were: camphor (30.4%), 1,8-cineol (25.2%), camphen (7.2%) α-pinene (4.5%).

P- 88 Essential Oils Analysis of Wild Populations of *Achillea wilhelmsii* koch. from Two Ecological Conditions of Iran

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Achillea wilhelmsii Koch. is an aromatic plant belonging to Asteraceae family that has a relatively wide distribution area in different parts of Iran. In this study, we compare the essential oils contents and constituents in two wild populations of two different ecological conditions (Fars and Khorasan Provinces, South West and North East of Iran respectively). The blooming herbs were collected and after drying, essential oils of the samples were extracted by "Clevenger apparatus". Essential oils constituents were determined by GC and GC-MS. Our results showed that the "Khorasan" population of *Achillea wilhelmsii* contains more essential oil than the "Fars" population (0.65 and 0.2 % v/w respectively). Thirty components of the "Khorasan" population that were 96.94% of total component determined successfully and the main components were: camphor (19.1%), cembrene (10.0%), 1,8-cineole (8.8%), α-pinene (8.1%) and linalool (7.5%). Thirty-four components (91.9%) of "Fars" wild population was determined and the main components were: isopentyl-isovalerate (9.5%), $\alpha-$pinene (8.8%), 1,8-cineole (8.7%), eudesmol (10-epi-gama) (5.6%), spathulenol (4.9%). In conclusion there are differences between these two populations as essential oils content and constituents as concerned.

P- 89 Characterisation of *Achillea* Populations Based on Morphological, Cytological and Chemical Traits

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A taxonomical evaluation of 4 taxa of Hungarian wild origin and 2 varieties of the *Achillea millefolium* aggregate, was carried out to determine their potential for breeding and cultivation in Hungary. We used a complex approach including morphological, cytological (microscope, flow-cytometry) and chemical (essential oil GC-MS) aspects.
Three of the taxa of tetraploid genom and high chamazulene contents (33-40% of the essential oil) proved to be *A. collina* Becker. Beside chamazulene, β-caryophyllene, caryophyllene-oxide and germacrene D proved to be the main components of the cultivars, while the strain of wild origin was characterised by high α-bisabolol content. It showed also a good production potential comparable with the selected cultivars. However, the accumulation level of the oil was highest in the variety 'Proa'. An octoploid population was identified as *A. pannonica* Scheele of typical morphological traits. The oil showed a wide sesquiterpene spectrum but was lacking of 1.8 cineole and β-pinene described in former references [1, 2]. Thus, we established it as a new chemotype of the species. Another population of wild origin proved to be a mixed one, consisting of both *A. collina* and *A. pannonica* plants. The sixth strain in the study was a tetraploid, azuleneless taxon. Other qualitative (i.e. presence of β-thujone, lack of germacrene D, humulene) and quantitative (i.e. low level of β-caryophyllene, high proportions of γ-muurolene) differences compared to *A. collina* were also obvious. According to the complex evaluation it could be concluded as *A pratensis* Saukel and Länger [3]. not described before in Hungary. The diverse performance of the populations calls the attention to the significance of controlled plant raw in phytotherapeutical products.

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P- 90 Antimicrobial Activity of an Essential Roman Chamomile Oil from France and Some of its Main Compounds

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The antimicrobial activity of an essential Roman chamomile oil (*Chamaemelum nobile* (L.) All. syn. *Anthemis nobilis* (L.) syn. *Ormenis nobilis* (L.) J. Gay ex Coss. et Germ., Asteraceae) from the Provence (France) was tested against various strains of Gram-(+)-bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-(-)-bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Salmonella* sp.) as well as against the yeast *Candida albicans* using a modified agar dilution and agar diffusion method [1]. In addition, some pure main and minor compounds (chemical composition obtained by means of GC- and GC-MS-analyses), such as isobutyl angelate (32.1%), 2-methylbutyl angelate (16.2%), isobutyl isobutyrate (5.3%), 2-methylbutyl isobutyrate (3.1%), prenyl acetate (1.4%) and 2-methylbutyl acetate (1.2%), were also studied for their antimicrobial effects. The Roman chamomile sample showed high antimicrobial activity against all strains of tested microbes (reference compounds: eugenol and 3 synthetic antibiotics). A similar result was found for 2-methylbutyl acetate and prenyl acetate. Surprisingly, antimicrobial effects against *Klebsiella pneumoniae* were shown by isobutyl angelate, 2-methylbutyl angelate and 2-methylbutyl isobutyrate as well as by isobutyl isobutyrate against *Staphylococcus aureus*. Therefore, we can state that the very high antimicrobial activity of the essential Roman chamomile oil from the Provence results of effects found for both, main and minor constituents of this oil.

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P- 91 Comparative Analysis of the Essential Oil of Artemisia herba-alba from Different Localities at Three Stages of Growth

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The seasonal and local variations in the composition of the essential oil of Artemisia herba-alba leaves have been analyzed.The aerial parts were collected at three stages of growth at different altitudes and different slopes of Hassan-abad Valley in the North of Iran. The essential oils were obtained by hydro-distillation method. The oils were investigated by capillary GC and GC-MS in order to obtain retention indices.
The essential oil yields were 0.62 to 1.16 precent. Among the 30 identified constituents (accounting for 92.89% of the oils), the major components identified included :1,8-cineole, β-thujone, borneol, camphor, camphene, terpinolene and bornyl acetate. Significant differences were observed in yield and in each of the components of the different samples. These differences show that ecological and phenological alternations have different effects on different pathways of biosynthesis.

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P- 92 Antimicrobial Activity and GC-MS Analysis of an Essential Davana Oil (*Artemisia pallens* Wall. ex DC) from India

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The antimicrobial activity of a sample of the essential Davana oil (*Artemisia pallens* Wall. ex DC, Asteraceae) from India as well as a distilled fraction of the oil were tested against several strains of Gram-(+)-bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), Gram-(-)-bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Salmonella* sp.) and the yeast *Candida albicans* using a modified agar diffusion and agar dilution method. As reference compounds, synthetic antibiotics and eugenol were tested. Some main and minor compounds identified using GC-FID and GC-MS were also tested in both assays: *Cis*-davanone (45.8%), bicyclogermacrene (9.6%), davana ether isomer 2 (3.9%), linalool (2.5%), phytol (2.1%), geranyl acetate (1.5%) and ethyl cinnamate (1.5%) were identified in the essential oil. In the fraction of the oil cis-davanone (72.2%), davana ether isomer 2 (5.2%), ethyl cinnamate (5.2%), davana ether isomer 3 (2.1%), bicyclogermacrene (2.1%), iso-davanone (1.9%) and davana ether isomer 1 (1.7%) were identified as main constituents. The essential davana oil showed high activity against the bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella* sp. as well as yeast *Candida albicans*, the fraction of davana oil showed the highest activity towards bacteria *Staphylococcus aureus*, *Enterococcus faecalis*, yeast *Candida albicans* and medium effects against *Klebsiella pneumoniae*, *Salmonella* sp. and *Escherichia coli*. With respect to the tested reference and main compounds, we can confirm the antimicrobial activity of the oil and the fraction to be dependent on both, main and minor constituents of the essential oil.

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P- 93 *Artemisia annua* L. Volatile Oil and its Mosquito and Pesticidal Activity.

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Artemisia annua L. is an annual herb, native to Asia. It has been used for many centuries for the treatment of fever and malaria. Its essential oil contains many terpenes such as artemisia keton, artemisinic alcohol and monoterpenes like linalool, hydrocarbons like myrcene (1). The most important is artemisinin which is now the potent plant derived compound in the treatment of the chloroquine-resistant and cerebral malarias (2). Many African countries suffer from malaria, began to introduce and cultivate this plant in a way to produce the active ingredient potent for treating malaria.
In Egypt, this plant was cultivated however in small scale to produce the active artemisinin.
The present study deals with the analysis of the essential oil, determine its constituents, and to determine artemisinin by GC/MS (3). The insecticidal activity of the volatile oil, hexane and alcohol extract was studied. The following table compiles the different constituents of the volatile oil

Table (1) Major constituents of Artemisia annua L. volatile oil

Compound	Content (%)
β-Pinene	5.17
Benzene,1-methyl-4-(1-methyl ethyl)-(CAS)	9.88
1,8-Cineol	4.62
trans- Caryophyllene	6.72
Germacrene-D	17.56
Bicyclogermacrene or γ-elemene	5.17
Cyperone	2.58
(+) Spathulenol	3.25
Di epicedren-1- oxide	10.14
Selinene	2.47

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P- 94 Studies on *Artemisia annua* Essential Oils: Composition, Bioactivity and Safety Aspects

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Artemisia annua L. was experimentally cultivated in Madeira Archipelago to evaluate the climatic conditions influence on the contents of artemisinin and its derivatives, the antimalaric substances produced by this plant.
In an integrated valorisation approach, we studied the essential oil as a by-product of artemisinin extraction which can find its own application as an addictive in aromatherapy and cosmetics industry, while the aqueous residue can be used in biological agriculture as an allelopatic agent.
For the present study, artemisia seeds were received from Brazil, from the hybrid CPQBAxPOP developed in UNICAMP in 2002. One-month-old *A. annua* seedlings, developed in nursery beds, were transplanted to experimental fields in several locations of Madeira and Porto Santo islands in May 2003, plants were harvested in September at budding stage and dried in field under direct sunlight to increase artemisinin contents, the main objective of the work.
Dry leaves (50 g each) were hydrodistilled in a Clevenger-type apparatus for 3 h and analyzed by GC and GC-MS. Twenty-five constituents of the oils were identified, with camphor being the major component in all samples. Plants cultivated in Madeira and Porto Santo show higher essential oil yields, higher camphor and lower 1,8-cineole contents than plants obtained from the same hybrid cultivated in Campinas, Brazil. In no case was Artemisia ketone detected a reassurance towards the safety of these oils. Mild antioxidant activity was determined by DPPH, ABTS and FRAPS methods and no toxicity was found in the brine shrimp lethality bioassay.

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P- 95 Volatile Constituents of Genepì Elixir and *Artemisia umbelliformis* Essential Oil.

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Wild alpine plants are being used in the production of liqueurs with bitter-eupeptic properties at artisan and industry level. The elixir commonly named Genepì is a typical product of the alpine mountains produced by alcoholic infusion of the little plants of some *Artemisia* species [1,2]. These are, in particular, *Artemisia genepi* Weber (the "male" Artemisia) also called *Artemisia spicata*; *Artemisia umbelliformis* (also called mutellina or laxa, white genepì); *Artemisia glacialis* (black genepì, "female" genepì). The indiscriminate picking of these plants has increased their rarity and decreased the commercial availability of the product. It is for this reason that plants such as *Achillea moschata* are used as substitute of *Artemisia* in the preparation of the Genepì liqueur. The flavour of a liquor, besides being one of the first consumer's perceptions, is of crucial importance in establishing its genuineness and quality, revealing the origin of the raw material and the preparation procedure used. Due to the lack of data in literature, the aim of the present study was, firstly, to evaluate the *Artemisia umbelliformis* volatiles profile, and secondly, to assess the quali-quantitative composition of some commercial brands of Genepì liquor, that have been compared to a lab-made sample. Different sample preparation techniques were used such as solid-phase microextraction (SPME) in the liquors analysis, and simultaneous distillation-extraction (SDE) in the essential oil isolation. Volatile constituents were analysed by means of: GC-qMS and GC-FID, for structure elucidation and absolute quantification; enantioselective (GC-Enantio) for enantiomeric ratio determination and olfactometric (GC-O) gas chromatographic evaluations, for the identification of odour-active components.

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P- 96 Essential Oil Composition from the Different Aerial Parts of *Asteriscus maritimus* (L.) Less. from Spain

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The genus *Asteriscus* Miller belongs to the Compositae family and is present in Europe with two species *A. aquaticus* (L.) Less and *A. maritimus* (L.) Less. *A. maritimus* is a perennial shrub not higher than 20 cm that grows over maritime rocks of the West Mediterranean region.
The essential oil composition of different aerial parts (leaves+stems, flowers and fruits) of *Asteriscus maritimus* has been analysed by gas chromatography and gas chromatography coupled to mass spectrometry. The different parts studied share most of the compounds although seasonal variations have been detected between the flower and fruit oils. The main constituents were found to be alpha-pinene (27.5%) and p-cymene (10.0%) in the leaves+stems oil, while the flower oil shows β-phellandrene (11.8%), fonenol (10.9%) and α-phellandrene (10.0%) as principal ones, and the fruit oil bisabolene (23.2%), α-pinene (15.6%) and p-cymene (12.3%). Monoterpene compounds were present in higher amount than the rest of constituents except in the fruit oil where sesquiterpene compounds were predominant.

P- 97 Essential Oil Composition of Two *Centaurea* Species from Iran

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The genus *Centaurea* of the Compositae family is represented by a very large number of species (about 581), distributed particularly in the North, North-western of Iran. *Centaurea zuvandica* is an endemic plant 1 in Iran. Taxonomically, this taxon is very difficult and needs further studies, mainly using modern cytological and chemical techniques.
The essential oil of two *Centaurea* from Iran, *Centaurea rhizantha* and *Centaurea zuvandica* (collected in the same area) was obtained from the aerial parts and investigated by combination of GC and GC/MS. The essential oil of *Centaurea rhizantha* contained mainly sesquiterpenes, caryophyllene oxide (28.5%), spathulenol (15.8%), β–eudesmol (4.5%), cedrenol (3.9%), apiol (2.9%) and other sesquiterpenes present in percentages of 0.4–3 %. Centaurea rhizantha also contained significant amounts of hydrocarbons specially 2-methoxy-4-vinylphenol (9.4%), 9, 12, 15-octatrienal (4.0%) and other hydrocarbons presented in percentage of 0.7 to 1.0 %. Monoterpenes were represented in percentages of 0.9 to 2.9%, and the diterpene phytol was present in a trace amount (0.7%). The main compounds found in the *Centaurea zuvandica* essential oils is monoterpenes contained, 1,8 cineol (65.7%), trans-pinocarveol (5.7%), p-cymene (4.4%) and other monoterpenes presented in percentages of (0.3-2%). *C. zuvandica* contained also sesquiterpenes like trans-iso elemicin (2.1%) and dillapiol (2.1%).

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P- 98 Composition of the Essential Oils of *Centaurea armena* Boiss and *Centaurea sessilis* Wild

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In Turkey, the genus *Centaurea* L. (Asteraceae) is represented by 182 species including 113 endemics, distributed particularly in the Southwest, Central and Eastern parts (1-2). The ratio of endemism is quite high (62.1%). Several members of this genus, such as *C. cyanus* L., *C. behen* L. *C. calcitrapa* L., are used in Anatolian folk medicine (3).
Aerial parts of *Centaurea armena* Boiss and *Centaurea sessilis* Wild, both are endemic in Turkey and distributed mainly in Eastern Anatolia, were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus and the oils trapped in hexane were analysed by gas chromatography-mass spectrometry (GC-MS).
Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to the series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library) (4-5), and in-house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (6-8), were used for the identification.
Seventy one compounds representing 90.2% and 82.5% of the essential oils were characterized from *C. armena* and *C. sessilis*, respectively. Hexadecanoic acid (31.5%), dodecanoic acid (6.0%), spathulenol (4.8%) and β-eudesmol (4.7%) were the main constituents in the oil of *C. armena* and hexadecanoic acid (23.4%), spathulenol (10.0%), caryophyllene oxide (4.8%) and salvial-4(14)-en-1-one (=mintketone) (4.2%) were the main constituents in the oil of *C. sessilis*.

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P- 99 GC-MS Analysis of Essential Oil Composition of *Echinacea purpurea* and *Echinacea pallida* Cultivated in Turkey

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The genus *Echinacea*, a herbaceous perennial of the family Asteraceae, although being a medicinal plant reputed to have immunamodulatory virtues, also has a strong aromatic nature (Thappa et al., 2004). The genus originated in eastern North America. Nine species of the genus are known and three are currently used in therapy for their medicinal properties: *Echinacea angustifolia*, *Echinacea purpurea* and *Echinacea pallida*. *Echinacea* is one of the best selling herbal medicines in United States and Europe, and represents 10% of the whole herbal market (Chicca et al., 2007).
The aim of the present study was to investigate and represent composition of the essential oils of two species; *E purpurea* and *E. pallida*, which were cultivated in the experimental farm of Selcuk University, Faculty of Agriculture in ecological conditions of Konya. Essential oil analysis was performed by GC and GC-MS. Two species' essential oil compositions were found to be significantly different from each other. The essential oil of *E. purpurea* was found remarkably rich in germacrene D (11.20 %), spathulenol (8.83 %), caryophyllene oxide (8.46 %) and 1,5-epoxysalvial-4(14)-ene (5.76 %). On the other hand essential oil of *E. pallida* was found rich in caryophyllene oxide (9.35 %), germacrene D (7.25 %) and α-cadinol (6.40 %). When compared with the previous work, results vary significantly (Chicca et al., 2007; Mirialili et al., 2006).

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P- 100 Volatile Constituents of the Essential Oils from Aerial Parts of *Senecio gallicus* Chaix Growing Wild in Iran

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The genus *Senecio* involves 21 species in Iran, among which five are endemic [1]. A literature survey reveals that there are some reports on the essential oils of species of the genus *Senecio* [2-6]. Identification the components of the aerial parts oil of *Senecio gallicus* Chaix was the main goal of this research which encouraged us to initiate the project. According to our knowledge, analysis the essential oils of the aerial parts of this plant has not been reported, previously.
The plant was collected during the flowering stage in May 2006 from Shahmirzad in Semnan province of Iran, at an altitude of 1550 msl. A voucher specimen has been deposited at the herbarium of Research Institute of Forests and Rangelands, Tehran, Iran. Air-dried aerial parts of *Senecio gallicus* Chaix (100g) were subjected to hydrodistillation for 3h using a standard Clevenger-type apparatus to produce essential oil. The oil were dried over anhydrous sodium sulfate and stored in a sealed vial at low temperature (4 ˚C) before analysis. The obtained oil was analyzed by a GC (Shimadzu GC-9A equipped with a SE30 fused silica column) and a GC/MS (Hewlett-Packard 6890/5973 fitted with a fused silica HP5MS capillary column) instrument. Twenty four components were identified constituting 98.04 % of the oil. The main components of the oil were α-pinene (9.8 %), sabinene (7.2%), β-pinene (4.2 %), myrcene (8.2%), α-phellandrene (3.8%), p-cymene (6.4%), β-phellandrene (12.2%), cis-ocimene (7.0%), δ-3-carene(1.7%), α-terpinolene (3.3%), β-caryophyllene (6.8%), α-humulene(1.6%), germacrene-D (17.9%), α-zingiberene (1.5%) and caryophyllene oxide (1.0 %).

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P- 101 Essential Oil *Senecio kotschvyanus* Boiss from Iran.

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The genus *Senecio* comprises twenty one species in Iran and some of them are endemic (1). Water-distilled essential oil of *Senecio kotscvhyanus* (Compositae), collected in June 2006 from Khoy Province of Azarbayejan was analyzed by means of GC and GC/MS. Twenty five compounds were found in the oil and twenty one of these were characterized. β-phellandrene (12.2%), α-pinene (9.8%), myrcene (8.2%), germacrene D (9.7%) and Z-β-ocimene (7.0%) were the main constituents in the oil of *Senecio kotscvhyanus*.

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P- 102 Composition of the Essential Oil of *Tanacetum canescens* (DC.) Rech. f. from Iran

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The genus *Tanacetum* is represented in the flora of Iran by twenty-six species including twelve endemics [1,2]. Reports of the microbiological properties in other *Tanacetum* species are also recorded in the literature. This activity is mainly due to a sesquiterpene lactone in *T. indicum* var. *tuneful* [3], and *T. argyrophyllum* and to terpenoid constituents in the oils of *T. cilicium*, *T. corymbosum* and *T. macrophyllum* [4].
The aerial parts of *T. canescens* were collected from Sanandaj in Kordestan Province, located in North-West of Iran during the flowering period in August 2006. Air-dried aerial parts of the *T. canescens* were ground and subjected to hydrodistillation, for 4 hours, using a Clevenger-type apparatus to produce yellowish oil in 0.3% (w/w) yield based on dry weight.
The oil was analysed by GC and GC-MS. Identification of the constituents of the oil was made by comparison of their mass spectra and retention indices (RI) with those given in the literature and those of authentic samples [5]. Thirty-six components, representing 94.1% of the total oil, were identified of which 1,8-cineole (22.2%), α-pinene (14.9%),1-borneol (9.0%), β-eudesmol (8.4%) and α-terpineol (6.1%) were the main compounds. The oxygenated monoterpenes faction comprises 53.1% of total oil of which 1,8-cineole and borneol are in appreciable percentage.

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P- 103 A New Possible Chemotype of *Tanacetum densum* (Lab.) Schultz Bip. ssp. *amani* Heywood

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T. densum species is represented in Turkey with four subspecies which are ssp. *sivasicum*, ssp. *laxum*, ssp. *eginense*, and ssp. *amani*. In our previous research we worked on the essential oil composition and biological activities of ssp. *sivasicum*, and ssp. *eginense* oils. We reported the main oil composition of ssp. *sivasicum* as 1,8-cineole (21.1%), camphor (19.2%), borneol (5.8%) and ssp. eginense as camphor (30.9%), 1,8-cineole (12.4%), camphene (10.6%), α-pinene (7 %) and an unknown compound (11.5%) [1]. According to previous work on the essential oil of ssp. *amani* from Elazig/Turkey, twenty three components were identified representing 83.1% of the oil. β-patchoulene (17.5%), camphor (15.6%) and 1,8-cineole (11.5%) compounds were given as the main components of the ssp. amani oil [2].
In this investigation essential oil of *T. densum* ssp. *amani* which is collected from Adana/Turkey was hydrodistilled from the aerial parts of the plant and examined using GC and GC/MS simultaneously. Eighty eight compounds were identified representing 90.7% of the oil. Main essential oil components were β-pinene (27.2%), 1,8-cineole (13.1%), α-pinene (9.7%), p-cymene (8.9%) and lavandulyl acetate (8.1%). Oil composition of the plant from Adana showed significant differences from the previously examined plant from Elazig. Oil from Elazig was rich in camphor and the sesquiterpene hydrocarbon β-patchoulene where as Adana oil was rich in monoterpenes. The only main common compound in both plants was 1,8-cineole which consisted almost the same amount in both oils.

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P- 104 Chemical Composition, Antibacterial and Antioxidant Activity of the Essential Oil of *Tanacetum polycephalum* Schutz. Bip.

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The chemical composition of the essential oil obtained by hydrodistillation from *Tanacetum polycephalum* was analysed by GC and GC/MS and 39 compounds constituting 94.02% of the oil were identified, the major components being borneol (28.30%), β-pinene (10.10%), α-pinene (6.5%), camphene (6%), α-terpineol (5.16%) and 1,8-cineol (5.10%). The essential oil exhibited remarkable bactericidal activity against *Staphylococcus aureus*, *Shigella flexnery*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus saprophiticus*, *Pseudomonas aeroginosa* and *Escherchia coli*. The antioxidant activity of the essential oil was evaluated using the DPPH test and 5-lipoxygenase test.

P- 105 Essential Oil Composition from the Leaves and Stem Bark of *Vernonia perrottettii* Sch. Bip. (Asteraceae) from Nigeria

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Vernonia is a genus of about 200 species of forbs and shrubs in the family Asteraceae. The plants are annual and herbaceous. Some species are sometimes known as ironweeds. Some species are edible and of economic value. There are several reports describing the pharmacological potentials of the extracts and compounds isolated from the genus *Vernonia*. These included anthelmintic, cathartic, analgesic and anti-inflammatory, anti-plasmodia, hypoglycemia and anti-mutagenic [1]. Vernonia oils have potential in industrial application [2]. The nutritive potentials of the plants have also been described [3, 4]. To the best of our knowledge there are no reports concerning the composition and biological potentials of the *Vernonia* species essential oils. Therefore, we decided to investigate on the volatile constituents of *Vernonia perrottettii*.
The essential oils from the leaves and stem bark of *V. perrottettii* have been extracted by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC/MS). The oils were dominated by sesquiterpenoid compounds. Quantitative but not qualitative differences have been found between the analyzed parts. The principal compounds from the leaves oil are germacrene D (34.5%), β-caryophyllene (28.1%), caryophyllene oxide (3.7%), α-humulene (3.5%) and, among the monoterpenes limonene (3.8%), while the oil from the stem bark showed β-caryophyllene (39.8%), germacrene D (16.0%), caryophyllene oxide (3.9%) among the sesquiterpenes, and limonene (4.1%) among the monoterpenes, as major compounds. The paper will discuss further the biological potentials of the oil extracts.

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P- 106 Volatile Compounds of Seeds of *Hesperis bicuspidata*, *H. bottae* and *H. podocarpa*

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It is a well known fact that interest in natural compounds is growing fast. As part of our ongoing research into screening the rich and diverse flora of Turkey, three species of *Hesperis* (Cruciferae) were investigated (1-2).
Crushed seeds of *Hesperis bicuspidata* (Wild) Poiret, *H. bottae* Fourn., *H. podocarpa* Boiss. were subjected to microdistillation and the oils were analzed by gas chromatography (GC-FID). Gas Chromatography/Mass Spectrometry (GC/MS) was used to characterise the compounds.
Identification of the volatiles was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library) (3-4), and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (5-7), was used for the identification.
Oils of all three species, *Hesperis bicuspidata*, *H. bottae*, *H. podocarpa*, were found to contain octyl acetate as the main constituent, respectively 44,4%, 29% and 42.4%.

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P- 107 Chemical Composition of the Essential Oil from Stems of *Calycanthus floridus* L. var. *oblongifolius* (Nutt.) D.E. Boufford & S.A. Spongberg from Iran

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The genus *Calycanthus* belongs to Calycanthaceae family that includes two to four species depending on taxonomic interpretation and two are accepted by the Flora of North America [1] . To the best of our knowledge this is the first report on the essential oil from stem of *Calycanthus floridus* L. var. *oblongifolius*. The aim of our study is to identify the constituents of the essential oil of stems of *Calycanthus floridus* L. var. *oblongifolius* at the flowering stage. The essential oil obtained by hydrodistillation of stems of *Calycanthus floridus* L. var. *oblongifolius* (Nutt.) D.E. Boufford & S.A. Spongberg , planted in Sabzevar, Iran, was analyzed by GC and GC/MS. Twenty compounds representing 93.8% of stem oil of *Calycanthus floridus* L. var. *oblongifolius* were identified. Among them 1,8-cineole (31.7%), bornyl acetate (12.6%), α-pinene (10.0%), elemol (9.0%), β-pinene (7.2%) and α-terpinyl acetate (6.8%) were the major ones. In this essential oil oxygenated monoterpenes (56.0%) predominated over monoterpene hydrocarbons (26.3%) and sesquiterpenes (11.6%). Nonterpene hydrocarbons were not found among the identified components.

References: 1. Flora of North America, http://www.efloras.org

P- 108 Comparison of Chemical Composition and Antioxidant Capacity of Glycosidically Bound and Free Volatiles from Clove (*Syzygium aromaticum* L. Merill et Perry)

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Comparison of chemical composition and antioxidant capacity of glycosidically bound and free volatiles from clove (*Syzygium aromaticum* L. Merill et Perry) was examined in this study. The volatile aglycones were extracted from their nonvolatile glycosidically bound compounds by enzymatic hydrolysis with β-glycosidasae enzyme, while free volatiles were extracted using Clevenger-type apparatus. The chemical composition analyses of volatiles were run on coupled system gas chromatography-mass spectrometry (GC-MS) with two different polarity column.

15 compounds were identified in volatile aglycone fraction, including aromatic compounds, aliphatic alcohols, acids and esters. The major volatile aglycone was phenylpropanoide eugenol (80.5 %). Other quantitatively important volatile aglycones were vanillin (7.2 %), chavicol (2.3 %) and benzyl alcohol (1.1 %). Free volatiles have only five identified compounds with eugenol as major (91.2 %). Eugenyl acetate, trans-caryophyllene, α-humulene and δ-cadinene were present in smaller percentages. Comparison between chemical composition of glycosidically bound and free volatiles showed only eugenol as identical.

2, 2´- diphenyl-1-picrylhydrazyl radical scavenging method (DPPH method) was used to evaluate antioxidant capacity of clove volatile compounds. Results were compared with commercial antioxidants BHT and α-tocopherol. They showed that antioxidant capacity decreased in the following order -tocopherol ≥ free volatiles > BHT > volatile aglycones. Better free volatiles antioxidant capacity compared with volatile aglycones capacity could be explained with higher eugenol content and eugenyl acetate present in them.

Lower volatile aglycones antioxidant capacity contributes to overall clove capacity and confirm clove's significant role among spice plants. This is a guideline for replacing commercial antioxidants with natural ones.

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P- 109 Chemical Composition of the Essential Oils of *Equisetum palustre* L. and *Equisetum telmateia* Ehrh.

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The essential oils obtained by hydrodistillation of dry sterile stems of *Equisetum palustre* L. and *Equisetum telmateia* Ehrh. from Serbia were analyzed for the first time by GC and GC/MS. In total, one hundred ninety-five compounds were identified in the both oils, 103 in *E. palustre* and 171 in *E. telmateia* oil, accounting for more than 95.3% and 98.0% of the total oils, respectively. The major constituents of both *E. palustre* and *E. telmateia* oils were hexadecanoic acid (15.7%, 33.8%), (E)-phytol (16.7%, 4.6%) and hexahydrofarnesyl acetone (10.6%, 13.1%), respectively. The oils were additionally marked by the presence of β-ionone (4.1%, 3.2%), α-ionone (3.1%, 2.5%), heneicosane (3.5%, 2.1%) and tricosane (6.6%, 3.4%). A rather large number of identified fatty acid derivatives (40; 64) and carotenoid (36; 57) derived compounds, accounted for 38.0 and 36.6% of *E. palustre* oil, and 56.2 and 33.8% of *E. telmateia* oil, respectively. Terpenoids and Maillard reaction products represented only minor constituents of the oils. The volatile profiles of *E. palustre* and *E. telmateia*, reflect the close phylogenetic relationship of these species. The essential oil of *E. arvense* [1] differed from the two examined oils in having a predominance of the carotenoid derived compounds (45.7%) over fatty acid derived compounds (4.9%), and a significant share of the volatile terpenoid fraction (47.3%).

Acknowledgements: This work was funded by the Ministry of Science and Environment Protection of Serbia (Project 142054 B).

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P- 110 Volatile Constituents of *Arctostaphylos uva-ursi* (Ericaceae)

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The crude drug of *Arctostaphylos uva-ursi* (L.) Spreng. (Ericaceae), also known as bearberry, contains three main groups of pharmaceutically relevant compounds, phenols, tannins and flavonoids, with arbutin (hydroquinone-D-monoglucopyranoside) being the main phenolic constituent. Besides being prized as a garden plant, the leaves of *A. uva-ursi* are used as a urinary antiseptic and an astringent [1]. The volatile constituents of the dry leaves of *A. uva-ursi* have never been investigated previously, and here we report on the chemical composition of the hydrodistilled essential oil. The GC and GC-MS analysis of the oil resulted in the identification of 262 components, accounting for 90.6% of the total oil obtained in 0.006% yield (w/w). The major constituents were α-terpineol (7.1%), linalool (6.6%), palmitic acid (4.1%), and (E)-geranyl acetone (3.7%) and (E) phytol (3.0%). Terpenoids (44.5%) constituted the main fraction of the oil with oxygenated monoterpenes (32.7%) as the most abundant compound class (sesquiterpenoids–8.1%, diterpenoids–3.5%). Fatty acid and carotenoid derived compounds attained also relatively high amounts in the oil (21.2 and 18.4%, respectively). Volatile profile of *Vaccinium arctostaphylos* L.[2], that belongs to a completely different genus of the same family, shows a great resemblance in both qualitative and relative quantitative compositions with the essential oil of *A. uva-ursi*.

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P- 111 A Detailed Analysis of the Essential oil of *Centaurium erythraea* (Gentianaceae) from Serbia

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Centaurium erythraea Rafn of the Gentianaceae family (common or European centaury), is known in Serbia as 'kičica', where it has enjoyed a wide reputation as a medicinal herb. Centaury attracted our attention because it is used in the pharmacopoeia of 23 different countries [1]. *Centaurium erythraea* Rafn has been the subject of several phytochemical investigations [2-4], but the chemical composition of its essential oil has never been studied previously. The volatile constituents of the aerial parts of *C. erythraea* obtained by hydrodistillation have been analyzed by GC and GC-MS. The yield of a yellowish highly fragrant essential oil was quite low (0.015%, w/w dry plant material). Two hundred and thirty one constituents identified accounted for 93.2% of the total oil. The major components of the oil were: neophytadiene isomer III (10.0%), carvacrol (7.9%), p-camphorene (5.6%), hexadecanoic acid (4.9%) and thymol (4.1%). The terpenoid fraction represented more then one half of the oil (14.2% monoterpenoids, 18.7% sesquiterpenoids and 22.4% diterpenoids), while the fatty acid derived compounds made up a third, and the carotenoid derived compounds only 5% of the oil. The oil was characterized by the presence of diterpenoids (e.g. cubitene, (3Z)-cembrene A) that have previously been reported as termite metabolites [5]. Another feature of the oil was the identification of erythocentaurin, 5-formyl-2,3-dihydroisocoumarin (previously isolated from this plant species [6]), among the volatile constituents, for the first time in an essential oil sample.

Acknowledgements: The authors acknowledge the Ministry of Science and Environmental Protection of Serbia for financial support (Project 142042).

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P- 112 Chemical Composition of the Essential Oil from Leaves of *Biebersteinia multifida* DC. Growing Wild in Iran

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The genus *Biebersteinia* belong to Geraniaceae family and it has one perennial herb species in Iran that is called *Biebersteinia multifida* DC. This species can be found in Syria, Armenia, Afghanistan, Lebanon and Central Asia besides Iran[1,2]. To the best of our knowledge this is the first report on the essential oil from leaves of *Biebersteinia multifida*. The aim of our study is to identify the constituents of the essential oil of leaves of *Biebersteinia multifida* DC. growing wild at the flowering stage in Northeast of Iran. In this work the essential oil obtained by hydrodistillation from leaves of *Biebersteinia multifida* DC. Growing wild in Khorasan province (Iran), was analysed by GC and GC/MS. Seven compounds representing 93.45% of leaf oil of *Biebersteinia multifida* were identified among them α-pinene (49.79%) and 6,11,14-trimethyl-2-pentadecanone (21.59%) were the major ones. In the identified compounds, α-pinene predominated over nonterpene hydrocarbons and other terpenes.

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P- 113 Composition and Insecticidal Bioactivity of the Essential oils of *Hypericum aegypticum* and *Hypericum fragile*

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The genus *Hypericum* (Guttiferae) comprises c. 370 species widespread in warm-temperate regions and tropical mountains [1]. Members of the genus *Hypericum* grow in a variety of habitats ranging from rocky sea-facing cliffs to damp biotopes around mountainous streams. In this study aerial parts of *H. aegypticum* L. (section Triadaenia) were collected from sea facing cliffs and rocks on the island of Kephallonia, Aghioi Theodoroi (13-7-2004) and of *Hypericum fragile* Heldr. & Sart (section Taeniocarpium) from coastall cliffs at Chiliadou Evia (14.7.2006). The essential oils were isolated by hydrodistillation and analyzed by GC and GC-MS. The main compounds of *H. aegypticum* oil were α-pinene (41.6%), β-pinene (12.3%) and sylvestrene (7%), while the major compounds of *H. fragile* oil were ishwarane (28.7%), nonacosane (18.7%) and (E)-caryophyllene (8.3%).
The isolated oils were tested for insecticidal activity. The LC50 of *H. aegypticum* and *H. fragile* essential oils was estimated by means of arenas in which a series of concentrations (ml/dl) was applied. The termites (*Reticulitermes balkanensis*) and the ants (*Crematogaster scutellaris*) were entered in the arenas and the proportion of dead insects was recorded at the end of 24 h for the termites and 48 h for the ants. The insect killing efficiency was higher for *H. aegypticum* oil for both insects.

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P- 114 Composition of the Essential Oil of *Hypericum fasciculatum*

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The genus *Hypericum* (Guttiferae) comprises a large number of species widely distributed in many countries in Midle Europe, North America and Eastern Africa. The interest in the genus *Hypericum* is increasing since its constituents posses therapeutic effects as antimicrobial, cytotoxic, anti-inflammatory, and antidepressant agents [1,2]. *Hypericum fasciculatum* is a shrub 1.5-2 m tall. Flowers mostly in 3-26 flowered cymes terminal or terminal and axillary on the branchlets, some sometimes solitary in the leaf axils. [3]. In this study we investigated the chemical composition of essential oil of *Hypericum fasciculatum* from Florida (2004).The essential oil was isolated from dry, aerial parts by hydrodistillation and analyzed by means of GC and GC/MS. The aerial parts of *H. fasciculatum* yielded 0.5% (v/w) of oil. Thirty-five compounds have been identified, representing (82.3%) of the total oil. The fraction of sesquiterpene hydrocarbons (52.1%) was dominated, followed by ogygenated sesquiterpenes (29.0%). The main constituents were γ-muurolene (9.5%), (E)-caryophyllene (8.7%) and spathulenol (8.6%).

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P- 115 Volatile constituents of *Ballota nigra* L. subsp. *anatolica* P. H. Davis, Growing Wild in Iran

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The genus *Ballota* (Lamiaceae) is comprised of about 90 species in widespread over the world. Remarkably, three species of this genus are found in the flora Iranica [1]. The genus *Ballota*, has been used in folk medicine as antiseptic, anti-inflammatory, anti-rheumatic, antioxidant and antimicrobial agent, also for nausea, vomiting and nervous dyspepsia [2]. *Ballota nigra* ssp. *anatolica* is an aromatic plant that grows wild in Golestan and Mazandaran province of Iran [3].
Ballota nigra subsp. *anatolica* were collected during the flowering stage from Shahnaajar village, Nowshahr, Mazandaran Province, located North of Iran in July 2006 and identified by Herbarium of Nowshahr, Botanical Garden, Research Center of Natural Resources of Mazandaran Province, Nowshahr, Iran.
The air-dried aerial parts of *B. nigra* subsp. *anatolica* were hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 0.3% (w/w) of yellowish oil. The components of the essential oil were identified by comparision of their mass spectra with those of a computer library or with authentic samples and confirmed by comparision of their retention indices as well as with data published in the literature [4].
Twelve compounds were characterized in the essential oil of *B. nigra* subsp. *anatolica*, representing 93.6% of the oil, of which germacrene D (18.7%), nerolidol epoxyacetate (15.8%), sclareol oxide (12.5%), linalyl acetate (11.7%), β-caryophyllene (10.5%) were found to be the major constituents.

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P- 116 Composition of the Essential Oils of *Calamintha sylvatica* Bromf. subsp. *sylvatica* and *Calamintha sylvatica* Bromf. subsp. *ascendens* (Jordan) P.W. Ball

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Calamintha Miller (Lamiaceae) is represented in Turkey by 9 species and altogether 13 taxa, 6 being endemic. The rate of endemism in Turkey is over 45% (1,2). *Calamintha* species are locally called as ''Güzel Nane, Dağ Nanesi, Miskotu,, Dağ Miskotu, Yabani Oğulotu'' and used as herbal tea (3-5).
In the present study, aerial parts of the *Calamintha sylvatica* Bromf. subsp. *sylvatica* collected from the following regions of the İzmit (A) province and *Calamintha sylvatica* Bromf. subsp. *ascendens* (Jordan) P.W. Ball collected from the following regions of the Adana (B) province.
A : İzmit: Maşukiye on September 7, 2004 (ESSE 14409)
B : Adana: Horzum on September 19, 1993 (ESSE 10375)
The oils were extracted from aerial parts of *Calamintha* species using Eppendorf Microdistiller®. Oils were analyzed by GC and GC/MS. Piperitone oxide-I (45.7 %), terpinen-4-ol (8.6 %), piperitenone oxide (5.5 %) and piperitone oxide-II (5.2 %) were detected as main constituents in sample A; Piperitone oxide-I (21.8 %), limonene (15.7 %), piperitenone oxide (10.5 %) and caryophyllene oxdie (4.0 %) were found as main constituents in sample B.

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P- 117 Essential Oil Composition of *Calamintha origanifolia* Boiss. (Lamiaceae) Growing Wild in Lebanon

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Calamintha species are known for their medicinal uses as antipyretic, diaphoretic, expectorant, sedative [1]. Some species are reported to possess strong antibacterial and antifungal activities [2]. *Calamintha origanifolia* Boiss. is a strongly aromatic, suffruticose, much branched species wild growing in the Horsh Ehden reserve that is located on the northern part of the Lebanese western mountain range, just below Cornet As Sawda, the highest mountain peak in Lebanon. Aerial parts of *C. origanifolia* Boiss were collected at the full flowering stage from plants growing wild on rocky soil at Oyoun Ouvghanch, 2200 m s.l., in June 2005. The oil was isolated by hydrodistillation [3]. The GC and GC/MS analyses on a HP 5MS column evidenced fifty constituents, representing 92.1% of the total oil. The oxygenated monoterpenes were the most abundant components, particularly those with p-menthane skeleton and their content represented 59.7% of the oil. The most abundant compounds of this fraction were pulegone (22.5%), isomenthone (12.2%) and piperitenone (9.6%). The presence of isomenthone can be considered a characteristic of the present oil because this compound is reported in other *Calamintha* oils in lower quantity [4]. The presence of pulegone and isomenthone in such high amounts is significative as these compounds show interesting biological properties, particularly antibacterial [2].

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P- 118 Chemical Composition of the Essential Oil from Stems and Aerial Parts of *Eremostachys labiosa* Bge. from Iran

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Eremostachys labiosa grows in Pakistan, Iraq, Afghanistan and Turkmenistan besides Iran is one of the 15 perennial species of the genus *Eremostachys* (family labiatae) are found in Iran and among these five are endemic [1]. Only one investigation has been carried out on the chemical composition of the essential oils of the genus *Eremostachys*, and this deals only with the aerial parts of *Eremostachys laciniata* (L.) Bunge [2]. To the best of our knowledge this is the first report on the essential oils from different parts of *Eremostachys labiosa*. The aim of our study is to compare the yields and to identify the constituents of the essential oils of stem and aerial part of *E. labiosa* Bge. growing wild at the flowering stage in Northeast of Iran. The pale yellowish colored oils were obtained by by 3-hours' hydrodistillation, using a Clevenger-type apparatus, of the stems and aerial parts in yields of 0.037% and 0.052% (w/w), respectively. GC and GC-MS revealed nine compounds, representing 94.41% of the stem oil. The major constituents were, α-phellandrene (28.6%), β-phellandrene (11.3%), α-pinene (10.1%) and tetradecane (10.0%). The aerial parts oil of the plant was characterized by higher amounts of 6,10,14-trimethyl 2-pentadecanone (22.3%), 1,8-cineole (21.7%), α-pinene (16.5%) and sabinene (7.3%) among the eleven detected components, which together comprised 90.5% of the total oil. The stem oil of the plant consist of monoterpenes and nonterpene hydrocarbons while aerial parts oil includes sesquiterpenes too. In both oils monoterpenes predominated over other hydrocarbons.

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P- 119 Chemical Composition of the Essential Oil from Flowers, Stems and Leaves of *Eremostachys macrophylla* Montbr. & Auch. from Iran

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The genus *Eremostachys* belong to labiatae family and 15 perennial species from this genus are found in Iran among them 5 species are endemic and *Eremostachys labiosa* grows in Pakistan, Iraq, Afghanistan and Turkmenistan besides of Iran [1]. Only one investigation has been carried out on the chemical composition of the essential oils of the genus *Eremostachys*, and deals only with the aerial parts of *Eremostachys laciniata* (L.) Bunge [2]. To the best of our knowledge this is the first report of essential oils from different part of *Eremostachys macrophylla*. The aim of our study is to compare the yield and to identify the constituents of the essential oils of flower, stem and leaf of *E. macrophylla* Montbr. & Auch. growing wild at flowering stage in Northeast of Iran. The colorless oils were obtained by hydrodistillation, using a Clevenger-type apparatus for 3 hours, from flowers, stems and leaves in 0.031% , 0.019% and 0.024% yield (w/w), respectively, and analyzed by GC and GC-MS. Twenty-four compounds representing 95.7% of the flower oil of the plant were identified. Among them, 1,8-cineole (19.0%), germacrene D-4-ol (10.6%), α-pinene (9.8%) and α-terpinyl acetate (9.0%) were the major ones. Twelve compounds representing 96.0% of the stem oil of the plant were identified. Among them 1,10 di-epi cubenol (33.4%), elemol (24.0%) and octadecane (8.0%) were the major ones. Ten compounds representing 91.8% of the leaf oil of the plant were identified. Among them α-pinene (30.0%), 1,10 di-epi cubenol (22.7%), elemol (13.3%) and bornyl acetate (11.0%) were the major ones. The flower oil of the plant consist of nearly equal amounts of monoterpenes and sesquiterpenes while in stem and leaf oil of the plant, sesquiterpenes predominated over monoterpenes.

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P- 120 Chemical Composition of the Essential Oil from Stems and Leaves of *Hymenocrater platystegius* Rech. f. from Iran

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Hymenocrater platystegius is one of 9 species of *Hymenocrater* (family Labiatae) found in Iran, of which 4 are endemic [1]. These plants are bushes with beautiful, colored sepals Although some investigations on the chemical composition of essential oils from the genus *Hymenocrater* were done a few years ago [2-4] to the best of our knowledge this is the first report on essential oils from different parts of *Hymenocrater platystegius*. The aim of our study was to compare the yields and to identify the constituents of the essential oils of the stems and leaves of *H. platystegius* Rech. f. growing wild in the flowering stage in Northeast Iran. Colorless oils were obtained by hydrodistillation, in a Clevenger-type apparatus for 3 hours, in yields of 0.027% and 0.041% yield (w/w), from the stems and leaves, respectively. Analysis by GC and GC-MS revealed 10 compounds representing 84.9% of the stem oil. Among these, α-pinene (17.6%), spathulenol (16.9%), cis-calamene (10.2%) and β-pinene (9.4%) predominated. The leaf oil of the plant was characterized by higher amounts of spathulenol (16.1%), α-pinene (15.6%), 1,8-cineole (12.9%) and β-pinene (9.0%) of the 17 detected components which comprised 89.4% of the total oil. Monoterpenes predominated over sesquiterpenes in both the stem and leaf oils.

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P- 121 Antifungal Activity of *Lippia alba* (Miller) N.E Brown Essential Oils from Different Colombian Regions

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Essential oils from *Lippia alba* (Miller) N.E Brown (Verbenaceae) plants, grown in different regions of Colombia, were obtained by microwave-radiation assisted hydrodistillaton and analyzed by GC-MS. The antifungal activities of the 10 *L. Alba* oils at 2% (v/v) and lower concentrations, were evaluated by determining the minimum inhibitory concentration (MIC), according to the standard microdilution protocols AFST-EUCAST and CLSI-M38-A [1]. Quality control strains of *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258, and the reference strains of *Aspergillus flavus* ATCC 204304 and *A. fumigatus* ATCC 204305 were employed. MIC ranges for these strains were: 0.0310 - 0.25% for *C. parapsilosis* ATCC 22019, 0.016 - 0.156% for *C. krusei* ATCC 6258, 0.029 - 0.167% for *A. flavus* ATCC 204304, and 0.004 - 0.313% for *A. fumigatus* ATCC 204305. MICs for itraconazole and amphotericin B were in the range of values established by the techniques. These data indicate that *Lippia alba* essential oils are active against *Candida* spp. and *Aspergillus* spp. and thus, they may be useful in the development of new antifungal drugs.

Acknowledgements: Colciencias, Grant 432-2004.

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P- 122 GC-MS Study of Secondary Metabolites from Two *Lippia origanoides* H.B.K. Chemotypes and Determination of their Radical Scavenging Activity

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Microwave-assisted hydrodistillation, simultaneous distillation-solvent extraction, and supercritical fluid extraction, were used to isolate secondary metabolites from two *Lippia origanoides* chemotypes cultivated in an experimental garden. Their chemical composition was determined by GC-MS (EI, 70 eV). Compound identification was based on their Kovàts retention indices and mass spectral comparison with those of the QuadLib2004, NIST02, NBS75K and Wiley138 databases. The radical scavenging ability was measured spectrophotometrically (at 734 nm) by means of the ABTS assay [1]. The main components identified in the chemotype I extracts were p-cymene (13%) and 1, 8-cineole (9%). The main components found in the chemotype II extracts were thymol (56%) and p-cymene (10%). Table 1 summarizes the main compositional differences of the two chemotypes, according to compound families.

Table 1. Comparison of relative amounts (%) of compound families in the two *Lippia origanoides* essential oils studied.

ESSENTIAL OIL	Monoterp.	Oxygen. Monoterp.	Sesquiterp.	Oxygen. Sesquiterp.	Phenols	Hydro-carbons	Other oxygen. Comp.
Lippia origanoides Chemotype I	25.8	3.4	3.4	0.6	46.2	14.0	6.7
Lippia origanoides Chemotype II	28.8	17.2	19.6	12.7	2.9	17.8	0.8

Acknowledgements: Colciencias, Grant 432-2004.

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P- 123 Composition and Antimicrobial Activity of the Volatile Oil of *Melissa officinalis* L. subsp. *officinalis* from Anatolia

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Melissa officinalis L. (Lamiaceae), or lemon balm, is natively grown in the region from Iran to East Mediterranean which includes Anatolia and Black Sea region. The plant is commonly used as a traditional remedy in different countries and it is also used in pharmaceuticals, food and perfume industries. *M. officinalis* possess many pharmacological activities such as sedative, antispasmodic, antimicrobial, antioxidant, analgesic etc (1,2).
Melissa officinalis L. is represented by three subspecies in Turkish flora: *M. officinalis* subsp. *officinalis*, *M. officinalis* subsp. *inodora* and *M. officinalis* subsp. *altissima* (3). We have previously studied the volatile oil composition of *M. officinalis* subsp. *inodora* (4). It is known that several subspecies of *M. officinalis* exhibit a very different chemical profile. The present report deals with the composition and antimicrobial activity of the volatile oil of *M. officinalis* subsp. *officinalis* from Anatolia for the first time. The leaves of the plant collected in eastern Anatolia at the flowering time. The volatile oil was obtained by hydrodistillation of the plant material with the yield 0,26% v/w and it was analyzed by GC and GC-MS. The main constituents of the oil were geranial (26,4%), neral (20,5%), caryophyllene oxide (15,5%) and β-caryophyllene (3,99%). The volatile oil of the present sample is extremely different compared with the oil of M. officinalis subsp. inodora.
The volatile oil of *M. officinalis* subsp. *officinalis* was tested for its antimicrobial activity using disc diffusion and microdilution assays. The oil has strong activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Candida tropicalis*.

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P- 124 Qualitative and Quantitative Evaluation of Lemon balm (*Melissa officinalis* L.) Essential Oil from Transylvania, Romania

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Lemon balm (*Melissa officinalis* L.), is a perennial herb in the mint family Lamiaceae. As a medicinal plant, lemon balm has traditionally been employed against catarrh, fever, flatulence, headaches, influenza, and toothaches. It has also been used as a carminative, diaphoretic, and sedative. Recent evidence suggests that lemon balm has a depressant or sedative action on the central nervous systems of laboratory mice. Oil of balm has also been shown to have antiviral, antibacterial, and antispasmodic activity. Lemon balm has been reported to be an insect repellent. It is also used for several purposes such as an additive in food, a herb tea and an ingredient in cosmetics.
We have been studied the volatile oil obtained from five populations originated from Transylvania region, Romania.
Composition of essential oil was determined by GC-MS and GC-FID. GC analyses were carried out with a Hewlett-Packard 6890 gas-chromatograph equipped with a flame ionization detector. The sample was analyzed on two fused silica capillary columns with bonded phases of different polarity. Compounds were identified using both chromatographic and mass spectroscopic criteria.
We have found significant variations between locations and populations in terms of yield and concentration of compounds. The essential oil rate ranged from 0.052% to 0.14%, and the main components were geranial (41,3%-56.7%) and neral (14.4%-23.4%). Our results, show that the essential oil from Transylvania has also a good olfactometric quality.

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P- 125 Essential Oil Composition of *Mentha aquatica* L. from North of Iran

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Mentha aquatica was collected from north of Iran (forests around of Rasht). The essential oil of *Mentha aquatica* L. was produced by steam distillation of its dry leaves at a yield of 1% (weight of essential oil/weight of dry leaves) and then analyzed by the aid of capillary gas chromatography method using two different polarity columns (DB1 & DB-WAX). Sixteen compounds were identified in this essential oil which consists of about 79.9% of total oil, among them menthofuran (50.4%), β̃caryophyllene (13%), germacrene D (9.1%) and 1,8-cineole (5.3%) had the highest percentages.

P- 126 Antimicrobial Activity of Essential Oils of Mint and Peppermint as well as Some of their Main Constituents

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The antimicrobial activity of essential oils of mint (*Mentha arvensis* L., Lamiaceae) from China, peppermint (*Mentha* x *piperita* L., Lamiaceae) from the USA and a menthone-rich extract of the essential mint oil was tested against various strains of Gram-(+)-bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-(-)-bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Salmonella* sp.) and against the yeast *Candida albicans* using agar dilution and agar diffusion methods. Additionally, pure main and minor compounds (chemical composition obtained by GC- and GC-MS analyses), such as menthol (mint 41.2%; peppermint 40.7%; extract 12.3%), menthone (20.4%; 23.4%; 58.3%), menthyl acetate (4.6%; 4.2%; 1.5%), 1,8-cineole (2.7%; 5.3%; 0.2%) and limonene (4.3%; 2.6%; 0.8%) were investigated for their antimicrobial effects. The mint sample showed high to medium antimicrobial activity against both strains of Gram-(+)-bacteria, *Candida albicans* and Gram-(-)-bacteria *Escherichia coli*, *Proteus vulgaris* and *Salmonella* sp. (reference compounds eugenol and three antibiotics). For the peppermint sample very high antimicrobial effects were found against *Klebsiella pneumoniae* and *Salmonella* sp. as well as medium ones against all other strains, except *Pseudomonas aeruginosa* (no effects). Similar results were obtained by testing the “menthone-extract” and pure menthone. Menthol showed only low to medium antimicrobial activities against Gram-(+)-and Gram-(-)-bacteria (no effects against *Escherichia coli*) and *Candida albicans*. In conclusion, antimicrobial activities of the essential oils of mint and peppermint as well as the mint-extract result from effects, found for both, main and minor constituents of these samples.

P- 127 Chemical Composition and Antibacterial Activity of the Persisch Mint (*Mentha piperita* L.)

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The genus *Mentha* (Labiatae) comprises 6 species, which have been widely distributed in Iran.1 Known as mint or peppermint, *Mentha piperita* L. is used for medicinal and food purposes. 2 Its cultivation has economic importance, due to its ability to produce and store essential oil, whose main constituent is menthol, used in oral hygiene products, pharmaceuticals, cosmetics, and foods. Menthol also has high antifungal and antibacterial potentials, thus becoming one of the most demanded substances by the scents and essences industry. 3 Because of this and other reasons, peppermint essential oil ranks high in terms of total sales volume. 4 Persisch Mint is a Natural Soft-Drink in Iran. For analysis of the Persisch Mint, a sample, subsequently, passed through C18 and Licrout en column. The components absorbed on the column were eluted by washing with Hexane and Methanol. The solvent was evaporated to small volume and analyzed using GC and GC/MS. Many components were identified in the oil Persisch Mint. All oils consist of monoterpenes, sesquiterpenes and aliphatic compounds.
Furthermore, the oils exhibited an interesting antibacterial activity against Gram-positive and Gram-negative bacteria. The following strains of gram negative and gram positive bacteria were provided from Persian Type Culture Collection (PTCC) and American Type Cultur Collection (ATCC). Each test was performed in 4 replications and the results analysed for statistical significance. Gentamicin and tetracycline with positive responses were used as controls for plates. Gentamicin and tetracycline served as positive controls on gram-positive and gram-negative bacteria.

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de Produção de Plantas Medicinais, Aromáticas e Condimentares. São Pedro, Brazil, pp.923.

P- 128 A Chemotaxonomic Study of Essential Oil Components in *Nepeta* (Lamiaceae) Species from Iran

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To evaluate the inter-specific relationship on the basis of essential oil constituents in *Nepeta* species (Lamiaceae) from Iran, 39 samples representing 27 previously known oil components and 12 current investigated, of which essential oil compositions of *N. bachtiarica* and *N. hormozganica* two endemic species to Iran reported for the first time, were analyzed using Hierarchical Cluster Analysis (HCA) and Principal Components Analysis (PCA). A total of 72 compounds were detected and identified [2]. Multivariate statistical analyses of oil components characterized three groups. Group 1 was formed by 11 species that constituted different disteroisomers of nepetalactone as major component. The second group clustered 8 species with 1,8-cineol as main component. Group 3 which divided into two subgroups, is comprised by rest species containing sesquiterpenoids and oxygenated monoterpenoids as major constituents except nepetalactone and 1,8-cineol. Principal component analysis confirmed clustering result [2,3].

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P- 129 Chemical Composition of Essential Oil of *Nepeta menthoides* Boiss. & Buhse from Iran

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The genus *Nepeta* (Lamiaceae) comprises about 250 species widespread over the world. *Nepeta* is represented in the flora of Iran by sixty-seven species [1] .Some *Nepeta* species are used in folk medicine as diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic, febrifuge, emmenagogue and sedative agents [2,3]. Many reports on phytochemical analysis of this species-rich genus, including essential oil analyses, are found in the literature.
Some differences in the oil composition were detected in several *Nepeta* oils [4-6], as well as differences in the oil composition of the same species from different localities [7].
The aerial parts of *N. menthoides* were collected during the flowering stage, grounded and subjected to hydrodistillation, for 4 hours, using a Clevenger-type apparatus to produce yellowish oil in 0.4% (w/w) yield based on dry weight.
The oil was analysed by GC and GC/MS. Identification of the constituents of each oil was made by comparison of their mass spectra and retention indices (RI) with those given in the literature and those authentic samples [8]
Fifty-two components were identified in the essential oil of *N. menthoides*, representing 93.8% of the oil, of which the 1,8-cineole (30.7%), geranyl acetate (9.86%), α-terpineol (5.83%), terpinen-4-ol (4.68%), manoyl oxide (6.22%) were found to be the main constituents.

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P- 130 Volatile Oil Constituents of Basil Wild Species (*Ocimum campecianum*, *O. kilimandscharicum*, *O. selloi*, and *O. tenuiflorum*)

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Essential oils extracted from basil (*Ocimum* spp.) by steam distillation are used to flavor foods, oral products, in fragrances and in traditional medicines. The genus *Ocimum* contains around 30 species native to the tropics and subtropics, with some species naturalized and/or cultivated in temperate areas.

In this paper, we characterize the volatile oils of four wild species of *Ocimum*, two from South America (*O. campechianum* and *O. selloi*), one from Africa (*O. kilimandscharicum*), and one from Asia (*O. tenuiflorum*), growing in a homogenous conditions at the USA.

Ten accessions of *O. campechianum* (3), *O. selloi* (2), *O. kilimandscharicum* (3), and *O. tenuiflorum* (2) were grown at Purdue University Research Station, W. Lafayette, IN, in a randomized complete block experimental design, with four plants in each of the three replications. The volatile oil was extracted using a clevenger apparatus and analyzed in a GC/MS.

The results showed a high percentage of essential oil in *O. campechianum* (1.4-4.1%) and *O. kilimandscharicum*, which presented an average of 5.1% of oil/100g dry weight, largely superior of all other *Ocimum* species.

The oil composition of the *O. kilimandscharicum* accessions (ot3 and ot51) presented camphor as major constituents, with 78.3 and 59.4% respectively. *O. campechianum* accessions showed 1,8 cineol (ot30=49.5%) and α-humulene (ot31=49.4%) as major constituents. Meyhyl chavicol (30-39%) was the majoritary constituent of both *Ocimum selloi* accessions and β-caryophyllene (33.8-66.8%) in *O. tenuiflorum*.

P- 131 Compositions of the Essential Oils of *Origanum onites*, *Satureja hortensis* and *Thymbra spicata* Grown in Turkey

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In the present study, the chemical composition of the essential oils from the aerial parts of the three Lamiaceae species, oregano (*Origanum onites*), wild savory (*Satureja hortensis*) and black thyme (*Thymbra spicata*), with commercial importance in Turkey, were evaluated.
The steam-distilled volatile oils (by Clevenger apparatus) of the *O. onites*, *S. hortensis* and *T. spicata*, grown in Izmir, Antakya and Konya (Turkey) were analyzed by GC-MS technique. The major components of *O. onites* oil were carvacrol (70.27±0.17%), linalool (11.85±0.10%), thymol (9.34±0.17%), p-cymene (2.93±0.02%) and γ-terpinene (2.61±0.02%). *S. hortensis* oil was mainly composed of carvacrol (56.95±0.15%), γ-terpinene (21.98±0.02%), p-cymene (14.19±0.02%), α-terpinene (2.88±0.01) and myrcene (2.27±0.08%). Carvacrol (69.2±0.3%), γ-terpinene (12.75±0.22%), p-cymene (8.58±0.13%) and β-caryophyllene (5.01±0.11%) were the major components of *T. spicata* essential oil. All the essential oils of the spices were characterized by high content of cavracrol, p-cymene and γ-terpinene.

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P- 132 Essential Oil Composition of *Origanum vulgare* ssp. *vulgare* from Corsica

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Origanum vulgare is the species with the highest distribution range of the genus, ranging from the Azores to Japan. In this work we present population analysis of the essential oil compounds from *O. vulgare* ssp. *vulgare* in Corsica, collected in June 2006.
Two main patterns were detected in the populations of Corsica, the sabinyl type with *cis*-sabinene hydrate as main compound predominantly present in only two populations, and the p-cymyl type with γ-terpinene, carvacrol and thymol as main compounds in all other populations. The plants of the p-cymyl type could be classified in a pure carvacrol, a pure thymol chemotype and a chemotype where both hydroxylated monoterpenes occured. In most of the populations all three p-cymyl chemotypes could be detected. The presence of thymol methyl ether and carvacrol methyl ether always concurrently appearing with thymol and carvacrol, respectively, was characteristic for *O. vulgare* from Corsica. A multivariate discriminant analysis of the essential oil compounds could not resolve the populations but distinguished the four regions from where the plants were collected.

P- 133 Essential Oil Composition of 11 Populations of *Origanum syriacum* from Syria

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Origanum syriacum is a commercially important *Origanum* species growing from Turkey to Egypt (Sinai) and cultivated or collected from the wild in several countries due to its high essential oil content and is valuable essential oil composition.
Eleven populations of this species were collected in Syria in full bloom in July 2006 and analysed by GC-MS and GC-FID. All plants analysed were pure p-cymyl types with main compounds thymol and/or carvacrol. Conspicuously high values of up to 24 % were observed for thymoquinone.
Discriminant analysis of the essential oil compounds revealed two distinct groups, a Northern group (populations 1 to 6) and a Southern group (populations 7 to 11) possibly indicating the border between *Origanum syriacum* var. *bevanii* in the North and O. syriacum var. *syriacum* in the South of Syria.

P- 134 Chemical Composition of the Essential Oil from Stems and Leaves of *Phlomis cancellata* Bge. from Iran

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Phlomis cancellata is one of 17 perennial species from genus *Phlomis* which belongs to family Labiatae.Ten of these species are endemic in Iran [1]. Several investigations of the chemical composition of essential oils from the genus *Phlomis* have been done recently [2,3], of which six were reported in 2006; one of these deals with aerial parts of *Phlomis cancellata* [3] . To the best of our knowledge the present report is the first on essential oils from the stems and leaves of *Phlomis cancellata*. The aim of our study is to compare the yield and to identify the constituents of the essential oils of stem and leaf of *P. cancellata* Bge. growing wild at flowering stage in Northeast of Iran. Pale yellowish colored oils were obtained by 3-hour hydrodistillation, using a Clevenger-type apparatus, of the stems and leaves, which gave yields of 0.019% and 0.032% (w/w), respectively. GC and GC-MS analysis identified 7 compounds that accounted for 94.8% of the stem oil of the plant. Of these, β-selinene (34.7%), germacrone (20.4%), germacrene B (17.0%) and γ-elemene (15.4%) were the major components. The leaf oil of the plant was characterized by higher amounts of germacrene D (40.5%), β-caryophyllene (18.6%) and bicyclogermacrene (10.1%) among the sixteen detected components, which comprised 98.6% of the total oil. Both stem and leaf oils of the plant consist of sesquiterpenes only, of which sesquiterpene hydrocarbons were the major constituents.

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P- 135 Essential Oil Composition and Antibacterial Activity of the Leaf and Flower of *Phlomis herba-venti* subsp. *pungens* from Iran

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The genus *Phlomis* (Labiatae) comprises 17 species, which have been widely distributed in Iran.1 Several Phlomis species are used in herbal medicine, e.g. for treatment of respiratory tract diseases or externally for treatment of wounds.2 The essential oils obtained from the dried leaves and flowers of *Phlomis herba-venti* subsp. *pungens* were analyzed using GC and GC/MS. Twenty-four components were identified in the oil of leaf subsp. *pungens* with germacrene D (31.1%), T-muurolol (11%) and α-pinene (7.1%) as main constituents. Twenty-six compounds were identified in the oil of flower subsp. *pungens* with germacrene D (39.2%), α-pinene (9.3%) and 2-pentadecanone (7.6%) as main components. All oils consist of monoterpenes, sesquiterpenes and aliphatic compounds.
Furthermore, both the oils exhibited an interesting antibacterial activity against Gram-positive and Gram-negative bacteria. The results showed that the sample of leaf exhibited better activity against all the tested micro-organisms. On the other hand, the oil of flowers did not show any activity against the Gram-negative bacterium *Escherichia coli* and *Pseudomonas aeruginosa* but this oil exhibited higher activity against *Staphylococcus aureus*.

Acknowledgments: We are grateful to the research councils of Islamic Azad University for their financial support.

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P- 136 Constituents of Essential Oil of *Rosmarinus officinalis* Grown in the North of Iran (Mazandaran, Kandelous)

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Rosmarinus officinalis is one of the most important medicinal plants which are growing in Iran. This plant was collected from Kandelous in the West Mazandaran province. The essential oils were obtained from dry leaves by hydro distillation. The percentage of oil was 92% v/w calculated on the dry weight , then analyzed by capillary GC and GC/MS. Main components were piperitone (27.7%), -pinene (17.6%), linalool (17.8%) and 1,8-cineole (8.6%).

P- 137 Essential Oil Composition and Biological Activities of Three *Salvia* Species Indigenous to South Africa

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Salvia species are traditionally used in South Africa to treat various conditions such as malaria, inflammation, tuberculosis and cancer. The composition of the hydrodistilled essential oil obtained from *Salvia dolomitica*, *S. muirii* and *S. radula* was determined by GC-MS. The oil of *S. dolomitica* was dominated by oxygen-containing monoterpenes (71.1%) with geraniol (19.6%); linalyl acetate (19.6%) and linalool (16.6%) being the major constituents. *Salvia muirii* oil mostly accumulated monoterpene hydrocarbons (53.0%) with 1, 8-cineole (23.2%) and α-pinene (22.3%) being the major constituents. The oil of *S. radula* was dominated by oxygen-containing sesquiterpenes with β-caryophyllene oxide (22.6%) and humulene epoxide (12.6%) being the major constituents. The three oils exhibited poor anti-oxidant (using the DPPH radical) (IC50 values > 100 µg/ml), and anti-inflammatory (using the 5-lipoxygenase assay) activities. The three oils exhibited moderate antimicrobial activity against *B. cereus* (MIC value: 9.3 mg/ml) and good to moderate antimalarial activity against the *Plasmodium falciparum* FCR-3 strain (4.8 µg/ml ≤ IC50 values ≤ 13.5 µg/ml). However, the oils displayed some degree of toxicity to human kidney epithelial cells (3.1 µg/ml ≤ IC50 values ≤ 22.9 µg/ml). Considering the toxicity associated with the essential oils, these plants should be used with caution.

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P- 138 Composition and Antimicrobial Activity of *Salvia amplexicaulis* Lam. Essential Oil

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Salvia amplexicaulis Lam. (Labiatae) is a perennial plant distributed in W. Anatolia and E. Balkans [1]. Aerial parts of this species were collected in S.E. Serbia at Mt. Rudina, Bosilegrad (alt. 1000-1200 m), during the full flowering period (August 2006). The chemical analysis of the essential oil was performed using GC-FID and GC-MS. Fifty-one compounds (90.2% of the total oil) were identified. The essential oil was characterized by a high amount of sesquiterpenes (81.1%) with germacrene D (14.8%), viridiflorol (10.6%), caryophyllene oxide (10.5%), (E)-caryophyllene (9.4%) and eudesma-4(15),7-dien-1-β-ol (5.2%) being the main components. The microbial growth inhibitory properties of the essential oil was determined against Gram-positive bacteria *Staphylococcus epidermidis* (ATCC 12228), *S. aureus* (ATCC 25923), *Micrococcus luteus* (ATCC 10240), Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCIMB 9111), *Pseudomonas aeruginosa* (ATCC 27853), and a yeast *Candida albicans* (ATCC 10259). The best inhibitory effect was detected against *M. luteus* (MIC 40 μl/ml), followed by *S. epidermidis*, *S. aureus* and *C. albicans* (MIC 80 μl/ml). Tested Gram-negative bacteria were more resistant (MIC> 80 μl/ml) than Gram-positive bacteria and *C. albicans*.

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P- 139 *Salvia aucheri* Bentham var. *canescens* Boiss. & Heldr.: Essential Oil Composition, Phenolics and Antioxidant Properties

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Salvia aucheri Bentham var. *canescens* Boiss. & Heldr.,a member of the family Labiatae, is a perennial plant endemic to Turkey. In the Flora of Turkey, more than 70 species and varieties of Salvia can be found. Many *Salvia* species and varieties grow wild or are cultivated in many parts of the world. The herbs and/or their essential oils are used commonly in the food, drug and perfumery industries.

The oil yields of dried plants (v/w) obtained by hydro-distillation were 0.9% and the essential oil composition of *S. aucheri* was analysed by GC–MS. Thirteen compounds were identified, representing 94% of the oil. The main constituents of the oils were: 1,8-cineole (44.4%), camphor (19.0%), α-pinene (6.4%), borneol (5.6%), camphene (5.8%) and β–pinene (5.1%), respectively. 1,8-Cineole and camphor were the major components of *S. aucheri* var. *canescens*. Antiradical activity (IC50) of the essential oil was found as 41.31=μg/ml.

In addition, phenolic components and antioxidant properties of extracts from *S. aucheri* were determined. Soxhlet extraction method with methanol was used. The extract yield (%) was 13.58. Main phenolic components were found as carvacrol (1523.73 mg/100g dried herb), rosmarinic acid (860.33 mg/100g dried herb) and (+)-Catechin (361.00 mg/100g dried herb). Total phenolic content and antiradical activity (IC50) of the extract were also determined as 112.14 mg GAE/g extract and IC50= 75.69 μg/ml. Further studies will be carried out on using food system as natural antioxidant.

P- 140 Influence of Continental Climatic Condition on Essential Oil Composition of *Salvia brachyodon* Vandas Transplanted from Adriatic Coast

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Salvia brachyodon Vandas is an endemic Dinaric species with narrow geographical distribution limited to only two localities, one in Croatia, Pelješac Peninsula, and another at the border of Herzegovina and Montenegro at Mt Orjen [1]. Our study was related to the essential oil composition of S. brachyodon transplanted from Adriatic Coast to continental climatic conditions in period of three years.
Data of the Croatian population of *S. brachyodon* show that the main volatile constituent was humulene epoxide II [2], while Orjen population had 1,8-cineole as main component [3,4].
GC-MS analysis of essential oils obtained from transplanted plants by hydrodistillation, show that sample collected one year after transplanting is sesquiterpene-type, while all further samples have 1, 8-cineole as main compound (22.2–42.3%). The total number of all identified components is over one hundred in four samples.
The results of essential oil analyses confirm the influence of environmental conditions on the nature of plant chemical composition that has the important role in plant adaptation.

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P- 141 Volatile Constituents of the Leaf of *Salvia glutinosa* L. from Iran

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The genus *Salvia* (Lamiaceae) comprises more than 700 species widespread over the world. In the Flora Iranica this genus is represented by fifthy-eight species, seventheen of which are endemic [1,2]. The genus *Salvia* is known for its medicinal value, antibacterial and antioxidant properties [3,4].
Salvia glutinosa was collected during the flowering stage from Kojur, Nowshahr, Mazandaran Province, located North of Iran in July 2006 and identified by Herbarium of Nowshahr, Botanical Garden, Research Center of Natural Resources of Mazandaran Province, Nowshahr, Iran. The leaves of plant were hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 0.2% (w/w) of a dark yellowish oil.
The oil was analyzed by GC and GC-MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [5].
Thirty-two compounds were characterized in the essential oil of *S. glutinosa*, representing 90.1% of the oil, of which phytol (15.8%), caryophyllene oxide (14.1%), β-caryophyllene (8.3%), humulene oxide (5.5%), α-humulene (5.2%), germacrene D (4.9%), spathulenol (4.7%) were found to be the major components.

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P- 142 Chemical Composition and Antimicrobial Activity of the Essential Oil of *Salvia macrochlamys* Boiss. & Kotschy, Growing Wild in Iran

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The genus *Salvia* (Lamiaceae) comprises more than 700 species widespread over the world. Remarkably, this genus is in the Flora Iranica represented by 58 species, of which 17 are endemic [1,2]. The genus *Salvia* is known for its medicinal value, antibacterial and antioxidant properties [3,4].
The aerial parts of *Salvia macrochlamys* were collected during the flowering stage from Marmishuo Hill in West-Azerbaijan Province, in July 2006 and were hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 0.35% (w/w) of yellowish oil.
The oil was analyzed by GC and GC-MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [5]. Thirty-four compounds were characterized in the essential oil of *S. macrochlamys*, representing more than 97.6% of the oil, of which β-caryophyllene (32.7%), 1,8-cineol (18.9%), caryophyllene oxide (13.6%), camphor (7.55%) and β-pinene (6.9%) were found to be the major components.
The essential oil showed high antibacterial activity against gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) and *Klebsiella pneumoniae* as gram-negative bacteria.

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P- 143 Chemical Composition of the Essential Oil of *Salvia multicaulis* Vahl.

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Salvia multicaulis is a perennial herb belongs to Lamiaceae family which widely grows in most regions of Iran. This plant is used as flavor and tea. The aerial parts of this plant were collected from Broujerd in Lorestan province. The air-dried samples were subjected to hydrodistillation using by Clevenger apparatus for 2.5h (yield: 0.3%). The essential oil was analyzed by GC and GC/MS. Forty-two constituents were identified, accounting for 90.96% of total oil. The main components of this oil were bornyl acetate (18.6%), borneol (17.2%), camphor (13.8%), camphene (5.6%) and β-caryophyllene (5.9%).

P- 144 Chemical Composition of the Essential Oils from Flowers, Stems and Roots of *Salvia multicaulis* Vahl from Iran

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The genus *Salvia* comprises 700 herbs and shrubs, growing in the temperate and warmer zone of the world. Fifthy-eight species are found in Iran, among which 17 are endemic [1]. Some species of genus *Salvia* are used as medicinal and ornamental plants. *Salvia officinalis* is one of the most widespread species and known as a spice, condiment and medicine [2-3].
The aim of our study is identification the constituents of volatile oils of flower, stem and root of *Salvia multicaulis* Vahl. According to our knowledge, this is the first report about analysis the essential oils of stem and root of *Salvia multicaulis*.
The yellowish colored oils were obtained by hydrodistillation, using a Clevenger-type apparatus for 3 hours, from flower, stem and root in 0.38, 0.29 and 0.18 % w/w yield, respectively, and analyzed by GC and GC-MS. Twenty one compounds representing 94.3% of flower oil of *Salvia multicaulis* were identified among them 1,8-cineol (25.3%), α-pinene (18.3%), camphor (12.4%), camphene (8.4%) and bornyl acetate (7.9%) were the major ones. The stem oil of the plant was characterized by higher amounts of 1,8-cineol (24.8%), camphor (24.2%), α-pinene (20.9%) and camphene (13.0%) among the eight components comprising 98.0% of the oil. Nine compounds representing 95.37% of the root oil of the plant were identified among them borneol (48.8%), camphor (17.2%), 1,8-cineol (8.0%), and α-pinene (6.6%) were the major constituents. In essential oils of flower and root monoterpenes predominated over sesquiterpenes while in the essential oil of stem, monoterpenes were only identified compounds.

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P- 145 Chemical Composition and Antibacterial Activity of Essential Oil from Leaves, Stems and Flowers of *Salvia reuterana* Boiss. Grown in Iran

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The essential oils obtained by hydrodistillation of the leaves, stems and flowers of *Salvia reuterana* (Lamiaceae) were analysed by GC and GC/MS. Germacrene D and β-caryophyllene were the major constituents in all three oils: (28.5%, 27.7% and 32.5%) and (15.5%, 11.4% and 16.6%), respectively. Bicyclogermacrene (10.2% and 13.2%) was also predominating in the stem and flower oils. The composition of the oils was mostly quantitatively rather than qualitatively different. All oils consisted mainly of sesquiterpenes and a small percentage of non-terpenoid compounds. In all three oils monoterpenes were less than 0.5%. Antibacterial activity was determined by measurement of growth inhibitory zones.

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P- 146 Antioxidant and Antimicrobial Properties of the Essential Oil of *Satureja cuneifolia* During Different Maturation Stages

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Satureja species are used in traditional medicine for their medicinal and aromatic properties such as anti-inflammatory, analgesic, sedative, antitussive and antipyretic. *Satureja* species are also widely used as herbal tea in the Turkey especially Mediterranean part (1-3).
The essential oils obtained by hydrodistillation from aerial parts of *Satureja cuneifolia* Ten., collected in three different maturation stages via pre-flowering, flowering and post-flowering, were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems, simultaneously. At the same time, the essential oils and main components were evaluated for its antimicrobial activity using a micro-dilution assay resulting in the inhibition of a number of common human pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and the yeast *Candida albicans*. Furthermore, the antioxidant capacity of the essential oils and major components were examined using an in vitro radical scavenging activity test. In addition, the effect on inhibition of lipid peroxidation of the essential oil was assayed using β-carotene bleaching method.
Thymol (42.5-45.2%), p-cymene (19.4-24.3%) and carvacrol (8.5-13.2%) were identified as the main constituent in all stages. The minimum inhibitory concentrations (MIC) of essential oils varied between 62.5-250 μg/ml within a moderate antimicrobial activity range. The essential oils obtained from *S. cuneifolia* in three different stages and its main components were interacted with 1,1-diphenyl-2-picrylhydrazyl (DPPH●) as a nitrogen centered stable radical, resulting in IC50 1.6-2.1 mg/ml. All the oils inhibited the linoleic acid peroxidation almost as same as BHT (93.54-94.65%). BHT and ascorbic acid were used as positive controls in the biological assays. Bioactivities of thymol and carvacrol were also tested.

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P- 147 Qualitative and Quantitative Comparison between Essential Oils of Winter (Perennial) and Summer (Annual) Savory

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The Essential oils of the aerial parts of different species of *Satureja* cultivated in Iran were extracted by hydro-distillation method. The results showed the significance differences on the quantity and quality of the essential oils. The largest amount of the essential oil (3%) was extracted from annual summer savory (*S. hortensis*) and the lowest amount (1.4%) was obtained from perennial winter savory (*S. montana*). The results of GC and GC/MS analysis of the oils showed there are different essential oil compositions among *Satureja* species. Sixteen and fourteen components were analyzed from the oil of annual and perennial savory respectively. The perennial winter savory (*S. montana*) accumulated more carvacrol (65.8%) than annual summer savory (*S. hortensis*) (38.0%).

P- 148 Antimicrobial and Antioxidant Activities of the Extracts of Two Turkish Endemic *Sideritis* Species

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Sideritis phlomoides Boiss.& Bal. and *Sideritis libanotica* Labill. are endemic species in Turkey and commonly used as herbal tea and folk medicine. In this study, these teas were ascribed to have antibacterial, antifungal and antioxidant properties in vitro.
The extracts yields obtained Soxhlet extractor with absolute methanol:acetone:water:acetic acid (40:40:9:1) were 12.56% for *S. phlomoides* and 15.10 % for *S. libanotica*. Total contents of phenolics, flavonol and flavanol of *S. phlomoides* and *S. libanotica* extracts were determined as 59.98 and 16.70 mg gallic acid equivalent (GAE)/g, 20.48 and 4.18 mg catechin equivalents/g and 15.55 and 3.94 mg rutin equivalents/g by using Folin-Ciocalteu colorimetric, Neu's reagent solution and vanillin colorimetric methods, respectively. Total phenolics of the *S. phlomoides* and *S. libanotica* extracts were found as 59.98 and 16.70, respectively. Antiradical activities of extracts at 100 ppm were determined were 50.99 % for *S. phlomoides* and 31.35 % for *S. libanotica* using the 1, 1-diphenyl-2- picrylhydrazyl (DPPH) method.
In this study, the extracts were determined for antimicrobial activity against fifteen microorganisms and paper disc diffusion method was used. *S. libanotica* extract was more effective than the *S. phlomoides* extract. The *S. libanotica* extract was exhibited the effective antimicrobial activity against *B. brevis*, *B. subtilis*, *B. subtilis* var. *niger*, *K. pneumonia*, *M. smegmatis*, *Ps. aeruginosa* and *Y. enterocolitica* and ineffective against other seven microorganisms. Additionally, *S. phlomoides* extract at 1% concentration was not shown any antimicrobial activity to the test microorganisms.

P- 149 Chemical and Micromorphological Characterization of *Stachys germanica* subsp. *salviifolia* (Ten.) Gams (Labiatae)

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Stachys germanica is a very polymorphous species, widely diffused in Europe, particularly in the Mediterranean basin [1,2]. It shows noteworthy morphological and ecological variability; in Italy three subspecies have been identified [3].
In this work we examined *S. germanica* subsp. *salviifolia* collected at Monte Morello, near Florence. The composition of the essential oil and the distribution, morphology, histochemistry and ultrastructure of the glandular trichomes were studied, in order to attain a deeper insight into this difficult taxon.
The essential oils from both leaves and inflorescences were obtained by hydrodistillation in a Clevenger-type apparatus and analysed by GC/MS. The major constituents of the essential oil of the inflorescences are limonene (24.0%), β-pinene (18.6%), γ̃muurolene (12.6%) and (E)-nerolidol (6.6%), while those of the leaves are γ-muurolene (37.6%), phytol (9.7%), β-bourbonene (3.4%) and β-ylangene (3.2%).
Glandular trichomes are both capitate and peltate. The latter (type A) are present on leaves and inflorescences and have the typical morphology and lipophile secretion described in the literature [4], but their basal cell is elongated to form a well developed stalk. The capitate hairs are of two different types: type B, localised on the whole plant; type C, present only on the inflorescences. Type B is a short capitate trichome, with both lipophilic and hydrophilic secretion, already described in the literature [4]. Type C is a long capitate hair characterised by a multicellular stalk and a secretory head of 6-8 cells, each bearing on the apex a small subcuticular space. Their secretion is mainly polysaccharidic, but with polyphenolic and essential oil fractions.
For the first time the essential oil composition of *S. germanica* from Italy, as well as the characterisation of the glandular trichomes, are reported.

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P- 150 Chemical Composition and Antibacterial Activity of the Essential Oil of *Stachys lavandulifolia* Vahl

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Stachys lavandulifolia is a grassy, permanent and self-propelling plant from the Labiatae family that grown wild in mountains of West and South-West of Iran. The aerial parts of this plant are used as anodyne drug in folk medicine. This plant was collected from Khoramabad mountains of Lorestan province in Iran and the air-dried epigeous parts were subjected to hydrodistillation method (in a yieid 0.8% W/W). Identification of essential oil constituents was made by GC and GC/MS. The antibacterial tests were carried out by drilling well and measuring the diameter of the inhibition zone.
The results show that a large proportion of the oil was composed of monoterpene compounds. 14 components were identified, accounting for 91% of the total oil. The major components are α-pinene (16.3%), myrcene (20.9%) and α-terpinene (20%). The results of the antibacterial activity showed that the oil was active against of *Staphylococcus epidermidis*, *Salemonella typhi* and *E. coli* (diameter of growth inhibition zones: 29, 31 and 25mm, respectively).

P- 151 Volatile Constituents of the Essential Oil of *Stachys obtusicrena* Boiss. from Iran

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The genus *Stachys* is represented in the flora of Iran by 34 species of which 13 are endemic [1].
Aerial parts of *Stachys obtusicrena* Boiss., a plant native to Iran, was collected from Isfahan Province at the time of flowering.
The aerial parts were air-dried at ambient temperature in the shade and hydrodistilled by using a Clevenger-type apparatus for 3 hours. The essential oils were analyzed by GC and GC/MS, and their constituents were identified by comparison of retention indices with those reported in the literature and by comparison of their mass spectra with the Wiley library or with the published mass spectra [2].
Nineteen compounds were identified in the oil of *S. obtusicrena* representing 90.0% of the total oil with α-pinene (34.6%) as the major constituent, followed by germacrene D (8.0%) bicyclogermacrene (7.8%) and β–caryophyllene (6.2%).
Thus, the oil of *S. obtusicrerna* consisted of three monoterpene hydrocarbons (37.0%), one oxygenated monoterpene (3.2%), ten sesquiterpene hydrocarbons (40.0%), four oxygenated sesquiterpenes (8.1%) and one aliphatic hydrocarbon (1.3%).

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P- 152 Compositions of Essential Oils and Trichomes of *Teucrium chamaedrys* L. subsp. *trapezunticum* Rech. fil and subsp. *syspirense* (C. Koch) Rech. fil.

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Teucrium chamaedrys L. is a member of Lamiaceae family and is represented in the flora of Turkey by six subspecies. Plants were collected during the flowering period from Erzurum (subsp. trapezunticum Rech. fil) and Aksehir [subsp. *syspirense* (C. Koch) Rech. fil] province of Turkey. *Teucrium chamaedrys* is suffrutescent perennial herb, often rhizomatous, 5-50 cm, very variable indumentum and corolla reddish-purple coloured and grows on rocky slopes, limestone slopes, steppe and the flowering time is from April to August (1).

In the Lamiaceae, glandular trichomes, which are often microscopic and secrete various types of compounds, are generally classified as either capitate (clavate) or peltate (subsessile), based on their morphological characteristics (2). The aerial organs of *T. chamaedrys* L. subsp. *trapezunticum* and subsp. *syspirense* bears numerous eglandular and glandular trichomes. Eglandular trichomes are simple, long-multicellular with cuticular micropapillae, and glandular hairs are peltate type. The peltate hairs, pale-yellow to colourless, consists of a basal cell, a short unicellular stalk and a secretory head, usually composed of 4-8-celled and secrete an essential oil which accumulates in the large space formed at the tip of the head between the raised cuticle and the apical cell walls. Peltate types on the calyx were denser than the leaves. Scanning electron microscopy (SEM) was used to determine the morphology of trichomes.

The aerial parts were subjected to microdistillation for the isolation of volatiles. The analysis was performed simultaneously by using a gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The major components were characterized as β-caryophyllene (18%), nonacosane (12%), germacrene D (11%), caryophyllene oxide (7%) and α-pinene (7%) for subsp. trapezunticum and caryophyllene oxide (23%), α-pinene (11%) and caryophyllenol II (5%) for subsp. syspirense, respectively.

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P- 153 Chemical Composition of the Essential Oil of *Teucrium chamaedrys* L. subsp. *chamaedrys* from Iran

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The genus *Teucrium* (Lamiaceae) is comprised of about 340 species widespread over the world. In the Flora Iranica, this genus is represented by 12 species [1]. Various species of the genus *Teucrium* are known as antiseptic, antipyretic, anti-inflammatory, antispasmodic, antinociceptive, anti-rheumatic, anti-helmetic, diuretic, hypoglycemic, diaphoretic and tonic in folk medicine [2].
Teucrium chamaedrys subsp. *chamaedrys* were collected from Marmisho Hill in west Azarbayjan Province, loctated in North-West of Iran, in July 2006 and identified by Herbarium of Agricultural Research Center of West Azarbayjan Province, Urmia, Iran. The air-dried aerial parts of *T. chamaedrys* subsp. *chamaedrys* were hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 0.25% (w/w) of yellowish oil.
The components of the essential oil were identified by comparison of their mass spectra with those of a computer library or with authentic samples and confirmed by comparison of their retention indices as well as with data published in the literature [3].
Forty-two compounds were characterized in the essential oil of *T. chamaedrys* subsp. *chamaedrys*, representing 94.1% of the oil, of which α-muurolene (15.3%), β-caryophyllene (15.0%), α-pinene (7.9%), Z-β-farnesene (7.6%), β-pinene (5.9%) and limonene (5.1%) were found to be the major constituents.

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P- 154 Chemical Composition of the Essential Oil of *Teucrium hyrcanicum* L., Growing Wild in Iran

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The genus *Teucrium* (Lamiaceae) is comprised of about 340 species widespread over the world. In Iran, there are 12 species, three of which are endemic [1]. Various species of the genus *Teucrium* are known as antiseptic, antipyretic, anti-inflammatory, antispasmodic, antinociceptive, anti-rheumatic, anti-helmetic, diuretic, hypoglycemic, diaphoretic and tonic in folk medicine, also it has been reported antioxidant and antimicrobial activity in the essential oil of them [2,3]. *Teucrium hyrcanicum* is an endemic plant related to Caspian sea area, in Iran.
The aerial parts of the plant were collected during the flowering stage from road of Khalkhal, 45 km to Asalem, Gilan Province, North of Iran, in July 2006 and were hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 0.2% (w/w) of yellowish oil. The oil was analyzed by GC and GC/MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [4]. Thirty-six components were characterized in the essential oil of *T. hyrcanicum*, representing 94.1% of the oil, of which hexahydrofarnesyl acetone (12.7%), linalool (11.7%), E-β-farnesene (10.7%), dihydroedulane (8.6%) and ar-curcumene (8.5%), were found to be the major constituents.

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P- 155 Antioxidant Activity of the Essential Oils, Essential Oils Fractions, Hydrodistillation-aqueous Phase Extracts and Soxhlet Extracts of Portuguese *Thymbra capitata* and *Thymus mastichina*

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Portuguese *Thymbra capitata* and *Thymus mastichina* a) essential oils (EO), b) EOs fractions, c) remaining hydrodistillation-aqueous phase solvent- (RHAPSE) and aqueous-extract (RHAPAE) and c) plant Soxhlet extracts (PSE) have been assayed for antioxidant activity.
EOs were isolated by hydrodistillation and analysed by GC and GC-MS. EOs fractions were obtained with 100% pentane (P100), 95% pentane:5% ether (P95:E5), 75% pentane:25% ether (P75:E25) and 100% ether (E100). PSEs were performed with hexane, dichloromethane and methanol. The antioxidant activity of the oils (100-1000mg.l-1) was assessed by the free radical scavenging (DPPH), reducing power determination, chelating effect, superoxide anion scavenging activity and by the TBARS assay.
Carvacrol (75%) was the main *T. capitata* EO component and 1,8 cineol (44%) dominated that of *T. mastichina*. Carvacrol (97-98%) also dominated P95:E5 and P75:E25 T. capitata EOs fractions and 1,8 cineol was particularly abundant in the *T. mastichina* P95:E5 EO fraction.
In general, the RHAPAE and methanolic-PSE extracts showed better reducing power and free radical-scavenging activity than the EOs or EOs fractions. Chelating effect was only detected for the methanolic-PSE extracts, namely at the highest concentration (1000mg/L). The best superoxide anion scavenging activity was detected with the RHAPAEs of both plants. With TBARS assay, both essential oils showed some inhibition of lipid peroxidation, at 1000mg/L, *T. capitata* oils showing the highest activity (80%).

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Keywords: Thymbra capitata, Thymus mastichina, essential oils, antioxidant activity.

P- 156 Bioactivities of Essential Oil and Extract of *Thymus argaeus* Boiss. & Bal.

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Thymus argaeus Boiss. & Bal., an endemic plant species for Turkey has been traditionally used as beverage and medicinal tea in different regions of Turkey. In this study, the essential oil components, antimicrobial and antioxidant effects of *T. argaeus* known as wild thyme were determined. The herb collected as wild was dried, then their essential oil was extracted using a Clevenger apparatus, and their methanolic extract was obtained using Soxhlet apparatus. *T. argaeus* had 0.8% of the essential oil. The main components of *T. argaeus* were linalool (49.89%), terpineol (15.02%), linalyl acetate (9.70%) and thymol (9.44%). Antimicrobial effect of the essential oil with the concentrations of 0.2, 0.5, 1.0 and 2.0 % were determined in vitro, against fifteen different microorganisms using agar diffusion method. The results showed that essential oil of *T. argaeus* had an antimicrobial effect against *A. hydrophila*, *B. brevis*, *B. cereus*, *M. morganii*, *M. smegmatis*, *P. mirabilis*, *S. aureus* and *S. cerevisiae*, and *A. hydrophila* was the most sensitive microorganism.
Again, total phenolic of the extract was 83.31±0.59 mg gallic acid equivalents (GAE)/g. Total flavanols and flavonols of the extract were 6.26±0.00 mg catechin equivalents (CE)/g and 28.81±0.21 mg rutin equivalents (RE)/g, respectively. The free radical scavenging activity of the extract was 83.18±0.42% by DPPH method. Antimicrobial activity of the extract was assessed (1, 2.5, 5 and 10% w/v) by the agar diffusion method against above fifteen microorganisms. While all concentrations of the extract were ineffective against only *B. subtilis*, *E. coli*, *K. pneumoniae*, *M. morganii* and *P. mirabilis*, they had antimicrobial activities against the other tested microorganisms. The most sensitive microorganism was *P. aeruginosa*. In conclusion, it can be concluded that the essential oils and the extract of *T. argaeus* could be used as natural antimicrobial agents in the food preservation practices

P- 157 Chemical Variability and Radical Scavenging Activity of *Thymus hyemalis* L. Essential Oil Cultivated at the Region of Murcia (Spain)

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Thymus hyemalis Lange, winter thyme, is an endemic shrub over the Southeastern Iberian peninsula, mainly Alicante, Murcia and Almeria. The chemical variability of essential oil from wild *T. hyemalis* of the Southeastern Iberian Peninsula has been reported by [1-3]. However, no bibliographic references have been previously published related to the radical scavenging activity of this thyme species essential oil.
Chemical variability of the essential oils from this species were analyzed by means of gas chromatography-mass spectrometry and assayed for their radical scavenging activities (%AA) (50µL/mL) on 2,2-diphenyl-1-picrylhydrazyl (DPPH•) according to the method described by Brand-Williams et al. [4]. The plants were grouped into the following groups: 3 pure thymol chemotypes with (60-69-75)%AA respectively; Thymol/Linalool (67%AA); Thymol/Borneol (60%AA); Thymol/ Linalool/Borneol/Verbenone (67% AA); Thymol/(E)-Sabinene Hydrate/Terpinen-4-ol (65% AA); 1,8-Cineol/Thymol/ α-Terpineol (56% AA); Myrcene/(E)-Sabinene Hydrate (38% AA); Carvacrol (69% AA); 3 of α-Terpineol/ Thymol (49-55-58 %AA); (Linalool/Thymol) (41%AA); Linalool /Thymol/Verbenone (44%AA); Linalool/ /Thymol (44% AA); Linalool/Linalyl acetate (41% AA); Linalool/ Thymol /1.8-Cineol (67%AA); Borneol/Thymol/Linalool (69% AA); Thymol/Cineol (66% AA); Thymol/Verbenone (53% AA).
From these results it can be concluded that the antiradical activity of winter thyme essential oil is not related directly to the presence of high concentrations of thymol, since shrubs with a 25% thymol relative concentration exhibited high decolouration capacity against the radical DPPH• than those with relative concentration of 46%.Thyme oil with combined chemotype reached better antiradical activities that those from pure chemotypes, as it can be stated by the oils rich in the pair of volatile components thymol and linalool.

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P- 158 Biological Activities and Chemical Composition of Essential Oil and Extract Isolated *Thymus revolutus* Čelak

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In our study, it was aimed to determine chemical composition and antioxidant activities in vitro of *T. revolutus* Čelak, endemic to Anatolia, (Lamiaceae) essential oil using hydro-distillation and methanol:acetone:water:acetic acid (90:90:18:2) extract using Soxhlet extractor were investigated.

The yield of the essential oil was 0.6% (v/w) and the essential oil composition was analysed by GC–MS. The major constituents of the essential oil were carvacrol (24.53%), thymol (15.39%), p-cymene (15.50%) and borneol (14.66%). Total contents of phenolics (38.68 mg gallic acid equivalents / g) flavonol (12.875 mg catechin equivalents / g) and flavanol (12.87 mg rutin equivalents / g) of extract were determined by Folin-Ciocalteu colorimetric, Neu's reagent solution and vanillin colorimetric methods, respectively. Antiradical activities were evaluated using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and were found as 11.40 % for essential oil and 48.91 for extract at 100 ppm.

At 1, 2.5, 5 and 10 % concentrations of the extract and 0.2, 0.5, 1.0 and 2.0 % concentrations of essential oil obtained *T. revolutus* were tested for antimicrobial activity against 15 species of microorganisms by using agar diffusion methods. The 5 and 10% concentrations of the extracts showed antimicrobial activities against many microorganisms except for *Aeromonas hydrophila*, *Bacillus cereus*, *Escherichia coli*, *Morganella morganii*, *Proteus mirabilis*, *Candida albicans* and *Saccharomyces cerevisiae*. *B. subtilis* var. *niger* was the most sensitive microorganism against the extract contaminations. Additionally, 2% concentration of the essential oil had antimicrobial effects against all tested microorganisms except for *Y. enterocolitica*. *B. cereus* was the most sensitive microorganism against the essential oil.

As a result, the extract and essential oil of *T. revolutus* could be used as a natural antioxidant and antimicrobial agent in food.

P- 159 Comparison of Essential Oil Composition of Three *Thymus striatus* Vahl Populations from Balkan Peninsula

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Essential oil profile of *Thymus striatus* Vahl, collected from three different natural habitats, was analyzed by capillary gas–chromatography coupled to mass spectrometry. According to the former studies of this species, environmental conditions have a great influence on chemical composition of the essential oils [1, 2, 3].
In the present work, two volatile oils were obtained from populations growing wild in continental Bosnian region, and third plant material originated from the Croatian Adriatic Coast.
The samples of *T. striatus* collected from different localities showed significant differences, both in qualitative and quantitative composition of their hydro-distilled essential oils. More than seventy chemical constituents were identified. The volatile oil from one continental population clearly belongs to the thymol-chemotype (40.5%), while the main compounds in the oil from the other continental population were sesquiterpene hydrocarbons 6α-hydroxygermacra-1(10),4-diene (14.5 %) and germacrene D (13.5 %). In contrast, the chemical composition of the essential oil of *T. striatus* from Adriatic Coast was characterized by high content of oxygenated sesquiterpenes with caryophyllene oxide (11.3 %) and α-elemol (11.1 %) as the most abundant constituents.

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P- 160 Characterization of the Volatile Oil of *Vitex agnus-castus* L. from Turkey

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Vitex agnus-castus L. is a member of Verbenaceae with its natural habitat ranging from the Mediterranean coastal region to Central Asia and is widespread in South, West and North Anatolia (1). *V. agnus-castus* is a typical medicinal plant with a long tradition in folk medicine. It is particularly used as a popular remedy for disorders of the menstrual cycle (2). In Turkey, the plant is used in folk medicine as a diuretic, carminative, sedative and an anaphrodisiac (3). The volatile oils obtained from leaves and fruits of the plant were found to be effective in gynaecological problems (4). The chemical composition of leaf and fruit oils of the plant exhibit differences according to the plant origin, furthermore some chemotypes of the plant were reported in previous studies (5, 6).
There is only one study on the leaf oil composition of *V. agnus-castus* from Turkey (7). The present work was focused on chemical composition of leaf (LO) and fruit oils (FO) of *V. agnus-castus* collected from two different region of Turkey. The volatile oils from the leaves and fruits of *V. agnus-castus* obtained by hydrodistillation were analyzed by GC-MS and their composition were compared. The volatile oil yields of leaf samples (LO1, LO2) were 0,64% and 1,14%; fruit samples were 0,81% and 1,28%, respectively. The leaf oils were rich in 1,8-cineole (16,2%), α-pinene (9,6%), β-caryophyllene (7,5%), sabinene (6,6%) and limonene (5,2%) (LO1); 1,8-cineole (19,3%), sabinene (13,3%), β-caryophyllene (9,0%), α-pinene (5,4%) (LO2), while the fruit oils contained β-caryophyllene (12,8%), α-pinene (8,8%), 1,8-cineole (4,6), limonene (4,6%) (FO1); β-caryophyllene (10,4%), 1,8-cineole (10,2), sabinene (7,6%), trans-β-farnesene (6,1%) (FO2).

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P- 161 Preliminary Investigation on the Volatile Constituents of *Vitex madiensis* from Gabon

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Vitex madiensis Oliv. (syn. Vitex camporum Büttn., Vitex barbata Planch. ex Bak., Vitex pobeguini Aubrév., Verbenaceae) is a shrub or small tree, ranging in height from 3 to 5 m, which grows in the sub-saharian savannah [1]. This species is widely used in the Central and West Africa for many purposes (food, medicinal, etc.) [2]. In Gabon, the species is known as "gépobwè" in the Mitsogo dialect [3] and the infusion of leaves is traditionally used to treat indigestions and parasitic contaminations.
The main previous chemical investigations on *Vitex madiensis* concern its non volatile constituents, such as phytoecdysteroids [4]; despite the large utilization of this species, there is no accurate information about its essential oil when the chemical compositions and / or the biological properties of essential oils obtained form several other *Vitex* species were studied.
The essential oil obtained by hydrodistillation of leaves of *Vitex madiensis* collected in Franceville (South Eastern Gabon) with 0.27% yield (w/w) was analyzed by a combination of GC and GC/MS. The chemical composition is complex, characterized by a majority of oxygenated terpenic compounds; only three components account for more than 10% of the mixture: linalol (13.9%), α–muurolol (10.8%) and $\alpha\tilde{}$–cadinol (15.8%); these results differ completely from those reported in the literature for the other species [5-9]. Considering the utilization of the leaves in traditional medicine, the essential oil was tested against three bacteria strains (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and *Aspergillus niger*. The inhibition effects observed are presented and discussed in relation to the possible use of the plant in drug formulation.

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P- 162 Composition and Antifungal Activity of the Essential Oil from Cameroonian *Vitex rivularis* Gürke Leaves

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Vitex L. is a taxonomically complex genus that includes 250 species with a pantropical distribution [1]. The genus includes various aromatic species used in traditional medicine [2,3]. The aims of the present work were to investigate the chemical composition and the antifungal activity of the essential oils of *V. rivularis* collected in Yaoundé, Cameroon (Africa). Two samples of essential oils were obtained from the leaves by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). The oils were characterized by high amounts of sesquiterpene hydrocarbons, being α-copaene, β-caryophyllene, γ-curcumene, ar-curcume and germacrene D the main compounds.

This composition with high amounts of sesquiterpenes is according to the composition of the essential oils of other African species: *V.ferruginea* subsp. *amboniensis, V. obovata* subsp. *obovata*, *V. obovata* subsp. *wilmsii*, *V. pooara*, *V. rehmannii* and *V. zeyheri* [4,5]. Contrarily, the European and Asian *V. agnus-castus* [6,7], the Asian *V. negundo*, *V. pseudo-negundo*, *V. rotundifolia* and *V. trifolia* [8,9,10,11] are compounded mainly by monoterpenes.

The minimal inhibitory concentration (MIC) and the minimal lethal concentration (MLC), determined according to the NCCLS protocol (M27-A and M38-P), were used to evaluate the antifungal activity against yeasts (*Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. guillermondii*, *C. krusei*, *Crytococcus neoformans*) and dermatophyte strains (*Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *T. mentagrophytes*, *Epidermophyton floccosum*). The oils exhibited significant antifungal activity against dermatophyte strains, with MIC values ranging from 0.16-0.64μL/mL.

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P- 163 Chemical Compositions and Biological Potentials of Leaf Essential Oils of *Callistemon salignus* Growing in KwaZulu-Natal Province of South Africa

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Essential oils were isolated from fresh and air-dried leaves of *Callistemon salignus* from three different districts in KwaZulu-Natal Province using hydrodistillation method. The GC and GC/MS analyses of the oils identify 1,8-cineole as the major component with percentage composition of 48.0-89.9%. Myrtenol, another prominent constituent was identified in the dry leaf oils from Empangeni and KwaDlangezwa (12.8-18.7%) alone while the fresh leaf oils had this component only in trace amount (~0.1%). Myrtenol was absent in the Durban leaf oils. The testing of the essential oils against 15 bacteria for antimicrobial activities showed various possible applications of the oils. Evaluation of the antioxidant potential reveals the oils to have moderately to strong antioxidant property. Most of the oil samples were effective in killing mosquito larva.

P- 164 Aromatic Plants of French Polynesia: Chemical Composition of the Essential Oil from the Leaves of *Eucalyptus citriodora* Hook

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Investigating the chemical composition of aromatic and medicinal plants of French Polynesia, fine chemical analysis of the essential oil of *Eucalyptus citriodora* collected at Pirae in Tahiti has been carried out by Gas Chromatography and Gas Chromatography/Mass Spectrometry.
Nineteen constituents were identified among which four oxygenated monoterpenes were predominant: citronellal (31.1%) and citronellol (24.3%) as major compounds accompanied by significant amounts of isopulegol (19.2%) and isoisopulegol (6.8%).
A comparison with oils from other subspecies reported in literature shows important qualitative and quantitative differences.

P- 165 Direct and Multiple Shooting in *Eucalyptus microtheca*

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Differentiation and regeneration was induced from juvenile explants of *Eucalyptus microtheca*. Isolated cotyledons, leaves and hypocotyls were cultured on modified MS medium with half-strength KNO3 and NH4NO3 and 3% sucrose supplemented with 12 different concentrations of α-naphthalene acetic acid (NAA) and Kinetin (Kin) and maintained under 16h photoperiod and 25°C temperature regime. After 22 days, hypocotyls treatment of 1 mg/l NAA + 1 mg/l Kin period expressed direct shoot initiation without callus initiation. Callus initiated from both cotyledons and leaves within first week after culture, and multiple shoots regenerated form within 25 days after culture. Cotyledons and leaves expressed regeneration in media containing 4 mg/l NAA + 0.5 mg/l Kin and 2 mg/l NAA + 1 mg/1 Kin, respectively. The frequency of regenerates indicated that was significantly difference between direct and multiple shoot regeneration.

P- 166 In Vitro Antimicrobial Essential Oil of *Melaleuca genistifolia* and *Acokanthera spectabilis*

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GC-MS investigation of the essential oils isolated from *Melaleuca genistifolia* (leaves) and *Acokanthera spectabilis* (flowers) revealed that *M. genistifolia* leaves contain more than 28 major compounds four of them are investigated to the first time. Concerning *A. spectabilis* it is the first time to be investigate its essential oil. It biosynthesizes more than 32 major compounds.

The results of the In vitro assays using a variety of essential oils isolated from *M. genistifolia* and *A. spectabilis* revealed a particularly antibacterial effect and affecting the viability of a broad spectrum of bacteria and yeast such as *Staphylococcus aureus*, *Aspergillus niger*, *Escherichia coli* and *Candida albicans*. In large scale, this investigation may be of interest in the pharmaceutical applications of *M. genistifolia* and A*. spectabilis* oils.

P- 167 Aromatic Plants of French Polynesia : Chemical Composition of the Essential Oils from the Leaves of *Psidium cattleianum* Sabine and *Psidium guajava* L.

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Investigating the chemical composition of aromatic and medicinal plants of French Polynesia, fine chemical analysis of the essential oils of *Psidium cattleianum* collected at Taravao (island of Tahiti) and *Psidium guajava* collected at Paopao (island of Moorea) have been carried out by Gas Chromatography and Gas Chromatography/Mass Spectrometry.
To the best of our knowledge there has been no report on the oil composition of the species *P. cattleianum*. Only few reports deal with the leaf oil composition of *Psidium guajava* L.
The comparison of the composition of the oils of the leaves of *Psidium cattleianum* and the leaves of Psidium *guajava* collected in French Polynesia is achieved in this paper.

P- 168 Volatile Constituents of Two Papilionaceae Growing Wild in Iran.

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Lathyrus rotundifolius and *Trifolium mazanderanicum* belonging to the family Papilionaceae. The genus of *Lathyrus* is represented in the flora of Iran by twenty two species, of which nine species are endemic of Iran. “Khelar-e-barggerd” is the Persian common name for *Lathyrus rotundifolius*, that is found wild in many mountainous regions in north of Iran. The genus of *Trifolium* is represented in the flora of Iran by forty-nine species, of which two species are endemic of Iran (*T. radicosum* and *T. mazanderanicum*). Shabdar-e-mazandarani is the Persian common name for *Trifolium mazanderanicum*.1, 2

The water distilled volatile oils from aerial parts of *Lathyrus rotundifolius* and *Trifolium mazanderanicum* two Papilionaceae species of Iran were analyzed by GC and GC/MS. Germacrene-D (50. 5%), germacrene-B (18.7%), γ-elemene (9.5%) and myrcene (7.4%) were the main component among the thirteen constituents characterized in the oil of *Lathyrus rotundifolius*, representing 98.1% of the total components detected. Thymol (41.4%), 8-cedrene-13-ol-acetate (40.9%) and p-cymen-8-ol (5.4%) were the main component among the ten constituents characterized in the oil of *Trifolium mazanderanicum* representing 100% of the total components detected.

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P- 169 Chemical Compositions of the Essential Oil from Aerial Parts of *Astragalus schahrudensis* Bge. from Northeast of Iran

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The genus *Astragalus* (Papilionaceae) contains about 800 species of perennial and annual plants in Iran and most of these are endemic [1,2]. The aim of our study was to identify the constituents of the essential oil of the aerial parts of *A. schahrudensis* Bge. at the flowering stage. The plant is endemic in Iran and is growing wild in the Northeast of Iran. Colourless oils (0.11% (w/w)) were obtained from aerial parts of *A. Schahrudensis* by hydrodistillation in a Clevenger-type apparatus for 3 hours. The essential oil was analyzed by GC and GC/MS. The aerial parts oil of *A. schahrudensis* was characterized by a high amount of benzyl benzoate (54.4%) among the fifteen components comprising 94.3% of the total oil detected. In aerial parts oil of *A. schahrudensis* nonterpenes predominated over monoterpenes and sesquiterpenes.

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P- 170 The Leaf Oil of *Daniellia ogea* L

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The investigation of the volatile compounds of the leaf oil of *Daniellia ogea* L. (Leguminosae) by capillary gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) led to the identification of 31 com¬pounds in the oil. The oil was found to be rich in sesquiterpenoids. The major compound was caryophyllene oxide (20.1%). Humulene oxide, oc-humulene and 3-selinene were also found in moderate amounts of 6.9%, 3.8% and 3.8%, respectively.

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P- 171 The Essential Oil Composition of *Trigonella disperma* Bornm. Leaves and Stems from Iran

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The genus *Trigonella* L. with close 135 species belonging to the tribe Trifolieae and family of Fabaceae. Most of the species are distributed in the dry regions around the E Mediterranean, W Asia, S Europe, N and S Africa, with only one species being present in S Australia. They are herbaceous or annual with pinnately trifoliate leaves, often exhaling an odour. In Flora Iranica, the genus is represented by ca. 27 species, most of them are endemic. The Iranian species have been divided to 4 main groups based on morphologic and pollen micromorphologic characters by us. *Trigonella disperma* Bornm. is a widespread species of the genus in Iran. In this research, the content and chemical composition of the hydrodistilled essential oils obtained from dried leaves and stems of *Trigonella disperma* Bornm. were studied by GC and GC/MS for the first time. 24 and 19 components were identified in the essential oils of the leaves and stems, respectively. The essential oils of the leaves and stems were similar in 12 components. The major constituents of the leaf oil were pentadecen (27.3%) and spathulenol (17.8%). The major constituents of the stem oil were (z)-6-decenyl acetate (38.5%) and butanoic acid,octyl ester (24.4%). Decanal (0.6%) and 2-Hexadecen-1-ol,3,7,11,15-tetramethyl (0.3%) have the lowest percentages in the oils of the leaves and stems, respectively. The essential oils show inhibitory activity mainly against Gram-positive bacteria.

P- 172 A New Fragrant Molecule from Himalayan Cedarwood Oil.

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Perfumery on the whole has its origin in the utilization of natural resources such as essential oils and natural extracts. But after a triumphant progress in the area of synthetic fragrant substance, natural ingredients are nowadays used only to a relatively small extent, e.g., in fine fragrance perfumery. However, several natural raw materials consist mainly of hydrocarbons that are not suitable as smelling substance. These hydrocarbons often possess complex carbon skeletons, which are difficult to synthesize, but offer excellent possibilities for functionalization with oxygen containing groups transformations that often lead to interesting new fragrant materials.
In the present work two sesquiterpenic ketones from Himalayan cedarwood oil were isolated which were found to be α-atlantone and γ-atlantone, respectively. The suceesful route involved the preparation of novel ester molecules of atlantone such as atlantol acetate and atlantol propionate. Stability study of these esters in functional products such as deodorant stick, face powder, lipstick, shampoo and household products such as soap & detergents were carried out.

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P- 173 Chemical Composition of the Essential Oil of *Pinus pinaster* from Corsica

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Pinus pinaster Ait. (Maritime pine) is one of the most common pines in the Mediterranean region. There are more than 4 millions hectares of maritime pine forest in France, Portugal, Spain, Italy, Morocco, Tunisia, and Algeria. Maritime pine is especially exploited for its wood and resin products. The use of pine needles to produce the essential oil is another possibility of augment profit of pine forests. Continuing our research on essential oils of conifers growing wild in Corsica, we report here on the chemical composition of the essential oils from needles of Pinus pinaster.

The composition of the essential oil of needles of 46 individual trees from Corsica was investigated by GC(RI). Among them, various samples, selected on the basis of their chromatographic profile, were also analyzed by GC-MS (2 samples) and by 13C NMR spectroscopy (15 samples). In order to carry out a detailed analysis, one oil sample was fractionated over SiO_2 and all the fraction of chromatography were analyzed by GC(RI) and NMR. In total, 71 constituents were identified and they accounted for 83.4-96.7% of the total amount of the oils.

Pinus pinaster needle oils were characterized by a high content of mono-, sesqui-, and diterpene hydrocarbons. $\tilde{\alpha}$-Pinene, germacrene D, (E)$-\beta$-caryophyllene and abietadiene were the major constituents, depending on the sample. Although the amount of the major components varied drastically from sample to sample, only one group was distinguished, by chemometric analysis within the essential oils (K-means clustering and Principal Component Analysis).

In conclusion, the chemical composition of the needle oil of *P. pinaster* from Corsica looks original by its high content of diterpene hydrocarbons (20.3-57.2%), not yet reported in the literature.

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P- 174 Composition of Essential Oil Obtained from Leaves and Inflorescences of *Aruncus vulgaris* Raf. from Serbia

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In the flora of Europe, there is only one species of the genus *Aruncus*, namely *Aruncus vulgaris* Raf. [syn. *Aruncus dioicus* (Walt) Fern.; *Aruncus silvester* Kost; *Spiraea aruncus* L.]. Up to now, data about polyphenolic constituents of *A. vulgaris* root, leaves and inflorescences (procyanidins; (-) epicatechine; p-coumaric, caffeic acid, gallic, ferulic, p-hydroxybenzoic acid, 3-O-mono and 3-O-diglycosides) are published (1,2,3). The antioxidant and antimicrobial activity of this *Aruncus* species is confirmed (1,4), yet there are no data published about the essential oil obtained from this plant. The results of GC, GC/MS analyses of essential oil of *A. vulgaris* from Serbia will be presented.
The samples of leaves and inflorescences were collected during July of 2005 at the mountain Tara, western Serbia. The air-dried plant material was cut and the essential oil was obtained by hydrodistillation using Clevenger type apparatus. The 1 % solution of the oil in ethanol was used for chromatographic analyses GC and GC/MS. The components of the oil were identified by comparison of their mass spectra to those from Adams, Wiley, NIST/NBS libraries. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1., DTRA/NIST, 2002). The main components of *A. vulgaris* leaves essential oil were: benzaldehyde (6.2%), linalool (23.9%), myristicin (8.4%), α-terpineol (4.6%) and geraniol (4.2%). The most abundant constituents of *A. vulgaris* inflorescences oil were: linalool (7.4%), phytol (31.1%), tricosane (15.3%) and β-damascenone (6.3%).

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P- 175 Chemical Composition and Antibacterial Activity of the Persisch Rose Water (*Rosa damascena* Mill.)

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The genus *Rosa* (Rosaceae) comprises 12 species, which are widely distributed in Iran1. Several *Rosa* species are used in herbal medicine, e.g. for neuropsychological refresher, cardiovascular and stomach tonique.2 Persisch Rose Water is a Natural Soft-Drink in Iran and prepared from natural and main tannin ingredients and contains oil essence called rose water oil. For analysis of the Persisch Rose Water, a sample, subsequently, passed through C18 and Licrout en column. The components absorbed on the column were eluted by washing with hexane and methanol. The solvent was evaporated to small volume and analyzed using GC and GC/MS. Many components were identified in the oil Persisch Rose Water. All oils consist of monoterpenes, sesquiterpenes and aliphatic compounds.
Furthermore, the oils exhibited an interesting antibacterial activity against gram-positive and gram-negative bacteria. The following strains of gram negative and gram positive bacteria were provided from Persian Type Culture Collection (PTCC) and American Type Cultur Collection (ATCC). Each test was performed in 4 replications and the results analysed for statistical significance. Gentamicin and tetracycline with positive responses were used as controls for plates. Gentamicin and tetracycline served as positive controls on gram-positive and gram-negative bacteria.

Acknowledgments: We are grateful to the research councils of Shiraz Narvan Co. for their financial support.

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P- 176 Chemical Composition and Antioxidant Activity of Conventional and Organic Damask Rose Oils

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Damask rose (*Rosa damascena* Mill.) oil is one of the most valuable base materials in the fragrance and cosmetic industry because of its special scent. The aim of this study is to determine the chemical composition and antioxidant properties of conventional and organic rose oils. Conventional and organic rose oils were water-distilled in factory conditions. Essential oil composition of oils were determined by GC-MS. Antioxidant activities of the oils were evaluated using the 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging (1), reductive potential (2) and phosphomolybdenum method (3). While 20 essential components were found in rose oils, the main essential components were citronellol, gereniol, nerol and nonadecane. In conventional rose oil, citronellol, gereniol, nerol and nonadecane were found as 34,50%, 24,23%, 10,87% and 10,75%, respectively, organic rose oil had 34.78% citronellol, 24.61% geraniol, 11.44% nerol and 9.56% nonadecene. Antiradical activities of conventional and organic rose oils were found as IC50=32.93 mg ml-1 and IC50=34.94 mg ml-1, respectively. Antioxidant capacities were 287.26 mg AAE g-1oil for conventional rose oil and 297.34 mg AAE g-1oil for organic rose oil. The reductive potential of the conventional and organic rose oils increased with increased concentration and were found as 0.077-0,271 and 0.088-0.322, respectively. As a conclusion, in spite of the valuable base material for cosmetic and perfumery industry, rose oils are not described a good natural antioxidant source. On the other hand regarding with essential oil composition and antioxidant properties there were no clear differences between conventional and organic rose oils.

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P- 177 Essential Oils from the Leaves and Barks of *Drymis brasiliensis* Miers, Winteraceae

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Drymis brasiliensis Miers (Winteraceae) is an arboreal species from South of Brazil, particularly found in its temperate forests. Known popularly as “cataia”, it has been used in the folk medicine as anti-inflammatory and analgesic drug, and for treating respiratory diseases and also as a alcoholic beverage “uisque-de-caiçara”. Its essential oil (EO) has shown antimicrobial properties against pathogenic bacteria and fungi. In this work we report the main compounds of its EO, obtained from the leaves (1.9%) and barks (0.75%) by hydro distillation for 3 h using the Clevenger-type apparatus. After dried over anhydrous sodium sulphate, the EO has been analyzed by GC-MS and its compounds identified by comparison of their mass spectral data and retention time (RT) with the corresponding data of authentic compounds and with the MS's own libraries and literature data. The main components identified were: α-pinene (RT 9.92; relative abundance 9.82 and 3.08% in the bark and leaves, respectively); β-pinene (RT 10.32; 10.32 and 4.94%); limonene (RT 10.59; 3.66 and 10.13%); 4-terpineol (RT 15.90; 10.6 and 4.3%); β-elemol (RT 27.93; 1.9 and 9.8%); spathulenol (RT 28.81; 11.0 and 6.34%) and drimenol (RT 33.84; 3.84 and 1.18%). Although further studies are needed, the results and presented in this work may be useful as a preliminary data to confirm the usefulness to give support for the popular usage of *Drymis brasiliensis*.

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P- 178 Headspace Volatiles of Three Turkish Plants

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Headspace volatiles of fresh flowers of *Stenbergia candida* Mathew et Baytop (Amaryllidaceae) and *Muscari muscarimi* Medikus (Liliaceae), and crushed bulbs of *Nectaroscordum siculum* (Ucria) Lindl. (Liliaceae) were analyzed by gas chromatography/ mass spectrometry. The volatiles were trapped by SPME in a dynamic headspace set up. *Stenbergia* flowers red - Polydimethylsiloxane (PDMS) , blue - Polydimethylsiloxane / Divinylbenzene (PDMS / DVB) and black - Carboxen / Polydimethylsiloxane (CAR / PDMS) fibres were used. (E)-β-ocimene (50-81 %) and nerol (2-19 %) were found as main components.
Volatiles of *Muscari muscarimi* were trapped by blue SPME fibre over a period of 3 days. Main components were identified as (E)-β-ocimene (tr-36 %), methyl salicylate (1-21 %), trans-methyl isoeugenol (4-22 %) and benzyl benzoate (7-56 %). Volatiles of crushed bulbs of Nectaroscordum siculum were trapped on an HS-SPME (Red fibre). Only two components were found as dibutyldisulfide (88.5 %) and 2-hexyl-5-methyl-(2H)-furan-3-one (11.5 %).
This is the first report on the headspace volatiles of these three plants.

P- 179 MIPAROM Trek: Collect and Analysis of Midi-Pyrénées Medieval Aromatic Plants

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A decade ago, several treks (NEBLINA, TASTETREK) were performed by companies of aromatic industry sector both in South-America and West-Africa in order to investigate the aromatic potential of rare exotic plants for novel flavors and fragrances formulations. Midi-Pyrénéees region (South-West of France) has a rich and unvalorized wild vegetal heritage, especially for aromatic and medicinal plants. In the framework of the AROMATIC Program (design of a new generation of natural WONF flavorings), the MIPAROM Trek was conducted in order to collect and analyse various regional medieval (aka forgotten) aromatic plants, e.g. *Achillea millefolium*, *Agastache foeniculum*, *Meum athamanticum*, *Chrysantemum balsamita*, *Myrrhis odorata*…. In the present paper, the first results of the biorefinery of one of the 20 investigated plants (*Achillea millefolium*) were reported. After collection of the aerial parts of the plant in Toulouse area, volatile compounds were isolated by hydrodistillation of leaves and flowers in a Clevenger-type apparatus. Major components and odor active compounds of essential oils were identified respectively by GC-FID and GC-MS and by GC-O using a 10 assessor's panel. If Sabinene, Nerolidol and Germacrene were the main components of the flowers oils, our extracts presented a chemiospecificity. In addition, interesting aromatic notes were for the first time described for leaves essential oil.

P- 180 Antifungal Activity of Some Oxygenated Acyclic Monoterpenes (OAMs) and OAM-rich Essential Oils Against 38 *Candida albicans* Clinical Isolates

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Of the 250.000 known species of yeasts and moulds 300 are pathogenic for humans and animals. Polyene antibiotics as well as fluconazole and ketoconazole are among the leading antifungal chemotherapeutics, nevertheless the occurence of multidrug-resistant fungal strains increase the demand for new antifungal compounds. In the present study the antifungal activity of 5 OAM-containing essential oils (3 of geranium, 1 of citronella and 1 of rose) and of their main compounds, the OAMs citronellol, citronellal, geraniol, nerol and linalool were examined against 38 clinical isolates of *Candida albicans*. The minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC) were evaluated using the NCCLS reference microdilution method. Furthermore, the chemical composition of these oils and the purity of the OAMs were investigated by GC and GC-MS analyses. Fungal growth inhibition for all essential oils and the OAMs could be detected at concentrations from 0.1% to 2% whereas fungicidal activity was proved at concentrations from 0.5% to 4%. The strongest antifungal activity against all 38 tested strains was found for the African geranium oil and decreased at the Chinese geranium oil, followed by the Bourbon geranium oil, the Turkish rose oil and the Chinese citronella oil. Deduced from the results obtained from the GC- and GC-MS analyses, the antifungal activity of the investigated essential oils was found to depend on their chemical composition. Oils rich in linalool (geranium oils) possessed a higher antifungal activity than the other tested essential oils.

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P- 181 Antifungal Activity of Eugenol and Various Eugenol-Containing Essential Oils against 38 Clinical Isolates of *Candida albicans*

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Antifungal activity of eugenol containing essential oils – pimento oil (*Pimenta dioica* (L.) Merr), bay oil (*Pimenta racemosa* (Mill.) J. W. Moore), clove oil (*Syzygium aromaticum* (L.) Merr. & M. Perry), and cinnamon oil (*Cinnamomum zeylanicum* Blume) was investigated against 38 clinical isolates of *Candida albicans* (12 oropharyngeal, 16 vaginal and 10 skin damaging strains). The strains isolated from skin infections were more susceptible to all of the investigated essential oils whereas the oropharyngeal strains were more resistible. The strongest antifungal activity against clinical isolates of oropharyngeal, vagina and skin strains of *Candida albicans* possesses clove oil, followed by cinnamon oil, pimento oil and bay oil. Anticandidial action of the investigated oils is significantly due to its major component eugenol, but also minor constituents of the essential oils, such as methyleugenol, chavicol and linalool, must have additional and/or synergistic effects to explain the obtained anticandidal data of each essential oil sample.

P- 182 Production of in Vivo and In Vitro Aromatic Plants for Antioxidant Essential Oils

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An efficient method to produce friable callus from *Salvia officinalis* (different genotypes) was performed to start with cell cultures. The study was carried on *S. officinalis* as well as other edible *Salvia* spp. Hairy roots culture were performed too.
Furthermore in vitro cultivation of four different cultivars of basil (*Ocimum basilicum* L. var. Dark Opal, Superbus, Red Rubin and Gecom) from seeds was performed.
The micropropagation and callus culture procedures were tested to induce in vitro production of biomass from germinated in vitro seedlings.
The produced in vitro plant material was compared with in vivo plants by GC-MS and SPME-GC-MS analysis. We studied several samples of the most common Italian sweet basil named "Genovese" grown in Ligurian areas (Albenga). The analyses were carried out on fresh samples both on the headspace and essential oils by SPME-GC-MS and GC-MS. In addition four different micropropagated cultivars of Basil (*Ocimum basilicum* L. var. Dark Opal, Superbus, Red Rubin and Gecom) were analysed in order to compare them with the corresponding adult plants cultivated in the same area.
The EO yields of *O. basilicum* in vitro plant material were lower than those obtained from the adult plants. However the *O. basilicum* callus was already able to produce the typical volatile constituents of *O. basilicum* adult plants.
Furthermore several varieties of *Salvia officinalis* were studied for their essential oils which are well-known as antioxidant too. Other *Salvia* spp. was considered for the selection of plant material since their essential oils did not show thujones among their constituents. Some of these species were studied for the first time.

P- 183 Antibacterial Properties of Some Essential Oils from Antalya / Turkey

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Five plant-oils were examined after their antibacterial properties. The plant-oils were of *Centaurium erythraea* (centaury), *Juniperus communis* L. (juniper), *Mentha piperita* (peppermint), *Nigella sativa* L. (fennel flower) and *Thymus vulgaris* (thyme). The testbacteria were *Bacillus megaterium*, *Escherichia coli* and *Lactobacillus plantarum* (ATCC 80,14). *Thymus vulgaris* showed the strongest effect. The most sensitive strains were *B. megaterium* and *L. plantarum*. All plant-oils showed antibacterial effect except *Centaurium erythraea*.
The major constituent of the oils are explained. The examination was carried out using agar hole diffusion method.

P- 184 Insecticidal Activity of Some Essential Oils Against *Oryzaephilus surinamensis* L. and *Tribolium castaneum* Hbst.

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Insecticidal and repellent activity of some essential oils against several insect pests has been documented. In the present study, the effects of essential oils of rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), peppermint (*Mentha piperita*), eucalypt (*Eucalyptus globulus*) and green cumin (*Cuminum cyminum*) were investigated on two important pest of rice during storage (*Oryzaephilus surinamensis* L. and *Tribolium castaneum* Hbst). The oils were extracted from dried mentioned plant using hydrodistillation. The treatments were different concentration of the essential oils (0.04, 0.2 and 0.4 ppm) as fumigation in a 25 ml glass jar with 8 adult insects. The experiment was conducted in Randomized Complete Block Design with four replications. LC50 and LC90 of each treatment were calculated and analyzed by Probit Software. The results indicated that toxicity of rosemary and Green Cumin was higher than others on the pests. There are not significant differences between the plants as LC50 and LC90 on *Oryzaephilus surinamensis* as concerned (LC50= 0.0042ppm and LC90= 0.309 ppm). *Rosmarinus officinalis* essential oils affect faster than *Cuminum cyminum* followed by eucalypt (LC50= 0.419 and LC90= 0.0915), peppermint and thyme (LC50= 0.37 and LC90= 4.69). Our results in *Tribolium castaneum* proved that oil of rosemary and green cumin are toxic and after 16 hour exposure mortality reach to 100%. In conclusion rosemary and green cumin essential oils are the best candidates and safe for control of *Oryzaephilus surinamensis* L. and *Tribolium castaneum* Hbst. and further research need on the formulation of the essential oils.

P- 185 Effects of Citral, Citronellal, Perillaldehyde, Carvacrol, Geraniol and Terpineol on Airborne Microbes.

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Airborne microbes are an indisputable cause of risk for human health, as they surround humans 24h a day. Actually all currently used air disinfectants have disadvantages. Therefore, selected fragrances, representing safe substances with antimicrobial potential [1,2] were tested for their antimicrobial capacity against airborne microbes. Since there are no relevant studies available dealing with the antimicrobial properties of aroma chemicals on airborne microbes, a new method of examination was developed, which has already been successfully applied on the testing of thymol, eugenol, t-cinnamaldehyde and linalool by our research group [3].
In the present study citral, citronellal, perillaldehyde, carvacrol, geraniol and terpineol were investigated. After determination of the total microbial count in the testing room by using an RCS Air Sampler, each aroma chemical was vaporized in five different concentrations (1:100, 1:200, 1:350, 1:1000 and 1:5000). After 15min the total microbial count was measured again using the same experimental set-up. With each of the tested substances a reduction of total microbial count in the air was achieved. The microbes were identified as *Micrococcus* sp., *Bacillus* sp., *coryneforms* and *Penicillium* sp. employing gram straining, motility checking (hanging drop) and API-analysis. In our study carvacrol was the most effective substance with an average reduction of total count of 63.08% (1:1000). The other tested volatiles performed total microbial reduction up to approximately 50%. Consequently it can be stated, that the aroma chemicals investigated in this work can be used for air disinfection as an alternative to established air disinfectants.

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P- 186 Essentials Oils as Natural preservatives in Cosmetics

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Antimicrobial and antioxidant activity of essential oils (EO's) has been known and used since centuries and it was established for numerous oils in in vitro investigations. On the other hand, only few papers present the in situ investigation of EO activity that measured in food or cosmetics is not as pronounced.
We assessed the possibility of replacing of synthetic preservative or a part of it in cosmetic formulations by amounts of EO's appropriate for their flavouring role. Commercial lemon oil, tea tree oil, lavender oil and Siberian fir oil are used. EO'S composition was investigated by GC-RI and GC-MS. MIC and MBC values against six microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida mycoderma* and *Aspergillus niger*) were assessed by bioimpedimetric method. EO's were also tested for their abilities to inhibit the lipid peroxidation in a linoleic acid emulsion. Tea tree oil showed the greatest inhibitory activity toward microorganisms (MIC 0.2-0.5 μL/mL, MBC 0.5-3.0 μL/mL) as well as was also the most effective as antioxidant, where the inhibition time increased with the concentration of this oil in the linoleic acid emulsion.
Challenge test was used for evaluation of EO's activity in tonic and O/W lotion. The results demonstrated that 0.5% of each EO in tonic showed long-lasting antimicrobial activity. The same amount of each EO in lotion was not satisfactory and usual cosmetic preservative have to be added although in the amounts lower than 0.5%. The nature of a formulation in which EO is incorporated as preservative brings considerable effect on its efficacy.
Potential synergistic effect of EO's in combination with cosmetic preservatives is being investigated.

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P- 187 The Pharmacological Interaction of Commercial Essential Oils in Combination with Conventional Antimicrobials

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Many essential oils are known for their antimicrobial properties and are considered valuable as natural therapeutic treatments. Concern has been raised when conventional medicine is used concomitantly with natural treatment regimens. This study reports the in vitro pharmacological interactions between a selection of popular essential oils (*Melaleuca alternifolia*, *Thymus vulgaris*, *Mentha piperita* and *Rosmarinus officinalis*) and conventional antimicrobials when used in combination to determine whether synergistic, antagonistic or indifferent profiles are noted. Isobolograms graphically display the interactions that the inhibitors have on microbial growth when combined in various ratios. Interactions of the essential oils when combined with ciprofloxacin against *Staphylococcus aureus* indicate mainly antagonistic profiles. The highest synergistic profile was observed when ciprofloxacin was combined with *Mentha piperita*. When tested against *Klebsiella pneumoniae* antagonism, synergy and additive profiles are noted, depending on the combined ratio. The *Rosmarinus officinalis* / ciprofloxacin combination displayed the most favorable synergistic pattern. The interactions of *Melaleuca alternifolia*, *Thymus vulgaris*, *Mentha piperita* and *Rosmarinus officinalis* essential oils with amphotericin B indicate mainly antagonistic profiles when tested against *Candida albicans*. The predominant antagonistic interactions noted here, suggests that some natural therapies containing essential oils should be used with caution when combined with antibiotics.

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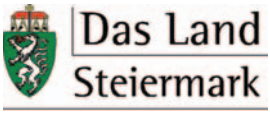

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