33rd International Symposium on Essential Oils

4 – 7 September 2002
Lisboa • Portugal

Program, Book of Abstracts and Participants List

FACULDADE • DE • CIÊNCIAS UNIVERSIDADE • DE • LISBOA

33rd International Symposium on Essential Oils

Faculty of Sciences of Lisbon, 4 to 7 September 2002, Lisbon, Portugal

Program,

Book of Abstracts

and Participants List

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Welcome Address

On behalf of the *Faculdade de Ciências da Universidade de Lisboa* I welcome you to our University and to the *33rd International Symposium on Essential Oils*. It is the first time that such an important meeting takes place in Portugal and we are very honoured to host it.

Looking at your programme for the next four days I was particularly impressed by the existence of a workshop devoted to the topic of "Essential oils and public opinion". In fact, nowadays, it is extremely important that scientists, besides communicating among themselves, dedicate a special attention to the communication with the public. It is crucial that the society at large understands the importance of science and the need to support a robust research program.

Despite the fact that you have to attend the interesting talks of your full programme, I hope that you still have some spare time to visit our beautiful city of Lisbon. I wish you all a very pleasant stay in Portugal.

Prof. Dr. Augusto Barroso
The Dean of the Faculty of Sciences of Lisbon

Acknowledgements

With the high patronage of His Excellency the President of the Republic, Dr Jorge Sampaio

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Scholarships

- The International Federation of Essential Oils and Aroma Trades (IFEAT) supported the Registration fees of the following students:

  Anamarija Partl - Zavod za farmaceutsku botaniku FBFa, Croatia
  Biljana Božin - Faculty of Natural Sciences, University of Novi Sad, Yugoslavia
  Carlos Ribeiro - Escola Superior Agrária de Beja, Portugal
  Eloisa Helena A Andrade – Coordenação de Botânica, Museu Emílio Goeldi, Brazil
  Gökalp İşcan - TBAM, Anadolu University, Turkey
  K. Termentzi – Aristotle University of Thessaloniki, Greece
  Lin C. Ming - Department of Vegetable Production - FCA/Unesp / Botucatu, Brazil
  M. C. Kalita - Dept. of Biotechnology, Gauhati University, India
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  Stéphanie Cozzani - Université de Corse, France
  Tiago Cunha Luis – Faculdade de Ciências da Universidade de Lisboa, Portugal
  Wellington de A. Gonzaga - Universidade Federal de Santa Maria, Brazil

- Estética Viva supported the Registration fee of the following student:

  Pedro António Santos – Faculdade de Ciências de Lisboa, Portugal

Organisation

Organising Committee

Prof. Dr Ana Cristina Figueiredo
Prof. Dr José Gonçalves Barroso
Prof. Dr Luis Gaspar Pedro

Symposium Venue

The Faculty of Sciences of Lisbon hosts the *33rd International Symposium on Essential Oils*.

Organising Committee Contact

33rd Intenational Symposium on Essential Oils
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WWW Information

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Prof. Dr Chlodwig Franz, University of Veterinary Medicine of Vienna, Austria
Prof. Dr Johannes J. C. Scheffer , Leiden University, The Netherlands
Prof. Dr Stavros Katsiotis, University of Aristotle, Greece

General Information

Symposium Office

The Symposium Office will be located in the Foyer of the Lecture Halls in the building C3 of the Faculty of Sciences of Lisbon.

Registration and Opening Hours:	Wednesday, September 4:	14:00 – 20:00
	Thursday, September 5 to Saturday, September 7:	09:00 – 18:00

Symposium Language

English will be the official language of the *33rd ISEO*. There is no simultaneous translation.

Badges

Participants and accompanying persons are requested to wear their Symposium badges for identification and admittance to Scientific and Social venues.

Oral Communications / Slide Preview

The Plenary Lectures are limited to 50 min, Oral Communications to 30 min and Short Oral Communications to 10 min, all with plus 5 min for discussion. A room for slide preview is available. Overhead and slide projectors as well as MS-Power Point presentation facilities are available. Slides should be handed in at the Symposium Office, in the Foyer of the Lecture Halls, with the corresponding number of the presentation and the name of the presenting author at least 1h prior to the beginning of the respective session.

Posters

Two poster sessions are scheduled, odd numbers on Thursday and even numbers on Friday. Poster boards measuring 90 cm wide and 120 cm height are available for poster presentation. The Registration Office provides fixation facilities. All posters will remain exhibited throughout the symposium and the authors are recommended to be present at their posters during posters sessions.

Poster Awards

Poster awards will be granted for Layout and Scientific Content.

Meals

Owing to the short time allotted to lunch, we would highly recommend you to take lunch in the Canteen. The Canteen serves meals from 12:00 to 14:00. Lunch vouchers are required at the Canteen. You can either find the lunch vouchers in your bag if you ordered them with your registration fee, or you can purchase them at the Symposium Office. Neither the Canteen nor the Campus coffee shops accept foreign currency.

There are also several restaurants, snack bars and coffee shops in the area around the University Campus.

Currency

All payments should be done in Euro.

Means of conveyance to the Symposium venue

1▪3▪7▪31▪31-A▪35▪36▪47▪50▪67▪68▪83▪85
Cidade Universitária ▪ Campo Grande
A few parking places will be available at the Faculty parking

Insurance / Liability

The Organisers are in no respect responsible for any accommodation problem, nor for any accident, injury, theft, loss and property damage to any person during the Symposium.

Scientific Program

Wednesday, September 4, 2002

14.00 - 19.00	**Registration at the Symposium Office**	Foyer of the Lecture Halls
17.00 - 17.45	**Opening Ceremony**	Main Lecture Hall
	Welcome Addresses	
	Prof. Dr Augusto Barroso, Dean of the Faculty of Sciences of Lisbon	
17.45 – 18.00	**Coffee Break**	Foyer of the Lecture Halls
18.00 - 19.00	**Plenary Lecture**	Main Lecture Hall
	Chair: Prof. Dr Karl-Heinz Kubeczka	
	Scents from rain forests – new results	
	Dr Roman Kaiser, Givaudan Dübendorf Ltd., Switzerland	
19.00 - 21.00	**Get-together Party**	

Thursday, September 5, 2002

09.00 – 10.00	**Plenary Lecture**	Main Lecture Hall
	Chair: Prof. Dr Johannes Scheffer & Prof. Dr Stahl-Biskup	
	Essential oils: sample preparation and analysis	
	Prof. Dr Patrizia Rubiolo, University of Torino, Italy	
10.00 – 10.30	**Oral Communications**	Main Lecture Hall
10.00 – 10.15	***Orange concentrated essences – manufacture by fractional distillation and pervaporation***	
	Dr Mércia Bettini, Flavor Tec., Brazil	
10.15 – 10.30	***Hydroponics - a novel way to create new aroma in living aromatic plants***	
	Dr Braja Mookherjee, International Flavour & Fragrances, USA	
10.30 – 11.00	**Coffee Break**	Foyer of the Lecture Halls
11.00 – 12.15	**Oral Communications**	Main Lecture Hall
	Chair: Prof. Dr Czeslaw Wawrzenczyk & Prof. Dr Wittko Francke	
11.00 – 11.30	***Rapid and reliable analysis of essential oils applying various vibrational spectroscopy methods***	
	Prof. Dr Hartwig Schulz, Institute for Plant Analysis, Germany	
11.30 – 12.00	***Highly enantioselective cyclodextrin derivatives and conventional inner diameter short capillary columns: an approach to improve separation of medium to low volatility compounds***	
	Prof. Dr Carlo Bicchi, University of Torino, Italy	
12.00 – 12.15	***Supercritical carbon dioxide extraction of essential oil from Satureja fruticosa Béguinot***	
	Dr José Coelho, Technical University of Lisbon, Portugal	
12.15 – 14.00	**Lunch**	Canteen/Local Restaurants

14.00 – 15.30	**Oral Communications**	Main Lecture Hall

Chair: Dr Brian Lawrence & Prof. Dr Hüsnü Can Başer

14.00 – 14.30 ***Volatile components of southern hemispheric epiphytic liverworts Frullania and Schusterella species (Frullaniaceae)***
Prof. Dr Yoshinori Asakawa, Tokushima Bunri University, Japan

14.30 – 14.45 ***Repeated measurement analysis as tool in essential oil research***
Dr Johannes Novak, University of Veterinary Medicine, Austria

14.45 – 15.00 ***Biotransformation of cuparene, plagiochilide, aristolene and nootkatone by some microorganisms***
Prof. Dr Yoshiaki Noma, Tokushima Bunri University, Japan

15.00 – 15.30	**Coffee Break**	Foyer of the Lecture Halls
15.30 – 16.30	**Plenary Lecture**	Main Lecture Hall

Chair: Prof. Dr Raimo Hiltunen

Biosynthesis of essential oils
Dr Edward Davis, Washington State University, USA

16.30 – 18.30	**Poster Session (Odd Numbers)**	Foyers of the Lecture Halls

Friday, September 6, 2002

09.00 – 09.50	**Plenary Lecture**	Main Lecture Hall

Chair: Prof. Dr Chlodwig Franz & Prof. Dr Gerhard Buchbauer

Essential oil biodiversity
Prof. Dr E. Stahl-Biskup, University of Hamburg, Germany

09.50 – 10.30	**Oral Communications**	Main Lecture Hall

09.50 – 10.15 ***Essential oils of Sideritis species of Turkey***
Prof. Dr Hüsnü Can Başer, Anadolu University, Turkey

10.15 - 10.30 ***Data bank of the Amazon aromatic plants and their essential oils***
Prof. Dr José Guilherme Maia, Museu Emílio Goeldi, Brazil

10.30 – 11.00	**Coffee Break**	Foyer of the Lecture Halls
11.00 – 12.00	**Plenary Lecture**	Main Lecture Hall

Chair: Prof. Dr Patrizia Rubiolo

Volatile signals: chemical structures and ecological aspects
Prof. Dr Wittko Francke, University of Hamburg, Germany

12.00 – 14.00	**Lunch**	Canteen/Local Restaurants
14.00 – 15.00	**Plenary Lecture**	Main Lecture Hall

Chair: Prof. Dr Carlo Bicchi

New trends in intellectual property relating to perfumery materials
Dr Maria Salvaterra-Garcia, Firmenich SA, Switzerland

15.00 – 15.30	**Coffee Break**	Foyer of the Lecture Halls

15.30 – 17.00	**Workshop**	Main Lecture Hall
	Chair: Prof. Dr Carlo Bicchi ***Composition of essential oils and public opinion: Problems and solutions***	
17.00 - 19.00	**Poster Session (Even Numbers)**	Foyers of the Lecture Halls
19.30 – 23.00	**Symposium Dinner**	

Saturday, September 7, 2002

09.00 – 10.00	**Plenary Lecture**	Main Lecture Hall
	Chair: Prof. Dr Yoshinori Asakawa & Prof. Dr Stanislaw Lochynski	
	Biological activities of the essential oils *Prof. Dr Gerhard Buchbauer*, University of Vienna, Austria	
10.00 – 10.30	**Oral Communications**	Main Lecture Hall
10.00 – 10.15	***Influence of Ylang-ylang oil on mental, emotional and human physiological parameters*** *Dr Tapanee Hongratanaworakit*, Srinakharinwirot University, Thailand	
10.15 – 10.30	***Adverse effects of essential oils and components: fact and fiction*** *Dr Maria Lis-Balchin*, South Bank University, UK	
10.30 – 11.00	**Coffee Break**	Foyer of the Lecture Halls
11.00 – 11.45	**Oral Communications**	Main Lecture Hall
	Chair: Prof. Dr Karl-Heinz Kubeczka & Dr Edward Davis	
11.00 – 11.15	***The essential oil of Australian Sandalwood (Santalum spicatum) – effects of different samples on Human physiology and subjective evaluation*** *Dr Eva Heuberger*, University of Vienna, Austria	
10.15 – 11.30	***Exploring the biological activity and phytochemistry of South African medicinal aromatic plants used in traditional healing rites*** *Dr A. M. Viljoen*, University of the Witwatersrand, South Africa	
10.30 – 11.45	***Composition and biological activities of essential oils of Fagara zanthoxyloides (dried fruits) from Cameroon as well as of Syzygium cuminii and Syzygium travancoricum (fresh leaves) from India*** *Dr Leopold Jirovetz*, University of Vienna, Austria	
11.45 – 12.00	**Closing Ceremony**	Main Lecture Hall
12.00 – 14.00	**Lunch**	Canteen/Local Restaurants
14.00 – 19.00	**Half-day excursion**	Half Day Tour to Lisbon

Social Program

Get-together Party

Wednesday – 4 September
The Get-together Party is included in the registration fee.

Symposium Dinner

Friday – 6 September
The Symposium dinner will take place at a local restaurant.

Half Day Tour to Lisbon

Saturday – 7 September

Departure from Symposium venue. Panoramic tour: the main avenues and squares of Lisbon. Walking tour in the typical old town to S. Jorge Castle to have a magnificent panoramic view over Lisbon downtown and the Tagus river.

The Belém district with the monuments connected with the Discoveries period: visit of the Jerónimos Monastery, masterpiece of the Portuguese Manuelin Style; visit to the Church and the Cloister, considered to be one of the most interesting ones in the world. Stop by the Belém Tower and the Discoverers' Monument.

Accompanying Persons' Program

Half Day Tour to Sintra

Thursday – 5 September

Departure from the Symposium venue. Sintra – recognized by the UNESCO as World Heritage – visit to the former Royal Palace of the 15th and 16th century: a beautiful collection of old glazed tiles, wooden painted ceilings and furniture. The picturesque old town: shopping possibility. Drive to the seaside resorts Cascais and Estoril and back to Lisbon, along the Atlantic Ocean and the Tagus river.

Full Day Tour to Évora / Arrábida

Friday – 6 September

Departure from the Symposium venue. Drive on the new Vasco da Gama bridge, one of the longest in Europe (ca. 17Km long), to the South bank of the Tagus river. The Alentejo Province – large plains with wheat and oak trees. Visit of the Museum-Town Évora: walking tour in the main streets and squares with nice marble fountains – the ruins of the Roman Temple, visit of the Cathedral (the Church, the Cloister and the Religious Art Museum) and the S. Francisco Church.

Time free for lunch. Panoramic tour on the Arrábida Mountains, with beautiful panoramic views: the Mediterranean vegetation and the Atlantic. Drive back on the Lisbon suspension bridge overlooking the town.

Plenary Lectures Abstracts

Scents from Rain Forests – New Results

Roman Kaiser

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Since the dawn of time, the secretive and fragile kingdom of Rain Forests, which covers 11 -13 % of all emerged land and shelters eight to nine tenths of all forms of known and unknown life, has harboured extraordinary biological treasures that deserve to be treated with the utmost care. According to a further estimate, 70 – 80 % of these species, i. e. around 60 % of the entire biodiversity, is located for ecological reason in the canopy region of the Rain Forests.

Convinced that this richness would also be reflected on the olfactory level, for the past years we have searched in the understory as well as in the canopy of Rain Forests for new attractive scents.

This communication continues the discussion of chemical, biological and olfactory aspects of such scents, which we encountered in the American, African, or Indoaustralian tropics, respectively.

The respective micro-samples have been trapped by applying our non-destructive headspace trapping method which we adapted to field experiments under extreme conditions. Complementary, a field-adapted “solid phase extraction” method (SPE) was applied if fragrant compounds had to be trapped from aqueous solutions.

Discussed among others are new highly unsaturated dodecan-5-olides identified in the flower scent of *Coryanthes* species (Orchidaceae), 3-methyl-4-alcanolides identified in that of *Passiflora chocoensis* (Passifloraceae) and *Lycaste deppei* (Orchidaceae), a new methoxy-methyl acetophenone identified as a main constituent of the flower scent of *Cyphomandra divaricata* (Solanaceae), new carotenoid-derived constituents in the flower scents of *Anthurium salvadorense* (Araceae) and *Palisota* species GHS06 (Commelinaceae) as well as unusual esters of 2-pentanol in that of *Hoya* vs. *sussuela* (Asclepiadaceae).

Essential oils: sample preparation and analysis

Patrizia Rubiolo

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The definition of essential oil, extremely precise and internationally accepted, is "An essential oil is the product obtained by hydrodistillation, steam distillation or expression (for *Citrus* fruit) of a plant".

Since essential oil components are generally medium to highly volatile compounds with medium to low polarity (terpenoids, phenyl propanoids and so on), GC is the technique of choice for their analysis.

In this definition, sample preparation is considered very important and fundamental although hydrodistillation or steam distillation are not the only techniques used to obtained the so-called "volatile fraction" of a plant. Therefore it is necessary to use a correct terminology to indicate the volatile or semi-volatile fraction of a plant, in particular it would be better to talk about the "volatile fraction" and to use the definition "essential oil" only when the sample preparation is distillation or expression.

This lecture will deal with the following main topics:

1. Alternative techniques to distillation: this part will mainly deal with advantages and disadvantages of SFE as an alternative technique to distillation to *extract* the volatile fraction in comparison to the classical steam- or hydro*distillation.*

2. High concentration capacity static headspace sampling techniques: this part will be focussed on recently introduced techniques such as headspace-solid phase microextraction (HS-SPME) and headspace sorptive extraction (HSSE) that have opened new perspectives in the analysis of the volatile fraction allowing us to run a static headspace sampling but at the same time concentrating trace components.

3. Recent improvements in GC analysis of essential oils: this part will concern the recent achievement in GC analysis of essential oils in particular with the so called "high speed (or fast) GC" with both narrow bore and conventional inner diameter short columns.

Biosynthesis of essential oils

Edward M. Davis and Rodney Croteau

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Institute of Biological Chemistry, Washington State University, Pullman, WA, USA

Monoterpenes comprise structurally diverse, major components of many essential oils, and the biosynthesis of the monoterpenes of peppermint (*Mentha* x *piperita*) have provided a model system for their study. Recent advances in the isolation of intact epidermal oil glands (peltate glandular trichomes) have permitted investigations of the biosynthesis and developmental regulation of metabolism of these C3 oxygenated *p*-menthane monoterpenes, and have provided an enriched source of mRNA for the generation of an expressed sequence tag (EST) library. The identification and heterologous overexpression of genes responsible for the biosynthesis of the universal monoterpene precursor, geranyl diphosphate, and for downstream steps leading to the primary peppermint monoterpene, menthol, have allowed detailed characterization of these catalysts and the transgenic manipulation of peppermint oil composition and yield.

Essential oil biodiversity

Elisabeth Stahl-Biskup

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The expression ”biodiversity“ was coined in the nineties of the last century by the biologist Edward O. Wilson who tried to describe the total variety of the world’s living organisms including all genes, species and ecosystems, and the ecological processes of which they are part. No wonder that an internet search “biodiversity” provides about 1,200,000 hits, a fact which reflects its broad meaning in biological contexts. Discussing essential oil biodiversity we focus on the chemical polymorphism in aromatic plants, which means the infraspecific variation of their essential oil patterns in qualitative and quantitative respects. The levels of examination can be represented by species, populations or even individuals. Essential oil polymorphism is due to the fact that the biogenesis of terpenoids as well as of phenylpropane derivatives are genetically controlled by several steps. Furthermore ontogenetic variability and environmental factors can be considered to influence the biosynthesis. Essential oil polymorphism is widespread in the plant kingdom; in 1975 Tétényi estimated 360 species of 36 families which were known to be chemically polymorphous.

As a result of the understanding that it is a fundamental requirement to analyse infraspecific differences, we encounter a flood of reports on polychemism. Therefore, nowadays the compilation of all species which show essential oil polymorphism would be a worthwhile but arduous undertaking. It is a fact that the Lamiaceae have always been an interesting object of polychemism research (Lawrence, 1980), the genus *Thymus* being one of the most frequently investigated and the object of the most detailed research regarding this phenomenon (Sáez and Stahl-Biskup, 2002). Since the early 1960s, one species, *Thymus vulgaris*, has been at the heart of ecological and genetic research on the evolutionary dynamics of genetic polymorphism. Its essential oil variation has a genetic basis and the presence of six distinct genetically-based forms has thus provided an attractive system to explore the ecological genetics of secondary compound variation and the population structure and spatial dynamics of genetic polymorphism in thyme (Thompson, 2002).

Nowadays taxonomists agree that the analysis of the chemical polymorphism can only be completed analysing a huge number of individuals producing an immense amount of quantitative data which can only be categorized and classified by computer-based multivariate statistical analyses. In this respect the genus *Thymus* again is a good example for demonstrating the methods to discover and to document essential oil polychemism. Special focus will be put on *Thymus* in south-eastern Spain, western Iberia and northern Europe. As a résumé it can be anticipated that in *Thymus* two forms of polymorphism are manifested: some species occur with only few chemotypes, other species show more than 7 or even an uncertain number of chemotypes. The highest chemical variability seems to be concentrated in species from the sections *Serpyllum* and *Thymus*.

Lawrence BM (1980) VIIe Congrès International des Huiles Essentielles, Octobre, Cannes-Grasse, 118-131.

Sáez F, Stahl-Biskup E (2002) In: *Thyme –The Genus Thymus* (eds: Stahl-Biskup E, Sáez F), Vol. 24, Series “Medicinal and Aromatic Plants – Industrial Profiles“ (ed. Hardman R), Taylor & Francis, London, pp 125 (in press).

Tétényi P (1975) Planta Med. **28**: 244-256.

Thompson JD (2002) In: *Thyme –The Genus Thymus*, pp. 44; Vol. 24, Series “Medicinal and Aromatic Plants – Industrial Profiles“ (ed. Hardman R) Taylor & Francis, London, pp 125 (in press).

Volatile signals: chemical structures and ecological aspects

Wittko Francke

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Molecular recognition is prerequisite to the beginning of life, and thus, "chemical signalling" is the oldest means for the transmission of information.

During evolution, principles of chemical communication may have been developed several times and for different reasons, typical elements being made up by secondary metabolites.

Most of the relevant compounds are represented by acetogenins, polyketides and terpenoids, the biosynthesis of which is not restricted to animals, but is also valid in plants and microorganisms, in terrestrial as well as in aquatic ecosystems.

Typical sex pheromones of moths are *de novo* produced medium chain unbranched, unsaturated, even numbered acetogenins with an oxygen containing functional group at one end of the chain. There are, however, secondary alcohols and polyenes, including the corresponding epoxides, showing uneven numbers of carbon atoms. This latter group represents degradation products of fatty acids, i.e. β-oxidation/decarboxylation or epoxidation/decarboxylation, respectively.

Replacement of acetate units by propanoate during the synthesis of a typipcal acetogenin will result in methyl branching which adds to the variability of principle structures since the position of the methyl group and the configuation of the stereogenic center may account for the species specificity of the compound and for its biological activity. The same is true for polyketides which are produced from propanoates, exclusively. Relevant substances are made up of 2-5 propanoate units showing several stereocenters at the branching points as well as at the hydroxy groups originating from the aldol-type biogenesis of the compounds. Stereochemical compositions play a particularly important role in the biological activity of such structures. The possible role of (endo)symbionts in their production and transformation is yet unknown.

A third large group of semiochemicals is represented by terpenes. They may be sequestered and used unchanged or oxidised and/or rearranged, however, they may also be produced *de novo*. The involvement of propanoate units in the principle biosynthesis of terpenes results in the formation of homomevalonate, which finally yields homoterpenes. Again, the additional methyl group gives rise to stereoisomers which enhance the specificity of the produced signal.

Sequestered food constituents which animals use as chemical signals, provide information about the quality of the environment. The more such compounds are altered through metabolic activities in the animal (finally leading to a totally *de novo* synthesis), the more they reflect the physiological state of the emitter. During biosynthesis of semiochemicals, the action of less specific enzymes will lead to the production of „byproducts" (stereoisomers, chain length) of the "actual signal" which may add a fine tuning of the bouquet or facilitate evolution through the introduction of a „disposable variant". In many cases, stereochemistry plays a major role in chemical communication: A well defined (correct) composition of positional and geometrical isomers or of enantiomers and diastereomers of attractants are decisive for an optimal biological activity. Closely related species may simply generate specific blends by producing different blends (including different stereochemical proportions) of the same compounds – which may even be mutually deterrent.

Concerning aspects of coevolution between a producer (biosynthesis and release) and a receiver (perception and transduction) the problem strongly resembles the story of the hen and the egg.

New trends in intellectual property relating to perfumery materials

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It has been traditional in all industries, and that of Perfumery is no exception, to follow innovation through the patent literature since, if patent law functions properly, it enhances the incentive to innovate.

The trend already observed a few years ago in the flavor and fragrance industry towards the patenting of more complex "systems", rather than just "molecules" as such, seems to have accelerated, although a few of the industry's players have remained strong in the research and protection of the latter.

Other recent patent trends relate to the protection of new products for manufacturing odorants through molecular biology methods.

Finally, outsiders to the industry are actively pursuing research and patenting of olfactory receptors, the use of which could in the future complement the traditional creation and formulation of fragrances and flavors.

These different tendencies will be discussed through some examples.

Biological activities of the essential oils

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A review is given on scientifically proven activities of essential oils. Essential oils (EO) as natural mixtures (sometimes up to more than 250 single volatile compounds) are very valuable natural products and used in many fields, e.g. fragrance and perfumes, cosmetics, aromatherapy and phytotherapy, spices and nutrition, sources of raw materials. Above all, EO are used as therapeutic agents against various ailments, disorders and psychosomatic complaints. Contrary to esoteric and holistic believes, the rather small and lipophilic fragrance compounds of an EO exhibit distinct physiological activities (besides their sensorial qualities) which can be shown in animal and human experiments.

The psychological effects, e.g. the creation of a sort of well feeling, happiness, etc. and especially the physiological/pharmacological activities are topic of this review. Own research results obtained with healthy subjects as well as experimental data found by other scientists are presented. In addition also some exemplary and interesting animal experiments are included in this report. Finally, some pros and cons regarding the therapeutically use of EO are mentioned.

Oral Communications Abstracts

Orange concentrated essences – Manufacture by fractional distillation and pervaporation

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The citrus juice processing generates large amounts of wastes. Almost 50% of the fruit weight finishes as waste in the form of peel, segment membranes, rags and seeds. Besides that, during juice concentration, volatile constituents contained on the vapour phase are condensed by the aroma recovery systems. The missing of these aromatic constituents results in a decrease of juice quality, unless these compounds are being reincorporated on it. The aroma recovery systems get two separated phases: essence oil (oil phase) and aqueous essence (water phase). The average yield of these products is very low. It's necessary around 2,000 kg of oranges to produce 1 kg of water phase and 8,000 Kg of oranges for 1 Kg of oil phase. These two products are rich in aldehydes, esters and other volatile compounds. Oil phase has a high concentration of terpenes and water phase is rich in alcohol. As the storage and transportation of these products are expensive, the evaluation of concentration processes of these essences is of high interest.

The aim of this work is to study the special fractions of these aromatic volatile compounds from the oil phase and water phase, obtained by fractional distillation and pervaporation. The fractional distillation is far the most used process, mainly after the development of efficient columns, which can reach 100 or more theoretical plates. Nowadays, it is possible to conduct efficient fractional processes. Relative to alternative treating techniques, pervaporation provides a system which is simple to operate and easy to maintain. The high reliability and versatility of pervaporation systems make pervaporation an attractive choice for applications involving the recovery or removal of volatile organics from water streams.

By fractional distillation of the oil phase, concentrated fractions with high contents of fragrant compounds as ethyl butyrate and valencene were obtained. For the concentration of the water phase, the two processes, fractional distillation and pervaporation, were carried out. Essences with ethanol content higher than 80% were obtained. Their smell and taste, proceeding from the original esters and aldehydes of the raw materials, such as acetaldehyde, seems to be very good.

The evaluation of these concentrated essences was performed by physicochemical and chromatographic analysis. Different amounts of these concentrated products were added to juices and soft drinks, showing wide acceptance by sensory evaluations.

Hydroponics - a novel way to create new aroma in living aromatic plants

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Both in ancient and present time, various people have grown plants hydroponically. It is know in scientific literature that hydroponically grown vegetables have different tastes due to their change in protein, carbohydrate and fat of the aroma contents. But to our knowledge, nobody has ever studied the fragrance composition of the hydroponically grown aromatic plants. We have grown various flowers, viz. Hyacinth, Rose, Jasmin, fruits, like lime, lemon, strawberry and many herbs and spice plants and have studied the composition of these hydroponically grown living flowering plants with those grown under the conventional soil method. It was found that these hydroponically grown flower, fruits, herbs and spices not only have superior growth patterns, but they also produce different aroma constituents than the conventionally grown plants.

As for example, in the case of Hyacinth, phenyl ethyl alcohol in conventionally grown Hyacinth is 3.3%, which has increased to 23% in hydroponically grown Hyacinth. Benzyl Tiglate, which is 12% in conventionally grown Hyacinth, is not detected in the hydroponically grown hyacinth. In the case of hybrid tea rose, the green note components, viz. cis-3-hexenyl acetate and hexyl acetate have decreased from 7% in conventionally grown rose to 0.3% in hydroponically grown rose. At the same time, Citral content, which is 10% in conventionally grown rose, decreases to 5% in the hydroponically grown rose. In the case of strawberry, ethyl acetate content which is 12% in conventional grown fruit decreases to 0.1% in hydroponically grown strawberry. Ethyl caproate, which is 35.9% in conventionally grown strawberry, has decreased to 9.7% in hydroponically grown strawberry. On the other hand, 2-nonanone, which is 3.6% in conventionally grown strawberry, has increased to 19% in hydroponically grown strawberry. In the case of Persian Lime, limonene content has increased from 11.6% in conventionally grown fruit to 23% in the hydroponic condition, where as Citral, which is 22% in conventional has reduced to 7% under hydroponic condition.

Present paper will describe in details the difference between the aroma chemistry of the hydroponically grown flowers, fruits, herbs and spices to those of the soil grown plants. In addition, the significance of the aroma of hydroponically grown flowers in the fragrance industry will be illustrated.

Rapid and reliable analysis of essential oils applying various vibrational spectroscopy methods

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During the last years near-infrared spectroscopy (NIRS) has been successfully introduced as an efficient tool for rapid and reliable analyses of various essential oils [Schulz *et al.*, 1999; Steuer *et al.*, 2001]. Usually the NIR spectra (range: 1100 to 2500 nm) are registered in the transflection mode using quartz cuvettes, equipped with a diffuse gold reflector (path length 0.2 mm). Since NIRS is an indirect analysis method, at first it is necessary to establish calibration equations for the individual essential oil substances (analytes). In this context standard GC methods are applied to obtain the reference data. In most cases statistical errors of the NIR predicted data (SECV) are not significantly higher than the relating standard deviations of the GC method. The individually developed calibration statistics can be used not only for rapid quality control purposes but they can be also successfully introduced to monitor the enrichment of valuable essential oil components during distillation or extraction processes.

The recent availability of attenuated total reflectance (ATR) technology for mid-infrared spetroscopy has made the handling of liquid samples very quick and simple. Whereas in the past MIR spectroscopy was frequently used as a qualitative technique for the identification and verification of pure essential oil substances, up to now there exist only a few applications in this area which use multivariate calibration algorithms to extract the quantitative data from the rich in information and well-structured MIR signals. Today portable Fourier-Transform (FT) IR spectrometers are available which need only sample amounts of several microliters. The simultaneous analysis of several oil components can be performed in some seconds [Schulz *et al.*, 2002].

Also NIR-FT-Raman spectroscopy, which is to be seen as a complementary method to MIR, has been successfully applied for quality analysis purposes of various essential oils [Schulz et al., 2002]. The spectra were processed with a PLS algorithm and the accuracy of the individual calibration models is generally characterized by the statistical parameters R^2 and SECV. Compared to the NIR calibration results generally a lower detection limit is reached due to the larger signals of individual fundamental vibration modes. All spectroscopic techniques described here have the potential to replace existing analytical procedures because they are capable of faster quality control measurements, and they allow on-line analyses during distillation or extraction processes to be performed. In addition to rapid analysis of isolated essential oils, partly the described spectroscopic techniques have also potential to determine non-destructively the amount and composition of essential oils directly in the fresh plant or in the relating drug without performing any clean-up procedures. Furthermore there exists the option to connect a Raman spectrometer with a microscope which offers the opportunity to map in-vivo the occurrence of selected essential oil substances in the plant matrix.

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Highly enantioselective cyclodextrin derivatives and conventional inner diameter short capillary columns: an approach to improve separation of medium to low volatility compounds

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This communication reports the results obtained by combining highly enantioselective cyclodextrin derivatives with conventional inner diameter short capillary columns in the enantiomer separation of medium to low volatility compounds.

Cyclodextrins (CDs) are successful enantioselective chiral selector used in the gas chromatographic (GC) separation of underivatized volatile enantiomers. The CD ring size and the substituents of the sugar units at C-2, C-3 and C-6 hydroxylated positions influence the CD enantioselectivity and chemical and physical properties.

6-tert-Butyl-Di-Methyl-Silyl (TBDMS)-β-CDs substituted in positions 2 and 3 with methyl (ME) or ethyl (ET) groups are among the most effective chiral selector for enantioselective GC and show enantioselectivities that very often are complementary. To increase enantioselectivity completely asymmetric ME-ET-6-TBDMS-β-CD were synthesised and tested as GC stationary phases, with the aim of developing CDs of as universal as possible application, and of evaluating how the simultaneous introduction of ME and ET groups in positions 2 and 3 of CD ring influences enantioselectivity.

The increased enantioselectivity of the new derivatives affords to analyse several racemates with shorter columns still obtaining enantiomer base line separation. Shorter columns not only contribute to shorten analysis time thus overcoming one of the main drawbacks of enantioselective GC with CD derivatives, but also concur to reduce the enantiomer elution temperature. This favours separation of enantiomers (in particular of those of medium-to-low-volatility racemates) because of the very low difference in energy involved in the enantiomer host-guest interaction whose discrimination is favoured by a lower temperature.

Supercritical carbon dioxide extraction of essential oil from *Satureja fruticosa* Béguinot

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Aromatic plants produce essential oils, which have important biological functions in plant metabolism. The quality of these oils is also very important in human consumption. The main health applications of *Satureja fruticosa* Béguinot are due to its digestive and gastric properties.

Supercritical fluid extraction (SFE) is a separation process, based on the solvent power of supercritical fluids, which can be controlled by changing the pressure and temperature (Palavra *et al*., 1993, 1995). With this technique it is possible to obtain solvent-free extracts and avoid the degradation of thermally labile components existing in the oils. Therefore, the natural odour and flavour of the plant are maintained, thus making SFE a very promising process for the separation of essential oils from herbaceous matrices (Palavra *et al*., 1997).

Flowers and leaves of *Satureja fruticosa* Béguinot, cultivated at Servicio de Investigacion Agroalimentario, Zaragoza, Spain, were used as matrices in supercritical CO_2 extraction followed by a two stage fractional separation. The process was carried out in a flow apparatus, provided with an extraction vessel (1 L) and two separators (0.27 L, each) at temperatures of 40 and 50 ºC and pressures of 90 and 100 bar.

The isolated essential oils were analysed by GC-MS and GC. The oxygen-containing monoterpenes, pulegone (46-56 %), piperitenone (10-13 %), piperitenone oxide (9-14 %) and isomenthone (7-9 %) were the major oil constituents.

The effect of the plant particle size, extraction time and solvent flow rate on the extraction yield and quality of the obtained oil is discussed.

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Volatile components of southern hemispheric epiphytic liverworts *Frullania* and *Schusterella* species (Frullaniaceae)

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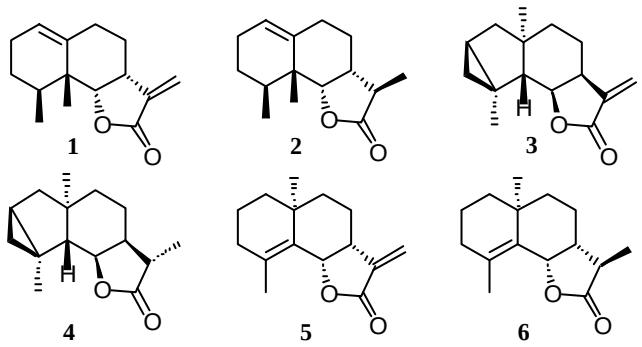
A number of liverworts and mosses are medicinal plants and are said to possess certain biological activity (cytotoxic, antimicrobial, antifungal, antidotal, antipyretic, anticancer, sedative, diuretic and antiseptic activity etc.) and effect (for cardiopathy, hematostatis, pulmonary tuberculosis and neurasthenia etc.) (Asakawa 1982, 1995 & 1999). Many liverworts also show characteristic fragrant odour and hot-taste.

There are ca. 300 of *Frullania* species (Frullaniaceae). Some sesquiterpene lactones isolated from *Frullania* species display potent allergenic contact dermatitis, piscicidal activity and cytotoxicity against certain cancer cell lines (Asakawa 1995). We are continuing to study the chemical constituents of the southern hemispheric epiphytic *Frullania* and *Schusterella* species from view-point of search on the biologically active substances and chemosystematics of the Frullaniaceae.



The volatile components of *Frullania* and *S. chevalierrii* have been analyzed by GC-MS. The major components were isolated by HPLC and their absolute configurations established by spectroscopy and X-ray crystallographic analysis. The present *Frullania* species are chemically divided into five groups: 1) Sesquiterpene lactone, 2) Sesquiterpene lactone-bibenzyl, 3) Bibenzyl, 4) 2-Alkanone and 5) the others. *S. chevalierrii* is closely related chemically to sesquiterpene lactone group of the *Frullania* species since it elaborates two eudesmanolides, β-cyclocostunolide and its dihydro derivative, as the major components.

The Argentine *F. brasiliensis* produces both new eremophilanolides (**1,2**) and eudesmanolides (**3-6**), along with a new 3,3',4-trimethoxybibenzyl. This species resembles chemically the European *Frullania dilatata* (Asakawa *et al.* 1976, Nagashima *et al.* 1999).

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Repeated measurement analysis as tool in essential oil research

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'Repeated measurement analysis' is a special statistical procedure to analyse groups of dependent variables, which are measurements of the same trait. By repeating essential oil analysis of individuals in time or space, the accuracy of the determination of either environmental or ontogenetical influences (repetition in time) or plant organ differences (repetition in space) is enhanced.

To demonstrate the capability of this method, two model experiments are presented. In one experiment, a population of marjoram (*Origanum majorana*) was sampled twice at the same stage of development but grown under different environmental conditions. In the second example, different plant organs of monks-pepper (*Vitex agnus-castus*) from individual plants of several accessions were analysed.

Biotransformation of cuparene, plagiochilide, aristolene and nootkatone by some microorganisms

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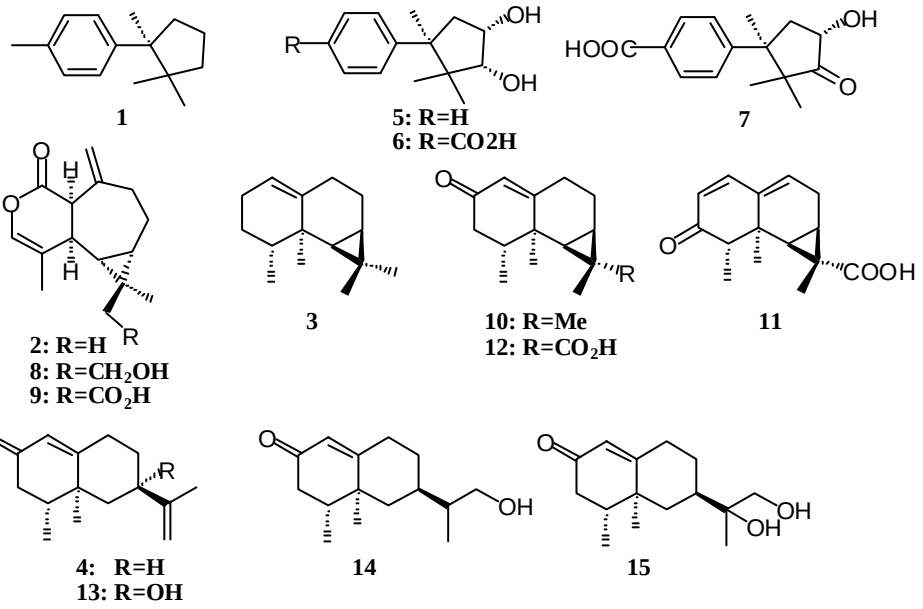
We are continuing to study the biotransformation of sesquiterpenoids by several microorganisms and mammals to obtain functional substances (Noma & Asakawa 1995; Hashimoto, Noma & Asakawa 2001).

The present paper concerns the biotransformation of four sesquiterpenenoids, (-)-cuparene (**1**) and plagiochilide (**2**) which are the major sesquiterpenes isolated from the liverworts *Marchantia polymorpha* and *Plagiochila fruticosa*, respectively, and aristolene (**3**) from the crude drug *Nardostachys chinensis* and nootkatone (**4**) from grape fruit by *Aspergillus niger*, *Bortyosphaeria dothidea* or *Chorella pyrenoidosa*.



(-)-Cuparene (**1**) was biotransformed to compounds **5-7** by *A. niger* as the major products. Gem-dimethyls of **1** was not oxidized at al. On the other hand, one of the gem-dimethyls of plagiochilide (**2**) and arisolene (**3**) was oxidized by the same fungus to give **8** and **9** from **2,** and **10-11** from **3**. Aristolene (**3**) was also converted to **12** by *C. pyrenoidosa* as the major component. 7-Hydronootkatone (**13**) was mainly obtained from nootkatone (**4**) by *Botryosphaeria dothidea*. On the other hand, 11,12-dihydroxynootkatone (**14**) and 12-hydroxynootkatone (**15**) were major products from **13** by *A. niger*.

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Essential oils of *Sideritis* species of Turkey

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Anatolian peninsula is one of the two main gene-centres of the genus *Sideritis* L. (Lamiaceae) together with the Iberic peninsula. While Turkey contains *Sideriti*s species belonging to Sect. Empedoclia (Rafin.) Bentham and Sect. Hesiodia Bentham, the Spanish *Sideritis* species belong to Sect. Sideritis. Species in Sect. Empedoclia are used for making tea in Turkey and Greece.

We have collected all the *Sideritis* species growing wild in Turkey and investigated their essential oil compositions by GC and GC/MS. The paper will present a comprehensive view of the *Sideritis* species of Turkey as regards their essential oil composition.

Data Bank of the Amazon aromatic plants and their essential oils

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As part of the inventory of the Amazon odoriferous flora we created a data bank based on the aromatic plants and their essential oils. Since 1980 we travelled all over 50,000 km, visiting numberless collection sites in all nine Amazonian states and at different ecosystems. Were collected over 2,500 aromatic part plants including leaves, stems, barks, trunk woods, rhizomes, fruits, flowers, resins, etc. For economic purpose the last 6 years of this work was addressed to collect fast growing plants (herbs and shrubs) occurring in areas of natural fields and secondary forests like savanas and cerrados. Their essential oils were hydrodistilled and analysed by GC-MS using WCOT SE-54 and DB-5 fused silica capillary columns. Individual components were identified by comparison of both mass spectrum and GC retention data with those of authentic compounds previously analysed and stored in the data system or cited in the literature (Maia *et al.* 2002; Adams 1995). Today, the Data Bank contains about 1250 registered specimens providing the following informations: families, scientific and common names and synonyms; collection sites; geographical distribution; habitat; popular uses; oil yields; part of plant furnishing the essential oils; botany, agronomic and ecological sights; GC and GC-MS ion-chromatograms; bibliography and photos. The Data Bank provide also information about the essential oil application according previous bioassays and fragrant and olfative perfurmer analyses. At present, from Data Bank were published over 100 scientific papers reporting the Amazon essential oils. It is available for oral presentation in Windows Access ambient.

Acknowledgements: supported by MCT-PPG7/European Community.

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Influence of Ylang-ylang oil on mental, emotional and human physiological parameters

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Ylang-ylang oil (*Cananga odorata*) is widely used as a fragrance in perfumery and cosmetic industries. In medicine the interest in the usage of ylang-ylang oil as therapeutically active agent has grown considerably. Especially in aromatherapy, ylang-ylang oil has been used as an antidepressant in the case of depression and nervousness as well as used for reducing the blood pressure in the case of hypertension. In spite of its widespread use, scientific evaluation of the effects of this fragrance on human is rather scarce. The aim of this study was to investigate the influence of ylang-ylang oil on mental, emotional, and human physiological parameters. The fragrance was administered by inhalation. Physiological parameters recorded were blood pressure, breathing rate, skin temperature, and heart rate. Self-evaluation was assessed in terms of alertness, attentiveness, calmness, mood, relaxation, and vigour. Additionally, the fragrance was rated in terms of pleasantness, intensity, and effect.

The present study revealed that ylang-ylang oil affected both physiological parameters and subjective mental and emotional conditions. Ylang-ylang oil caused a significant decrease of blood pressure, which are likely to represent a sympatholytic effect, i.e. dilation of blood vessels and lowering blood pressure. Furthermore, ylang-ylang oil caused a significant increase of subjective attentiveness, which seem to show a harmonizing effect of the oil. Correlational analyses revealed interactions between physiological parameters and self-evaluation in all groups.

These findings give an evidence for the usage of the ylang-ylang oil in aromatherapy such as furnishing a reduction of blood pressure or a relief of depression and stress in humans.

Adverse effects of essential oils and components: Fact and Fiction

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Many essential oils and extracts used in the food and cosmetics industries are already banned or severely restricted due to their toxic properties (wormseed, wintergreen); carcinogenicity (calamus and sassafras); sensitisation (benzoin, Peru balsam); photosensitization (bergamot, expressed). Components like cinnamaldehyde are also restricted due to sensitisation. Extensive research by RIFM has been conducted on the allergic effects of fragrances on skin, many of which have been shown to cause dermal allergic reactions, but they have rarely tested the effects of inhaling fragrance chemicals. An estimated 5.72 million in the US have skin allergy to fragrance. Around 25% of the general population report some sensitivity to chemicals and of these more than 80% report that exposure to fragrances has a major impact on their quality of life. Symptoms include: headaches, dizziness, nausea, fatigue, shortness of breath, difficulty with concentration, and allergy-like symptoms. Fragrances trigger or may actually cause asthma.

Some sceptics (mainly in the cosmetics business) suggest that reactions to fragrance could be psychological i.e. a conditioned response, which could include elements of paranoia, stress-induced psychosomatic symptoms, hypochondriasis, hysteria, and suggestibility.

Due to secrecy laws, adverse reactions to fragrances may be difficult or impossible to link to particular fragrance chemicals. This is complicated by synthetic organic chemicals, which constitute more than 80-90% of the raw materials used in flavour and fragrance formulations. Chemicals that appeared in more than half of cosmetic products included limonene (showed carcinogenicity in male rats, but not in mice or female rats), linalool, ß-phenethyl alcohol, and ß-myrcene, these other chemicals have not been tested for carcinogenicity. The frequently-occurring α-pinene is however a mutagen. With time, more components are being tested and also found to be carcinogenic or allergenic. The worrying problems are that: when a person is exposed to an odour, the sensation appears to diminish over time as the person adapts to the odour, while sensory irritation occurs in an opposite manner; the higher the number of chemicals being combined, the lower their individual concentrations need to be to cause an adverse reaction and children may be more susceptible than adults.

Is the proposed new EU legislation justified in requiring the labelling of products containing known sensitisers above a certain level like: benzyl alcohol, benzyl salicylate, cinnamal, citral, coumarin, eugenol, geraniol, isoeugenol, anisyl alcohol, benzyl benzoate, benzyl cinnamate, citronellol, farnesol, limonene and linalool; the synthetics: amyl cinnamal, hydroxycitronellal, butylphenyl methylpropional, hexyl cinnamal, methyl 2-octynoate and alpha methyl ionone as well as the naturals, oakmoss and treemoss? Is it the occurrence of these components or their oxidised forms which are sensitisers? Is it the same whether the components are from natural essential oils or the synthetic equivalents, which may have different enantiomeric proportions and have different biologically activities? Does it just make it more difficult for perfumers, flavour chemists and for aromatherapists, who may be severely restricted in what they can use? Or, does it benefit the consumer?

The essential oil of Australian Sandalwood (*Santalum spicatum*) – Effects of different samples on human physiology and subjective evaluation

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The biological properties of the essential oil of Australian Sandalwood (*Santalum spicatum*), e.g. bacteriostatic (Beylier, 1979; Viollon *et al.*, 1996) and anti-inflammatory activity (Mt Romance Pty Ltd., 2002), are very similar to those of the essential oil of East Indian Sandalwood (*Santalum album*). Also, the use of Australian Sandalwood oil in traditional medicine very much resembles that of East Indian Sandalwood oil. Recently, our group was able to show that topical administration of East Indian sandalwood oil has relaxing and harmonizing effects on humans (Hongratanaworakit *et al.*, 2000). However, no such investigations have been carried out with Australian Sandalwood oil. Therefore, the aim of the present study was to elucidate the effects of Australian Sandalwood oil on human physiological parameters and self-evaluation after percutaneous absorption.

In our study three samples of Australian sandalwood oil with varying compositions of the same constituents (dissolved in peanut oil) and a placebo substance (peanut oil) were administered topically for 30 minutes to healthy human subjects while physiological data (skin temperature, skin conductance, breathing rate, blink rate, heart rate, muscle activity and blood pressure) were recorded. Inhalation of the substances was prevented by breathing masks. At the beginning and at the end of the application subjects were asked to rate their mental and emotional condition in terms of relaxation, vigour, calmness, attentiveness, mood, and alertness.

Statistical evaluation showed subtle changes of both physiological and subjective parameters after the administration of Australian Sandalwood oil in comparison to the placebo substance. Moreover, differences in effectiveness were revealed between the three samples of Australian Sandalwood oil.

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Exploring the biological activity and phytochemistry of South African medicinal aromatic plants used in traditional healing rites

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Despite the technological advancement in modern medicine, many people in South Africa rely on traditional healing practices. It is estimated that there are approximately 200000 traditional healers ('sangomas' and 'inyangas') in South Africa. South Africa also harbours one of the six floral Kingdoms in the world and this biodiversity has provided the traditional healer with an impressive 'natural pharmacy' from which plants are selected as ingredients to prepare herbal preparations. Aromatic plants are often used in these preparations in the form of infusions or aromatic plant material may be burnt and the smoke inhaled. Ten of the most widely used indigenous medicinal aromatic plants have been selected to investigate their chemical composition and biological activity in an attempt to establish a scientific basis for their frequent use. The decongestant and anti-microbial properties of essential oils explain the use of aromatic plants in treating respiratory ailments. Using time kill methods the minimum inhibitory percentage (MIP) has been determined for various indigenous species on pathogens responsible for respiratory ailments (e.g. *Klebsiella pneumoniae, Cryptococcus neoformans, Bacillus cereus*).

Although the action of essential oils on bacteria and fungi are well documented, almost no information is available on the antimycobacterial properties of mono- and sesquiterpenoids. A preliminary screening of anti-mycobacteria was performed using *Mycobacterium smegmatis*, a rapid-growing, non-pathogenic species, in an agar plate-based format. All oils showed inhibitory activity to varying degrees. Susceptibility of *Mycobacterium tuberculosis* H37*rv* to the oils was tested by the BACTEC radiometric method. This method measures metabolism of a radioactive substrate into $^{14}CO_2$ as a rapid indicator of growth of the organism. Inhibitory activity of the oils was reported at concentrations between 0.125% and 1.0%.

Many traditional uses of aromatic plants hint at possible anti-inflammatory activity, hence vernacular names such as 'fever tea' or 'fever-berry'. This information prompted us to investigate the *in vitro* inhibition of prostaglandin-synthesis. For the anti-inflammatory assay the essential oils were resuspended to a concentration of 1.0 % in DMSO and anti-inflammatory activity determined using the cyclooxygenase-2 radiometric assay.

Aromatic plants are documented for use in patients with "fever" or "flu-like" symptoms; which are the clinical symptoms used to describe a malaria infection. Thus, preliminary results of the *in vitro* antimalarial activity of the essential oils against a chloroquine-resistant *Plasmodium falciparum* strain will be discussed.

Composition and biological activities of essential oils of *Fagara zanthoxyloides* (dried fruits) from Cameroon as well as of *Syzygium cuminii* and *Syzygium travancoricum* (fresh leaves) from India

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The composition of the essential leaf oil of *Fagara zanthoxyloides* (syn. *Zanthoxylum zanthoxyloides*, *Z. xanthoxyloides*, Rutaceae) from Cameroon and the essential fruit oils of *Syzygium cuminii* and *Syzygium travancoricum* (Myrtaceae) from India were analyzed by GC, GC-MS and olfactometry.

As main compounds of the essential *F. zanthoxyloides* oil α-pinene, *trans*-β-ocimene, citronellol, sabinene, myrcene, limonene, citronellyl acetate, α-terpinolene, α-phellandrene, geraniol, terpinen-4-ol, *p*-cymene, methyl citronellate and β-pinene were identified.

Pinocarveol, α-terpineol, myrtenol, eucarvone, muurolol, myrtenal, geranyl acetone, α-cadinol and pinocarvone as well as *trans*-β-ocimene, *trans*-β-caryophyllene, α-humulene and α-farnesene were found to be the dominating constituents of the *S. cuminii* and the *S. travancoricum* oil respectively.

The above mentioned essential oils were tested for their antimicrobial activities against *Bacillus cereus*, *Bacillus sphaericus*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphyllococcus aureus*.

The results of these antimicrobial activity testings and additional correlations of such effects of some single compounds (Jirovetz *et al.* 2000, 2001 and 2002; Lis-Balchin *et al.* 1998), identified in the essential oils of *F. zanthoxyloides*, *S. cuminii* and *S. travancoricum*, are disussed in this presentation.

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Posters Abstracts

P. 1. Volatiles from fruit and leaf oils of *Spondias* species growing in the Amazon

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The species *Spondias mombin* L is originated from Amazon basin, though it is also known in the Northeast Brazil, Central America and West Indies. Its fruits are known as "taperebá" and "cajá-pequeno". Its wood barks, leaves and flowers are used in the local folk medicine. The fruits of *Spondias dulcis* Robinson are known as "cajarana" and the species was originated from the South Pacific. Now, it is widespread in many tropical countries, including Brazil. The fruits of *Spondias purpurea* L. are known as "umbu" and the species occurrence is the Northeast Brazil. All three *Spondias* fruits are used for preparation of ice creams, juices and aromatization of pies and liqueurs.

The volatiles of fruits and leaves of *S. dulcis*, *S. mombin* and *S. purpurea* were obtained by hydrodestillation and analysed by GC-MS. Individual components were identified by comparison of both mass spectrum and GC retention data with those existing in the data system libraries and cited in the literature (Maia *et al.* 2000, Adams 1995). The major constituents identified in the oils of *S. dulcis* were α-thujene (fruit: 44.8%), α-pinene (leaf: 62.9%) β-pinene (fruit: 19.7%; leaf: 13.8%) and α-terpineol (fruit: 10.2%). The oils of *S. mombin* were dominated by α-copaene (leaf: 27.1%), β-caryophyllene (fruit: 19.3%), δ-cadinene (leaf: 11.9%), (E, E)-α-farnesene (fruit: 11.1%), ethyl hexanoate (fruit: 10.1%), α-selinene (leaf: 9.4%), (Z)-β-ocimene, (fruit: 8.9%), caryophyllene oxide (leaf: 8.8%) and ethyl butyrate (fruit: 8.2%). The main components found in the fruit oil of *S. purpurea* were limonene (60.8%), Δ^3-carene (7.7%) and terpinolene (4.1%).

Acknowledgements: supported by MCT-PPG7/European Community.

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P. 2. Volatiles from *Lippia grandis* Schau.

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The genus *Lippia*, belonging to the Verbenaceae, comprises about 200 species of herbs, shrubs and small trees growing in South and Central America and Tropical Africa. Of these, the chemical composition of the essential oils of about forty species has already been reported (Terblanché and Kornelius, 1996). The main compounds previously identified were limonene, *p*-cymene, α-pinene, camphor, β-caryophyllene, linalool and thymol. The species *Lippia grandis* Schau. is a shrub reaching 2.5 m, known as "erva-do-marajó" and occurring at natural fields of the Amazon Basin. Their leaf tea is used for liver ailments and menses disorders. Previously, we report a preliminary analysis of the essential oil of *L. grandis* (Silva *et al*. 1973). Now, we are reporting the volatile composition of five chemotypes of *L. grandis* collected at different locations and soil types at Southeast of Pará state (chemotypes A and B), Northeast of Roraima state (chemotypes C and D) and West of Maranhão State (chemotype E). Voucher specimens from chemotypes of *L. grandis* have been deposited in the herbarium of Museu Emílio Goeldi, in the city of Belém, Pará state, Brazil. The chemotype A was dominated by 1,8-cineole (22.2%), (E)-nerolidol (21.1%), α-pinene (17.4%) and β-caryophyllene (8.2%); The chemotype B by thymol (52.6%), methyl thymol (14.7%), *p*-cymene (21.1%) and γ-terpinene (8.9%); the chemotype C by thymol (67.4%); the chemotype D by carvacrol (21.2%), thymol (19.3%), *p*-cymene (14.0%) and β-caryophyllene (12.1%); and the chemotype E by 1,8-cineole (56.0%) and α-terpineol (13.7%).

Acknowledgements: Supported by MCT-PPG7/European Community.

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P. 3. Composition of the essential oil of *Hypericum glandulosum* Aiton grown on Madeira and its glandular structures

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Nine *Hypericum* (Hypericaceae or Gutiferae) species grow on the Madeira archipelago, three of which are endemic (Tebbs 1994, Vieira 1992). *Hypericum glandulosum* Aiton (= *H. joerstadii* Lid) is a rare endemic species of Madeira, Porto Santo and Canaries Islands, currently known in Madeira as "malfurada" or "hipericão". The plant is a small evergreen shrub up to 1m heights growing in rocky areas, particularly in the ravines in eastern Madeira. The infusion of the whole plant is used as a diuretic, to treat urinary bladder, kidney and liver diseases and against lithemia (Rivera and Obón 1995).

Scanning electron microscopic studies on vegetative and reproductive organs of *Hypericum glandulosum* revealed three different types of glandular structures: translucent glands or cavities containing essential oil, that occur exclusively at the leaves and two types of black nodules (internal and external) containing hypericin that are found at leaves calix and corola.

The aerial parts of *H. glandulosum* were collected during the flowering phase at Serra de Água, Madeira. The essential oils were isolated by distillation-extraction for 3h and analysed by GC and GC-MS.

The sesquiterpene hydrocarbons (46%) and non-terpenoid compounds (30%) were the main fractions of *H. glandulosum* oil, whereas the monoterpene fraction amounted to 15%. An unidentified sesquiterpene hydrocarbon (25%), β-caryophyllene (19%), *n*-undecane (13%) and *trans*-2-hexenal (11%) were the dominant components of this oil.

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P. 4. Quality of supercritical fluid extract from *Levisticum officinale*

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Lovage (*Levisticum officinale* Koch, Apiaceae) drugs and extracts are well known spices for food- and liqueur industry and are used medicinally for their stomachic, carminative and antirheumatic properties. As various chemical substances are involved both in the culinary value and the bioactivity to obtain high quality extracts of lovage has a great value. Supercritical fluids have been shown to exhibit several advantages in the extraction of natural products from plant matrices. The major effort on supercritical fluid extraction has to date been to evaluate whether or not it can replace traditional organic solvent extraction methods.

The effect of two factors (pressure and temperature of the extractor vessel) were investigated in the ranges of 150 - 450 bar and 40 - 60^0C by designed experiments using $C0_2$. The 3^2 full factorial design was performed once, with three repetitions in the centre of the design. The extraction yield was used as dependent variable. It was concluded that increasing the pressure and/or temperature the extraction yield rises. The extracts contained the characteristic bioactive components of *Levisticum officinale*. Sixteen volatile constituents were separated by GC from which five were identified: butylphthalide, 2-butylidenephthalide, Z-ligustilide, 3-butyl-4.5-dihydrophthalide, E-ligustilide. The major component in each SFE extract was Z-ligustilide, which was always present in more than 70 %. The characteristic phthalide derivatives were also proved by HPLC and UV spectra. The effect of the extraction pressure for the yield of fatty acids and β-sitosterol was highly significant. Their satisfactory recovery was achieved if the pressure and temperature were higher than 300 bar and 50^0C respectively. Fatty acid composition of SFE extracts was typical for Apiaceae species. HPLC analysis proved the presence of some minor compounds as coumarin derivatives beside the main Z-ligustilide. By comparison of retention times and on line UV spectra with authentic samples the linear furanocoumarin imperatorin, isoimperatorin, begapten and the simple coumarin umbelliferon were identified. Efficiency and selectivity of SF $C0_2$ and traditional extractions support the view that qualitatively new products can be gained in function of various phytochemicals, which may be beneficial in the industrial and therapeutical use.

Acnkowledgements: The work was supported by FKFP-0169, ETK-T-08-158/99 (OTKA T 0300344)

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P. 5. An unique *n*-propyl sesquiterpene from *Eryngium creticum* L. (Apiaceae)

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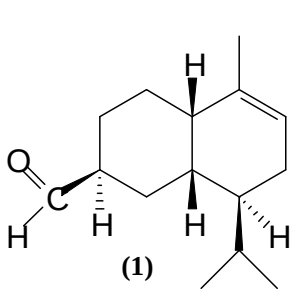
The sesquiterpenes cadinanes and muurolanes are known to bear isopropyl substituent connected to their perhydronaphthalene cores. Recently, the muurolane derivative, muurol-9-en-15-al, **(1)** has been isolated from *Eryngium maritimum* (Kubeczka, 1998). In a continuation of our previous studies (Kubeczka, 1988, 1999 and Ayoub, 2001) on the essential oils of *Eryngium* we describe herein the isolation, from *E. creticum*, of a new perhydronaphthaline derivative with a *n*-propyl substituent at its position no. 1. It was identified as 1,2,4a,5,6,7,8,8a-octahydro-4-methyl-1-propyl-naphthalene-7-carbaldehyde, **(2)**. The compound possesses an unique sesquiterpene carbon skeleton for which we suggest the name eryngane **(3)**. The isolated terpenoid **(2)** itself is therefore, renamed, erng-9-en-15-al. We were able, in addition, to isolate the new natural methyl ketone eicos- 8,11-dien-18-ol-2-one, **(4).** Structures of compounds **(2 & 4)** were established by conventional methods of analysis and confirmed by DEPT, COSY, HMQC and HMBC as well.



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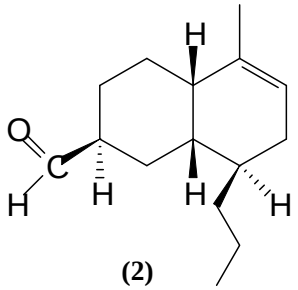

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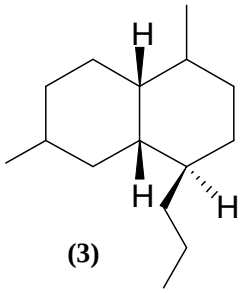

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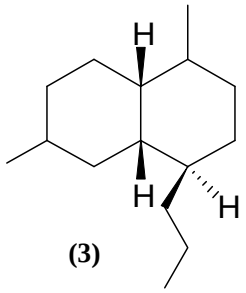

(3)

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P. 6. Chemical constituents of the essential oil of *Achillea pachycephala* Rech.f., a species endemic to Iran

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Achillea is a large genus belonging to the family *Asteraceae*. It is native to Europe and Asia, and can also be found in North America. Ninteen species of the genus *Achillea* are found in Iran, among which seven are endemic (Mozaffarian, 1996). The genus *Achillea* is well known for medicinal properties, in particular, *A. millefolium is* used as a popular antipyretic, because of its analgesic and anti-inflammatory properties (Chandler *et al.*, 1982).

Chemical studies on several *Achillea* species have resulted in the isolation of sesquiterpene lactones, phenolic and acetylenic compounds. Several authors have studied the composition of the oils of different *Achillea* species (Rustaiyan *et al.*, 1998, 1999).

Water distilled essential oil from the herbal parts of *A. pachycephala* Rech. f., which is endemic to Iran, was analysed by means of GC/MS in combination with retention indices. Thirty-three components were characterized in the oil representing 96.9% of the total compounds detected with 1,8-cineole (27.6%) and camphor (27.4%) as major constituents.

The oil was characterised by a large amount of oxygenated monoterpenes (78.7%), and a lesser amount of monoterpene hydrocarbons and sesquiterpenes (15.7% and 2.5%, respectively).

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P. 7. Scents from Amazon aromatic plants

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Over the last 20 years, human incursion into the Brazilian Amazon resulted in the degradation of more than 600000 km^2 of the natural forest, an area bigger than France. Rosewood, threatened by a half-century of predatory extraction, contributes with ca. 5000 fallen trees per year. Rosewood oil of international commerce currently originates from a destructive process, from a endangered species in which the wood is chopped and extracted by steam distillation (Barata, 2001a).

In consequence of environmental regulations and economic factors, the Brazilian Rosewood industry are progressively pushing away towards establishing plantations of this specie. To face this problem, the author established collaboration with commercial rosewood distillers to establish plantations system trials. In consequence, since the year 2000, ca. 20000 Rosewood trees have been planted in consortium with thousands of others aromatic native plants (Table 1). The goal is to develop new essential oils derived from leaves by coppicing the trees.

Research & Development of the essential oil of the Aromatic Plants is in progress, under a project that shall impact Environmental, Economical, Social and Technological areas in Amazon. A pessimistic estimation considers that 30ha of rosewood plantation shall produce ca.1000 kg of essential oil under the coppice of 3-5 year-old trees, representing a reasonable cash flow to local producers.

Experiences with rosewood plantations in Amazon have been published (Barata, 2001b).

This presentation reviews the results achieved of recent research on the potential for a new essential oils from Amazon Aromatic Plants. Steam distillation has been made using laboratory extractors and chemical composition was done by GC-MS chromatography.

Table 1. Rosewood and other Aromatic Plants species cultivated by consortium in Amazon

Commom Name	Scientific Name	Family	Class	# Trees	Years[1]
Rosewood	*Aniba rosaeodora*	Lauraceae	Tree	20000	5
Macacaporanga	*Aniba fragrans*	Lauraceae	Tree	1000	5
Preciosa	*Aniba canelilla*	Lauraceae	Tree	2100	5
Puxuri	*Licaria puchuri-minor*	Lauraceae	Tree	NR	5
Andiroba[2]	*Carapa guianensis*	Meliaceae	Tree	5900	4
Copaiba	*Copaifera* spp.	Caesalpiniaceae	Tree	720	50
Cumaru	*Dipteryx odorata*	Fabaceae	Tree	980	10

1. Number of years to adult stage. 2. Not an aromatic plant. Produces insecticide substances.

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P. 8. Activity of the essential oils of citronella (*Cymbopogum winterianus*) and basil (*Ocimum basilicum*) on the dry wood termite *Cryptotermis brevis*

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The termite *Cryptotermis brevis*, commonly known as "dry wood termite", is usually found in home environments, where it attacks furniture and wooden built structures. The conventional control method of termites is based on the use of chemicals, which are highly toxic for humans and other living beings. For this reason, our work aimed at verifying the effect of citronella and basil essential oils on *C. brevis*, analysing the consumption of substrate (filter paper) impregnated with the oils, and termite mortality. The oils were assayed on concentrations ranging from 94 to 471 $\mu g/cm^2$. In all tests, 1 mL of essential oil solutions was applied to the dry filter paper disks, using blank and acetone-impregnated filter paper disks as controls. 50 worker termites were placed in each Petri dish. Daily observations of the use of substrate and insect mortality were carried out during a 25-day period. After that period, the determination of the effective dose that produces a 50% reduction in the feeding (ED_{50}) and the median lethal concentration (LC_{50}) were carried out by the PROBIT method. Higher feeding inhibition effects were observed when basil essential oil was used, resulting in ED_{50} (56 $\mu g/cm^2$). Citronella oil also showed anti-feeding activity, but its ED_{50} was 167 $\mu g/cm^2$. The toxicity effect of the essential oils was measured by the mortality rate of the termites. The toxic effect of the oils was LC_{50} 262 $\mu g/cm^2$ for basil and 290 $\mu g/cm^2$ for citronella.

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P. 9. Repellence and toxicity of wax of orange oil in the alternative control of drywood termites

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Cryptotermis brevis is the most common drywood termite. It has been a wide geographic distribution, occurring in all tropical and subtropical areas around the world. In Brazil, it occurs from south to northeast, being the second most important plague among termites in southeast of Brazil. Searching alternatives to prevent and control the infestation by termites, we can emphasise natural products derived from plants. Despite this, the objective of this study was to evaluate the repellence and toxicity of a natural product, which constitutes a residue in the citrus industry, resulting from the cold-pressing process: the wax of orange oil, *Citrus sinensis* (L.) Pers. Another product generated by the cold-pressing process in the citrus industry, wax of lime oil "Tahiti", *Citrus aurantifolia* (Christ.) Swingle, was used as a parameter for comparison of the tested products. The bioassays were carried out in Petri dishes. Two wooden blocks (*Pinus* sp.) (3.0 x 3.0 x 0.5 cm) and 25 termites were placed in each Petri dish, being one of them treated with wax and other used as control (without treatment). The bioassays were done in triplicate and the results were evaluated for 30 days. The results showed that both waxes tested caused repellence to the insects with an index of mortality of 6.0%.

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P. 10. The essential oil of *Pentapleura subulifera* Hand.-Mazz.

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Pentapleura subulifera Hand.-Mazz. (Lamiaceae) is a monotypic genus growing in South Eastern Turkey, Northern Iraq and Western Iran (Davis 1982).

Water-distilled essential oil from the aerial parts of *Pentapleura subulifera* Hand.-Mazz., was analysed by GC and GC/MS. Fifty three components were characterised representing 95.9% of the oil with 1,8-cineole (39.0 %) and T-cadinol (11.1 %) as main constituents.

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P. 11. Chemical and microbiological studies of main compounds of *Thymi herba et extracta*

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Thyme (*Thymus vulgaris* L., *Lamiaceae*) is listed to aromatic plants containing essential oil (1.0 – 2.5%). The essential oil of *Thymus vulgaris* L. would normally be assigned to the aromatic alcohol-rich group. The main phenolic components of the volatile oil of thyme are thymol and carvacrol in varying amounts (thymol 0.2 - 66.5 %, carvacrol 0.2 - 72.0 %). These compounds determine antibacterial (Agnihotri, 1996; Didry, 1993) and fungicidal (Zambonelli 1996) actions of thyme and its volatile oil. On the other hand, in some countries a fundamental part of many galenicals are thyme extracts, and the studies of them were the aim of our investigations.

In the first part we determined the content of essential oil, thymol, luteolin and rosmarinic acid, in the raw material and in a liquid and dry extract. The second part has included an analysis of antiseptic activity of: thyme oil, thymol, luteolin, rosmarinic acid, thyme ethanolic extract and infusion, as well as of its commercialised liquid and dry extract.

The material for research was supplied by PHYTOPHARM, Klęka, Poland. The plant material had been collected in June 1998, at the flowering stage, near Piotrków Trybunalski. It was dried at 35°C. The liquid extract (*extractum fluidum*) was obtained from *Thymi herba* according to DAB X, while the dry extract is *extractum spirituosum siccum*. The essential oil was obtained by steam distillation, using a Deryng apparatus, and was dehydrated with anhydrous sodium sulphate and stored at 4-6°C. The content of thymol was determined by GC method (Shimadzu GC-17A). The contents of luteolin and rosmarinic acid were determined by TLC-photodensitometry (Shimadzu CS-9301 PC). Microbiological actions were investigated against five bacteria and yeast, *Candida albicans*, where a disc diffusion method was used.

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P. 12. Genetic diversity and variability of essential oils from Tunisian *Mentha pulegium* L. populations

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Pennyroyal (*Mentha pulegium*, Lamiaceae) was exploited for a long time for the quality of its essential oils, used in medicine, cosmetics, perfume and fragrance (Simon, Chadwick and Craker 1984). The overexploitation of natural populations has lead to great genetic erosion (Voirin *et al.* 1999). Measures of conservation of the species and the elaboration of improvement programs were necessary to ease natural populations.

The analysis of the genetic diversity of populations, by isoenzymatic markers, and the detection of variations in chemical composition of the essential oils could help to the selection of interesting cultivars (Lewis and Crawford 1995; Kang *et al.* 1998).

Fifteen wild *Mentha pulegium* populations, prospected in Tunisia, were surveyed. Eight isozyme systems (PGI, PGM, GOT, ICD, ADH, MDH, LAP and 6-PGD) were analysed by horizontal electrophoresis to access the genetic diversity.

The variation of essential oils composition, within and among populations, was analysed by gas chromatography (GC) and (GC/ MS). Eight major components (pulegone, menthone, carvone, isomenthone, 1-pinene, 1-limonene, menthol...) were considered.

The species maintained high levels of polymorphism within populations. Particular alleles: Adh1-a, Adh1-b, Adh1-d, Icd1-a, Mdh1-a, Mdh3-a and Pgi2-a emerged as a genetic markers for some populations, relating of their geographical localisation. The essential oils composition varied between individuals within the same site and between sites.

There is a positive relationship between the distribution of the isozymic markers (unique alleles) and the variability of the chemical components. Isozyme markers may play an important role in rapid identification of appropriate chemotypes, to promote their exploitation in industrial fields. They could be also used to facilitate conservation and improvement actions in *Mentha pulegium*.

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P. 13. Genetic diversity, mating system and essential oils in *Rosmarinus officinalis* L., *Myrtus communis* L. and *Mentha pulegium* L.

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The majority of medicinal and aromatic plants is used to extract essential oils which are exploited in various fields (traditional medicine, perfumery, pharmacy, insecticides, fungicides). However, the bulk of the treated material is still harvested wildly. The over exploitation of spontaneous species has led to the rarefaction and the extinction of many of them (Vinay, 1996; Khiari and Boussaid, 2002).

Systematic cultivation of species can therefore be introduced in order to conserve biodiversity, protect threatened taxa and promote the culture of interesting varieties.

The quality of essential oils has to depend on the whole process beginning with the selection of material and ending with the final product proposed to the consumer.

Plant selection programs require first the assessment of the genetic diversity and that of the variability of the essential oils in natural populations taking into account the species mating system. These studies may help in the detection and improvement of performant genotypes and contribute to elaborate conservation strategies (Soltis and Soltis, 1991).

Three medicinal and aromatic species: *Rosmarinus officinalis, Myrtus communis* and *Mentha pulegium* growing spontaneously in Tunisia were surveyed in this study.

12 to 15 populations collected in different geographical sites represented each species. We analyzed the polymorphism of eight isozyme markers by starch gel electrophoresis. The variation of fourteen essential oil components has been studied by gas chromatography. The mating system in each species was also analyzed in order to interpret the genetic variability.

High levels of genetic variation were observed within populations and unique alleles were detected in *Mentha pulegium* and *Myrtus communis* in relation with geographical sites (Ben Fadhel, 2002; Messaoud, 2002). However, a substantial differentiation between populations was observed, which is a result of low gene flow due to habitat destruction.

Despite their high morphological similarities, individuals within populations exhibited high levels of variation in the composition of their essential oil. This may indicate an important genotype richness, which could be used to promote appropriate varieties.

Isozyme and chemical variations observed in populations may be due to the mating system. In fact, species analyzed were allogamous. So, hybridizations between individuals occurred. It is important to take this parameter into account to ensure efficient selection and conservation.

The presence of particular alleles, revealed by electrophoresis, was in relation with that of some essential oil components. So, isozyme markers could be considered as valid predictors of the chemical composition of plants.

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P. 14. Biological activities of basil (*Ocimum basilicum* L.) essential oil

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Oxygen-derived free radicals and lipid peroxidation (LP) are widely accepted as major contributors to the ethiology of atherosclerosis and its chronic disorders, including coronary heart disease, stroke and ishemic dementia (Knight, 1995). Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely used for many years to retard lipid oxidation. However, concern about the safety of synthetic antioxidants together with consumer's preference for natural products has resulted in increased research on natural antioxidants. Since ancient times, aromatic plants have been added to different types of food to improve the flavour. Furthermore, it was recognized that the deterioration and rancidity of food was also prevented, what is primarily due to the antioxidant and antimicrobial ability of plant constituents. The essential oils of many aromatic plants are widely used in cosmetic, pharmaceutical and foodstuff industries, due to their strong antimicrobial activity. Recently, for some terpenes (geraniol, tocotrienol, perillyl alcohol, β-ionone, *d* –limonene) have been found to be effective, non-toxic dietary antitumor agents and hold promise as a novel class of antitumor drugs for human cancer (Karlson et al, 1996).

In this study, the results of the free radical scavenging capacity (RSC) and the antibacterial activity of basil (*Ocimum basilicum*) essential oil are reported.

The essential oil was isolated from dried aerial parts of basil (cultivated in northern part of Vojvodina) by hydrodistillation. The composition of essential oil was evaluated by GC-MS. RSC was assessed using DPPH (2,2 –diphenyl, 1-picryll hydrazyl)-method (Kirby and Schmidt, 1997) and measuring $OH^{\bullet}$ -radical induced degradation of 2-deoxyribose. Antibacterial activity of essential oil (20 and 50% solution in *n*-hexane) was tested against 13 bacterial strains, by standard antibiogram test.

The examined essential oil strongly reduced free radical formation of DPPH (IC_{50}=0.285 µg/ml). The inhibition of 2-deoxyribose degradation was 47.1% for pure oil, 50.0% for 50% solution and 44.1% for 20% solution of essential oil. The RSC measured by DPPH-method was dose-dependent. The most effective antibacterial activity of 20% solution of essential oil was on *Shigella sonei* (multiresistant strain from Public Health Institute, Faculty of Medicine, Novi Sad) and of 50% solution on *Bacillus subtilis* ATCC 10707. Both examined solutions also exhibited inhibitory activities on multiresistant strains of *E. coli, Salmonella typhi,* and *Shigella sonei.*

The obtained results suggest that basil (*Ocimum basilicum*) could serve not only as flavour agent, but also as a safe food supplement in preventing deterioration of foodstuff products. Furthermore, consumption of food prevented with natural basil essential oil is expecting to prevent the risk of many free radicals dependent diseases.

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P. 15. Antimicrobial activity of the *Vanillosmopsis erythropappa* (DC.) Sch.Bip. essential oil

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Vanillosmopsis erytropappa Schultz-Bip. (*Eremanthus erytropappus* (DC.) Mac Leish/ Asteraceae) is a Brazilian tree used for construction purposes or firewood. The essential oil from its trunk wood has been widely explored by chemical and pharmaceutical industry as source of α-(-)-bisabolol. This compound, typically associated with camomile, is used in cosmetic products to provide soothing effect due to its anti-inflammatory activity. In this work we have investigated the antimicrobial activity of the essential oil of this plant against drug-resistant microorganisms. The trunk wood of *V. erytroppapa* was collected from mature trees growing in Barão de Cocais (Minas Gerais) and extracted with hexane. The extract was submitted to antimicrobial essay and showed activity against *Staphylococcus aureus* at concentration of 2%. Quantitative composition of the active oil was calculated from GC/ MS peaks, which showed the presence of several compounds mainly α-(-)-bisabolol (43,6%), β-bisabolene (27,5%), α-humulene (18,0%) and α-bisabolol oxide B (17,0%). Column cromatography of the active oil on silica gel with toluene - ethyl acetate (93:7) yielded 10 different fractions, which were assayed for antimicrobial activity. The results revealed that a fraction constituted by β-bisabolene and α-humulene are highly effective against *Staphylococcus aureus*.

P. 16. Breath-by-breath nosespace analysis while eating banana by Proton-Transfer-Reaction Mass-Spectrometry

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The aroma (odour) of food products is related to volatile organic compounds (VOCs) that are released from foods and reach the olfactory epithelium in the upper part of the nose. VOCs can reach the olfactory epithelium from two distinct directions. Either they are sniffed through the nose, via the orthonasal pathway and enter the nostrils from the front. Alternatively, they reach the olfactory receptors through the oral cavity and the pharynx, via the retronasal pathway. When food is eaten, the retronasal path leads to olfactory perception.

Here we report on the in-mouth aroma while eating banana. By analysing banana we have chosen to explore a solid food whose aromatic composition is known. Headspace and nosespace air are sampled using Proton-Transfer-Reaction Mass-Spectrometry (PTR-MS) technique. Nosespace air is collected via two glass tubes fitted into the nostrils. Then, the air is introduced into the drift-tube of the PTR-MS device. Headspace differences between unripe and ripe banana are shown. Furthermore, the time-intensity evolution of selected aroma compounds, the phenomenon of swallowing and the after-odour, will be discussed.

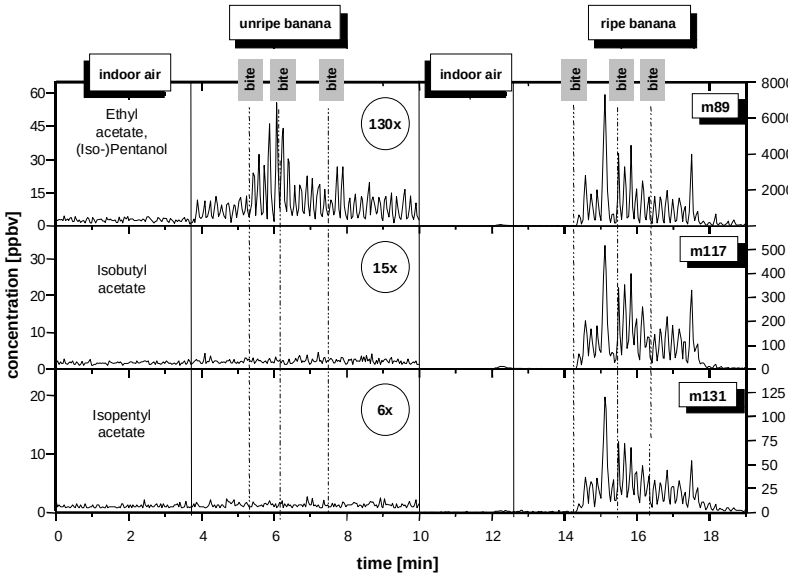

Figure: *In-vivo breath-by-breath analysis of nosespace with PTR-MS while eating banana. Three selected masses are shown. The top trace shows mass 89. Two compounds contribute to this ion mass intensity: Ethyl acetate and isopentanol. The middle trace represents Isobutyl acetate, which has a typical banana note and is the single most important key flavour compound of banana. The bottom trace represents isopentyl acetate, which also has a banana odour note. On the left, the trace are shown while eating an unripe banana, while the right traces correspond to volatiles released in the mouth with eating a ripe banana.*

P. 17. Quantitative structure activity relationship for synthetic sandalwood fragrance compounds

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QSAR is a modern throughout preferred approach to drug design. The main concept of QSAR techniques is to quantify the relationship between chemical structure and biological activity of potential drug candidates. Choices as to the set of compounds, the molecular descriptors, the mathematical form of the relationship between the biological activity and the descriptors as well the statistical method used to analyse the data are most important considerations in QSAR practice.

The lack of information of 3D-structures of the human odorant receptor and the better understanding of physicochemical mechanism of olfaction cause the need of development of new theoretical models for odorant molecules.

East Indian sandalwood oil is an appreciated constituent of perfume compositions because of its excellent odor properties. Its limited availability coupled with the high price is the reason of the incessant search for synthetic products with similar scent. Among all of these substitutes, the group of α-campholenyl derivatives achieved an odor most similar to the natural sandalwood. The unflagged interest in invention of new sandalwood odor substituents and particulary the synthesis of optically pure products are the foundations of providing this research.

A QSAR study for a set of α-campholenyl derivatives with known configuration of their chiral centers was performed. The relationship between the biological activity and structural, electronic and lipophilic properties of the molecules is described by the best model, which was found after applying Multiple Linear Regression. The model is evaluated by cross-validation and successfully applied on a set of test compounds for prediction of their odor thresholds.

The results help to expand the understanding of the interaction between sandalwood molecules and the still unknown odorant receptor site.

P. 18. SPME applied to the study of volatiles from Rosemary submitted to stress and ageing

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This work reports on the profile of volatiles from Rosemary leaves submitted to stress and ageing. Rosemary was grown in Campestre da Serra, South of Brazil. Solid Phase Micro Extraction (SPME) with 100 μm polydimethylsiloxane fiber and GC/MSD/IT were used to obtain the chromatographic profile of Rosemary volatiles.

Chromatographic analysis showed different results when in *in vivo* Rosemary leaves were compared with those, which had already been cut. The composition of volatiles was monitored through 28 days. The leaves were drying at room temperature (25 °C) and the plant was submitting it to stress conditions as without water. The compounds limonene, α-terpinene, γ-terpinene, terpinolene, are not present in chromatograms of *in vivo* Rosemary, suggesting that these compounds are encapsulated in reservoir bags where essential oils are accumulated. Other compounds like α–bisabolene, δ–2-care-4-ol have been detected only after plant cutting.

This monitoring allows the ideal time for Rosemary storage after harvesting to be determined. Geraniol, ocimene, verbenol, myrtenol, geranial, δ–3-caren-2-ol, myrtenyl acetate, bornilene and other less volatile compounds disappear within 120 hours after harvesting. The terpenes α-pinene, camphene, β-pinene, β-terpinene, terpinolene, 1,8-cineole, γ-terpinene and caryophyllene remain for 28 days after harvesting.

Results obtained so far, allow us to conclude that some compound appear only in stress conditions, such as lack of water or environment change. Echinenone and α–bisabolene were detected shortly after a transplant to a ceramic pot and did not appear afterwards, indicating that their presence is associated with plant non-equilibrium. The increase of quantity of the Rosemary essential oil and the appearance of β-pironene, *p*-mentone and *p*-cimene was observed when the plant was left without water for 3 days.

P. 19. Influence of seasonality and leaf position on leaf biometry and essential oil yield of *Lippia alba* (Mill) N. E. Br. ex Britt & Wilson

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This work relates variations in leaf biometry and in yield of essential oil of *Lippia alba* as a function of the season of the year and position of the leaves on the branch. Thus the parameters of seasonal variation (spring, summer, autumn, and winter) were considered concerning location of leaves in three parts of the branch (apical, median, and basal). Extractions of essential oil were accomplished through hydrodistillation. For biometric analysis of the leaves, samples were collected of the third median of the blade. The assay was carried out at São Manuel Experimental Farm (Unesp/Botucatu/Brazil), Department of Plant Production. The cuttings were maintained in a greenhouse, and after 60 days transplanted into a definitive location with a 1.0 X 0.60m space utilized for each. The plots are composed by four seasons (Summer'98/99, Autumn'99, Winter'99 and Spring'99), a completely randomized block was employed for the experimental design using 6 replications and different leaf positions (apical, median, basal). Samples were taken from 12 plants. On each plant, 10 leaf pairs were collected from basal (nodes 1-9; old leaves from 7.5-9.0 cm^2 in area), middle (nodes 10-18; mature leaves from 9.0-9.5 cm^2 in area) and apical regions (nodes 19-28; young leaves from 6.0-6.5 cm^2 in area). According to plot, all plant samples were collected during different seasons: summer'98/99 (December/January); autumn'99 (March), winter'99 (June); and spring'99 (September). Tukey (5% ratio) was used to evaluate essential oil yields with respect to season and leaf position. Histological sections were prepared from samples obtained from medial region of the leaf blade taken from the three plant positions. The samples were fixed in Karnovsky solution (12), dehydrated in an alcohol series, and embedded in glycolmethacrylate resin. Sections (8-µm in thickness) were stained with 0.05% toluidine blue in acetate buffer, pH 4.7 (15), and permanently mounted in Entelan synthetic medium. Quantitative data are based on 24 individual counts for each treatment (station X leaf position). A completely randomized block was employed for the experimental design using 12 treatments and 6 replications. The statistical design was performed at 5% significance. Tukey and Scheffé tests were used to evaluate the means of each station and leaf position. Essential oil extraction was performed by hydrodistillation of fresh leaves (100 g) in a Clevenger type apparatus for 1h and the extract analyzed by GC/MS. An analysis of variance (Tukey 5% ratio) was calculated for the percentage values of the oil yield at each station and leaf position. Data shown in the tables are mean values of four replications. The highest yield of oil was obtained in summer, in leaves located in the apical part of the branch; in these leaves during the same season, the total thickness of the blade was greater, resultant of greater development of the palisade parenchyma and epidermal cells of the adaxial face.

P. 20. Seasonal variation, leaf position on the stem and drying effect on biomass production and essential oil yield and composition of *Lippia alba* (Mill) N. E. Br. ex Britt & Wilson (Verbenaceae)

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According to the ethnobotanical data, *Lippia alba* (Mill.) N. E. Br. ex Britt. & Wilson, Verbenaceae, commonly known as "erva cidreira brasileira" or "falsa Melissa", is one of the most important plants widely used by the population, mainly through the leaf tea, due to its antispasmodic, sedative and stomachic activities among others (Ming, 1992). This work aimed to study the best harvest time for biomass production and essential oil yield and composition of the different plant parts (apical, medial and basal) related to the seasonal variation (spring, summer, autumn and winter). The oils were analysed by GS/MS (Shimadzu, QP-5000) and the components identified by comparison of their mass spectra with those of a MS computer library and/or published in the literature as well as by co-injection with authentic standards Applying phytochemical tests over fresh and dry mass, the chemical component percentages of essential oils were calculated and identified as follows: citral (neral and geranial), β-myrcene, β-caryophyllene and β-elemene. These results have also been found by Gomes (1993), Correa (1992), in the same species collected at different sites of Brazil. The secretory structures and chlorophyllaceous parenchyma holding essential oils were identified and quantified using microchemical tests through light (LM) and scanning electron microscopy (SEM). Essential oils were extracted by hydrodistillation with a Clevenger apparatus for both fresh and dry mass obtained in field and lab conditions respectively. The seasonal experiment in the field was carried out making possible the compoundable analysis employment for different harvest times in relation to different parts of plant (apical, medial and basal) as well as possible interactions between harvest time and parts of plant. This kind of multivariate analysis is possible, as there is a correlation among the three-plant parts and essential oil yield. The statistical design and methodology were performed at 5% significance in agreement with Morrison (1976). A completely randomised block design was employed for the drying experiment with five treatments and four replications. Tukey and Scheffé tests were used to evaluate the means of each temperature and analyse the differences between the two environments (natural and artificial) respectively, both at 5% significance.

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P. 21. Composition of the essential oil of *Teucrium lusitanicum*

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Continuing our research on the composition of the essential oils of *Teucrium* species, we now report on the composition of the essential oil of *Teucrium lusitanicum* Schreber [*T. polium* subsp. *vicentinum* (Rouy) D. Wood] from Portugal. This species grows spontaneously in W and S Iberian Peninsula and NW Africa (Valdés *et al*. 1987). In Portugal occurs only in W Algarve.

The aerial parts of plants from four populations were collected during the flowering period in Sagres and Cabo de São Vicente (Algarve - Portugal) and submitted to water distillation for essential oils isolation.

The oils were analyzed by GC and GC-MS, using two fused silica capillary columns with different stationary phases (polyethylenoglycol and polymethylsiloxane). Components were identified by GC retention indexes and mass spectra.

Sixty-five compounds were identified, representing more than 90% of the total oils. Although qualitative compositions are similar, important quantitative differences were found. (Table 1).

Table 1. Major compounds (>5% in at least one sample) and grouped components of the essential oil of *T. lusitanicum*

Compound	**% in samples**				
	A	**B**	**C**	**D**	**Average(Min-Max) %**
α-Pinene	8.2	2.5	0.8	8.5	5.0 (0.8-8.5)
Sabinene	9.6	3.2	2.1	7.7	5.7 (2.1-9.6)
β-Pinene	10.5	4.7	2.5	11.9	7.4 (2.5-11.9)
β-Myrcene	7.3	2.8	2.5	4.1	4.2 (2.5-7.3)
Limonene	11.5	1.2	3.0	2.3	4.5 (1.2-11.5)
Terpineol-4	1.9	2.7	5.5	2.8	3.2 (1.9-5.5)
Germacrene D	3.1	5.3	6.0	1.0	3.9 (1.0-6.0)
δ-Cadinene	2.0	4.1	5.3	7.3	4.7 (2.0-7.3)
Elemol	6.1	11.2	12.0	2.6	8.0 (2.6-12.0)
T-Cadinol	5.4	5.2	5.5	6.2	5.6 (5.2-6.2)
α-Cadinol	4.2	4.9	4.7	9.1	5.7 (4.2-9.1)
Grouped components					
Monoterpene hydrocarbons	49.8	15.7	12.4	36.2	28.5 (12.4-49.8)
Oxygen containing monoterpenes	6.8	11.0	15.6	10.9	11.1 (6.8-15.6)
Sesquiterpene hydrocarbons	14.3	30.1	32.2	20.2	24.2 (14.3-32.2)
Oxygen containing sesquiterpenes	20.5	34.2	32.9	25.8	28.4 (20.5-34.2)
Other compounds	1.2	1.6	1.9	0.6	1.3 (0.6-1.9)
Total identified	92.6	92.6	95	93.7	93.5 (92.6-95.0)

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P. 22. Essential oil from leaves of *Cryptocarya moschata* Nees (Lauraceae): constitution and population variability

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Cryptocarya moschata, the most common and numerous *Cryptocarya* species from Brazil, is a tree with 20-30 meters high and a very important alimentary source to mastofauna, mainly primates, as *Bracchyteles arachnoides* ("mono-carvoeiro" or "muriqui"), and cracid birds, as *Pipile jacutinga* ("jacutinga"), that eat their leaves and fruits. Previous phytochemical studies revealed the occurrence of several styrylpyrones in barks (Cavalheiro and Yoshida, 2000). Flavonoid glycosides and styrylpyrones are found in leaves, and intraspecific variability on contents of styrylpyrones in these organs was reported (Nehme *et al.* 2002). In previous studies about essential oils from *Cryptocarya species*, the occurrence of linalool was described in leaves of *C. aschersoniana* and *C. moschata*, and β-myrcene, 1,8-cineol and stereoisomeric linalool oxides were found only in the former (Naves *et al.* 1963).

To complement the phytochemical investigation on *C. moschata* for further ecophysiological studies, a detailed description of the components of essential oils from leaves was made, using GC-MS, retention index, chromatographic fractionation and ^{13}C NMR spectroscopy. Seventy compounds were identified, with predominance of isomeric sesquiterpenes (MW 204). The more intense peaks observed on the chromatogram obtained by GC-MS were attributed to germacrene-D, β-caryophyllene, bicyclogermacrene, α-copaene, δ-cadinene, spathulenol, α-humulene, linalool, caryophyllene oxide and β-cubebene. In addition, forty trees of three populations of *C moschata* from Atlantic Rain Forest of São Paulo State – Brazil, were analyzed by GC-FID, in order to assess intraspecific variability on essential oil components. The cluster analysis led to identification of two groups with different essential oil profile, characterized by quantitative differences of (on the) main components. Intra and interpopulational variability were verified. Essential oil group with predominance of β-caryophyllene (19-34%), germacrene-D (14-25%) and bicyclogermacrene (10-25%), named CGB group, is more frequent at South populations, and essential oil group with predominance of germacrene-D (26-41%), β-caryophyllene (9-20%), and bicyclogermacrene (9-18%), named GCB, is more frequent at North population, indicating a clinal variation in quantitative profile of essential oil components from leaves of *C. moschata*. These results are concordant with intraspecific chemical variability found to styrylpyrones from leaves of the same individuals of *C. moschata* (Nehme 2001; Nehme *et al.* 2002).

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P. 23. Contribution to the composition of the essential oils from herb and fruits of Greek coriander

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Seeds of *Coriander sativum* L (fam. Umbelliferae), well known as an important ingredient of many ethnic foods, produce a commercial valuable essential oil utilized widely in beverages, condiment and in the perfumery industry, as well as in pharmaceutics.

The essential oils of Greek coriander herb and seeds were studied, considering the influence of the maturity stages, the comminution of the seeds and the distillation time applied, on their yield and quality.

The main constituents of the oils, analysed by GC/MS, were 2-decenal for the herb, and linalool for the seeds. Significant variability in the essential oil content and composition were noticed in the seeds samples from different ontogenetic stages, resulting in an increase of the linalool content from 44.5 to 82.5%, throughout the whole maturity period.

The treatment of the seeds affected also significantly the obtained essential oils. The intact seeds yielded higher linalool amounts (max 82.5%) than the comminuted ones (max 71.7%). The latter were richer in oxygenated monoterpenes when shorter distillation times were applied.

P. 24. *Foeniculum vulgare* Mill. from different origin: variation of essential oil content and main components

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The aim of our investigation of Korean and Hungarian origin fennel was to examine the variation of essential oil content and its main components by hydrodistillation and capillary GC method with standards during the ontogenesis and plant growing seasons from 1999 to 1999. The essential oil contents of the shoots (2^{nd} year old) and that of the roots (2^{nd} and 3^{rd} year old) exhibited a higher accumulation level in the Hungarian population at the leaf-rosette-stage. The essential oil content varied according to vegetation years and phenological phases and the maximum essential oil values were measured (10.0-12.8% in the Hungarian and 7.1-11.8% in the Korean taxon) in the two-year old plants of seeds. We observed the maximal essential oil content of seeds at a certain phase, when the dry matter content was between 22 and 26%. From this stage, the increase of dry matter content and the decrease of essential oil content have a significant linear correlation (r>0,5) with each other in both taxa. Anethole (60.9-82.2%) proved to be the main component of the over-ground organs, while dillapiol (53.9-92.6%) was the major compound of the roots in each year and in both populations. Intra-specific differences concerning the reproductive organs are reflected first of all in levels of anethole (maximum values by 12-50% higher in the Hungarian) and of α- and β-pinenes (more in Hun. By 0.9-3.2%). Korean population can be characterised as a high anethole - low methyl chavicol chemoform of the anethole chemovariety (fenchone<15%; anethole> 68%; methyl chavicol<3,2%) respectively.

P. 25. Extraction of *Satureja montana* L. essential oil with supercritical carbon dioxide

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Aromatic plants are important raw materials for the perfumery, pharmaceutical and food industries. Essential oils from these plants are traditionally obtained by hydrodistillation and solvent extraction, although these techniques have several disadvantages, such as the heat instability of essential oils and the difficulty to remove the solvent from the extract.

With supercritical fluid extraction (SFE) it is possible to obtain solvent-free extracts and to avoid the degradation of thermally labile components (Palavra *et al*., 1993, 1995, 1999). Therefore, the natural odour and flavour of the initial material are maintained.

Satureja montana L. is an aromatic plant that grows in arid, sunny and rocky habitats. The corresponding essential oil, obtained by hydrodistillation, can reach about 1.8% (w/dw). The principal use of this plant is in the treatment of stomach ailments due to its carminative and desiccative properties.

Flowers and leaves of *Satureja montana* L., grown at Servicio de Investigacion Agroalimentario, Zaragoza, Spain, were used as matrices for supercritical CO_2 extractions, followed by two stage fractional separations. The oil was obtained with a flow apparatus, with an extraction vessel (1L), and two separators (0.27L) in the pressure and temperature ranges of 90 to 100 bar and 40 to 50 ºC, at the selected separations conditions, and analysed by GC and GC-MS. The essential oil was dominated by the monoterpene fraction, carvacrol (56-71%) being its main component. The amount of the sesquiterpene components was always lower than 2%.

The most convenient conditions of extraction and separation, to obtain the oil from this plant, are discussed.

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P. 26. Madeira Laurel essential oils

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Laurus azorica is a *Lauraceae* endemic of the Macaronesia (Press *et al.*, 1994) confined to the archipelagos of Azores, Canaries and Madeira. In Madeira, *Laurus azorica* is the most abundant tree in the thermo-Mediterranean (altitude 14-700m in the South coast, 0-300m in the North coast) and meso-Mediterranean (700-1500m in the South coast and 300-600m in the North coast) bioclimatic steps.

Essential oils may be obtained from different morphological parts of the plant; the better studied are the oils from leaves and berries. A fixed (lipidic) oil is expressed from the ripe berries and used in traditional medicine as anti-infective, anti-rheumatic, blood depurative, haemostatic and apoplexy preventive (Rivera *et al.*, 1995) This oil is much richer in volatiles (11.7%) than any other known vegetable oils (<5%).

Comparative studies on leaves and berries, complemented with results on the chemical composition of essential oils from stems and the fixed oil are presented. Three different locations in Madeira Island were used for plant material collection: Ponta do Pargo, Ribeiro Frio and Chão de Louros.

Volatiles were obtained by hydrodistillation with a Clevenger type apparatus, during 4 hours. Yields ranged from 0.19% in stems to 9.1% in the fixed oil. Over this lipidic product the hydrodistillation was left to proceed for two other (uninterrupted) periods of 4 hours until no more volatiles were obtained; two new portions (1.7 and 0.8% yield, respectively) were thus isolated. These were found to be very poor in monoterpenes but richer on sesquiterpenes and oxygenated compounds. Sample collection at regular intervals over a long time distillation proved to be a useful method for the crude separation of compounds for further NMR analysis.

Studies over the composition of foliar essential oils are in good agreement with literature results, showing a dominance of the monoterpene fraction, with α-pinene (13-15 %) as the major compound, followed by 1,8-cineole (9-10%) and β-pinene (7-8%).

The ratio hydrocarbons/oxygenated compounds presented a significant variation with collection location; correlation of this ratio with botanical diversity is still to be proved.

The phenylpropanoid elemicin was found in amounts varying from 2 to 9%, with the most expressive values on the stems of trees from Chão de Louros (Camacho *et al.*, 2001). This compound was previously detected (1.7%) in *Laurus azorica* leaves from Madeira Island although it was absent from samples collected in Azores archipelagos (Pedro *et al.*, 2001).

Chemical composition of the first fraction obtained by distillation from the expressed oil correlates well with the volatiles obtained from the ripe berries, suggesting that even though the oil production involves the boiling of the berries, the process does not affect the aroma of the product.

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P. 27. Compositon of the essential oil of *Micromeria juliana* (L.) Bentham ex Reichenb. *(Lamiaceae)*

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Micromeria juliana (L.) Bentham ex Reichenb. belongs to the section *Eumicromeria* (Boissier, 1879), tribe *Mentheae,* subfamily *Nepetoideae* (Cantino *et al*., 1992).

This species grows in the Mediterranean region, spreading westwards to S.E. France, and in C. Portugal (Chater and Guinea, 1972). In Yugoslavia, *M. juliana* is distributed in Kosovo and Montenegro (Silic, 1979).

The object of our investigation was the essential oil composition of *M. juliana* collected from three different localities in Western Montenegro: Moraca canyon, Cijevna canyon and Mt Orjen (*c*. 500 m).

The samples were gathered in the flowering period. The essential oils were extracted from dry aboveground parts of the plants by hydrodistillation. The yield of essential oils was lower than 0.1%.

The analyses of the oils were carried out using GC/MS. The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and mass spectra with those obtained from authentic samples and/or the MS library (Adams, 1995).

The main component in all oils was caryophyllene oxide. However, the contribution of certain other major components varied.

The population from Moraca canyon had a high content of caryophyllene oxide (18.1%), carvacrol (18.1%), *o*-cymene (10.8%) and *p*-cymene (5.3%). Traces of isomenthone and pulegone were found.

In the essential oil from the Cijevna canyon population there was a considerable presence of caryophyllene oxide (20.4%), (E)-caryophyllene (7.1%), α-cadinol (6.6%) and borneol (6.3%). Isomenthone, pulegone, thymol and carvacrol were found in traces, while no *o*-cymene was detected in the essential oil of this population.

The Mt. Orjen population contained essential oil rich in caryophyllene oxide (15.9%), isomenthone (10.1 %), pulegone (8.1%), thymol (7.3%), (E)-caryophyllene (5.6%), *allo*-aromadendrene (5.3%) and germacrene D (5.2%). There were traces of borneol and carvacrol, while there was no presence of *o*-cymene.

The species *M. juliana* is similar to other species of *Eumicromeria* section, according to essential oil characteristics.

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P. 28. Chemical Composition of the essential oil of *Teucrium polium* ssp. *capitatum* L. (Labiateae) from Corsica (France)

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The genus *Teucrium* exhibit more than 300 species, which were distributed around the world (1). Among them, *T. polium* (L.) has been used in folk medicine (2). Therefore, several studies reports the identification of components extracted *from T. polium* (2-3). Concerning the chemical composition of the essential oil of *T. polium*, three compositions were reported in the literature (4-5). In Corsica, the essential oil obtained by steam distillation of the aerial parts from *Teucrium polium* ssp. *Capitatum*, grown wild in the stony ground of the island, takes an interest in perfume industries. To our knowledge, the chemical composition of the essential oil of *T. polium* subsp. *capitatum* was never reported in the literature.

In the present work, the essential oil of *Teucrium polium* ssp. *capitatum* L. from Corsica, was investigated using combination of capillary GC, GC/MS and Carbon-13 NMR spectroscopy after fractionation by column chromatography. More than eighty compounds, accounting for 81% of the total amount were identified. The main components were α-pinene (19.9%), p-cymene (7.9%) and β-pinene (5.0%).

α-Agarofurane, β-dihydroagarofurane, 4α-hydroxydihydroagarofurane, 3β,4β-oxydo-agarofurane and 6,7-bisepoxycalamenene-seco, were identified as minor components and to our knowledge, this is a first report on these compounds in the essential oil of *Teucrium polium*.

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P. 29. Effects of *Thymus vulgaris* L. (Thyme) essential oil as an *in vivo* dietary supplement on chicken intestinal microflora

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Thyme oil possesses antimicrobial properties *in vitro*, due to the presence of phenolic terpenes in *Thymus vulgaris* L. (Dorman & Deans, 2000; Helander *et al.*, 1998). Terpenoids may also act as neutraceuticals against certain antibiotic-resistant bacteria (Nascimento *et al.*, 2000), by reducing the bacterial load. This paper describes the effects on the gut microflora when thyme oil was added to chicken diets in the presence or absence of supplementary dietary enzymes.

Using a factorial design, 240 female broiler chickens (Ross 308) were reared on litter from 0-42 days, arranged as 10 birds per pen and 6 pen replicates of 4 dietary treatments. All birds were reared on a basal wheat/soya bean meal ration (ME=12.88MJ kg^{-1}; CP=190.6g kg^{-1}), either with or without a thyme oil supplement at 5g kg^{-1}. Both diets were then fed with and without an enzyme at 0.5g kg^{-1} (xylanase=2776 IU & β-glucanase=117 IU). Caecal contents were sampled at 19 & 42 days, serially diluted and plated within 12 hrs onto MRS, MacConkey No.3, Wilkins Chalgren, Perfringens OPSP, charcoal cefaperazone deoxycholate (CCDA) and Slanetz-Bartley agar media (Oxoid Ltd., UK). After transformation to log_{10}, all counts were analysed by GLM in Genstat Release 5.

Table Effects of *T. vulgaris* supplementation in the diet on chicken intestinal microflora at 19 days

Treatment	Inclusion	Lactic Acid Bacteria	Coliforms	Total Anaerobes	Cl. perfringens
Oil	+ve	10.03	8.88[a]	10.63	2.54
	-ve	9.47	9.94[b]	10.73	2.62
	s.e.d.	0.352	0.451	0.320	1.062
	Sig	NS	0.028	NS	NS
Enzyme	+ve	9.58	9.56[a]	10.99	1.53[a]
	-ve	10.03	8.76[b]	10.95	4.06[b]
	s.e.d.	0.264	0.338	0.240	0.797
	Sig.	NS	P<0.05	NS	P=0.006
N		3	3	3	3

All counts are expressed as log_{10} colony forming units per gram sample. Significance comparisons are separate for each treatment factor and are based within each column

The trial birds were compromised by a coliform infection, unrelated to treatment from study days 1-10, with 7.4% mortality. At 19 days, thyme oil reduced the numbers of coliforms, whereas the enzyme reduced *Cl. perfringens*, indicating separate modes of action. No significant effects were observed at 42 days due to either treatment (not shown), and no treatment interactions were present.

These results indicate the protective potential of dietary thyme oil and enzymes in birds with an immature bacterial flora. Bacterial count reduction will aid recovery, representing reduced potential either for re-infection or the transfer of infection to other birds. The bacterial count variations masked any effects due to treatment at 42 days. Inclusion of more thyme oil in diets may compensate for volatilisation (only 60% was recovered), but may prove unpalatable, thus terpene mixtures may be more effective. In conclusion, terpenes show potential for dietary inclusion, although more detailed studies are required to elucidate the length of the protective period.

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P. 30. Seasonal variation of the leaf essential oil from two populations of *Pittosporum undulatum* Vent. grown in the Portuguese mainland

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The genus *Pittosporum* comprises about 200 species distributed throughout the world. Three species of this genus are described for Portugal: *P. coriaceum* Dryander ex Aiton [Mocano, Pitosporo], *P. undulatum* Vent. [Árvore do incenso] and *P. tobira* (Thunb.) W. T. Aiton.

P. undulatum is an evergreen 4-8m tree with white-creamy flowers in loose umbellate cymes, orange capsule when ripe and wavy edged glabrous leaves. Nowadays considered an alien weed, it was introduced in most countries because of its ornamental value, with its perfumed white-creamy flowers, orange capsules and fast-growing leaf canopy (Palhinha 1946, Goodland and Healey 1996). *P. undulatum* is abundant in several hill forest areas in the Portuguese mainland and it is one of the most frequent species in wet hill forest near Lisbon.

The aerial parts of *P. undulatum* were collected fortnightly during six months at the Parque de Saúde de Lisboa. Two populations were considered: pruned (pP) and non-pruned trees (npP). The essential oils were isolated from deep-frozen aerial parts by hydrodistillation to estimate the oil yields, and by distillation-extraction to determine their percentage composition, and analysed by GC and GC-MS.

The essential oils isolated from the leaves of pruned and non-pruned trees of *P. undulatum* were obtained in yields ranging from 0.05% to 0.15% (v/w) and from 0.03% to 0.20% (v/w), respectively.

Monoterpenes were dominant in all oils (>85%), the monoterpene hydrocarbons constituting the main fraction of both populations (pP >65%, npP >67%). Table 1 gives the percentage ranges for the main components. Sesquiterpenes occurred in a relative amount <8% in pP and <10% in npP, bicyclogermacrene being the dominant component of this fraction, Table 1.

Table 1: Percentage range of the main components from the two populations of *Pittosporum undulatum* studied.

Percentage range	pP	npP
α-Pinene	4 - 8	5 - 11
Sabinene	13 - 36	13 - 25
Myrcene	4 - 6	3 - 5
α-Terpinene	3 - 6	3 - 5
Limonene	12 - 36	14 - 37
γ-Terpinene	4 - 10	4 - 7
Terpinen-4-ol	8 - 24	10 - 16
Bicyclogermacrene	2 - 3	2 - 4

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P. 31. Chemical composition and antimicrobial activity of the essential oil of *Eucalyptus globulus* Labill., Myrtaceae, from Montenegro

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Essential oils, due to their bioactive components, are indeed promising to be used as effective antibacterial, antifungal and antioxidant agents. With the growing interest in the use of essential oils in both food and pharmaceutical industries, a systematic examination of plant extracts has become increasingly important (Baratta *et al*, 1998).

The *Eucalyptus* genus comprises over 500 species of aromatic trees and shrubs. Previous research suggests that some species counteract influenza viruses; others are antimalarial or highly active against bacteria (Ody, 1994). Of all Australian eucalypts, *Eucalyptus globulus* Labill., Myrtaceae (Tasmanian Blue Gum) is the species most widely introduced overseas and has been established especially throughout the Mediterranean region. It contains great amount of essential oil (*Oleum eucalypti*), up to 3.5% (w/w), which is used in medicine, aromatherapy and perfumes (Bremness, 2000).

In this study, fresh leaves of *E. globulus*, collected in March 2002 from the southern part of Montenegro (Boka Kotorska Bay), were extracted by hydrodistillation in a Clevenger-type apparatus for 2.5h. The obtained oil was dried over anhydrous sodium sulphate and stored at 6°C until analysed.

The yield of obtained oil was evaluated gravimetrically and chemical composition was determined by gas chromatography-mass spectrometry (GC/MS).

Diffusion technique on Mueller-Hinton agar was applied to test sensitivity of certain human pathogenic bacteria and fungi to *E. globulus* essential oil. The essential oil (5, 10, 15, 20 and 30μl) was poured on the Mueller-Hinton base prepared as required.

In this paper the following microbial strains, isolated from patients treated at the hospital or under out-patient regime, were examined: *Staphylococus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus* sp., *Streptococus pyogenes, Morganella morganii, Providientia stuarti, Enterobacter cloacae, Acinetobacter baumannii, Citrobacter freundi, Salmonella infantis* and *Candida albicans.*

In addition, reference antibiotic disks such as oflaxacin, ampicillin, erythromycin, tetracycline, amixacin, ceftriaxon, imipenem etc. were used for comparison (provided by the Institute for Serums, Vaccines and Diagnostic Preparations - Torlak, Yugoslavia).

Eucalyptus essential oil yield was 1.8% (w/w) on a fresh weight basis, whereas GC/MS analysis resulted in the identification of a total of 11 constituents, being eucalyptol (85.8%), α-pinene (7.8%) and β-myrcene (1.9%) the main components. Other identified compounds in gained oil were β-pinene, α-phellandrene, γ-terpinene, linalool, pinocarveol, terpinen-4-ol and α-terpineol.

The results of testing antimicrobial activity are shown in diameters of inhibition zones. The gained results revealed that the essential oil of *E. globulus* has very strong antimicrobial activity, especially against *Streptococus pyogenes, Escherichia coli, Candida albicans, Staphylococus aureus, Acinetobacter baumannii* and *Klebsiella pneumonas.*

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P. 32. Comparative headspace-SPME and hydrodistillation of two *Artemisia* sp. from Turkey

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Fresh plants of *Artemisia spicigera* C. Koch and *A. scoparia* Waldst. et Kit. (Compositae) were subjected to Headspace-SPME (HS-SPME) and the same plants were air-dried and hydrodistilled to obtain their essential oils. Headspace volatiles were analyzed by GC/MS. The oils were analysed by GC and GC/MS.

Camphor (37.5% and 43.9%) was found as main constituent in both SPME and oil samples of *A. spicigera*. However, in the headspace-SPME samples of *Artemisia scoparia*, capillene (a diacetylene) (53.1%) was found as the main constituent while β-pinene (20.8%), β-caryophyllene (16.4%), (Z)- β-ocimene (16.4%), myrcene (12.8%) and limonene (11.0%) were main components in the oil.

P. 33. The chemical composition and antimicrobial properties of South African *Plectranthus* (Lamiaceae) species

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The genus *Plectranthus*, a member of the mint family, has a cosmopolitan distribution of 300 species worldwide of which 50 are indigenous to South Africa. Many members of this highly aromatic group of plants are used extensively by the Zulu to treat various respiratory disorders. Guided by both traditional use and chemotaxonomic criteria, 12 indigenous species were selected, the leaves hydrodistilled and the essential oils analysed by gas chromatography (GC) and GC coupled to mass spectrometry. Antimicrobial assays incorporating 4 bacteria, 2 yeasts and 2 fungi were performed on the essential oils.

Disc diffusion assays on *Bacillus cereus* showed *P. grandidentatus* being the most active essential oil exhibiting inhibition (at 50 mg/ml) comparable to that of the Neomycin control. Three species showed promising activity against *Bacillus* species; *P. ciliatus* accumulates bicyclogermacrene (17%) and spathulenol (16%), *P. hadiensis* produces T-cadinol (27%), α-fenchone (18%) and cubenol (15%) as major compounds while *P. porphyranthus* produced cubenol (36%) as the major metabolite. A TLC bioautographic assay indicated that several essential oil compounds contribute to the observed antimicrobial activity.

P. 34. Detoxification of terpinolene by *Botrytis cinerea*

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In the course of our microbial transformation studies of monoterpenes, detoxification of terpinolene by the plant pathogenic fungus *Botrytis cinerea* resulted in the formation of two hydroxylated metabolites: 2,3-dihydro-3β,6β-dihydroxy-terpinolene (39 %) and 2,3-dihydro-1α,3α-dihydroxy-terpinolene (20 %), respectively. Terpinolene showed good levels of antifungal activity while both the metabolites were inactive against the other tested plant pathogenic fungus *Cladosporium herbarum*. The antifungal activity of the substrate and the metabolites were tested at a concentration of 10 microgram per ml using a bioautographic assay.

P. 35. HS-SPME of *Osyris alba* L. flowers

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Osyris alba L. (Santalaceae) was subjected to HS-SPME (Head Space- Solid Phase Micro Extraction) and subsequent GC/MS analysis due to the strong fragrance of volatiles emitted by the fresh cut flowering branches. SPME with polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre resulted in the identification of thirteen hexyl and hexenyl derivatives with a fruity, pear type, leafy green odour.

P. 36. Effect of nitrogen on *Pimpinella anisum* hairy root growth and essential oil production

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Pimpinella anisum hairy root cultures have been maintained, for over six years, in SH liquid medium (Schenk and Hildebrandt, 1972), in darkness, on orbital shakers (80 rpm) at 24ºC (Santos *et al.*, 1998), and subcultured every three weeks. Different nitrogen sources, NO_3^- / NH_4^+ ratios and nitrogen amounts were assayed (Table 1) in order to study their effect on the anise hairy root growth and the essential oil production.

Table 1. NO_3^- / NH_4^+ ratio and nitrogen source of the different media assayed.

Media	NO_3^-/NH_4^+	Nitrogen source
SH	25/3	KNO_3 / $(NH_4)_2HPO_4$
SHa	25/1	KNO_3 / $(NH_4)_2SO_4$
SHb	39/21	KNO_3 / NH_4NO_3
SH^+	25/3	$Ca(NO_3)_2.4H_2O$ / $(NH_4)_2HPO_4$
SH^{++}	0/3	$(NH_4)_2HPO_4$

The hairy root cultures growth was determined by the dissimilation method (Schripsema *et al*., 1990) and by dry-weight and fresh-weight determination. Morphological characterisation was performed by scanning electron microscopy. The essential oils from each of the culture types were isolated by distillation-extraction and analysed by GC and GC-MS. Both for the growth and essential oil composition studies, hairy root samples were collected periodically along the growth cycle.

In comparison to SH-grown hairy root cultures, all other culture conditions assayed led to a slower growth, a higher tendency to browning and to development of *callus* tissue.

The dominant components of the constitutive essential oil from the SH anise hairy root cultures were *trans*-epoxypseudoisoeugenyl 2-methylbutyrate (>69%) and β-bisabolene (>9%). The former component was found to be dominant also in the SHa, SHb and SH^+ grown hairy root cultures (>27%). The dominant component of the oil from the hairy root cultures maintained in SH^{++} was octyl isobutyrate, in a percentage higher than 12%. This compound occurred in a similar percentage in the essential oils isolated from the cultures maintained in SHa (13%), SHb (13%) and SH^+ (12%), but in a much lower relative amount (1%) in the oil from the SH anise hairy root cultures.

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P. 37. Composition of the volatiles from *Gennaria diphylla* (Link) Parl. (Orchidaceae) grown on the Madeira Island

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Gennaria diphylla (Link) Parl. [= *Peristylus cordatus* (Willd.) Lindl.] is the only species of the genus. It is a very unusual terrestrial orchid, which characteristically possesses only two cordate leaves at the base. The plant is 8 to 45cm height with a glabrous inflorescence bearing 8 to 46 flowers. In the Madeiran archipelago, this orchid can be found both on Madeira, in the higher parts of Porto Santo and also on Deserta Grande. On Madeira, *G. diphylla* grows in the Laurisilva, but can be seen also at lower altitudes. This species can also be found in Southern Spain, Sardinia, Corsica and Tunisia, Canary Islands and in the Portuguese mainland where it grows in small populations sheltered by *Corema album* along the coastal woods (Pena and Cabral 1997, Neiland 2000).

The aerial parts of *G. diphylla* were collected during the flowering phase at Montado do Sabugal, Madeira. The essential oils were isolated from the inflorescences, by distillation-extraction for 3h as well as by headspace sorption on charcoal filters for 48h, and analysed by GC and GC-MS.

The essential oils isolated by distillation-extraction possessed very different qualitative and quantitative compositions compared to those isolated by headspace sorption. Of the thirty-two components identified in the essential oil isolated by distillation-extraction, only eleven were detected in the volatiles isolated by headspace sorption. Conversely, of the eighteen components identified in the volatiles isolated by headspace sorption, seven were not detected in the former oil.

The non-terpenoid compounds were the main components (79%) of the oils isolated by distillation-extraction, whereas the monoterpene fraction amounted only to 7%. *n*-Octacosane (19%) and *n*-heptacosane (13%) were the dominant components of this oil.

Oxygen-containing monoterpenes were the major compounds of the volatiles isolated by headspace sorption, amounting to 67% of the total oil. Among those 5-methyl-5-vinyl-tetrahydrofuranyl derivatives were the dominant components (61%), *cis*-arbusculone (28%) and lilac alcohol (26%) being the main volatile components of this fraction.

The considerable differences between volatile compounds isolated by distillation-extraction and headspace sorption are related to the isolation procedure used. On the one hand the large amount of hydrocarbons detected in the distillation-extraction oil is probably due to the decomposition of the leaf cuticle waxes. On the other hand, charcoal traps are known to be ineffective in trapping the full range of floral fragrance compounds, moreover, the trapping time influences the relative amount of the fragrance components. In addition, some compounds, namely lilac aldehydes, decompose on charcoal during collection and storage, as it has been found for other orchid species (Patt *et al.* 1988).

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P. 38. Essential oils from cultivated plants of *Hypericum androsaemum* L.

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Plants from *Hypericum androsaemum* L. were cultivated by March 1998 in an experimental farm from Direcção Regional de Entre Douro e Minho (DRAEDM), at the northern region of Portugal, by planting slips of about 15 cm long obtained from wild plants growing near Ponte de Lima (Facha).

The cultivated plants were pruned by July 1999 (S1), November 1999 (S2) and June 2000 (S3) and the respective aerial parts were subjected to hydrodistillation in a Clevenger-type apparatus over one hour. The essential oils were analysed by GC and GC-MS. The identification of the compounds was performed with the help of mass spectra libraries and confirmed, whenever possible, by comparison with the relative retention times and mass spectra of authentic standards.

Identification was achieved for 90, 98, and 97% of the essential oils from the samples S1, S2, and S3 respectively, in a basis of the total area of the peaks plotted in the respective GC chromatograms and reports. More than seventy compounds were identified, majority distributed by five groups: monoterpene hydrocarbons (MH), oxygen-containing monoterpenes (MO), sesquiterpene hydrocarbons (SH), oxygen-containing sesquiterpenes (SO) and other hydrocarbons (H). The percentages of each one of these compound groups varied from one sample to another. In any case however, SH was the main compound group accounting for 58, 77, and 43% of the essential oils from the samples S1, S2, and S3 respectively. Following this sequence, the relative amounts of each one of the other four main compound groups were: MH (17, 7, and 29%); SO (6, 3, and 4%); H (4, 4, and 13%); MO (0.5, 0.3, and 0.5%). The ten main compounds and respective percentages (%) in each one of the three samples were the following ones: S1 - β-caryophyllene (12.5), γ-elemene (8.5), β-gurjunene (6.1), limonene (5.9), β-pinene (4.2), terpinolene (3.9), γ-muurolene (3.8), *n*-nonane (2.9), 2-hexenal (2.5), *trans*-β-ocimene (1.4); S2 - γ-elemene (17.9), β-gurjunene (15.5), β-caryophyllene (15.1), 2-hexenal (5.3), γ-muurolene (4.4), β-elemene (2.7), β-pinene (2.2), limonene (2.1), 1-octene (1.6), terpinolene (1.4); S3 - limonene (15.4), β-caryophyllene (9.4), γ-elemene (8.0), 2-hexenal (7.9), β-gurjunene (7.6), 1-octene (7.3), β-pinene (6.4), *n*-nonane (3.6) α-pinene (3.2), γ-muurolene (2.1).

Variations in essential oil composition could be induced by different physiological or environmental factors whose variation over the vegetative cycle may influence the compounds turnover. The attack of the cultivated plants by some kind of organisms constitute another type of factors that contingently could influence the essential oil composition. On the *H. androsaemum* cultivated plants, here reported, we identified two contaminant organisms; a rust fungus - *Uromyces* sp. - and an aphis - *Aphis gossypii* Glover. The populations of these contaminants appeared by April of 1998 and 1999, rising over the following four months. It remains speculative however, any interpretation inherent to the respective effects on the essential oil composition, because no type of control was made.

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P. 39. The effect of phytohormones on the essential oils produced by in vitro shoots of sage (*Salvia officinalis* L.)

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In vitro shoot cultures of sage (*Salvia officinalis* L.) were established as previously described (Santos-Gomes and Fernandes-Ferreira, 2002). 2,4-Dichlorophenoxyacetic acid (2,4-D) at 0.1 or 0.05 mg/l, combined with one cytokinin: benzyladenine (BA) or zeatine (ZEA) or kinetin (KIN), at 1.5 mg/l, and 2.0 or 4.0 mg/l, in the case of KIN, were tested in the evaluation of the effect of the phytohormones in the production of essential oils by these type of cultures. From the four types of primary explants tested (nodal shoot segments, inter-nodal shoot segments, leaves, and root segments, all of them excised from aseptic seedlings), only the nodal shoot segments were efficient in promoting direct shoot regeneration through organogenesis. Concentration of 0.05 mg/l 2,4-D has promoted higher number of shoots *per* explant and higher linear growth than 0.1 mg/l 2.4-D concentration, except when combined with KIN at 1.5 mg/l. In this case, differences were not weighty. However the percentage of explants that originated shoots did not change by the variation of 2,4-D concentration from 0.1 to 0.05 mg/l but was influenced by the variation of the KIN concentration. The increase in KIN concentration from 1.5 to 2.0 and 4.0 promoted multiple shoot formation.

After the fifth subculture to the same medium and same supplementation conditions, the shoots grown over 33-35 days were subjected to hydrodistillation in a Clevenger type apparatus over one hour. The essential oils were analysed by GC and GC-MS. The identification of the compounds was performed with the help of mass spectra libraries and confirmed, whenever possible, by comparison with the relative retention times and mass spectra of authentic standards. The quantification was performed according the methodology previously described (Santos-Gomes and Fernandes-Ferreira, 2001).

The highest content of essential oil (~24 mg/g of biomass dry weight) was obtained from shoots grown on Murashige and Skoog (1962) medium supplemented with 0.05mg/l of 2,4-D and 2.0mg/l of KIN. From the three cytokinin tested, KIN was the most effective in the essential oil production, specially when combined with 0.05 mg/l 2,4-D. The phytohormone supplementation influenced the relative amounts of the main group of compounds. However, at the individual level, the percentages of the major compounds were not greatly influenced. Camphor was the major compound (20-24%) followed by α-thujone (13-16%), β-pinene (9-15%), β-thujone (~7%), camphene (6-7%), manool (4-8%), α-humulene (5-7%), 1,8-cineole (4-6%), α-pinene (4-5%), and limonene (~2%). These composition parameters are majority within the ranges of the profile defined by standard ISO 9909 for essential oils of sage (Bruneton, 1999) with exception for the levels of α–thujone (somewhat low) and perhaps, β-pinene and manool, which are not referred by this author as taking part of the same standard profile.

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P. 40. Glandular trichomes and volatile oils from two *Plectranthus* species

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The genus *Plectranthus* L'Herit belongs to the Lamiaceae, known for their aromatic and medicinal properties. It is a large genus of about 350 species distributed through Africa, Asia and Australia, with over 40 species of indigenous to Southern Africa (Codd, 1985). *P. ecklonii* and *P. verticilatus*, ornamental plants in Europe, occur in South Africa along forest margins of woodlands in semi-shady places where frost is not too severe. This study compares the morphology and distribution of the trichomes of these two species, as well as the histochemistry of the secretory products and the composition of the essential oils produced.

Glandular and non-glandular trichomes are spread over the vegetative and reproductive organs. The glandular trichomes observed are of the same types as described previously for the Lamiaceae – peltate and capitate. However, on *P. ecklonii* and *P. verticilatus*, they have *in vivo* an uncommon and characteristic orange-to-reddish colour and, unlike the other peltate trichomes present in the Lamiaceae, never develop a large subcuticular space. They exhibit a lobated shape, somewhat flattened-to-depressed in the centre, even during the secretory phase. Capitate trichomes have an ovoid unicellular head and a short cell or an elongated two-to-three-celled stalk slightly enlarged at the base. In the short-stalked trichomes the secretion, mainly polysaccharidic, is probably exuded by micropores, whereas in the long-stalked a heterogeneous secretion stores temporarily in a subcuticular space, being released by cuticular rupture.

Glandular trichomes occur on both leaf sides. Peltate trichomes are more numerous on the abaxial surface, where they are particularly concentrated at basal intervein areas. Short capitate trichomes predominate along the veins of the adaxial surface. The reproductive organs, namely the calyx and corolla, exhibit scarce long-stalked capitate trichomes in addition to those already reported. Peltate trichomes are also abundant on the stamens, between the two anther lobes and on the basal portion of the style, between the four lobes of the ovary.

The aerial parts of *P. ecklonii* and *P. verticilatus* were collected, during the flowering and the vegetative phases at the Botanical Garden of Lisbon. The essential oils were isolated separately from flowers and from leaves by hydrodistillation, for yield determination, and by distillation--extraction for analysis by GC and GC/MS. All the oils were obtained in a yield <0.05% (v/w). In *P. ecklonii* monoterpenes represented the main fraction of the oil from the flowers, amounting to 74%, α-pinene (35%), sabinene (16%) and β-pinene (10%) being the main compounds. The oils from the leaves of this species, both from the flowering and the vegetative phases, were dominated by octen-3-ol, representing 49% and 62%, respectively. Monoterpenes represented only 7% and 2% of the total oils, respectively. In *P. verticilatus* all the oils were dominated by octen-3-ol that attained 41%, 84% and 85% in the oil from the flowers and in those from the leaves collected during the flowering and the vegetative phases, respectively. The monoterpene fraction amounted to 35% in the flower oil, limonene (15%) and sabinene (10%) being the most representative compounds, but accounted only 10% and 3% of the oils from the leaves collected during the flowering and the vegetative phases, respectively.

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P. 41. *Plectranthus laxiflorus*: morphology and histochemistry of glandular trichomes and essential oil composition

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Plectranthus laxiflorus, an aromatic perennial herb that emanates a characteristic citronella-like scent, occurs in Southern Africa along forest margins and on shady stream banks (Codd, 1985). In local medicine it is reputed to be antiseptic and analgesic. Infusions from leaves are commonly used for coughs and colds. Powdered leaves are also employed by Zulus as an enema for feverishness, abdominal upsets and influenza (Watt and Breyer-Brandwigk, 1962). Pursuing our studies on the genus *Plectranthus*, this work reports on the morphology, distribution and histochemistry of the glandular trichomes of *P. laxiflorus* and the composition of its essential oil.

Four distinct types of glandular trichomes were identified on the vegetative and reproductive organs. Unlike the majority of the peltate trichomes reported for *Plectranthus* species, which *in vivo* have a characteristic orange-to-reddish colour and never develop a large subcuticular space, the peltate trichomes of *P. laxiflorus* are colourless and become nearly spherical during the secretory phase. Capitate trichomes are divided in three subtypes according to the dimension of the stalk and the secretion process. In addition to the short and long-stalked trichomes, similar to the others previously described in the Lamiaceae, an unusual type of capitate trichomes (ellipsoidal trichomes) are reported for the first time in this family. They consist of an elongated four-to-twelve-celled stalk, which bears an ellipsoidal head cell that stores the secretion in the vacuoles.

Peltate trichomes are abundant at the intervein areas of the abaxial leaf surface, whereas ellipsoidal ones predominate on the leaf base and along the veins and margins. Short capitate trichomes are numerous on the adaxial surface above the veins. The flower shows all the trichome types here reported. Ellipsoidal trichomes are densely arranged on the abaxial surface of the upper corolla lip and on the calyx veins and teeth. However, glandular trichomes are absent on the stamens and carpels. The histochemical characterization of the secretion reveals the presence of terpenoids, flavonoids and polysaccharides as the main constituents.

The aerial parts of *P. laxiflorus* were collected, during the flowering and the vegetative phases at the Botanical Garden of Lisbon. The essential oils were isolated separately from flowers and from leaves by hydrodistillation and by distillation-extraction, and analysed by GC and GC/MS. All the oils, isolated from the flowers (F), leaves from the flowering phase (LF) and leaves from the vegetative phase (LV), were obtained in a yield <0.05% (v/w). Monoterpenes represented the main fraction, amounting to 55% (F), 70% (LF) and 51% (LV). The identified sesquiterpenes attained 27% (F), 26% (LF) and 39% (LV). The main components were α-phellandrene (10%), *trans*-β-ocimene (7%), citronellal (7%) and citronellol (6%) in the oil from the flowers, α-phellandrene (24%), *trans*-β-ocimene (15%), germacrene-D (10%) and β-phellandrene (10%) in that from the leaves of the flowering phase, and α-phellandrene (21%), germacrene-D (19%), *trans*-β-ocimene (9%) and β-phellandrene (9%) in that from the leaves of the vegetative phase. These results are in close agreement with those previously reported for the oils isolated from flowers and leaves collected during the flowering phase of the plant (Figueiredo *et al*., 2000).

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P. 42. *Pimpinella anisum* hairy root cultures: growth and essential oil production in a two-phase system

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In an attempt to avoid the toxicity of some essential oil components, and of making it easier to collect the volatiles secreted by *in vitro* cultures, the growth and essential oil composition of *Pimpinella anisum* hairy root cultures has been determined in a two-phase system. This system is based on the addition to the culture medium of an adsorbent that temporarily accumulates the products secreted by the cells. In this experiment Miglyol 812N (a non-toxic triglyceride immiscible with water) was added to the culture medium (5% v/v) before autoclaving.

The hairy root cultures growth in bubble-column bioreactors containing 1 litre of SH (Schenk and Hildebrandt 1972) medium, both with and without Miglyol (control cultures), was determined by dry-weight and fresh-weight determination, over a period of five weeks. The essential oils from each of the culture types were isolated by distillation-extraction and analysed by GC and GC-MS. Both for the growth and essential oil composition studies, the hairy roots were weekly collected along the growth cycle, and the essential oils isolated separately from the hairy roots and culture medium.

The control cultures and those grown with Miglyol showed comparable growth patterns, attaining a specific growth rate of 0.24/day and 0.22/day, respectively.

The dominant component of the oils isolated from the control cultures was the phenylpropanoid *trans*-epoxypseudoisoeugenyl 2-methylbutyrate (*trans*-EPIE2MB), attaining a maximum of 1.12µl/g d.w. β-Bisabolene (0.47µl/g d.w.), zingiberene (0.23µl/g d.w.), dodecanal (0.13µl/g d.w.) and *trans*-pseudoisoeugenyl 2-methylbutyrate (*trans*-PIE2MB; 0.11µl/g d.w.), were the other four main components of the oils. The same components were found to be the dominant ones in the Miglyol-grown cultures, but sometimes in lower concentrations; *trans*-EPIE2MB attained a maximum of 1.07µl/g d.w., β-bisabolene 0.19µl/g d.w., zingiberene 0.10µl/g d.w., dodecanal 0.05µl/g d.w. and *trans*-PIE2MB 0.09µl/g d.w.

Some of the volatiles isolated from the Miglyol culture medium such as dodecanal (0.019µl/L), zingiberene (0.032µl/L) and β-bisabolene (0.49µl/L) attained, particularly at the end of the growth cycle, higher concentrations than those obtained with the control culture medium (0.005µl/L, 0.012µl/L and 0.022µl/L, respectively). Interestingly, while compounds such as *trans*-anethole, that is usually found in trace amounts in the oils from the control hairy roots and not detected in the control medium, attained a maximum of 0.031µl/L in Miglyol culture medium, *trans*-EPIE2MB was only detected in amounts below 0.008µl/L throughout the culture period.

In conclusion, although *P. anisum* Miglyol hairy root cultures revealed comparable growth to control cultures and were even less prone to oxidation on harvest, there was no *de novo* synthesis and a smaller amount of volatiles was recovered from these cultures. The larger amounts of some volatiles recovered from medium of the Miglyol hairy root cultures was probably due to their higher adsorption to Miglyol than to the hairy roots, in this way not reaching toxic levels to the cells.

Acknowledgements: The authors are grateful to CONDEA Chemie GmbH for a generous gift of Miglyol.

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P. 43. Study and comparison of two mandarin essential oils from southern Brazil

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Only a detailed knowledge on the composition of the oils obtained by different cultivars of mandarin can provide the choice of the cultivars which will produce the best oils (Calvarano *et al*., 1989; Dellacassa *et al*., 1992; Cotroneo *et al*., 1994; Dugo *et al*., 1999; Verzera *et al*., 2000). To exemplify this topic, this study reports the composition of the essential oils of two varieties of mandarins (*Citrus reticulata*, Blanco), commonly called "Caí" and "Montenegrina," growing in Southern Brazil. Mandarin fruits were collected in a small scale experimental area located at Aripê Citrus, Montenegro, from 4-5 years old trees and processed during the 2002 Season (February-May). The volatile fractions were obtained by hydrodistillation of fresh mandarin peels using a Clevenger-type apparatus. The oil composition was analyzed by HRGC and HRGC-MS. Repeatability of the measuring system showed variation coefficients under 5% for all the components reported. The essential oil components were identified by comparison of their linear retention indices on methyl silicone. Comparison of fragmentation patterns in the mass spectra with those stored on databases (Jennings and Shibamoto, 1980; Adams, 1995) was also performed.

Sixty-seven components were identified in the oils from both mandarin varieties, which were similar in composition. Nevertheless, some characteristics such as the content of the main components with commercial interest, particularly dimethyl anthranilate and α-sinensal, make the oils from both varieties different from each other.

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P. 44. Characterization of fragrance notes used in perfumery by GC-MS

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Gas chromatography (GC) associated with mass spectrometry (MS) is currently the best analysis method of complex mixtures, namely essential oils and perfumes.

A perfume has a well-defined structure that is usually designed according to the fragrance pyramid (Figure 1). The perfume composition is therefore based on three kinds of odour notes:

- **Top notes**, which are responsible for the first impression of a perfume when it is smelled for the first time, such as citrus notes;
- **Middle notes** that become evident some time after the application, mostly floral and spicy notes, constituting the heart of the perfume and defining its unique character;
- **Base notes** make up the long-lasting scent that in the end smoothly fade away, working as well as fixative materials, which include vanilla, musk, amber and sandalwood

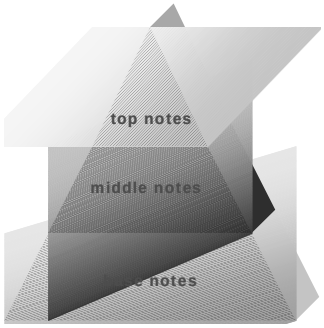

Figure 1 *– The Fragrance Pyramid*

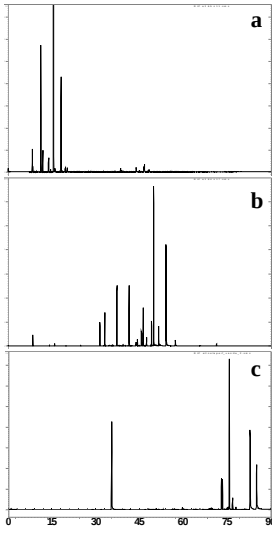

Figure 2 *– GC-MS chromatograms of lemon (a), geranium (b) and vanilla (c).*

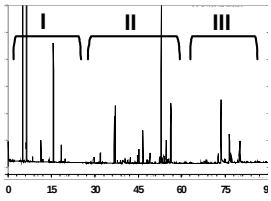

Figure 3 *– GC-MS chromatogram of a perfume with triangular structure.*

The retention time of the fragrance chemicals using a GC capillary polar column is related to the evaporating profile of a perfume when applied on the skin or on the smelling strips that are used for olfactory tests.

In this work, the fragrance notes of lemon, rose geranium and vanilla are characterized by GC-MS using a polar column (CP-Wax 52 CB), representing a top, middle and a base note, respectively (Figure 2). In general, it can be observed that top notes are the first to evaporate and therefore present the lowest retention times. On the other hand, the base notes have the highest retention times since they are the ones that last longer until they evaporate completely. When considering a perfume composition, it can be also identified the three areas, corresponding to the three kinds of odour notes, as can be seen in Figure 3 : I – top; II – middle and III- base note.

The characterization by GC-MS analysis of fragrance notes can provide a significant insight into the perfume composition, enabling to break down its structure into top, middle and base notes, as well as to evaluate the balance of the fragrant materials in the final composition. This way it is possible to establish patterns in perfume composing and GC-MS is a fundamental tool to help the perfumer in this work.

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P. 45. Composition and antibacterial activity of the essential oils from *Zanthoxylum rhoifolium*

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Zanthoxylum rhoifolium (Rutaceae), locally called “mamica-de-porca", “mamica-de-cadela", "juvevê" is a plant that grows in South America (Brazil, Uruguay, Paraguay and Argentina). It has been used in Brazilian folk medicine against a variety of diseases. As a continuation of our chemical studies on Rutaceae plants (Morel *et al.*, 1997), we now report on the volatile constituents of leaves, fruits and flowers of *Z. rhoifolium*. The oils were analysed by GC and GC/MS. The identification of the chemical constituents was based on comparison of their relative retention times and mass spectra with those obtained from authentic samples and/or the Wiley and NBS/NIST libraries and those published by Adams (1995).

Fourteen constituents were identified representing *ca* of 96% of the total oils. β-Myrcene (32%) and limonene (38%) were the most abundant components in the oil of the fruits, while β-myrcene (65%) was the main component in that from the flowers. Germacrene-D (34%) and bicyclogermacrene (23%) were the most abundant components in the oil from the leaves. As revealed by Bioautography (Homans 1970), the antibacterial studies showed that the oils of leaves and fruits were active against *S. aureus* ($Gram^+$) and *K. pneumonial and S. setubal* ($Gram^-$) bacteria, while the oil of the flowers was inactive (see Table 1). The microorganisms used in the antibacterial assays have been maintained at the Departamento of Chemistry of the Universidade Federal of Santa Maria, RS, Brasil. For the antimicrobial assays, 50.0, 25.0, 12.5, 6.25, 3.12, 1.06 μg of the oils were applied to pre-coated TLC plates. The inoculum was prepared by culturing each organism in tryptone soya agar (TSA, Oxoid) at 37ºC to a turbidity equivalent to McFarland 0.5 standard ($1.5x10^8$ CFU/mL). One microliter of each diluted inoculum (10^4 - 10^6 CFU) was applied onto Mueller Agar (MHA-DIFCO). Amoxicillin was used in order to control the sensitivity of the test organisms.

Table 1 Antibacterial activity: minimum amount required for inhibition on bacteria growth on TLC plates (μg)

Material	***S. aureus***	***S. efidermidis***	***K. pneumonial***	***S. setubal***	***E. coli***	***M. luteus***
Leaves	3.12	NA	1.06	12.50	NA	NA
Fruits	12.50	NA	50.00	50.00	NA	NA
Flowers	NA	NA	NA	NA	NA	NA

NA: No active.

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P. 46. Changing of terpene composition in single peltate trichomes of *Salvia officinalis* leaves by direct liquid, non-equilibrium SPME application

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A new non-equilibrium SPME method was developed for rapid extraction of single oil glands from sage and compared with solvent extracts and the essential oil. Differences between ageing of single peltate trichomes on three insertions of *Salvia officinalis*, an immature apical young leaf, an expanding, and a fully developed leaf were studied. Chemical variation between the leaves showed high sesquiterpene and bornyl acetate contents in the young leaf and high camphene and camphor contents in the old leaf, according to Croteau *et al.* (1981). The intermediary, still expanding leaf was used to carry out an age assignment of single oil glands. The basal region of the intermediary leaf contains oil gland compounds in high conformity with the young leaf trichomes, the remaining oil glands showed inconsistent accumulation patterns.

The oil glands of sage appear as opaque or as transparent heads. Chemical differences in their essential oil composition were found in the content of sesquiterpenes only, whereas Tirillini (1999) showed strong qualitative differences between them. Opaque glands showed a significant higher sesquiterpene rate than the transparent glands.

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P. 47. Essential oil of *Nepeta haussknechtii* Bornm. from Iran

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The genus *Nepeta* comprises about 250 species distributed in the world, of which 67 occur in Iran.

N. haussknechtii plants were collected, during the flowering period, at Chalous, North of Iran, in June 2001. Air dried aerial parts of the plants were grounded and submitted to hydrodistillation, for 4 hours, using a Clevenger-type apparatus. The oil was pale greenish and had a pleasant odour, with a yield of 0.15% (w/w). The oil was analysed by GC/MS.

Twenty-two components, representing 90% of the total oil, were identified of which 4aβ, 7α, 7aβ-nepetalactone (35.3%), diisooctyl 1,2-benzene dicarboxylate (16.3%) and 1,8-cineol (15.0%) were the main constituents.

P. 48. Composition of the essential oil of *Satureja isophylla* Rech. f. from Iran

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The genus *Satureja* comprises 12 species occurring in Iran. The aerial parts of *S. isophylla* were collected, during the flowering period, at Niknam village, Chalous, North of Iran, in June 2001.

The dried aerial parts of the plants were hydrodistilled, for 4 hours, using a Clevenger-type apparatus to yield 0.1% (w/w) of oil. The oil was yellowish and had a pleasant odour. The chemical composition of the essential oil of *S. isophylla* was analysed by GC/MS. Twenty-six components, representing 95% of the total oil, were identified, with α-eudesmol (24.2%), β-caryophyllene (12.1%) and camphor (9.5%) as main constituents.

P. 49. Utilisation of essential oils and fatty acid esters with the formulation of the "solvent/active" for application in phytosanitary industry

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Solvent extraction, co-hydrodistillation and hydrodistillation were compared as methods to extracts essential oils from *Origanum dictamnus, Origanum vulgare, Carum carvi, Acore calamus.*

Associating "hydrodistillation and solvent extraction" as traditional techniques of essential oils extraction, co-hydrodistillation process was obtained. Traditionally water is used as solvent in hydrodistillation process, nevertheless in the latter ethyl ester of fatty acid was found to be a good extraction solvent.

Gas chromatography-mass spectrometry analysis (GC-MS) showed that the composition of the isolated essential oils varies depending on the period of harvest the plants and conditions of co-hydrodistillation (solvent/raw matter report).

The products obtained from solvent extraction and co-hydrodistillation with solvents of vegetal origin named "solvent/active " will be tested for application on phytosanitary industry.

P. 50. GC/MS analysis of the oil from the berries of *Juniperus oxycedrus* L. subsp. *oxycedrus* grown in Spain

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The composition of the essential oil obtained from the berries of *Juniperus oxycedrus* L. subsp. *oxycedrus* was analised by GC/MS. Sixty-three compounds have been identified from which α-pinene (68.0%) and myrcene (12.1%) were the main constituents. We have also detected germacrene D (4.4%), abietadiene (1.0%), limonene (0.9%) and manoyl oxide (0.9%)

From 63 identified components in the oil of the berries of this Juniper, 32 were monoterpenes which represented 88% of the oil, 22 were sesquiterpenes summing 8% and 9 were diterpenes amounting 3.2% of the oil.

Other studies on the essential oil of *J. oxycedrus* have been done by different authors. Motl *et al.* (1960) cited ylangene and viridiflorol and De Pascual Teresa *et al.* (1977,1978) detected for the firs time β- and γ-bulgarenes, but we have not found these compounds. Guerrera Hernández *et al.* (1987) cited monoterpenes camphene, sabinene, α-terpineol, 1,8-cineole, γ-terpinene, terpinolene, terpinen-4-ol, camphor, linalool, and the sesquiterpene α-copaene. We have detected these monoterpenes and also several sesquiterpenes like α-cubebene (0.4%), β-caryophyllene (0.4%), δ-cadinene (0.5%) and α-cadinol (0.3%) and diterpenes like manoyl oxide (0.9%), abietadiene (1.0%) and pimarinal (0.4%). It is worth mentioning the presence of germacrene D (4.4%) as the major sesquiterpene found in our oil. This compound had not been mentioned before in the literature.

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P. 51. The bioactive essential oil of *Heracleum sphondylium* subsp. *ternatum* (Velen.) Brummit

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The essential oil of *Heracleum sphondylium* subsp. *ternatum* (Velen.) Brummit (Umbelliferae) was isolated from crushed seeds by means of hydrodistillation and analyzed by GC and GC/MS. Major components were identified as 1-octanol (50.3%), octyl butyrate (24.6%) and octyl acetate (7.3%). Furthermore, antimicrobial activity of the oil was evaluated using micro-dilution broth and agar diffusion methods. The bioactive constituent of the essential oil was determined as 1-octanol by using the bioautography assay.

P. 52. The ultrastructural features of plastids relative to synthesis of naphthoquinones in leaf glands cells in *Droseraceae* plants

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Different naphtoquinones are taxonspecific for genus of *Droseraceae* family. Plumbagin was found in *Drosera* (Durand, 1971), *Drosophyllum* (Durand, 1974), *Dionaea* and *Aldrovanda* (Culham, 1994) but 7-methyljuglone is peculiar to *Drosophyllum* and *Drosera* (Culham, 1994).

The most colored glands of unstimulated leaves were fixed with 3% glutaraldehyde or 2.5% glutaraldehyde with 2% formaldehyde in 0.1 M phosphate buffer pH 7.2 at room temperature and postfixed with 2% osmium tetraoxyde. After dehydration in alcohols material was prepared for electron microscopy by standard procedure.

Plastids in secretory cells of above-listed genera are leucoplasts. Their volume densities are similar within the error of calculations and come to 2.2%. In secretory cells of slime glands of *Drosophyllum lusitanicum* there are 9.0 plastids per cell section; one plastid section area is as small as 0.45 μm^2. Stroma is condensed; it contains light thylacoids with vacuole-like dilatations. Peripheral reticulum consists of one or two elements parallel to plastid membrane. Tubular complex is frequent. Plastoglobuli are small and rare. Sometimes short elements of reticular envelope occur near plastids.

In secretory cells of tentacles of *Drosera rotundifolia* there are 2.2 plastids per cell section; one plastid section area is 0.53 μm^2. Stroma is condensed, contain light thylakoids with local dilatations. In some plastids tubular complex forms dense accumulations. Periferal reticulum consists of single elements. Plastoglobuli are large and numerous. Plastids are enclosed in reticulum.

In secretory cells of digestive glands of *Dionaea muscipula* there are 3.7 plastids per cell section; one plastid section area is 0.15 μm^2. Stroma is light; it contains tubular complex, single thylakoids, large plastoglobuli of different electron density, and small starch grains. Reticular envelopes near plastids were not found.

In secretory cells of digestive glands of *Aldrovanda vesiculosa* there are 1.2 plastids per cell section; one plastid section area is 0.87 μm^2. Stroma is light. Thylakoids have small dilatations; they assembled in loose network. Tubular complex was not found. Plastoglobuli are small and rare. There is no reticular envelope around plastids but sometimes lipid bodies were found nearby plastid membrane.

On the strength of these data some considerations were made. Reticular envelopes around plastids in *D. lusitanicum* and *D. rotundifolia* cells indicate synthesis of secondary methabolites. Presence of reticular envelope distinguish both species containing juglone from that ones without it. Therefore it may be leucoplast reticular envelope which is related to juglone synthesis. Condensed stroma of *D. lusitanicum* and *D. rotundifolia* plastids may be due to high concentration of carbohydrates in cytoplasma. *D. lusitanicum* has maximal plastid number per cell section at the minimal area of plastid section among tested species therefore it has maximal total area of plastid surface. It may provide for greater intensity of synthesis and transmembrane transport of methabolites. In fact *D. lusitanicum* synthesizes plumbagin in greater amount then some other representatives of *Droseraceae* (Durand, 1974).

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P. 53. Diurnal variation in the concentrations of volatiles in leaves and florets of *Thymus vulgaris* and *Salvia officinalis*

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The major volatiles of *Thymus vulgaris* and *Salvia officinalis* leaves and florets were identified and quantified in order to detect diurnal oscillations in the concentration of volatiles in the tissues. This trial was conducted in an attempt to determine the optimal time of the day for harvest of the essential oil under Scandinavian conditions. The samples were collected at the initiation of flowering at 4 h intervals throughout the 24 h period. Leaves, open flowers and floral buds were extracted separately prior to GC-MS analysis.

In order to determine the optimal developmental stage for harvest of the essential oil, the entire plants were harvested from 1 m^2 plots at 14 d intervals from pre-flowering till maturation of the seeds. The theoretical yield of essential oil pr. Ha was evaluated after steam distillation.

Quality aspects of the oils together with the yield levels are discussed in order to evaluate the possibility for essential oil production in Scandinavia.

P. 54. Chemical composition of the essential oils of *Anthemis altissima* L.

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Anthemis, is one of the most important genera of the Compositae family. The genus *Anthemis* is represented by 37 species grown wildly in Iran (Rechinger, 1982). *Anthemis nobilis* has been known from Roman times. It has been used as antispasmodic and sedative in folk treatment of digestive and rheumatic disorders (DerMarderosian, 2001). The essential oil of *A. nobilis* possess anti-inflammatory and sedative properties (Rossi *et al.*, 1988). *A. cotula* showed antimicrobial activity against both gram-negative and gram-positive microorganisms (Quarenghi *et al.*, 2000).

In the present work we report constituents of the essential oil of the flowers, leaves and stems of *Anthemis altissima* L. growing wild in Iran. The essential oils were isolated separately from flowers, leaves and stems of *A. altissima* and analyzed by GC-MS. A total of 123 components were identified in the oils of flowers, leaves and stems. The main components of the oil of both the flowers and leaves were β-caryophyllene (18.9%, 10.7%) and caryophyllene-oxide (9.0%, 17.0%), respectively. Nonterpenic compounds were the main components of the oil of stem. Palmitic acid (30.4%) and linoleic acid (27.9%) were the main compounds of the stem oil.

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P. 55. Seasonal variation in yield and composition of essential oil from *Artemisia vulgaris* L. growing wild in Poland

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Artemisia vulgaris L., the common mugwort, occurs wild in Europe and Asia. The herb has been used as a digestive in folk medicine and a flavouring agent in food and beverages. It is regarded as a thujone-containing plant although there are some chemotypes lacking this ketone.

The aim of the presented work is to investigate the yield and chemical composition of the mugwort essential oil during vegetation. Plants were collected in 3-weeks intervals from June to September 2001 in three places in Poland. The essential oils were obtained by hydrodistillation from the air-dried material and separated to fractions by vacuum distillation and flash chromatography. The chemical constituents were identified by GC-RI, GC-MS and ^{1}H-NMR.

The yield of the oil was low at the beginning of vegetation period (0.03%), increased up to 0.19% at the stage of buds (mid-July) than decreased gradually to 0.10% in full flowering and to 0.04% after flowering. Simultaneously with change of the yield great seasonal variation was observed in the chemical composition of the oil. Up to stage of bud the sesquiterpene hydrocarbons were the main constituents of all essential oils, germacrene-D (20-30%), β-caryophyllene (13-20%) and α-humulene (6%), being the major components. On the bud emergence, the composition of the oils changed suddenly and monoterpenes became the major constituents. 1,8-Cineole (10-15%), camphor, borneol and terpinen-4-ol (each 3-5%) were the main oxygenated monoterpenes and α-pinene and sabinene (each more than 5%) were the main monoterpene hydrocarbons in both stage of buds and full flowering. Other important components of mugwort essential oil were camphene, β-myrcene, artemisia ketone, α-copaene, β-elemene, bicyclogermacrene, spathulenol, β-caryophyllene oxide, T-muurolol, α-cadinol. It is noteworthy that even traces of thujones, which are considered as neurotoxic and limited by food regulations, were not found in mugwort essential oil.

P. 56. Phytopesticidal and repellency effect of four ethnobotanicals from N. E. India against mosquitoes and their GC/MS analysis

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The use of botanicals as an alternative to the chemical compounds is gaining tremendous momentum because of its multifarious advantages. The essential oils of four species (*Acorus calamus, Cymbopogon flexuosus, C. winterianus* and *Ocimum americanum*) used traditionally by the various tribes of N.E. India have been subjected to bioassay against two mosquitoes species (*Culex quinquefasciatus* and *Aedes aegypti*) by the standard bioassay procedure (WHO/VBC/81.807). The essential oils were extracted using a Clevenger apparatus. Of the four species that were subjected to bioassay, *A. calamus* showed the highest potency in terms of pesticidal activity, with 80% mortality towards *A. aegypti* and 60% mortality in the case of *C. quinquefasciatus* at 25 ppm after 24 hrs. *C. flexuosus* also exhibited high potency, with 60% and 55% mortality against *A. aegypti* and *C. quinquefasciatus* respectively, within 6 hrs at 100. ppm. *C. winterianus* showed moderate efficacy with 70% and 20% mortality against *C. quinquefasciatus* and *A. aegypti* respectively, within 24 hrs at 50 ppm. The oil of *O. americanum*, which was reported to have pesticidal activity against some insects (Tawatsin *et al.* 2001), did not show any toxic effect to the target organisms. However, it showed a growth regulatory activity, with complete inhibition of pupal development even at 10% concentration. Similar effects are also seen for the other species, except for *A. calamus*, where 10% of *C. quinquefasciatus* and 15 % of *A. aegypti* formed pupae showed no subsequent adult formation.

The repellency effect of 55% and 60% was observed for *O. americanum* and *C. flexuosus*, after up to 4hrs, whereas *A. calamus* and *C. winterianus* exhibited 100% repellent activity after 1hr only. *C. winterianus* however showed a 70% repellency effect for *A. aegypti* and 80% for *C. quinquefasciatus*, after 3hrs.

The GC/MS analysis of *C. winterianus* oil showed 59 constituents, with citronellal constituting 26.5%, citronellol 11.4%, geraniol 20.3%, geranyl acetate 5.2%, nerol 0.5% and eugenol 0.5%. In the oil of *A. calamus* 40 constituents were analysed, with β–asarone as the major component, amounting to 82.6%. The analysis of *O. americanum* revealed 53 compounds of which thymol, constituting 52.9% of the total oil, was the main component. The essential oil of *C. flexuosus* showed 10 major compounds, with geranyl acetate (76.0%) being the main constituent.

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P. 57. Essential oil of *Juniperus horizontalis* Moench. cultivated in Spain: seasonal variation of leaf and berry oil

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The volatile oils from leaves and green unripe-berries of *Juniperus horizontalis* Moench., an ornamental prostrant shrub (Peña Arribas and Peña Arribas, 1998), cultivated under gardening conditions in a central park in Madrid (Spain) were analysed by means of capillary GC and GC-MS in combination with Kovats indices. A seasonal investigation of both leaves and berries oils was also performed. Significant differences in yields and in chemical composition from a quantitative and qualitative point of view were detected when comparing all the oil samples analysed. Among the almost 60 components investigated, moterpenes were dominant which is in accordance with data reported in a previous publication (Fretz, 1976). The sesquiterpenic fraction, relatively small, is characterized by high percentages of both cadinane-type sesquiterpenes and elemol/eudesmol-type; these results stand in sharp contrast with those previously published by Von Rudloff (1975). In addition, a small amount of diterpenes has also been detected.

The autumn-collected leaf oil was characterized by a high amount of monoterpenes namely: β-myrcene (16.9%), endobornyl acetate (19.4%) and sabinyl acetate (14.3%) followed by limonene (9.4%) and sabinene (9.3%) while, in decreasing order, α-thujene (3.7%), α-pinene (3.2%), β-thujone (2.4%), terpinen-4-ol (2.3%), camphene (2.0%), γ-terpinene (1.3%) and α-terpinolene (1.3%) were found to be in moderate amounts. Elemol (2.3%), δ-cadinene (1.9%) and α-cadinol (1.5%) were the main sesquiterpenes found.

On the other hand, the leaf oil chemical composition of the same plant collected in springtime of the following year has shown a significant increase of sabinene (19.2%) limonene (25.8 %), γ-terpinene (4.2%), terpinen-4-ol (6.6%) and cadinol (4.0%) whereas β-thujone and endobornyl acetate have decreased to 2.4% and 4.4%, respectively.

Furthermore, berries oil was found to contain a very big amount of limonene (28.6%), terpinen-4-ol (11.6%) and myrcene (18.9%) when compared to autumn-leaf oil but has shown a slight variation with the springtime-leaf volatiles except with regard to terpinen-4-ol.

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P. 58. Effect of essential oils for prevention of steel corrosion

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Corrosion of steel in concrete is one of the damage-causes for the durability of the concrete structures. At present, several techniques are used for the prevention of the steel corrosion. For example, as for the prevention methods of the corrosion, "painting the surface of steel with the resin", "using the steel of the corrosion-resistant material" or "using the material of FRP (fiber reinforced plastic)" and so on are done. However, these are not excellent methods for prevention of the steel corrosion at present. Therefore, it is necessary to propose a new opinion of prevention of the corrosion of steel in concrete.

In this study, the steel corrosion in the saltwater environment was evaluated by the electrochemical technique. In salt water, the steel corrosion without being processed by essential oils was compared with being processed by essential oils on the steel surface.

From these examined results, this study is discussed about the prevention effect of essential oils against the corrosion of steel.

P. 59. Glandular trichomes and essential oil of *Salvia glutinosa* L.

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The aerial parts of *Salvia glutinosa* L. bear indumentum with two types of trichomes: simple and multicellular nonglandular trichomes, and stalked and sessile dense glandular trichomes. Glandular trichomes are extremely long-stalked and dense on the stem and calyx surfaces. But, sessile glands are rare on the stem, calyx and leaf adaxial surfaces and dense on abaxial surface of the leaf. Secretion accumulates in a subcuticular space and is released to the outside by cuticle rupture. Water-distilled essential oil from dried aerial parts of *S. glutinosa* was analysed by GC and GC/MS. The main constituent was found as 1-octadecanol (11.0 %).

P. 60. Comparative Study of the Essential Oils of *Cymbopogon schoenanthus* ssp. *proximus* from Central Sudan

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There are seven wild species of *Cymbopogon* in Sudan: *C. caesius, C. commutatus, C. excavatus, C. giganteus, C. nervatus, C. sennarensis* and *C. schoenanthus* ssp. *proximus* (Andrews, 1956).

Cymbopogon schoenanthus (L.) Spreng ssp. *proximus* (Hochst.ex A.Rich.) Maire and Weiller (Poaceae), Camel's Hay, known in Sudan as Maherib and in Arab Countries as Halfa Bur is a well known aromatic and medicinal plant of North and Central Sudan. In Sudan this plant is used as antispasmodic and for treatment of stomach and kidneys pain.

The chemical composition of Maherib oil (*Cymbopogon schoenanthus* ssp. *proximus*) was studied by Modawi (1975), El Gamal and Wolff (1987). In our present study, we analyzed the oils isolated by hydrodistillation from leaves collected at different regions of Central Sudan, i.e., El Damer (North Central), El Obeid (West Central) and El Gedaref (East Central). The oil yield is.

The oils obtained in a yield of 3.5%, 1.2 % and 2.1 % respectively, were analysed by GC/ MS. Eleven components were reported for the first time. Pipertione, which identification was accomplished, with CNS excitatory, was the main component, ranging from 60.8% to 89.7 %. The major constituents were piperitone (60.8%), Δ–2-carene (11.4%) and elemol (6.4%) for El Obied sample, and piperitone (89.7%), α -terpineol (1.7 %) and β eudesmol (1.6%) for ElGedaref sample. Eldamer sample was dominated by piperitone (63.0%), elemol (11.1%) and Δ–2-carene (8.6%)

The essential oils from central Sudan are predominately monoterpenoid in character (75.5-93.8%), sesquterpenoids being the only significant at 18.4 % of El Damer sample.

Upon comparing the composition of essential oils of the Mahereib Sudanese populations with that from plants with Indian origin, considerable intraspecific variations were noted (Shahi and Tava, 1993).

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P. 61. Variation in essential oil content and composition of *Levisticum officinale* Koch. from different origins

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The variation in the essential oil content of lovage (*Levisticum officinale* Koch.) from Korean and Hungarian origin was studied from 1997 to 1999. Different plant organs and different times of harvest during the vegetative and reproductive periods were also considered. The essential oils were isolated by hydrodistillation and their composition determined capillary GC method with standards. The essential oil content showed significant differences between the two populations on the vegetative organs. The essential oil level of the leaves and roots was considerably higher in the Korean population at full flowering and waxy ripening stage but essential oil content of the roots was significantly higher in the Hungarian taxon at leaf rosette stage. We observed the essential oil accumulation tendency was mainly dependent on plant organs and intra-specific taxon during the vegetation period.

Butylidene-phthalide was proved to be the main component of the oil in both population roots (50.9-73.3%), while dimethyl-acetate was showed as a major compound on the over-ground parts (56.7-62.0%). The qualitative composition of the essential oil in the reproductive organs concerning the identified compounds was the same as the vegetative parts with the main component α-phellandrene (4.8-28.1%) and butylidene-phtalide (9.7-16.1%). The quantitative composition showed some changes during the ontogenesis phases. Most characteristic ones are the decreasing proportion of dimethyl-acetate (from 7.3% to 1.1%) and the appearance of α-pinene (from 0.5 % to1.5 %) only after fruit setting in both populations.

P. 62. Microbial transformation of (-)-carvone

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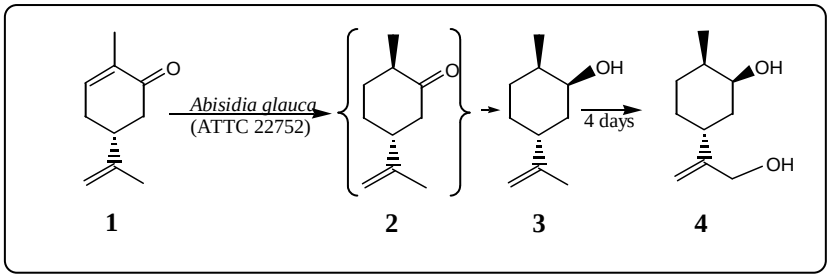
The enantiomeric monoterpene (–)-carvone (**1**) was subjected to microbial transformation studies using the fungi *Abisidia glauca* ATCC 22752. After 4 days of incubation a new diol: 10-hydroxy-(+)-neodihydrocarveol (**4**) was formed. The absolute configuration and structure of this crystalline substance was identified with the aid of X-ray refractometry and supported by spectroscopic techniques such as MS, IR and NMR. Antimicrobial activity of the substrate and metabolite was also investigated against human pathogenic microorganisms.



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*Part of the PhD thesis of Demirci F (2000), Eskisehir, Turkey.

P. 63. Quantitative data of plastidome in secretory canals of *Eleuterococcus senticosus*

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Eleuterococcus senticosus Maxim. is a wide-spread species in the Russian Far East. Its young leaves, bark and roots are fragrant and are used by local population for non-alcoholic drinks, beer and sweets production and in seasonings, the essential oil of *E. senticosus* is used in the perfumery. Monoterpenes represent up to 0.8% of the secretion isolated from the roots and shoots (Wild useful plants of Russia, 2001). Leucoplasts are the most specific feature of epithelial cells in secretory canals, already in the meristematic zone. They become dominant in epithelial cells at the stage of secretion. As plastidome is proved to be an important compartment that takes part in monoterpene biosynthesis (Cheniclet and Carde, 1985) we have studied their morphological properties. Plastids in the secreting cells are elongated, have rather dense stroma and no ribosomes. One or two large inclusions enclosed in a membrane are present in plastids. We distinguished two membrane systems in plastids: well developed peripheral reticulum and vesicular membrane system located in the central part of plastid. Plastoglobuli are numerous and are associated with the vesicular membrane system. Starch grains are often present and they are electron dense as the starch grains of the plastids in parenchyma cells.

The quantitative parameters were: linear and square dimensions, the number and partial volumes of membrane systems, plastoglobuli, starch grains and inclusions. Five plastid types were found in secreting cells of *E. senticosus* according to the quantitative data. They differed by partial volume of the main plastidome features. Depending on plastid type the stroma occupied from 30.9% to 48.1%. Moderately dense inclusions were present in most plastids but occupied different partial volumes ranging from 2.9% to 30.0%. Electron comparatively dense inclusions bordered by a membrane were present only in 3 plastid types, but occupied, when present, a relatively large volume from 39.0% up to 47.7%. The partial volume of starch grains had negative correlation with the dense inclusion volume. They were absent in plastids with the largest partial volume of dense inclusions and in the plastids that had no such inclusions ranged from 7.4% to 33.3% per plastid volume. We interpret the described plastid types as a developmental succession and propose that the cells at the same spatial canal level have different developmental or functional status.

Plastids appear to participate actively in the secretory processes. They are always sheathed by endoplasmic reticulum. At least one, sometimes two electron dense inclusions, that we interpret as terpenes, and mitochondria are adjacent to plastids at secreting stage.

In the senescent part of the canals plastids are smaller, rounded, and they have neither vesicular membrane system nor starch grains. The inclusions occupy the most part of plastid volume. There are no associations between plastids, electron dense droplets of secretion and mitochondria.

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P. 64. Evidence of cell divisions in the epithelial cells of *Pinus sylvestris* secretory canals

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Secretory canals are traditionally regarded as a character of great importance for the *Pinus* genera. They produce oleogumresin that is valued for its pharmaceutical and technical properties. The canals in *Pinus sylvestris* are present already in the buds and produce the secretion that takes part in buds protection during winter. Within a bud all the needles primordia are formed. In spring the bud starts to grow very rapidly. It elongates from the size 5-10 mm to 50-300 mm during 2-3 weeks. The bud grows both due to cell divisions in meristematic zones and to the cell linear dimensions increase. During the shoot growth the canal size increases greatly both in diameter and length. There may be two mechanisms of canal growth. The first is: the epithelial cell number grows only due to apical meristeme activity and below the apex the canal grows due to cells elongation. The second mechanism assumes that secretory cells may divide below the apex being already differentiated. In the literature we have found no evidence of cell division during the active secretion stage.

Longitudinal sections of *P. sylvestris* shoots, fixed in a mixture of glutaraldehyde and paraformaldehyde in June, when the shoot elongation is most evident, were examined using light and electron microscopes. Already in a bud the epithelial cells of the secretory canals have the features common for active secreting terpenoidogenic cells with hypertrophied leucoplastidome. Endoplasmic reticulum is present mostly in cisternal form and its membranes are associated with plastid envelope.

At a distance 3-4 cm from the apex canal lumen has maximal diameter, the cells surrounding it are moderately vacuolated. A large elongated or lobed nucleus occupies the central part of secretory cells. Plastids are located on both sides of the nucleus, they are very long, flexed or have invaginations. Plastid stroma is dense. Numerous osmiophilic droplets, which we interpret as terpenes, are located within plastid envelope. Different mitotic stages have been registered in the epithelial cells. The dividing cells had all the features of actively secreting cells. The number of such divisions found in different shoot samples allows regarding them as normal phenomenon.

The data obtained provides direct evidence of canal elongation due to both linear dimensions increase and cell divisions of differentiated epithelial cells. The capacity to divide at the stage and in the course of active secretion may be regarded as a unique feature in secretory canal epithelial cells. The main reason of this event may be a high growth rate of *P. sylvestris* shoot.

We acknowledge the Russian Academy of Sciences for the financial support of the present study according to the 6th young scientists supporting program grant N 245.

P. 65. Essential oil of laurel (*Laurus nobilis* L., Lauraceae) from Montenegro

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Laurel, bay laurel, *Laurus nobilis* L. (Lauraceae), is an ornamental tree, indigenous to the western as well as in the southest part of Europe. The main products are leaf of laurel and essential oil obtained from the leaf by steam distillation. The leaf is chiefly used as spice in culinary and the essential oil in the food technology. Rarely, leaf of laurel is used as a traditional drug for digestive disorders. The leaf of laurel contains approximately 1-3 % of essential oil, sesquiterpenoid lactones and isoquinoline alkaloids. The main constituents of this oil are 1,8-cineole (30-70 %), linalool (3-17 %) and methyleugenol (2-8 %).

The essential oil of laurel is one of the main export products of Yugoslavia. The main area for collected leaves of wild growing laurel is the southeast part of Adriatic coast in Montenegro. The essential oil is produced by many little, private distilleries without standardised technology of distillation. Mainly, the essential oil is obtained by steam distillation of the young shoots, not pure leaves. Besides this, the request for quality of this essential oil still does not exist as an ISO or AFNOR standards. The yugoslavian standard (JUS) is from the 1968. All this constitutes a major problem for laurel essential oil producers. This work deals with our effort to define the quality of leaf essential oil as one of the most export drugs from Yugoslavia and Montenegro. Besides, we made the comparison of the composition of essential oils obtained from diferent parts of laurel tree.

The autumn of 2000 was very weak with high daily temperature (up to the 20 ^{o}C) unusual for that part of the year. The laurel trees were in the second flowering phase just in period of October to November, the usual time for collecting leaves for commercial purpose. The samples of young shoots, bark and fruits were collected from the same tree, what was done at two different localities (Budva and Bar) in the Adriatic coast of Montenegro. The air-dried plant material was pulverized and the essential oil was obtained by hydrodistillation using a Clevenger-type apparatus. The content of essential oil was as following: 1.4 % in young shoots, 0.75 % in bark, 1.5 % in the separated leaves and 0.7 % in separated stems (locality Budva).

The chemical composition of these oils was determined by GC (FID) and GC/MS techniques. The main constituents of all investigated oils were, as it is usual for laurel, 1,8-cineol, methyleugenol and α-terpinyl acetate. Besides, α-pinene, β-pinene, sabinene and linalool were also present. It was interesting and important for commercial samples of laurel essential oil that there was no significant difference among the essential oils obtained from young shoots and separately from leaves and stems.

The main constituents of the essential oil obtained from the bark (from the trunk and branches) were 1,8-cineol (26%), methyleugenol (10%) and α-terpinyl acetate (9%). By comparison with the essential oil obtained from the leaves of laurel, the bark oil contained more methyleugenol, and the same level of α–terpinyl acetate but less 1,8-cineole, as well as α-pinene, β-pinene, sabinene and linalool.

The essential oil obtained from dry flowers of laurel contained the same components but in lower concentration: 1,8-cineol (15,7%), α-terpinylacetate (6.5%), methyleugenol (3.9%), sabinene (1.6%), linalool (1.4%), β-pinene (0.4%) and α-pinene (0.3%). Besides, the flower oil contained higher concentration of *trans*-caryophyllene (9.5%) and γ-muurolene (7.1%).

P. 66. Comparative study of the volatile compounds of different types of poppy seeds and -oils by SPME-GC-MS-Detection and by Olfactorimetry

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The opium poppy (*Papaver somniferum*) is said to be the oldest recognised medicinal plant in the world and it is still very important, as it furnishes a most important painkiller, morphine. Analysis of the remains from the Neolithic age verified that poppy was known to cavemen living in the territories of Spain, France, Germany, Austria and Hungary 4-5 thousand years BC.

The poppy, more than any other medicinal species, has been used and misused by humankind from the early days of history. Even today, the production and sale of this plant is politically charged. In Europe, especially in Hungary (6000-10 000 ha), Czech Republic (7000-8000 ha), Slovakia (7000-8000 ha), Romania (3000-5000 ha) and the Netherlands (1000-1500 ha) poppy is cultivated for its edible and oil producing seed. The poppy never lost its therapeutic and economic importance.

Although numerous investigations have been carried out with respect to the constituents of poppy capsules and seeds, very little was yet known about the volatile components of poppy seeds and -oils, which contribute to their characteristic odour.

In this work, the distribution of components in the headspace of different types of poppy seeds and -oils from white, blue and grey-varieties and the influence of different storage conditions on their odour are shown. For this work SPME-GC-MS-Detection and Olfactorimetry were used.

P. 67. Variability of methyleugenol and estragole of Basil varieties in field and greenhouse cultivation

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There are two opinions of the Scientific Committee on Food of the European Commission (Council of Europe, 2001, 2002) which point out, that estragole und methyleugenol have been demonstrated to be genotoxic and carcinogenic. Therefore the existence of thresholds cannot be assumed and the Committee could not establish a safe exposure limit. But consequently, reduction in exposure and restrictions in use levels are indicated. It is known, that both substances can be constituents of basil essential oils. The content of estragole in these oils can be up to 40 %, that of methyleugenol up to 90 %.

Comparisons of varieties from 2001 show that the composition of the basil essential oils are not only determined genetically. They are also affected substantially by the cultivation conditions. The differences between the contents of methyleugenol of all varieties are particularly explicit when field and greenhouse cultivation are compared (Tab. 1). Estragole types were not contained in the 2001 assortment.

Tab.1: Contents of methyleugenol in the basil essential oils of different varieties under field and greenhouse conditions

	Contents of methyleugenol [%] in the essential oils of the varieties							
	Genova	**Bavires**	**Aton**	**Bageco**	**Genua Star**	**Sanremo**	**Pesto**	**Genoveser**
Field cultivation	1.1	0.6	0.7	0.0	0.5	0.0	0.7	0.3
Greenhouse cultivation	32.4	8.0	30.3	20.1	23.2	3.3	25.5	27.1

The results from 2001 are to be complemented by systematic investigations of the variability and ontogenesis from the 2002 assortment of 12 varieties cultivated in the field and in the greenhouse.

Council of Europe – Scientific Committee on Food (2001) Opinion of the Scientific Committee on Food on Methyleugenol (4-Allyl-1,2-dimethoxybenzene): SCF/CS/FLAVOUR/4 ADD1 FINAL 26 September 2001

Council of Europe – Scientific Committee on Food (2001) Opinion of the Scientific Committee on Food on Estragole (1-Allyl-4-methoxybenzene): SCF/CS/FLAVOUR/4 ADD2 FINAL 26 September 2001

P. 68. Volatile constituents of *Sanguisorba officinalis*

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Sanguisorba officinalis L. belonging to the Rosaceae, subfamily Rosoideae is a semi-rosette shrub up to 100 cm with composite 1 to 2 cm dark red-brown ovate-oblong flower-heads. The rosette leaves are 20 to 40 cm long and consist of 7 to 15 ovate leaflets.

The plant is widespread in the northern, temperate regions of Europe, temperate Asia and North America and has been formerly used in folk medicine for staunching wounds. Phytochemical studies performed on the herb and the roots including the rhizomes led to the identification of numerous hydrolysable and condensed tannins, triterpene glycosides, sterols, and flavonoids including flavonoid sulphates, which seem to be characteristic of the genus *Sanguisorba*.

Since no previous reports concerning the chemical composition of the volatile constituents of *S. officinalis* were found, we have investigated the volatiles from the roots including the rhizomes and the fresh leaves obtained by hydrodistillation by means of GC and GC-MS.

In the yellowish root oil numerous constituents mainly belonging to monoterpenoids (34.7 %), sesquiterepenoids (16 %), and several fatty acids (16.8 %) were identified.

In contrast to the root oil the small amount of volatiles obtained from the leaves consisted mainly of degradation products of chlorophyll (phytol and phytadienes), aliphatic alcohols and aldehydes, alkanes and fatty acids. Terpenes were almost completely lacking.

P. 69. Analysis of a historical oil, Ninde oil (*Aeolanthus gamwelliae* Taylor) revisited

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Aeolanthus gamwelliae Taylor (Ninde) (Lamiaceae) is an aromatic plant native to northern Zambia. In 1930s, water and steam distilled oil from flowers of this plant was commercially available due to its high geraniol content. The cultivation of Ninde plant was also initiated in the region in the same years. Long ago, the cultivation practices have come to an end and the oil is not, at present, a commodity of commerce.

The oil subjected to this study was obtained from the collection of Natural Resources Institute (NCI), which had been produced by water and steam distillation in 1934/35.

The oil was analysed by GC and GC/MS. Forty one compounds were characterised representing 95% of the oil with geraniol (66%), geranyl acetate (12.5%), geranial (4%) and geranyl formate (3.7%) as main constituents.

P. 70. Leaf essential oil of *Laurus nobilis* L. from different localities of Croatia

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Bay laurel, sweet laurel, *Laurus nobilis* L., an evergreen shrub or small tree native to the Mediterranean region and Asia Minor, has been admired for its beauty and aromatic leaves since ancient times. Currently, the plant is both cultivated and collected from the wild. Dried leaves are used to flavour meats, fish, vegetables, and soups and also as an ingredient in pickling spice. The essential oil, distilled from the leaves, with bactericidal and fungicidal properties (Schnaubelt, 1998) is used in perfumery and for flavouring food products (Duke, 1987). This is the first report on the Croatian bay leaf essential oil.

The leaves were harvested at three different localities in October 2001 (Table 1.). The dried leaves were crushed to powder and the oil was isolated by hydrodistillation, for 3 hours, in a modified Clevenger-type apparatus. The oil yield varied from 0.87 – 1.53 % (mass/dry weight). The isolated oils were analysed by GC-MS using two columns with different stationary phase polarity (HP-20M and HP-101). The most abundant oil compounds are listed in Table 1.

Table 1. Comparative chemical composition of *Laurus nobilis* L. leaf essential oils - main components (%)

	Localities		
Compound	**Opatija**	**Rijeka**	**Krk-Kras**
1,8-Cineole	32.8	30.9	33.6
Sabinene	8.7	10.2	10.7
α-Terpinyl acetate	10.2	8.6	9.1
Linalool	7.3	8.8	4.7
Methyl eugenol	4.8	5.6	8.8
Eugenol	3.4	3.9	2.8
α-Terpineol	3.5	4.6	3.7
Terpinene-4-ol	3.3	2.7	4.6
α-Pinene	3.3	3.8	4.7
β-Caryophyllene	1.6	2.2	0.7

Our results are comparable with those reported for Portugal (Roque, 1989), Turkey (Lawrence, 1990) and Italy (Caredda *et al*., 2002) bay oils.

Caredda A, B Marongiu, S. Porcedda, C Soro (2002) *J. Agric. Food Chem.* 50:1492-1496.
Duke JA (1987) *Handbook of Medicinal Herbs*: 271-272.
Lawrence BM (1990) *Parfum. Flavor.* 15: 67-68.
Roque OR (1989) *J. Ess. Oil Res.* 1: 199-200.
Schnaubelt K (1998) *Advanced Aromatherapy*: 35-38.

P. 71. Steam-volatile leaf oils of some Western Australian species of the family Myrtaceae

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The volatile leaf oils of nine Western Australian species have been extracted by means of cohobative steam-distillation and analysed by GC/MS.

The leaf oils of *Agonis obtusissima* F. Muell., *Eremaea pauciflora* (Endl.) Druce, *Kunzea pulchella* (Lindl.) A. S. George and *Malleostemon tuberculatus* (E. Pritzel) J. W. Green have not been previously investigated whilst *Thryptomene australis* Endl. and *Thryptomene kochii* E. Pritzel have received only cursory attention. Additional data have been obtained for *Melaleuca uncinata* R. Br., *Eucalyptus erythrocorys* F. Muell. and *Eucalyptus stoatei* C. Gardner.

The oils of *Kunzea pulchella* (both the red and the white flowered forms) contain globulol as the main constituent (83-88%). The oils of *Thryptomene australis* are rich in geranic acid (*ca* 52%) and α-pinene (22%) whilst *Thryptomene kochii* contains α-pinene as its main constituent (58-60%). *Eremaea pauciflora* oil contains 1,8-cineole (22%) and the three isomeric eudesmols (α-, β-, and γ-, 26%). *Agonis obtusissima* contains substantial amounts of α-pinene (12%), *trans*-β-ocimene (16%) and globulol (39%). *Malleostemon tuberculatus* oil contains α-pinene (33%), 1,8-cineole (21%) as well as E,E-farnesol (6%) as its main constituents.

The oil of *Melaleuca uncinata* obtained from bushes growing near Jaurdie contains about 89% of α-pinene and represents the 4th chemical form of this species postulated by Watson (1943/44). The three other forms are characterised by: 1,8-cineole and the three eudesmol isomers; 1,8-cineole and no eudesmols (Brophy *et al.*, 1990); and terpinen-4-ol (Brophy and Lassak, 1992).

The composition of the leaf volatiles of *Eucalyptus erythrocorys* and *Eucalyptus stoatei* obtained by steam-distillation differ in the former case, but are comparable in the latter, with results published on vacuum extracted oils (Bignell *et al.*, 1994, 1996).

Bignell CM, PJ Dunlop, JJ Brophy, JF Jackson (1994) *Flavour Fragr. J.* 9: 113.
Bignell CM, PJ Dunlop, JJ Brophy, JF Jackson (1996) *Flavour Fragr. J.* 11: 145.
Brophy JJ, EV Lassak (1992) *Flavour Fragr. J.* 7: 27.
Brophy JJ, EV Lassak, DJ Boland (1990) *Flavour Fragr. J.* 5: 43.
Watson EM (1943/44) *J. Roy. Soc. Western Australia* 30: 83.

P. 72. Influence of extraction methods on the composition of essential oils

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The aim of this presentation is to show our results on comparison of composition of the essential oil fractions obtained by traditional steam distillation and supercritical fluid extraction. The latter technique avoids the degradative heat processes, hydrolysis, isomerisation and racemisation. It is a special separation technique to obtain extracts and fractions of natural origin for therapeutical or cosmetic uses (Chouchi *et al.* 1995, Bicchi *et al.* 1999).

The plant material for the various extraction methods was selected from the *Asteraceae* and the *Lamiaceae* families. For the supercritical fluid extraction (SFE) carbon dioxide was used as supercritical solvent. The extracts were collected by stagewise precipitation in two separators. The waxy product and extract rich in essential oil were collected in the 1st and in the 2nd separator respectively. The traditional water steam distillation (SD) was carried out according to the Hungarian Pharmacopoea 7th ed. and the GC analysis performed on capillary silica fused columns coated with DB-1701 and the specific chiral columns coated with Rt-β DEXm or Rt-β DEXsm.

Comparing the composition of steam distilled oils with that of volatile SFE fractions the following general characteristics were established. The SFE fractions were richer in monoterpene-esters and poorer in alcohols than the traditional essential oils (clary sage, lavander, dragonhead). Regarding the distribution of the monoterpene and sesquiterpene compounds, the SFE fractions contained sesquiterpene hydrocarbons in higher percentage than the distilled oils (*Salvia triloba*). Moreover, the proportion of sesquiterpenes increased in SFE fractions collected successively in time (*Salvia officinalis*) similarly to the ratio of oxygenated monoterpenes to monoterpene hydrocarbons (*Rosmarinus officinalis*). In more cases it was verified that a part of mono- and sesquiterpenes were present originally in bounded form (glycosides) in plants. So they appeared only in essential oil fractions after previous acidic treatment (*Thymus*, *Origanum*, *Satureja* species) [Oszagyán *et al*., 1996, Lemberkovics *et al.*, 1998]. During the supercritical extraction the azulenogene sesquiterpene lactones did not transform to azulenes in camomile and yarrow, but the non volatile SFE fraction of some *Asteraceae* plants (feverfew, blassed thistle) contained sesquiterpene-γ-lactons of unchanged structure.

Acknowledgements:The work was supported by Hung. Nat.Sci.Found (OTKA T 30034).

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Lemberkovics É, Á Kéry, B Simándi, G Marczal, M Then (1998) *Proceedings of Supercritical Fluid Material and Natural Products* 2: 567-572.
Oszagyán M, B Simándi, J Sawinsky, Á Kéry (1996) *J.Essent. Oil.Res.* 8: 333

P. 73. Indumentum features and essential oil composition of the Hungarian *Salvia* species

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In this work we report on the anatomical features of the indumentum of four *Salvia* species (Lamiaceae), *S. officinalis L., S. sclarea L., S. pratensis L., S. nemorosa L,* namely the structure and position of glandular and covering trichomes, as well as on the composition of their essential oils.

The chemical composition of the oils was investigated in the different plant organs (leaf, calyx and petal) of the various species. The plants used in this study were cultivated in the garden of the Ecological and Botanical Research Institute of the Hungarian Academy of Sciences. The microscopical studies were carried out by Axioscop apparatus. The essential oils were isolated by the methods described in the Hungarian Pharmacopoea and analysed by gas chromatography.

The indumentum of each organ of the *Salvia* species investigated is composed by glandular and covering trichomes. The characteristic Labiatae-type glandular trichomes occurred along with other glandular trichomes, composed of a unicellular head. Numerous glandular and covering trichomes can be seen on the calyx and petal. Their structure is similar to those of *S. officinalis*. A few of glandular trichomes are on the petals, too. A lot of whip-like trichomes are present, but conical unicellular trichomes are rare. The big covering trichomes, having short basal cells and a sharp terminal cell are typical of the *S. sclarea* leaf. Some conical covering trichomes are also found. The leaf of *S.pratensis* is poor in trichomes. There are rarely multicellular covering and glandular trichomes with unicellular head.

Comparing the essential oil composition of the fresh and dried plant organs of the four *Salvia* species, differences exist only in *S. officinalis*. The dominant components in the fresh leaf oil are the sesquiterpenes α-humulene (27,9%) and β-caryophyllene (13.0%). In dried leaf oil the monoterpene thujone isomers were the main ones. The qualitative oil composition of the three organs does not differ from each other, although some qualitative differences are recorded. The thujone isomers are dominant (17-20%) in the leaf and calyx oils but they represent only 5.9% of the petal oil. The main component in petal oil is the pharmacologically active β-pinene (24.4%). The oil from *S. pratensis* is dominated β-caryophyllene (7,1%) and γ-muurolene (24,0%). Their ratio in the calyx and petal oils is similar, but in the leaf oil this is reversed.

The structure of covering trichomes and the percentage composition of the oils provide a good basis for the differentiation of *Salvia* species.

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P. 74. Comparative pharmacology of essential oils with a floral to pungent odour

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Essential oils and absolutes with a floral odour like lavender, geranium and jasmine all have a spasmolytic effect on the guinea-pig ileum *in vitro* which is most likely mediated through the secondary messenger cAMP. The oils of a number of floral-smelling *Pelargonium* species are also similar, but the more pungent, camphoric species, though still exhibiting a spasmolytic effect, show a mechanism which is more likely to be through calcium channel blockade.

Peppermint oil is also similar to the florals, as is one of the so-called pungent Tea tree oils from New Zealand, Manuka (*Leptospermum scoparium*). However the other tea tree oil from New Zealand, Kanuka (*Kunzea ericoides*) and the Australian tea-tree (*Melaleuca alternifolia*) has an initial spasmogenic action followed by a spasmolytic response. Valerian oil, which can only be described as pungent again behaves spasmolytically like the floral oils but appears to act through calcium channels like the pungent *Pelargonium* oils. Oils like citrus, frankincense, nutmeg, dill and fennel show a very strong contraction. Two 'catty-like' Buchu oils, *Agathosma betulina* and *A. crenulata* showed differences in the mode of action although both showed an initial spasmogenic action followed by spasmolysis. The former alone showed an action via cAMP.

The mode of action on smooth muscle appears to be directly related to the chemical composition of the oils. Spasmolysis is effected by EOs with high concentrations of alcohols, aldehydes, esters, ketones and sesquiterpenes, regardless of the actual component. The spasmogenic effect is effected by EOs with high concentrations of monoterpenes. The actual mechanism of the spasmolytic effect is more complicated but seems to involve the floral or pungent odours of the oils.

The pharmacological effect on the uterus *in vitro* is remarkably constant, showing a decrease in spontaneous contractions by all EOs studied. This raises the question of safety when using oils during childbirth as they could invoke cessation of uterine contractions and therefore prove a danger to the baby's life.

The action on skeletal muscle, using the rat isolated phrenic nerve diaphragm preparation or chick biventer muscle, is more complex. Some EOs show a decrease in the twitch response alone, like lavender. Some like dill, frankincense and nutmeg show a contracture with a decrease in the twitch response, others like thyme show a contracture without the effect on the twitch response. Camphor caused an increase in the twitch response and angelica root showed no response.

All these aspects will be discussed and illustrated.

P. 75. New odoriferous terpenoids containing bicycle [3.1.0] hexane skeleton using (+)-3-carene as a starting substrate

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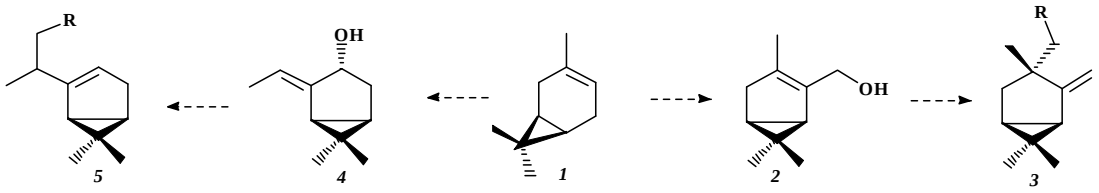
This paper is a continuation of our investigations on elaboration of simple methods for the synthesis of new chiral odoriferous compounds when using monoterpene hydrocarbon (+)-3-carene **1** (a major constituent of Polish turpentine, the essential oil from *Pinus sylvestris L.*) as a starting material.

In our previous paper we reported syntheses of odorants with preserved carane system (Lochyński, 2002), as well as those with di- or trimethylbicyclo[3.1.0]hexane system substituted at C-2 (Lochyński, 2001) and C-3 positions (Lochyński, 1997).

Here we present further study starting from allylic alcohols **2** and **4**, which were subjected to the Claisen rearrangement (orthoacetate modification) giving γ,δ-unsaturated esters and their derivatives **3** and **5**.

All newly obtained terpenoid compounds possess interesting olfactory properties. Odor characteristics will be presented with special emphasis on structure-activity relationship.



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P. 76. Aromatic species with economic potential on the cerrado- Goiás, Brazil

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The Brazilian Cerrado (Savannah) is rich in plants species that have economic potential, most of them unexplored. The increase of agricultural areas plus uncontrolled extractives actions is gradually reducing the natural areas where medicinal species grow wild. O Biome Cerrado (Brazilian Savannah) has 2 million of km2, representing 23% of Brazilian territory. It is the second vegetation type of Brazil. Unfortunately, on last the 30 years this Biome has been losing its genetic material unknown scientifically mainly because of devastation. Local groups use the plants and sell products made from it on local markets making perfumes, crafts and liquors extracted from flowers, leaves, roots and bark.

A pilot research project is in course at the Public School Zeca de Farias working with 180 children and their families to increase the popular interest on this matter. Besides the educational purposes, this project focuses on economic interest in collecting seeds and creating greenhouses. The population of this school is originally from rural areas and this pilot project may help them to reconnect with their roots. The methodologies used are interviews and field work, collecting seeds and information about the place where is the mother tree, popular name, its use and how to use it and what part of the plant can be used. The plants, which show the most interest for the population, will be planted in the school ground and/or in the city.

Alto Paraíso is located at the Northeast portion of the State of Goiás, in the microregion denominated Chapada dos Veadeiros, occupying an area of 2603,4 km2. The altitudes vary between 1180 and 1200 meters in the São Bartolomeu river basin. It is part of the territory São Jorge's District and the Rural Town of the Moinho. Due to its altitude the climate it is characterised by presenting soft summers and winters, with temperatures varying between 18ºC and 20ºC and the distribution of rains is seasonal.

A lack of economic alternatives pushed the small landowners to sell their properties to entrepreneurs who buy their land to introduce business forcing them to live in the city. The tourism, an important potential on the region, is make with no planning. The National Park, which has tourism and research potential, has lost its protected territory. In 1961 was approximately 625 thousand hectares, in 1981 was reduced to 65 thousand hectares.

Species	**Popular Name**	**Active Principles**	**Use**
Caryocar brasiliense Camb.	pequi	several substances were on essential oils like 1,8 cineole	liquors, home made food
Achyrocline satureoides D.C.	macela	essential oils and flavonóides	Aromatics pillows and shampoos
Lychnophora ericoides Less.	arnica	essential oils	Perfumery and cosmetics
Spiranthera odoratissima A. St. Hill.	manacá	essential oils	dry flowers, tea and source of jasmine
Brosimum gaudichaudii Trec.	mama-cadela	furonocumarinas	aromatic for pipe, food and medicine

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2 Agronomo e Membro do Cerrado *in Vitro*/FINATEC

P. 77. Seasonal essential oil variation of *Aniba canelilla* (H.B.K.) Mez

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Aniba canelilla is a high tree known as "casca-preciosa" (precious bark) and an important and historical species in the Amazon region because it was confused with cinnamon-trees during the voyage of Pizzaro and Orellana from the Andes to the Amazon estuary about 1540, and during the Humbolt and Bonpland's 1800 expedition to find the "famous cinnamon" of the Orinoco River. This work was part of a project to identify biogeochemical markers of mineral soils in the Amazon, based on essential oil analysis (Taveira, 1991).

The essential oils of leaves, stem bark and trunk wood of *Aniba canelilla*, collected in the rainy and dry season from different soil types, were obtained by hydrodistillation and analysed by GC-MS.

The odoriferous principle of leaf, bark and trunk wood of *Aniba canelilla*, responsible for the cinnamon odour, is 1-nitro-2-phenylethane, rare in the nature. We observed that methyleugenol is also an important volatile constituent in the oil of *A. canelilla*. The percentage content of these two compounds was depending of the season time. In the rainy period the 1-nitro-2-phenylethane reach values near 95%, while methyleugenol remain below 18%. By contrast, in the dry period 1-nitro-2-phenylethane decrease to 39%, while methyleugenol reach 45%. The leaf oils produced by specimens collected at different soil types in the dry season showed the lower percentage contents for 1-nitro-2-phenylethane (39.0%) and methyleugenol (0.5%). On the other hand, the mono- and sesquiterpene compounds present in the same oils showed the higher percentage contents (13.5% and 43.2%, respectively). The presence of small quantities of benzaldehyde, benzene acetaldehyde, benzonitrile and benzene acetonitrile identified mainly in the essential oils of leaves of *A. canelilla* is unusual and noteworthy, and they could be the end-products of phenylalanine degradation. This latter, probably the origin of 1-nitro-2-phenylethane and methyleugenol and both compounds seems to be biologically interchangeable depending on the impact of extrinsic factors.

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P. 78. *Stachys sylvatica* L.: secreting trichomes and essential oil

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Stachys sylvatica L. is a perennial herb belonging to the Stachys section (Ball, 1972) and is widespread in all the Italian peninsula in mountain and moist places. The whole plant emanates an unpleasant smell and is covered by non glandular and glandular trichomes, which give it a velvety look. The plant is used for medicinal purposes in Italian folk medicine (Gastaldo, 1987).

In this work the different types of trichomes present on the plant (leaves, stem and flowers) and the essential oil composition (determined for the first time), are reported.

The trichome study was carried out using light and electron microscopy (S.E.M and T.E.M). Peltate trichomes, which are considered characteristic of Labiatae species secreting essential oil (Werker, 1993; Hallahan, 2000), are lacking. Instead, capitate trichomes occur as three types, as follows:

1) short capitate, characterised by one cell stalk, two secreting cells and a small subcuticular space;

2) long capitate, characterised by a stalk constituted of two or three cells, four secreting cells and a large subcuticular space containing the secretion;

3) very long capitate, characterised by a stalk constituted of three or more cells, six or more secreting cells, each of them bearing a small subcuticular space on the apex. This type of trichomes is probably characteristic of the *Stachys* genus. Indeed, it has been described only for some species of this genus (Falciani *et al*., 1995). The number of the secreting cells, which constitute the head, depends on the species considered; however, the trichome morphology is the same in all the species examined. These trichomes usually are present on the inflorescence.

Concerning the substances secreted by the different types of trichomes, the histochemical analyses indicate that essential oils are present in all the trichomes. Moreover, in the short capitate trichomes a polysaccharidic secretion is present too.

The ultrastructure observations are consistent with the histochemical data. Indeed, both types of long capitate trichomes show plastids with dense stroma and numerous tubules containing electrondense material and smooth endoplasmic reticulum, i.e. typical organelles of a lipophile secretion (Bourett *et al*., 1997; Ascensao *et al*., 1998).

The oil analysis was carried out, separately on leaves and inflorescences, by Gas Mass Spectrometry. Preliminary results indicate a similar oil composition for both parts of the plant analysed. Germacrene D is the main component.

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P. 79. Essential oils from *Pothomorphe umbellata* and *Pothomorphe peltata*

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The genus *Pothomorphe* Miq. belongs to the Piperaceae family and is composed by two species: *Pothomorphe peltata* (L.) Miq. and *Pothomorphe umbellata* (L.) Miq. These medicinal plants occur in Brazil, being *P. peltata* from the western and Amazon region, and *P. umbellata* from the southern region of the country. *P. peltata*, whose synonyms are *Hecheria peltata* Kunth (L.) and *Piper peltata*, is commonly named "caápeba do Norte" or "malvarisco" (Yunker, 1973); there is a popular use of its leaves as an anti-inflammatory, cholagogue, choleretic, diuretic, vermifugal and anti-malarial (Miliken,1997). *P. umbellata*, also known as *Hecheria umbellata* (L.) Kunth and *Piper umbellatum* L. is commonly named "caápeba do Sul" or "paripoba" (Yunker, 1973), and is traditionally used as cholagogue and choleretic being recommended by the Brazilian Pharmacopoeia (Silva, 1929). Due to the similarity in their morphology, these plants are often mistaken for one another. However, morpho-anatomic studies report the following differences between them: in the insertion of the petiole, and in the occurrence and distribution of trichomes and stomata (Moraes, 1999).

Phytochemical studies of the essential oil from the leaves from *P. umbellata* collected in Brazil shows the occurrence of phellandrene, asarone and dillapiole (Hammer and Johns, 1983). The chemical composition of *P. peltata*, from the city of Belém, Pará State, showed the predominance of sesquiterpenes (Moraes, 1999). We describe herein the chemical composition of the leaf essential oils from *P. peltata and P. umbellata* as a contribution to the characterization of these species and in order to improve their use as medicinal plants.

P. peltata and *P. umbellata* were grown at the experimental farm at Instituto Agronômico in Campinas city - São Paulo State, southern Brazil. The *P. peltata* seeds came from the Amazon region (city of Belém Pará State). The essential oils were extracted by steam distillation of the leaves for *ca* 75 minutes. The analyses were performed using a GC-MS unit (Shimadzu, QP-5000), equipped with a fused silica capillary column DB-5 (J & Scientific, 30 m x 0.25 mm x 0.25 μm). The carrier gas was Helium (1.7 mL/min), with the following temperature program: 50°C - 280°C at 4°C/min. The injector and detector temperatures were 240°C and 230°C, respectively. The identification of the oil components was made by comparison of their mass spectra with those from an instrument library (Nist62.lib), and/or from literature along with the retention indices.

The chemical profile from these two plants were quite different; *P. umbellata* showed the predominance of monoterpenes (92,3%) while *P. peltata* possessed *ca*.100% of sesquiterpenes. The most abundant components in the oil from *P. umbellata* were β-myrcene (4.2%), α-phellandrene (2.8%), Δ-3-carene (77.7%) and terpineole (3.2%); the main sesquiterpenes were β-caryophyllene (0.9%), δ-cadinene (0.9%) and γ-cadinene (0.5%). The major components of the oil from *P. peltata* were β-caryophyllene (49,1%), germacrene-D (30,2%) and *trans*-β-guaiene (7,5%).

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P. 80. Essential oil constituents as taxonomic markers in *Ocimum* spp.

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The species *Ocimum minimum* L. and *O. basilicum* L. are related to each other and commercially known as cultivars of a same species, *O. basilicum*, popularly referred to as basil. But papers such as from Grayer *et al.* (1996a) and Grayer *et al.* (1996b) reported that in crossing experiments of these two materials, there was a production of no viable seeds, evidencing the fact that these plants should be two different species, both rich in essential oil. The cross-polinization, very frequent in *Ocimum* spp, leads to a large number of sub-species and great genetic variability (Marotti *et al.*, 1996). Plant secondary metabolites can be influenced by genetic, physiological and environmental factors. The aim of this work is to contribute to the chemosystematic of the *Ocimum* genus, and for this purpose, essential oils profiles were determined for the leaves of both species (*O. minimum* and *O. basilicum*) cultivated under two conditions (organic mineral substrate and hydroponics system) and collected during the inflorescence phase, at two different dates.

The essential oils were extracted by steam distillation for 90 minutes. The extracts were analyzed by GC-MS (Shimadzu, QP-5000), with a fused silica capillary column DB-5 (J & Scientific, 30m x 0.25mm x 0.25μm). Helium was used as carrier gas (1.0 mL/min), and the oven temperature was programmed to increase from 60°C to 280°C at 3°C/min. The injector and detector temperatures were 220°C and 240°C, respectively. The identification of the oil constituents was confirmed by GC-MS, trough the comparison of the instruments library (Nist62.lib), data from literature (McLafferty and Stauffer, 1989) and retention indexes (Adams, 1995). The statistical analysis was processed using Minitab- Statistical Software – 13 Demo.

The essential oils presented linalool as the major component, followed by α-*trans*-bergamotene, germacrene D, γ-cadinene, and cubenol. Among the cited substances, it is observed a significant difference in the quantity of linalool and α-*trans*-bergamotene, between the studied species. The linalool content in *O. minimum* varied from 44.3 to 54.3%, while in *O. basilicum* varied from 22.7% to 37.4%. For α-*trans*-bergamotene, the inverse result is observed: 6.6% to 9.5% for *O. minimum*, and 13.2 to 19.7% for *O. basilicum*. The variations within a same species were due to differences in the collecting time and growing system. The stepwise regression analysis, α=0.15, showed that the difference in the α-trans-bergamotene content explains 81.4% of the differences between the studied species and adding the linalool and γ-cadinene contents, it is possible to explain 89.84% and 96.60%, of the difference between the species, respectively.

These results, associated to the phenotypic differences between *O. minimum* and *O. basilicum*, support the conclusion that linalool, α-*trans*-bergamotene and γ-cadinene can be used as taxonomical markers for the studied species.

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P. 81. Comparative Study of the Essential Oils of Three *Stachys* Species Grown Wild in Iran

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The genus *Stachys* (Lamiaceae) is found in mild regions of the Mediterranean and in Southwest Asia. The genus *Stachys* consists of about 200 species, 34 are described in the flora of Iran, of which 13 are endemic (Mozaffarian 1996). *Stachys* species have several folkloric uses, e.g. the leaves of *S. officinalis* L. Trev. are used as a cholagogic, carminative and to relieve headaches. *Stachys* species have also been studied in biosystematic and chemotaxonomic studies in which flavonoids, quinones, iridoids, phenolic acids and diterpenoids were reported. The oils of the genus *Stachys* hve been the subject of only a few studies.

As apart of our research on aromatic flora of Iran, we investigated the chemical composition of the oils isolated by hydrodistillation from the aerial parts of *Stachys pilifera* Benth., *S. acerosa* Boiss. and *S. benthamiana* Boiss., which are endemic to Iran, by means of GC/MS in combination with retention indices.

In *S. pilifera* oil, 30 components representing 88.9% of the total oil were identified. *cis*-Chrysanthenyl acetate (25.2%) and *trans*-verbenol (19.7%) were the major compounds.

S. acerosa oil contained also *cis*-chrysanthenyl acetate (41.0%) and linalool (23.5%) among the 20 constituents characterised comprising 92.1% of the total components detected. Germacrene-D (16.8%), linalool (16.6%) and β-caryophyllene (11.0%) were the main constituents among the 20 characterised comprising 91.2% of the total components detected in the oil of *S. benthamiana*.

As can be seen the above information two oils (*S. pilifera* and *S. acerosa*) were rich in oxygenated monoterpenes, while the oil *S. benthamiana* consisted mainly of sesquiterpenes.

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P. 82. The effects of external factors on the quality and quantity of the camomile essential oil

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German camomile [*Chamomilla recutita* (L.) Rauschert] is one of the most important plants that are used widely in pharmaceuticals and cosmetics.

This research was performed to obtain the optimum growth conditions, the effects of soil texture and nutrient on flower crop and essential oil. In addition influence of IAA and different climates and different harvesting times on the quality and quantity of chamomile oil, was investigated.

The results show that the best soil texture for camomile growth is loam. This soil type leads to a flowering time decreasing of 29 days and a flower crop increasing of 376.9 %, in relation to plants cultivated under standard conditions.

IAA affects on plant growth and the quality of oil show that the flowering time was decreased in 20 days and essential oil yield and chamazulene content increased 58.3% and 33 %, respectively.

Cultivation of the plant in different climate influences affected also the camomile essential oil yield.

Different harvesting time (07:00; 12:00; 19:00 hours) showed that the best time for harvesting is at 12:00 hours.

P. 83. Composition of the essential oil from the leaves of *Pittosporum undulatum* from the Azores

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The composition of the essential oil obtained by hydrodistillation from the leaves on the flowering phase of *Pittosporum undulatum* Vent. (Sjogren, 1984) growing on San Miguel island (Azores) was investigated, with constituents being identified by GC/MS analysis (Van Den Dool, 1962; Davies, 1990).

The oil was found to contain monoterpenes, sesquiterpenes, diterpenes and alkanes, of which the sesquiterpenes calamenene (41.4%), farnesol (10.9%), spathulenol (5.6%) and β-selinene (5.2%) and the diterpene (8β,13β)-kaur-16-ene (10.7%) were the major components.

Tests for the detection of antithrombin activity (Medeiros, 2000) of the crude oils also revealed that these had even better activity than heparin.

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P. 84. Composition and antimicrobial activity of the essential oils from leaves and flowers of *Hedychium gardnerianum* from the Azores

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The composition of the essential oils, obtained by hydrodistillation, from the leaves and flowers of *Hedychium gardnerianum* Sheppard ex Ker Gawler (Sjogren, 1984) growing on San Miguel island (Azores) was investigated. The compounds were identified by GC-MS analyses (Adams, 1995). There are studies about the composition and characteristics of the essential oils of various *Hedychium* species (Gottlieb, 1959; Haggag, 1972), but only the composition of oils from *H. gardnerianum* rhizomes has been examined (Weyerstahl, 1998). The oils in the leaves and flowers were rich in α-pinene, β-pinene and α-cadinol. Their potential antimicrobial activities were tested against *Staphylococcus aureus*, *S. epidermis* and *Pseudomonas aeruginosa* by the agar diffusion method (Tanaka, 1992). The oils had the highest activity against *S. aureus* and *S. epidermis* but had no activity against *P. aeruginosa*.

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P. 85. Glycosidic derivatives of volatile components for cosmetic applications

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Years after the first positive identification of geraniol β-D-glucoside in rose flowers (Francis and Allcock 1969), glycosidically bound forms of several volatile components have been identified in various other natural sources: these structures may be regarded as non-volatile flavour and fragrance precursors.

On the other hand use of natural ingredients in cosmetic preparations are more and more appreciated. For example, essential oils are not only used in cosmetics for perfuming purpose but also for their biological properties (antioxidant, antielastasic…) (Etienne, 1998).

Synthesis of glycosidic derivatives of the three main constituents of the "rose scent": citronellol, geraniol and 2-phenylethanol is described. L-Rhamnose was selected for its ability to target keratinocyte lectins.

These components should find interesting applications in cosmetic formulation, owing to a better hydrophilic lipophilic balance as well as a progressive release of "the rose scent" by cutaneous microflora action.

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P. 86. Antioxidant activity of some *Thymus* species grown in Portugal

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Lipid peroxidation in biological membranes leads to cellular damage, which is involved in the development of chronic diseases that are life limiting. These include atherosclerosis, heart diseases, carcinogenesis and ageing processes. The oxidation of lipids in foodstuffs also affects their quality due to the volatile compounds formed, limiting the shelf life of fresh and processed food. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), gallats and *tert*-butylhydroquinone (TBHQ), and natural antioxidants such as tocopherol and ascorbate derivatives, are largely used in food processing. In addition to these compounds essential oils from various aromatic plants have also been the subject of an increasing interest as antioxidants more health friendly. In this work, the chemical analysis and the antioxidant activity of the essential oils isolated from *Thymus mastichina*, *Th. zygis*, *Th. caespititius* and *Th. camphoratus* were analysed. The antioxidant index percentage was calculated using the formula (1-t/c) x 100, where c is the absorbance value obtained in a fully oxidised control assay and t is the absorbance of the test sample, when a modified thiobarbituric acid reactive species (TBARS) assay was used. The antioxidant activity was carried out in the absence and presence of the radical inducer 2,2'-azobis(2--amidinopropane) dihydrochloride (ABAP). Different concentrations of essential oils (62.5, 125, 250 and 500 ppm) were used. Egg yolk was used as substrate. In the absence of ABAP, BHT was found to be the most efficient antioxidant, at all the tested concentrations. The antioxidant index percentages obtained with the essential oils, in concentrations of 250 ppm and 500 ppm, isolated from *Th. caespititius* and *Th. zygis* revealed to be as important as α-tocopherol and even superior when compared with BHA. The essential oils of *Th. mastichina* showed the less effective antioxidant effect. In the presence of ABAP, α-tocopherol and BHA were the most effective antioxidants, with similar antioxidant index percentages at 500 ppm. Within the essential oils, only *Th. zygis* showed the better antioxidant activity, reaching the highest AI% at 500 ppm, but significantly below the values achieved with α-tocopherol and BHA. The oils from *Th. mastichina* in a concentration range of 62.5 to 250 ppm and those from *Th. caespititius* at a concentration of 62.5 ppm showed pro-oxidant activity (Table 1).

Table 1. Antioxidant index percentage (AI%) of the essential oils, BHT, BHA and α-tocopherol using TBARS assay with and without ABAP

		500 ppm	250 ppm	125 ppm	62.5 ppm
Without ABAP	*Thymus mastichina*	39	32	17	10
	Thymus zygis	83	73	57	11
	Thymus caespititius	76	73	43	3
	Thymus camphoratus	52	38	16	14
	BHT	88	82	79	74
	BHA	66	65	61	60
	α-tocopherol	74	75	71	0
With ABAP	*Thymus mastichina*	5	-10	-15	-20
	Thymus zygis	53	33	11	0
	Thymus caespititius	42	26	2	-36
	Thymus camphoratus	37	34	9	7
	BHT	69	66	51	42
	BHA	83	74	64	55
	α-tocopherol	82	76	67	51

P. 87. Antioxidant activity of the essential oils of *Thymbra capitata, Thymus mastichina* and *Th. camphoratus* using sunflower and peanut oils as substrates

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The autooxidation of lipids in foodstuffs affects their quality, as it is responsible for the development of rancidity. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), gallats and *tert*-butylhydroquinone (TBHQ) are synthetic antioxidants commonly added to foods to prevent autooxidation. Nevertheless, these substances have been questioned because they show some toxicity and carcinogenicity. The demand for natural antioxidants is growing in order to render food products safer for mankind. Essential oils are receiving attention as potential antioxidants, due to their importance in food preservation.

In the present work, the chemical composition of the essential oils isolated from the leaves of *Thymus mastichina*, *Thymus camphoratus* and *Thym*bra *capitata* grown in Portugal and collected during the vegetative phase of the plants, was examined by GC and GC/MS. The essential oils (0.1 g/100 g) were also tested in order to evaluate their antioxidant ability on different substrates (peanut and sunflower oils). BHT and BHA were used as reference compounds for comparison. A blank assay was prepared under the same conditions, without adding any antioxidant. The rate of oxidation was followed by periodic determination of the peroxide index in the oils stored at 60 ºC, for 78 days.

The essential oils isolated from *T. capitata* were dominated by carvacrol (71.9 %), whereas 1,8-cineole (49.4 %) was the major component of the essential oils from *Th. mastichina*. In contrast, linalool (16.6 %), linalyl acetate (15.2 %) and 1,8-cineole (10.8 %) were the three main compounds detected in the oils from *Th. camphoratus*.

For peanut oil, BHT was found to be the most effective antioxidant leading to a peroxide index value of 52.3 meq O_2/kg at the end of the assay. Concerning the essential oils, during the same time, values ranging from 142.3 meq O_2/kg (*Th. camphoratus*) to 152.0 meq O_2/kg (*T. capitata*) were found. For sunflower oil, considering the end of the experiment (78th day), neither BHT (193.0 meq O_2/kg) nor BHA (204.3 meq O_2/kg) revealed to be effective antioxidants. The lowest peroxide vaues, 170.7 and 172.3 meq O_2/kg, were found for oils of *Th. mastichina* and *Th. camphoratus*, respectively. It is also noteworthy the better performance of all tested essential oils in preventing the oxidation of peanut oil, when compared with that recorded for sunflower oil, that, to some extent, can be related to the lipid composition of these two substrates (Table 1).

Table 1. Antioxidant effect (peroxide index, meq O_2/kg oil) of the essential oils, BHT, BHA and blank on peanut and sunflower oils, stored in the dark at 60ºC, at day 78th.

	Peanut oil	**Sunflower oil**
Thymus mastichina	145.0	170.7
Thymus camphoratus	142.3	172.3
Thymbra capitata	152.0	181.7
BHT	52.3	193.0
BHA	155.7	204.3
Blank	172.3	200.7

P. 88. Chemical composition and radical scavenger capacity of fennel (*Foeniculum vulgare* Mill.) fruits essential oil and fractions

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Fennel (*Foeniculum vulgare* Mill, Apiaceae) is a well-known medicinal plant, native to the Mediterranean, but extensively cultivated all over the world (Wichtl, 1994). In phytotherapy the fruits (*Foeniculi fructus*) and essential oil (*Foeniculi aetheroleum*) are used in catarrhs of the upper respiratory tract, as a secretomotor, secretolytic, and antiseptic expectorant, as a spasmolytic and carminative in mild digestive disorders. Externally it is used as an eye lotion (decoction) and in functional visual disorders (Wichtl, 1994; Blumenthal, 1998). In the present study we investigated the radical scavenger capacity (RSC) of fennel fruit essential oil and fractions obtained from it.

The ground fennel fruits were submitted to steam distillation in a Clevenger apparatus. The obtained Essential Oil (F) was submitted to liquid chromatography in a glass column with teflon stop (60 cm; 3 cm i.d.) filled with silica gel. The sample was chromatographed with 200 ml pentane, 200 ml pentane: Et_2O (1:1), and 200 ml Et_2O. Fractions of 50 ml were collected, obtaining 12 fractions. The fractions were analysed by TLC on silica gel sheets, and similar fractions were put together obtaining 6 fractions (F1, F2, F3, F4, F5 and F6). The chemical composition of the oil and its fractions was analysed by GC/MS. The oil and the obtained fractions were investigated on their RSC measuring spectrophotometrically the disappearance of $DPPH^{\bullet}$ at 515 nm according to the procedure described by Soler-Rivas *et al.*, 2000.

According to the results of the GC/MS analysis, the fennel fruit essential oil (F) comprise phenylpropanoids (76.1%), oxygenated monoterpenes (16.8%) and monoterpene hydrocarbons (6.2%), with *(E)*-anethole (72.3%), fenchone (16.4%) and methyl chavicol (3.8%) as the main compounds. The obtained fractions showed the following chemical profile: F1- monoterpenoid fraction with limonene as the main compound; F2 – phenylpropanoid fraction with *(E)*-anethole as the main compound; F3 – same as fraction F2; F4 – aromatic fraction with *p*-anisaldehyde as the main compound; F5 – fraction with similar amounts of oxygenated monoterpenes, benzyl derivatives and phenylpropanoids, with *(E)*-anethole and *p*-anisaldehyde as the main component; F6 – phenylpropanoid fraction with *(E)*-anethole as the main compound. The essential oil of fennel fruits showed very low RSC reducing the $DPPH^{\bullet}$ radical for 3.00% at the concentration of 125 μg/ml, while *(E)*-anethole as the main compound had even a smaller RSC. The fractions tested at the same concentration levels had RSC of 0.00, 0.00, 1.03, 20.20, 30.14 and 21.57% for F1, F2, F3, F4, F5 and F6, respectively.

According to these results it can be concluded that the carriers of RSC in the Fennel essential oil are the oxygenated monoterpenes, and that the reduction of the phenylpropanoid and benzyl derivatives content has a positive impact on RSC.

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P. 89. Essential oil of *Stellaria media* L. as possible contact dermatitis allergen

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Stellaria media (L.) Vill (Caryiophyllaceae) is a plant widespread all over the world, and considered as one of the commonest weeds. In folk medicine is used as diuretic, astringent, secretolytic and cough alleviating (Fournier, 1948). Although the medicinal properties of this plant is known for a long time, there are only limited reports on its chemical composition, mainly related to the flavonoid compounds (Budzianowski and Pakulski, 1988, 1991). In recent clinical study some cases of contact allergic dermatitis, type of erythema multiform, associated with this plant are described (Jovanović and Poljacki, 2001). Plant contact dermatitis, is primary provoked by the formation of the hapten (electrophhiles)-protein (nucleophiles) conjugate. Among natural haptens, sesquiterpene lactones are of particular significance, responsible for allergenic reaction of Compositae plants. However, the other terpenes such are limonene, Δ^3-carene, terpineol, α- and β-pinene, could also act as contact dermatitis allergens (Benezra et al., 1985). Taking into account that lower terpenoids, abundantly presented in essential oils, could be responsible for allergenic reaction of *Stellaria media*, in the present study the chemical composition of essential oil was investigated.

The essential oil, isolated by hydrodistillation from the dried aerial parts of *Stellaria media*, collected in Vojvodina (North Serbia), was obtained in a yield of 0.02%, and analysed by TLC and GC/MS. Forty-three compounds were identified, representing 93.9% of the total oil, montorepenes being the major components. Among them, 1.8-cinole was dominant (25.3%), but considerable ratio of *p*-cymene (12.4%), nepetalactone (13.4%) and camphor (9.1%) was also found. Among sesquiterpenes, (*E*)-caryophyllene and (*E*)-caryophyllene oxide were the most abundant ones. The obtained results suggest that monotepenes, especial 1,8-cineole and the iridoide nepetalactone, could be responsible for the allergenic properties of *Stellaria media*. A detailed dermatological study using the essential oil and target compound is in the course.

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P. 90. Morphometry of glandular trichomes and yield of essential oils from *Lippia alba* (Mill) N. E. Br. ex Britt & Wilson (Verbenaceae) in function of the season and location of the leaves on the stem

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The present work had as objective verifies possible variations in the morphometrical of the trichomes main glandular revenue and essential of oil *Lippia alba* in function of the station make year and position of the leaves any branch. The parameters seasonal variation were considered (spring, summer, autumn and winter) and the location of the leaves in the axis stem (apical, medium and basal). Extractions of essential oil were accomplished through hydrodistillation. For the structural characterisation of the glandular trichomes, samples of the blade were included in resin glycol methacrylate and processed conventionally for analysis in through light (LM). The analysis morphometrical of the glandular trichomes was accomplished starting from eletronmicrografic obtained in scanning electron microscopy (SEM), digitate and analysed through the system of analyses KS300. The obtained data were submitted to statistical analyses (Tukey 5%). The largest revenue of the oil was obtained in the summer, in the leaves of the part apical of the branch; in these leaves, in the same station, the number of trichomes peltate was significantly larger, in both surfaces foliate; independent of the station and of the location of the leaves, the abaxial epidermis presented larger amount of glandular trichomes. They happened variations with relationship to the diameter and area of the head of the trichomes peltate between the different stations and location of the leaves, however, such variations were not significant. In the conditions of this work, there was a positive correlation between the amount of peltate trichomes and the revenue of essential oil.

P. 91. Composition of the essential oil of *Lonicera nummularifolia* Jaub & Spach

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The genus *Lonicera* belonging to the Caprifoliaceae family is represented by six species grown in Iran. *L. nummolarifolia* (syn: *L. persica*) is distributed in Anatolia, Iraq, Afghanestan and many parts of Iran (Rechinger, 1982). There are many reports on the pharmacological activity of *L. japonica.* The aqueous extract of *L. japonica* showed anti-inflammatory effects (Lee, 2001). Polyphenolic compounds isolated from *L. japonica* thurb inhibited platelet activation and showed cytoprotective effect on hydrogen peroxide-induced cell injury (Chang, 1992).

In this study the essential oil of *Lonicera nummulariifolia* Jaub & Spach was analysed by GC/MS. The yield of the oil was 0.04% (w/w). Eighty-three compounds represent 98.83% of the total oil. The main monoterpene component was linalool (2.9%), which can be seen in *L. japonica,* as the main component (Ji, 1990). The essential oil of plant consisted mainly of sesquiterpenes (80%). The main constituents of the oil were spathulenol (21.6%), α-muurolol (14.1%), E-caryophyllene (6.8%), α-cadinol (5.4%) and bicyclogermacrene (5.1%).

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P. 92. Metabolism of (+)- and (-)-limonenes to respective carveols and perillyl alcohols by CYP2C9 and CYP2C19 in human liver microsomes

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Limonene, a monocyclic monoterpene, is present in orange peel and other plants and has been shown to have chemopreventive activities. (+)- and (-)-limonene enantiomers were incubated with human liver microsomes and the oxidative metabolites thus formed were analysed using gas chromatography-mass spectrometry. Two kinds of metabolites, (+)- and (-)-*trans*-carveol (products by 6-hydroxylation) and (+)- and (-)-perillyl alcohol (products by 7-hydroxylation), were identified and the latter metabolites were found to be formed more extensively than the former ones with liver microsomes prepared from different human samples. Sulfaphenazole and flavoxamine and antibodies raised against purified liver cytochrome P450 (P450 or CYP) 2C9, that inhibit both CYP2C9- and 2C19-dependent activities, significantly inhibited microsomal oxidation of (+)- and (-)-limonene enantiomers. The limonene oxidation activities correlated well with contents of CYP2C9 and activities of tolbutamide methyl hydroxylation in liver microsomes of 62 human samples, while these activities did not correlate with contents of CYP2C19 and activities of S-mephenytoin 4-hydroxylation. Of 11 recombinant human P450 enzymes (expressed in *Trichoplusia ni* cells) tested, CYP2C8, 2C9, 2C18, 2C19, and CYP3A4 catalysed oxidation of (+)- and (-)-limonenes to respective carveols and perillyl alcohols. Interestingly, human CYP2B6 did not catalyse limonene oxidations, whereas rat CYP2B1 had high activities in catalysing limonene oxidations. These results suggest that both (+)- and (-)-limonene enantiomers are oxidised at 6- and 7-positions by CYP2C9 and CYP2C19 in human liver microsomes. CYP2C9 may be more important than CYP2C19 in catalysing limonene oxidation in human liver microsomes, since levels of the former protein are more abundant than CYP2C19 in these human samples. Species-related differences exist in the oxidation of limonenes in CYP2B subfamily in rats and humans.

P. 93. Volatile components from the roots of *Scrophulia ningpoensis* Hemsl

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Scrophulariae radix (Genzin in Japanese) is the dry roots of *Scrophulia ningpoensis* HEMSL. (Scrophulariaceae) and is cultivated as a medicinal plant in China. The plants have been used for the treatment of fever, swelling, constipation, pharyngitis, neuritis and laryngitis in the Chinese traditional medicine. Various iridoid glycoside and iridoido-related aglycones were isolated from *S. ningpoensis* and identified. [1 - 3)] In our previous papers, we isolated four cinnamic acid derivatives as suppressive compounds of SOS-inducing activity, which is one of the DNA repair systems, from this plant[4)]. The volatile components of several Chinese crude drugs have been investigated in our research on the flavour compounds or flavour ingredients.[5 - 8)] However so far, there is no report of the volatile components from *S. ningpoensis*, and odour has not yet been reported for this Chinese crude drug. In this report, the composition of volatile components from the radix of *S. ningpoensis* was investigated.

The composition of the volatile oil from the radix of *Scrophilia ningpoensis* has been investigated by capillary GC, GC-MS. The oil contained 103 volatile components of which 3.5 % were terpenoids. The main constituents were fatty acids, such as palmitic acid (25.4%), linoleic acid (10.0%), α-linolenic acid (6.1%), γ-linolenic acid (4.8%), *cis*-palmitoleic acid (3.9%), *trans*-oleic acid (3.5%), *cis*-oleic acid (2.7%), and *trans*-palmitoleic acid (1.2%).

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P. 94. Chemical analysis and antifungal activity of the essential oil of *Calea clematidea* (Asteraceae)

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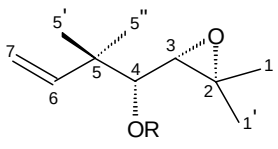
The genus *Calea* comprises *ca*100 species growing in the tropical and sub-tropical America, as herbs and shrubs. *Calea clematidea* Baker, Asteraceae, is a native shrub found in the southern region of Brazil and in Uruguay (Burkart, 1974). In Rio Grande do Sul, it is traditionally used by local people as anti-influenza agent and for the treatment of catarrh. In this work, we describe the study of the essential oils of the leaves and flowers of *Calea clematidea* from the state of Rio Grande do Sul, Brazil, a plant which has not been investigated previously.

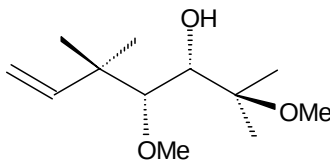
The chemical composition of the essential oils of *Calea clematidea* Baker obtained by hydrodistillation of the leaves and flowers was analysed by GC and GC/MS and assayed for their antifungal activities. The essential oil of the leaf had a high content of a new natural epoxi-terpenoid, named clemateol (*ca* 70%) (**1**), with minor amounts of *o*-vanillin (6.5%), spathulenol (4.2%), α-terpinene (4.0%), germacrene-B (2.9%), yomogi alcohol (1.8%), (*E*)-caryophylene (1.7%), *m*-cymene (1.6%), and α-gurjunene (1.5%), while the essential oil of the flowers was characterized by a higher content of thymol methyl ether (*ca.* 80%), with minor amounts of clemateol (4.8%) and *o*-cymene (4.7%). The antimicrobial activity of the oils was also evaluated against dermatophytes for their possible use in pharmaceutical preparations for topical applications. The oil of the leaves showed a moderate antifungal activity (MIC>3 mg/mL) against *Trichophyton tonsurans*, *Trichophyton rubrum*, *Trichophyton menthagrophytes* var. i*nterdigitale*, *Epidermophyton floccosum*, *Microsporum gypseum*, *Microsporum canis* and *Microsporum nanum.*

On alkaline hydrolysis, clemateol (**1**) afforded 2,5,5-trimethyl-2,3-epoxy-hept-6-en-4-ol (**2**)**,** identified by co-TLC, IR, ^{1}H- and ^{13}C-NMR. A Horeau determination of the absolute configuration indicated (*R*) configuration for the hydroxyl-bearing C-4 of compound **2**. To determine the absolute configuration of C-3 of **1**, the alcohol **2** was initially converted to the corresponding methyl ether **3** which was subsequently subjected to TCNE-catalysed alcoholysis in methanol to give **4** as a single stereoisomer (Masaki, 1993). The absolute configuration of C-3 of compound **4** was also determined as (*R*) by the horeau's method. Therefore, the stereochemistry of **1** was determined as (3*R*) and (4*R*).

In general, the essential oils have been reported to inhibit fungal growth. The results show that *Calea clematidea* essential oil obtained from the leaves, clemateol and compound **3** exhibited a moderate fungistatic and fungicidal property against dermatophytes. The oil of the flowers had no effect on the microorganisms tested.



1 R = $COCH_3$
2 R = H
3 R = OCH3



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P. 95. Essential Oils of Two Marchantiales Liverworts: *Targionia lorbeeriana* and *Asterella blumeana*

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Liverworts are known to be a rich source of terpenoids and phenolic compounds, which constitute the oil bodies. From these plants, a high diversity of substances has been isolated, including new structural types, bioactive compounds and unusual fragrances (Asakawa 1995).

Since only a few species of liverworts have been chemically investigated (less than 5% of known species), these plants constitute a phytochemical treasure where many interesting substances remain to be isolated. However, advances on the study of the chemistry of liverworts are hindered by the difficulty in collecting a sufficient quantity of pure material of certain species. Their small size and the fact that bryophytes usually grow mixed with other species and strongly attached by their rhizoids to the soil, make their purification a difficult and a time consuming procedure (Asakawa, 1995).

In vitro cultures of liverworts can be a good alternative to obtain biomass for the phytochemical analyses, since several studies with different species, suggested that the ability of *in vitro* grown liverworts to produce secondary metabolites, is similar to those found in field grown plants (Becker, 1994).

Recently we reported bioactive sesquiterpene lactones from the wild and *in vitro* grown *Targionia lorbeeriana* (Neves *et al*. 1999 and 2001) and triterpenoids from *in vitro* cultures of *Asterella blumeana* (Neves *et al*. 1998).

In this communication we describe the research on essential oils of *Asterella blumeana* and *Targionia lorbeeriana* by GC and GC-MS analysis of liverworts hydrodistillates.

The composition of essential oils of wild and *in vitro* grown *Targionia lorbeeriana* was compared. In both cases the monoterpenyl ester *cis*-2-methylene-3-(1-methylethenyl)-cyclo-hexanylacetate constituted the major compound. A significant number of compounds produced in the open were maintained in *in vitro* cultures, although a considerable number of other ones were not detected. Instead, under *in vitro* conditions, some new compounds were found which do not accumulate under wild conditions.

Essential oils produced by *in vitro* grown gametophytes, *calli* and suspension cultures of *Asterella blumeana* were evaluated. Monoterpene hydrocarbons constituted the majority of essential oils of plantlets, followed by oxygenated monoterpenes and oxygenated alkanes. Undifferentiated cultures were found to be unable to produce volatile terpenenoids.

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P. 96. Comparative aroma compound analysis of different essential oils of *Lippia rugosa* from Cameroon using GC-FID, GC-MS and olfactometry

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Lippia rugosa A. Chev. (syn. *L. adoensis* F.TW.A., *L. nigeriensis* Moldenke) is a robust woody perennial plant of the *Verbenaceae* family, up to 12 feet high, with large oblong-lanceolate bluish-green leaves, pleasant aromatic flowers, small, whitish in branched inflorescence. The upper surface of the leaves are rough to the touch, venation prominently rugosa, reticulate and the plant is found in the Savannah area of the Adamaoua province (Cameroon). *Lippia multiflora*, another species of the about 200 different *Lippia* species is common in western Africa, differs from *L. rugosa* by the upper surface of the leaves, which is smooth to the touch and venation obscure. The plant, locally named "Gossolhi", is used for medicinal purposes against many diseases, such as indigestion, rheumatism, fever, cough and jaundice.

Five essential *L. rugosa* oils from Cameroon were obtained by steam distillation and evaluated by professional perfumers for their aroma as follows: fresh old leaves: sweet, floral; fresh young leaves: sweet, floral (strong geraniol note); fresh flowers: sweet, floral with herbal- and spicy side-notes; dried leaves: fresh-fruity (strong limonene note), sweet, floral, weak woody and stems: sweet, floral, fresh-fruity side-notes.

The composition of the five essential oils of *Lippia rugosa* from Cameroon was investigated using GC-FID, GC-MS and olfactometry. As main compounds especially monoterpenes and sesquiterpenes were identified. Linalool, geraniol, geranial, limonene, myrcene, neral and linalool oxides as well as β-caryophyllene, *trans*-β-farnesene, germacrene-D, T-muurolol and caryophyllene oxides were found in higher concentrations.

A correlation of aroma compounds responsible for the characteristic odor impressions of each sample is additionally given.

P. 97. Contribution to the *Hypericum* L. genus chemotaxonomy in the mainland Portugal

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In recent years *Hypericum perforatum* L. (*Guttiferae)* has been studied worldwide. Pharmacological activities, including antidepressant, antiviral and antibacterial, provide supporting evidence for several of the established traditional uses reported (Barnes *et al*., 2001). In Portugal three species of *Hypericum* L. genus (*H. androsaemum* L., *H. perforatum* L. and *H. undulatum* Schousb. ex. Willd.) have been used for a long time.

Following previous phytochemical analysis of this genus (Nogueira *et al*., 1998, 1999), a comparative chemotaxonomical study of the *taxa* from the mainland Portugal is presented. This study relies on taxonomical characters, analysis of the aromas and chemical composition of essential oils of the following species: *H. androsaemum* L., *H. pulchrum* L., *H. montanum* L., *H. tomentosum* L., *H. pubescens* Boiss., *H. elodes* L., *H. perfoliatum* L., *H. linarifolium* Vahl., *H. humifusum* L., *H. undulatum* Schousb. ex. Willd., *H. perforatum* L., *H. calycinum* L. and *H. hircinum* subsp. *majus* (Aiton) N. Robson.

The analysis of the aromas was performed by olfactometry, using a sensor array detection device equipped with a multi-element array of 32 chemical sensors (Hodgins, 1997). The chemical characterization of essential oils was carried out by gas chromatography (GC) and GC coupled to mass spectrometry. The results were treated by multivariate analysis, classification and ordination techniques, namely cluster and principal components analysis.

Significant differences were found in the aromas and in the major components of *H. androsaemum* essential oils, by comparing to other *taxa*. *H. tomentosum* appears also as a separate group based alone on the essential oils characters. The group including all *taxa* but *H. androsaemum* and *H. tomentosum* reveals some compositional diversity of aromas and essential oils well correlated with morphological taxonomical characters.

Barnes J, LA Anderson, JD Phillipson (2001) *J. Pharm. Pharmacol.* 53: 583-600.
Hodgins D (1997) *Techniques for Analyzing Food Aroma* R Marsili Ed. Marcel Dekker New York 11: 331-371.
Nogueira T, F Duarte, F Venâncio, R Tavares, M Lousã, C Bicchi, P Rubiolo (1998) *Silva Lusitana* 6: 55-61.
Nogueira T, F Duarte, R Tavares, MJ Marcelo Curto, J Capelo, AC Freitas (1999) *Flavour and Fragrance Journal* 14: 195-199.

P. 98. Stereoselective formation of (1*R*, 2*S*, 4*R*)-(+)-*p*-menthane-2,8-diol from α-pinene by *Aspergillus niger*

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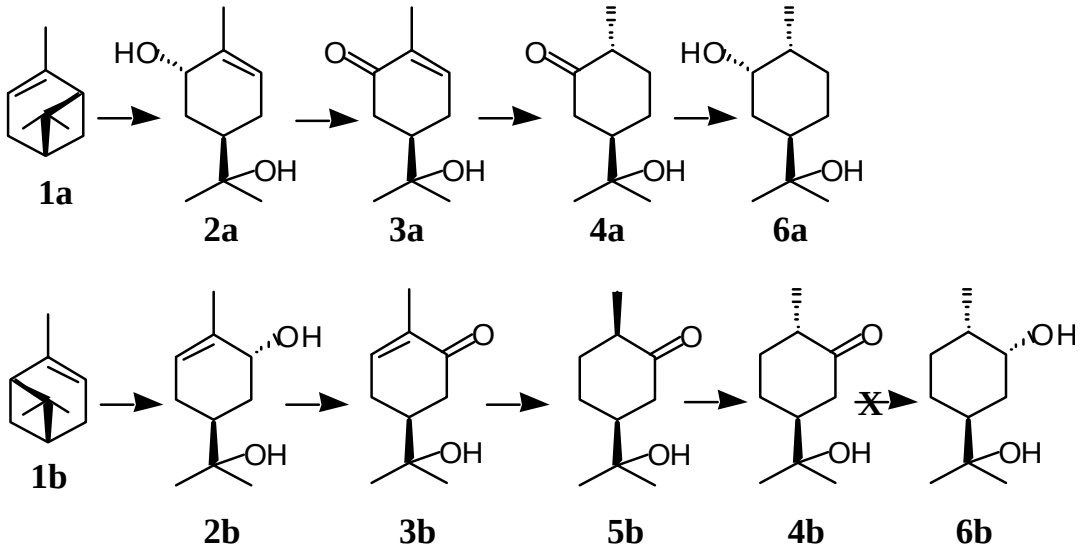
The biotransformation of pinenes has been investigated from view point of environmental sciences between microorganisms and terpenodoids in cedar and pine forests in Japan and production of functional substances (Noma & Asakawa 1994, 1995, 1998, 2000). *Aspergillus niger* converted (-)-α-pinene (**1a**) to (1*R*, 2*S*, 4*R*)-(+)-*p*-menthane-2,8-diol (**6a**) *via* (-)-*trans*-sobrerol (**2a**), (-)-8-hydroxycarvotanacetone (**3a**) and (+)-8-hydroxycarvomenthone (**4a**), together with (-)-vervenol, (-)-verbenone and (-)-α-terpineol. On the other hand, (+)-α-pinene (**1b**) was biotransformed enantio- and diastereoselectively except for the formation of (-)-8-hydorxyisocavomenthone (**5b**).



The present paper deals with the enantio- and diastereoselectivity of (-)-, (+)- and racemic *trans*-sobrerols (**2a**, **2b** & **2ab**) and (-)-, (+)- and racemic *cis*-sobrerols, (-)-, (+)- and racemic 8-hydroxycarvotanacetones(**3a**, **3b** & **3ab**) and (-)-, (+)- and racemic 8- hydroxy carvomenthone (**4a**, **4b** & **4ab**) by *A. niger*.in order to clarify the stereoselective formation of **6**. It was clarified that the stereoselective formation of **6** from **1** is due to the high substrate specificity of reductase towards the reduction of **4a**.

Noma Y, Asakawa Y (1994) In: *Biotechnology in agriculture and forestry. Medicinal and aromatic plants* VII (Bajaj YPS ed.) Vol. 28, p. 185-202. Springer Berlin, Heidelberg.

Noma Y, Asakawa Y (1995) In: *Biotechnology in agriculture and forestry. Medicinal and aromatic plants* VIII (Bajaj YPS ed.) Vol. 33, pp. 62-96. Springer Berlin, Heidelberg.

Noma Y, Asakawa Y (1998) In: *Biotechnology in agriculture and forestry. Medicinal and aromatic plants* X (Bajaj YPS ed.) Vol. 41, p. 194-237. Springer Berlin, Heidelberg.

Noma Y, Asakawa Y (2000) *Current Topics in Phytochemistry* Vol. 4, 63-78, Research Trends, Trivandrum, India

P. 99. Aromatic and medicinal plants of Sudan

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Sudan is the largest country in Africa with a land area of 2.5 million squares Km. It has three different climatic zones namely northern deserts, semi-arid central region and the tropical swamp and rain forest region of the south. Therefore Sudan enjoys a vast, diverse flora consisting of 3173 species of flowering plants belonging to 170 families and 1280 genera.

Medicinal and Aromatic plants and their derivatives are on sale in special shops called "Atareen" but there is a very little cultivation of aromatic plants in Sudan. They are collected from plants that grow wildly and therefore supply is irregular.

The poster covers examples of essential oils-containing or fragrance-bearing species. It is presented in a form table indicating the plant family, plant name (Latin, English and Arabic), plant habit and habitat (photograph or line drawing for the species) part used and type of the essential oil, fragrance or flavouring agent present in this plant part.

The main objective of this poster is to give an idea of the potentiality of investing on aromatic plants and their cultivation and utilisation in Sudan.

Andrews, F. W. (1950, 1952 and 1956), The Flowering Plants of Aglo-Egyptian Sudan. (Three Volumes)

P. 100. Investigation of essential oil content of crude drugs from lovage (*Levisticum officinale* Koch.)

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The essential oil content of species of the *Apiaceae* family and among them the essential oil content of *Levisticum officinale* is influenced by different biological and technological factors (Bylaite et al., 1998; Stahl-Biskup and Witchmann, 1991; Szebeni and Galambosi, 1992; Székely et al., 2001). Our investigations were focused on determining the role of some factors on the essential oil content and composition of different crude drugs of lovage. These factors were the followings: plant organ, age of plant, phenophase and harvesting time.

The experimental populations were grown under open field conditions at the Experimental Station of the Department in Budapest. We investigated one-, two-, and three-year-old populations of *Levisticum officinale* KOCH. of unknown origin from our gene-bank collection in 2001. Average leaf samples were collected in ten days' interval from July till November in case of one- and two-year-old populations and root samples were taken from the end of August till the end of October in ten days' interval from each population. Seed samples were taken in the two-year-old population in five different phenophases (green fruit, milky fruit, waxy fruit, ripe fruit and over ripe fruit stage). Essential oil content of crude drugs was determined by hydrodistillation. The main components of the oil were analysed by capillary gas-chromatography using standards for identification.

Our results proved the significant effect of the age of the plantation and a difference in oil accumulation also between plant organs, which is in harmony with former findings (Bylaite et al., 1998; Szebeni and Galambosi, 1992). In case of root samples the highest essential oil content was found in the three-year-old population (0,5-0,7% d.w.), compared with the two- and one-year-old populations (0,2-0,5% d.w.; 0,1-0,3% d. w., respectively). In case of leaf samples the effect of the age was not significant (between 1,3-2,4%). The highest volatile oil contents were found in the leaves in the second decade of August. In case of the roots the maximum was reached in the end of October (one- and three-year-old stands) and in the end of August (two-year-old stands), which requires further investigations. As for the fruits, the highest volatile oil content was found at green stage (5,9% d. w.).

The effect of harvesting time, age of plantation and phenophase on the accumulation of the main essential oil components (phtalide component in root, α-terpinyl-acetate in leaves and β-phellandrene in the seeds) could not be proved.

Bylaite E, Venskutonis RP, Roozen JP (1998) Influence of Harvesting Time on the Composition of Volatile Components in Different Anatomical Parts of Lovage *J. Agr. Food Chem.* 46: 3735-3740.

Stahl-Biskup E, Witchmann EM (1991) Composition of the Essential Oils from Roots of some Apiaceae in Relation to the Development of their Oil Duct System *Flavour Fragr. J.* 6: 249-255.

Szebeni-Galambosi Zs, Galambosi B (1992) Growth, Yield and Essential Oil of Lovage Grown in Finland. *J. Ess. Oil Res.* 4: 375-380.

Székely G, Bernáth J and Zámboriné Németh É (2001) Floral-Biological Characteristics and Fruit Development of Fennel World Conference on Medicinal and Aromatic Plants, 8-11 July, Budapest, Hungary, Abstracts: 125.

P. 101. Comparison of fruit development features from fennel populations of various age

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Fennel (*Foeniculum vulgare.* Mill) is one of the important species of the Apiaceae, which is used in large amount in phytotherapy and food industry worldwide and in Hungary as well.

In this respect fennel cultivar (*Foeniculum vulgare* subsp. *capillaceum* var. *vulgare)* 'Soroksári' and a population of genebank origin ('F86') were analysed on individual level. In the course of 2001 fruit development of plant stands being in the first, second and third vegetation cycles was examined.

In order to characterise the fruit development processes, fruits from the 1st range umbels were taken and analysed, continuously. The sampling was conducted from the start of the fruit development (from the end of fertilisation) until the full ripening.

Essential oil content of the fruits was determined by hydrodistillation in Clevenger-apparatus according to the method of Ph. Hg. VII. Relative percentage of the oil constituents was calculated from the GC (Shimadzu GC-B14) peak areas in percent of the total area.

It is a characteristic phenomenon of the essential oil accumulation, that its quantity and quality in the fruits change in relation to the increase of total dry matter during the ontogenesis. The essential oil yield is higher at the beginning of fruit development, then decreases along with the formation of endosperm (Bernáth *et al*., 1999, Gleisberg, 1960).

The essential oil content of fruits either collected in the first, or in the second vegetation cycles is the highest in green seed stage and it shows a decreasing tendency in case of waxy and ripe seed stages, in close agreement with the data published in the literature (Kattaá, 1996, Bernáth *et al*., 1999). However, in case of investigated populations, the maximum oil content in the third vegetation years was measured in full ripe stage.

According to our investigation, the content of anethole and methyl chavicol shows an increasing tendency during the fruit development, while that of fenchone, limonene, α-pinene and β- pinene decreases.

Our results on the content of anethole, methyl chavicol are partially identical to those reported in the literature, mainly because the accumulation level of the components highly depends on the taxa.

Acknowledgements: The research was supported by Hung. Nat. Sci. Found (OTKA T-034289).

Bernáth J., Németh, É. Petheő F., Mihalik, E. Kálmán, K. Franke R. (1999): Regularities of the essential oil accumulation in developing fruits of fennel (*Foeniculum vulgare* Mill.) and its histological background, J. Essent. Oil Res. 11:431-438.

Kattaá A., 1996: Morphological and production-biological study of fennel (*Foeniculum vulgare* Mill.) and caraway (*Carum carvi* L.) populations of different origin, PhD. Dissertation, Univ. Hort. and Food Industry, Budapest, pp. 60.

Gleisberg, W., R. Hartrott, 1960: Die Reifungsvorgang in der Fenchelfrucht Garten- beauwissenschaft, 7: 245-218.

P. 102. Determination of the growing location of marjoram (*Origanum majorana* L.) samples by comparison of essential oil profiles

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A canonical discriminant function analysis (CDA) was applied on essential oil compounds profiles to determine the growing location of marjoram samples.

Twelve out of 21 essential oil compounds were integrated into two discriminant functions as the best predictors for geographical differences. The stepwise integration allowed the determination of the essential oil compounds mainly responsible for the geographical variation, namely myrcene, terpinene-4-ol, γ-terpinene and *p*-cymene.

The two discriminant functions themselves showed a strong association between the locations and the predictors. The two functions allowed a correct classification of 95% of samples (crossvalidated).

The high positive classification rate demonstrates the CDA's ability as quality assurance tool enabling authenticity control.

P. 103. Volatile components of the New Zealand liverwort *Hymenophyton flabellatum* (Hymenophytaceae)

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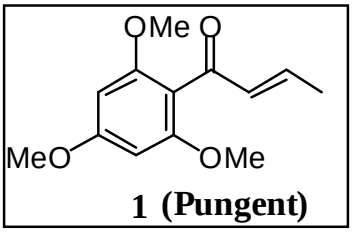
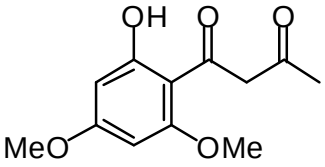
The New Zealand liverwort *Hymenophyton flabellatum* (Hymenophytaceae) grows on shaded wet soil, humus and old logs in forest, usually in shade, and on banks beside streams. It contains 1-(2,4,6-trimethoxyphenyl)-but-2-en-1-one (**1**), responsible for the characteristic hot taste of this liverwort, together with β-caryophyllene (Asakawa *et al*. 2001). Further fractionation of the volatile components of the ether extract of *H. flabellatum* resulted in the isolation of five 1-(2,4,6-trisubstituted phenyl) but-1-one derivatives (**2-6**) and three (**7-9**) (Toyota *et al*. 2002). The structures of the newly isolated compounds were elucidated by ^{1}H and ^{13} CNMR spectral analysis.

Compound **1** has been found in the Japanese fern, *Arachinoides standishii* (Tanaka et al. 1980), and compound **1** and **5** in the aquatic Angiosperm, *Dysophyla verticillata* (Chakrabarti & Chakraborty 1988). As far as we are aware this is the first record of the isolation of phenylbutenones and chromones in liverworts.



1 (Pungent)



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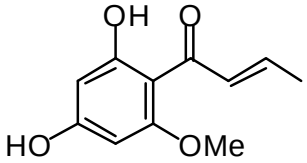

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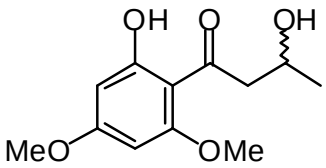

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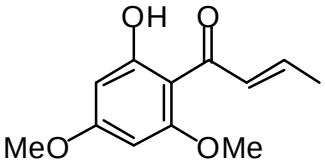

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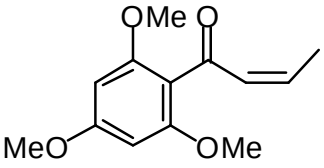

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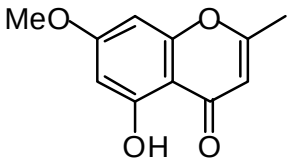

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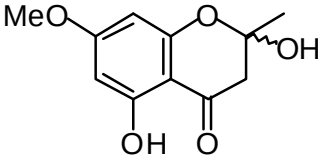

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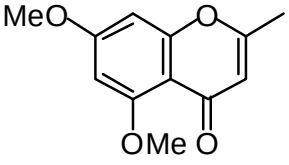

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Asakawa Y, Toyota M, Oiso Y, J Bragins (2001) *Chem. Pharm. Bull.*, **49**, 1380-1381.
Chakrabarti A, Chakraborty DP (1988) Phytochemistry **27**, 3683-3684.
Tanaka N, Maehashi H, Saaito A, Murakami T, Saiki Y, Chen C-M, Iitaka Y (1980) *Chem. Pharm. Bull.* **28**, 3970-3077.
Toyota M, Omatsu I, Asakawa Y (2002) *Chem. Pharm. Bull.* (to be submitted).

P. 104. The essential oil of *Cyclotrichium longiflorum* Leblebici

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The genus *Cyclotrichium* (Boiss.) Manden. et Scheng. is represented in Turkey by six species (Davis 1982, 1988). *Cyclotrichium longiflorum* Leblebici was described in the 1st supplement of the Flora of Turkey as a new record for Turkey (Davis 1988). The plant material was collected from South Eastern Turkey.

Water-distilled essential oil from the air-dried aerial parts of *Cyclotrichium longiflorum* Leblebici was analysed by GC and GC/MS. Isopinocamphone (34.8%), β-pinene (17.3%) and pinocamphone (8.4%) were found as major constituents.

Davis PH, *Flora of Turkey and the East Aegean Islands*. Vol. 7, p. 346-9, University Press, Edinburgh (1982).
Davis PH, *Flora of Turkey and the East Aegean Islands*. Vol. 10, p. 208, University Press, Edinburgh (1988).

P. 105. Seasonal variation in the chemical composition of *Cistus albidus* L. from Spain

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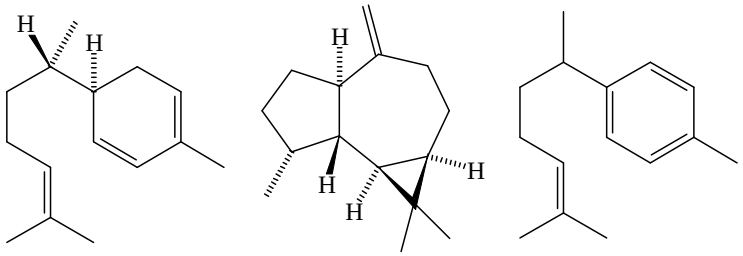
The genus *Cistus* L., belongs to the Cistaceae family and, it comprises 12 species in Spain. This genus is widely distributed in Mediterranean area and their species are considered as pioneers in disturbed environment. *Cistus albidus* L., is an evergreen woody shrub up to 1.5 meters. It grows in calcareous and siliceous soils and it is well represented all throughout Spain although it is more common in the mid South (1).

The terpene content and emission of *Cistus albidus* have been previously studied under field conditions (2), but as far as we know this is the first report about the seasonal variation on the chemical composition of the oil of this species extracted by hydrodistillation and analysed by Gas-Chromatography (GC) and GC-Mass spectrometry.

We have analysed the chemical composition of two samples of the same population of *Cistus albidus* gathered in Spain, one on winter and the other one on spring season. The samples were divided in leaves, stems, seeds and flowers according to the season of the year. The seed oils of this species were the part of the plant with less yield and constituents. All the samples were richer in sesquiterpenes than in monoterpenes. We have found quantitative but not qualitative differences.

The principal compounds of the winter leaves and stems oils were identified as α-zingiberene (14.8%), aromadendrene (10.5%), *ar*-curcumene (10.5%) and guaiol (6.5%) while the essential oil from the spring leaves and stems showed as main constituents aromadendrene (9.2%), *ar*-curcumene (8.8%), α-zingiberene (8.0%), guaiol (7.0%), (*E*)-caryophyllene (2.4%) and α-*trans*-bergamotene (1.8%). Finally the flower oils also contained these components but *epi*-α-muurolol (8.4%) and γ-selinene (5.4%) were identified as the principal ones.

Main constituents



α-Zingeberene Aromadendrene ar-Curcumene

1.- Castroviejo, S.; Aedo, C.; Cirujano, S.; Laínz, M.; Mntserrat, P.; Morales, R.; Muñoz Garmendia, F.; Navarro, C.; Paiva, J.; Soriano C. 1993. *Flora Ibérica, plantas vasculares de la Península Ibérica e Islas Baelares.* Real Jardín Botánico, C.S.I.C. Madrid.

2.-. Llusia, J. and Puñuelas J. 2000. *Seasonal patterns of terpene content and emission from seven Mediterranean woody species in field conditions.* American Journal of Botany. 87, 133-140.

P. 106. Essential oil composition of the seasonal heterophyllous leaves of *Eryngium vesiculosum* Labill from Australia

Jesús Palá-Paúl[a], Joseph J. Brophy[b], Lachlan M. Copeland[c], M[a] José Pérez-Alonso[a], Arturo Velasco-Negueruela[a]

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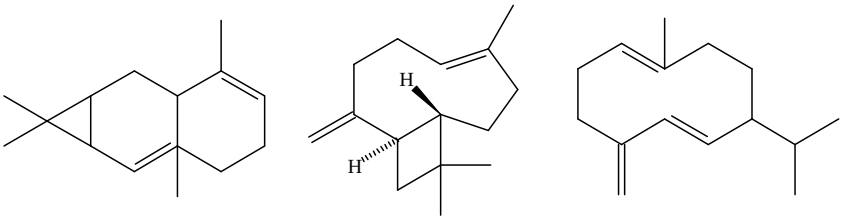
Eryngium vesiculosum Labill., commonly named prostrate blue devil or prickfoot, is a perennial herb of coastal sands, lake margins and river beds in Australia and New Zealand (1). It has different leaves according to the seasons of the year. Summer leaves are laminoid and prickly while winter ones are linear entire and fascicular (2). Two samples of each Australian season of the same population have been gathered from the Northern Tablelands of New South Wales, Australia. The essential oil of the flowers and both kinds of leaves have been analysed by GC and GC-MS. We have found quantitative but not qualitative differences. The principal compounds of the winter leaves were identified as β-caryophyllene (20.3%), germacrene-D (19.2%) and α-humulene (8.8%) while the summer ones showed bicyclogermacrene (22.2%), β-caryophyllene (15.6%), germacrene-D (15.8%) and α-humulene (8.1%) as major constituents. Bicyclogermacrene has changed its minor percentage composition in winter leaves oil (2.4%) to a principal compound in the other ones (22.2%). The flower oil contained practically the same composition of the summer leaves. Although we have previously reported the essential oils of some *Eryngium* species (3), this is the first report about the chemical composition of the flower and the different sort of leaves of *E. vesiculosum*.

Main constituents



Bicyclogermacrene β-Caryophyllene Germacrene D

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P. 107. *Agastache foeniculum* (Pursh) O. Ktze. essential oil composition and its antibacterial activity

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Agastache foeniculum (Pursh) O. Ktze. is a perennial herb native to North America which is also cultivated as a decorative plant or used as a spice due to its anise-like aroma. Leaves are opposite, almost glabrous, whitish underneath, flowers purplish in terminal spike-like inflorescence.

It is closely related to east Asian (China, Japan, Korea) species *A. rugosa* (Fisch et Mey.) O. Ktze. which is one of the main medical herbs in Chinese traditional medicine and was scientifically surveyed as such, for example as antimycotic and anti-HIV drug (Blaszczyk *et al*., 2000; Kim *et al*., 1999, 2001).

The aim of this study is to determine the main essential oil components of *A. foeniculum* grown in Croatia and to test the antibacterial activity of the essential oil. Test bacteria were: *Bacillus subtilis*, *Staphilococcus aureus*, *Klebsiella* sp., *Seratia marcescens*, *Escherichia coli*, *Citrobacter* sp. and *Proteus mirabilis*. The plates were incubated 24 h at 37°C except for *S. marcescens*, which was incubated at room temperature. Blind control was used to monitor normal bacterial growth under the same conditions.

The oil was obtained from dried plant material by hidrodistillation using a modified Clevenger apparatus for 4 hours. The analysis of the essential oil by GC and coupled GC-MS showed the main components to be estragole (92.5%) and limonene (4.2%) while all other components were present in less than 0.5%. In some former studies (Fuentes-Granados *et al*., 2000) myrcene was found as the third main component, present in our sample only in 0.05%. Total oil yield of the flowering plant was somewhat less than 2% of the dry plant weight.

Preliminary antibacterial study, performed using the technique of antibiograms, showed strong activity against *B. subtilis*, some activity against *S. aureus* and *Klebsiella* sp., discoloration and growth inhibition of *S. marcescens* and strong growth inhibition, without specific zones of inhibition, against *E. coli*, *Citrobacter* sp. and *P. mirabilis*. From these results, it can be concluded that the volatile components strongly inhibit the growth of different bacteria, rather by completely killing the bacteria due to the diffusion through the substrate. Further studies on other bacteria, but also some pathogenic fungi, would be interesting.

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P. 108. Comparison of essential oil composition and antibacterial activity between some Croatian wild growing plants and commercially available oils

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Three common aromatic species growing wild and commonly planted in Croatia - Dalmatian sage, rosemary and lavender - were chosen to compare their essential oil yield and composition against some commercially available oils of the same or related species.

Wild growing plants were collected in May/June on the south Croatian island Korčula; lavender and sage were collected during flowering period. Lavender was determined as *Lavandula latifolia* Med. which grows wild in Croatia but is very rare. Rosemary was determined as *Rosmarinus officinalis* L., which grows wild on some south Croatian islands in the typical rosemary garigue. Previous literature data state that although the same species, rosemary can vary significantly in the oil composition (Bego 1995; Schnaubelt, 1998, 1999). Dalmatian sage, *Salvia officinalis* L., is native to east Adriatic coast where it grows on rocky grasslands, but was spread throughout the whole Mediterranean. Oils were obtained by hidrodistillation using a modified Clevenger apparatus.

Two sets of commercially available oils were used for comparison of the essential oil composition - one set of oils which stated they are produced from Adriatic plants and another set of imported oils. The oils were analysed by GC to screen main components.

Antibacterial activity of the oils from wild growing plants was tested using the imported oils as a comparison and control for monitoring normal bacterial growth. The test bacteria were *Escherichia coli* and *Staphilococcus aureus*, and all the plates were incubated for 24 h at 37°C.

Wild growing plants showed the following oil characteristics: lavender yielded 5.5% essential oil from dried flowers and the main components were linalool (30.8%), borneol (18.4%) and 1,8-cineole (17.0%), while the total esters were only 6.7%, from which 5.6% was linalyl-acetate; rosemary had an oil yield of 2.7%, having as main components 1,8-cineole (34.8%), borneol (13.8%) and α-pinene (12.3%); and sage had an oil yield of 2.0%, with thujones (34.5%), 1,8-cineole (20.1%) and camphor (11.3%) as main components. Commercial samples of lavender oil showed the following results: imported lavender, declared as *L. angustifolia*, had very low linalyl-acetate (less than 6.0%) and the major components were 1,8-cineole (16.0%), linalool (40.0%), borneol (10.0%) and camphor (4.0%), while the Adriatic lavender (lavandine) had 8.0% linalyl-acetate, with major components being linalool (47%), 1,8-cineole (8.5%), borneol (8.5%), terpinen-4-ol (7.5%) and only 2.0% camphor. Adriatic sage oil was constituted of 44.0% thujones, 14.0% camphor and 11.0% 1,8-cineole, and imported sage of 29.0% thujones, 20.5% camphor and 14.5% 1,8-cineole. Adriatic rosemary oil showed 46.0% 1,8-cineole, 12.0% α -pinene, 6.5% borneol and 7.0% camphor, while the imported oil had 46.0%, 1,8-cineole, 12.0% α -pinene, 6.0% borneol and 6.8% camphor, which are practically the same results.

Antibacterial tests showed higher activity for imported rosemary oil on *E. coli*, but lower on *S. aureus*; almost the same activity against both bacteria by lavender and the strongest bactericidal activity of both sage oils, especially against *S. aureus*.

This study reveals the necessity of perpetual testing of each oil batch against target bacteria.

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P. 109. Composition of the essential oil of *Crithmum maritimum* L. grown in Turkey

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Crithmum maritimum L. (Apiaceae), the only species of the genus, can be found along the coast of Europe, Africa, Asia and Australia. In Turkey fresh leaves and young branches are used as a condiment and pickle and, in popular medicine, as an appetizer and diuretic (Baytop, 1984; Özcan, 2000). According to Guenther (1950), early studies on *C. maritimum* oil showed that all parts of the plant contain volatiles, but the yield and the chemical composition of the oils varied considerably depending on the site of growth and soil conditions. A study of the seasonal variation in the composition of the essential oil of *C. maritimum* showed major fluctuations in the relative amounts of several components, mainly sabinene (7-42%) and γ-terpinene (26-55%) (Barroso *et al.*, 1992). Other reports on essential oils from *C. maritimum*, collected in Italy and Portugal, showed also different compositions (Flamini *et al*., 1999; Pateira *et al*., 1999).

The essential oil isolated from plants collected at Bolu-Abant, in Turkey, showed sabinene (27%), limonene (24%), γ-terpinene (19%) and terpinene-4-ol (9%) as the main components (Baser *et al*., 2000). Senatore *et al*. (2000) reported on the composition of the oils from plants collected at Antalya and at Mersin, Turkey. The former showed β-phellandrene (30%), methylthymol (25%), *cis*-β–ocimene (14%) and *p*-cymene (13%) to be the major components while the latter was characterised by γ-terpinene (24%), dillapiole (21%), β-phellandrene (14%) and sabinene (12%). In another study, Özcan *et al*. (2001) found sabinene, γ-terpinene, methylthymol, terpinen-4-ol, dillapiole and *p*-cymene as major components.

The young leaves and the branches of *C. maritimum* were collected, during the first week of June 2001, at late vegetative phase, in icel (Sipahili and Yesilovacik), at sea level. The plant material was submitted to hydrodistillation using a Clevenger-type apparatus and the oil yields were 0.20% for Sipahili (S) and 0.23% for Yesilovacik (Y). The monoterpene hydrocarbon fraction was dominant in both oils, attaining 90% (S) and 80% (Y) of the total oils. The main components were γ-terpinene (S- 36%; Y- 88%), β-phellandrene (S- 21%; Y- 22%), sabinene (S- 13%; Y- 9%) and *p*-cymene (8% in both oils). The oxygen-containing monoterpenes attained 10 and 9%, respectively, and were dominated by methylthymol (S- 8%; Y- 9%). The only sesquiterpene detected in both oils was germacrene D (S- 0.2%; Y- 0.3). Dillapiole, the only component in the phenylpropanoid fraction, was detected in trace amounts in the oil from Sipahili but attained 10% in the oil from Yesilovacik.

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P. 110. Essential oils of *Origanum vulgare* subsp. *hirtum* growing wild in Turkey

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Wild marjoram (*Origanum vulgare* L.) is a perennial bushy herb, which grows wild in Western and Southern Turkey and in other Mediterranean countries (Davis, 1982).

Origanum species have been used in medicine and as spice and condiment since antiquity, mainly because of their essential oils, which contain considerable amounts of carvacrol and thymol. The volatile aromatic compounds are employed in the food industry as a flavoring. The oil is used in perfumery for its spicy herbaceous notes. *O. vulgare* is well known as a plant with a medicinal value and as such is accepted officially in a number of countries.

The aerial parts of *O. vulgare* subsp. *hirtum* were collected at two different locations, Büyükeceli (İçel; sea level) and Delikkaya (İçel; 400 m). The essential oils, isolated by hidrodistillation from the air-dried aerial parts, using a Clevenger-type apparatus, were obtained in yields of 3.0% and 4.7% (v/w), respectively. GC and GC/MS analyses lead to the identification of twenty-eight components, accounting for more than 99% of the total oils. Monoterpenes constitute the main fraction, amounting to 98% in both oils, with carvacrol (51% and 63%), linalool (28% and 2%), γ-terpinene (6% and 10%) and *p*-cymene (5% and 9%) as the main components, respectively.

As reported previously (Fleischer and Sneer, 1982; Sezik *et al.*, 1993; Başer *et al.*, 1994; Özcan and Chalchat, 2002) carvacrol (up to 79%), *p*-cymene (20%) and γ-terpinene (up to 16%) are the main components of *O. vulgare* subsp. *hirtum* oil.

Our results fit into the above-mentioned reported data, except for the content of linalool (28%) in the oil isolated from the plants collected at Büyükeceli.

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P. 111. Essential oils from *Pterospartum tridentatum*

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Pterospartum tridentatum WK. & Lge is a small genus of the *Papilionoideae* subfamily included in the *Cytiseae* tribe, subtribe *Genistinae* (Talavera *et al*. 1999). It is a shrub up to 100cm, with yellow flowers, alternate branches and coriaceous winged stems, which shows a characteristic anatomy and interesting epidermal features (Teixeira and Pereira 2001).

This plant is widely distributed in central west Iberian Peninsula and it is used in popular medicine in Portugal, namely for the control of *diabetes mellitus* type 2. Phytochemical studies performed on the aqueous extract of the flowered stem apices of *P. tridentatum* led to the identification of flavonoids (Vitor and Paulo 2001), which revealed some *in vitro* antioxidant activity (Vitor, pers. comm.).

The essential oils of *P. tridentatum* were isolated by hydrodistillation, from aerial parts of the plant collected during the flowering phase, to determine the oil yield, and by distillation-extraction to determine the constituents percentage composition. The analyses of the essential oils were performed by GC and GC-MS.

An yellowish oil was obtained from *P. tridentatum* in a yield of <0.05%. *cis*-Theaspirane (13%), *trans*-theaspirane (12%), octen-3-ol (11%), geraniol (5%), *n*-heptanal (5%), *n*-nonanal (5%), *cis*-rose oxide (4%) and linalool (4%) were the most representative components of the oil.

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P. 112. Investigations on drug production and essential oil properties of thyme (*Thymus vulgaris* L.) in different growing areas of Hungary

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Three populations of thyme (*Thymus vulgaris* L.), located in two regions of Hungary (in Budapest and in Transdanubia) were studied in detail regarding morphological, production biological and chemical properties during two vegetation periods (2000-2001).

The aim of our studies was to find individuals possessing high dry herbal mass and/or high level of essential oil yield including increased total amount of phenolic compounds (thymol + carvacrol), which may serve as a basis for developing new, highly productive Hungarian varieties in the future.

In the eastern Transdanubian region a younger and an older population were grown, while in Budapest we examined a population of the same age as the older Transdanubian population, paralelly. Twenty plants per each population were investigated individually. Each population existed on loose sandy soils differing mainly in the proportion of calcium carbonate (being the lowest in Budapest) and in macro element content (the young Transdanubian population was the best supplied).

Regarding morphological features, plant height changed by age, by harvesting period and by growing site. Higher values could be measured in Budapest, at first harvest and in younger population. Plant size diameter was affected only by growing site: Budapest appeared to be the better area in this respect.

The variability regarding individual dry mass production was considerable influenced by growing region, by age of the plants and by cutting period. We established that at the time of the first harvest the yields were about two-fold if compared with the second cutting.

Based on the Hungarian and international standards we have been found that most of the individuals examined in Budapest fulfil the required minimal essential oil content of 1.2 ml/100g. Moreover, our selection purpose, regarding 2 ml/100g essential oil level in average, could be reached or exceeded by some plants grown in Budapest, either. We detected the most constant essential oil composition in this site as well, where the total amount of phenolic compounds and that of the thymol were highly outstanding. The proportion of thymol and carvacrol changed considerably in the Transdanubian growing region not only individually, but also by cutting period. The essential oil yield was mainly affected by the dry mass.

According to high productivity and high accumulation level of essential oil, containing appropriate content of phenolic monoterpenes, several individuals has been selected in Budapest for further evaluation.

P. 113. In vitro evaluation of the antioxidant effect of the volatile compounds from *Satureja montana* L.

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Widely used artificial antioxidants, such as butylated hydroxytoluene and butylated hydroxyanisole are very effective in their role as antioxidants (Madsen *et al*. 1995). However, their use in food products has been failing off due to their instability, as well as due to a suspected action as promoters of carcinogenesis (Namiki 1990, Pokorny 1991). For this reason, there is a growing interest in the studies of natural additives as potential antioxidants. The antioxidant properties of many aromatic herbs are reported to be effective in this role mainly due to the presence of hydroxyl groups in their phenolic compounds (Herrmann *et al.* 1981, Brraco *et al*. 1981, Kramer, 1985).

Savory (*Satureja montana* L.) is an aromatic plant growing wild in the Mediterranean region of Croatia. With regard to the presence of phenolic compounds in its essential oil, savory is known to possess some biological, especially antibacterial, activity. As a part of an investigation of natural antioxidants from Dalmatian aromatic plants, in this paper we report the detailed study of chemical composition and antioxidant activity of the savory volatile compounds in order to find the fraction or component with the highest protection against lipid oxidation. The analyses of the total essential oil, as well as of its fractions, were made by capillary gas chromatography coupled to mass spectrometry (Hewlett-Packard GC/MS system). The evaluation of antioxidant power was performed *in vitro* by the β-carotene bleaching method (Pratt, 1980).

The antioxidant activity of volatile compounds from *Satureja montana* L. was compared with that of pure compounds. Antioxidant power decreased in the order BHT > α-tocopherol > CHO fraction > total essential oil > phenolic fraction > thymol > carvacrol > CH fraction. The CHO fraction was more potent than total essential oil and other fractions, but less effective than synthetic reference standard BHT and comparable in activity with natural antioxidant α-tocopherol. The fact that CHO fraction was more effective as antioxidant than phenolic fraction or its pure constituents thymol and carvacrol suggests that synergy among minor oxygen containing compounds plays a crucial role for antioxidant power of the savory essential oil. Also, the concentration of the oil influenced its antioxidant power, too. The control sample without addition of antioxidant oxidised most rapidly and descending bleaching rates were demonstrated for the increased concentration.

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P. 114. Marquesas Islands sandalwood concrete and biodiversity conservation of a forest species

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Santalum insulare (Bertero ex A. DC.) is an indigene sandalwood species from Marquesas Islands where 2 varieties have been distinguished by taxonomists: *S. insulare* var. *marchionense* and var. *deckeri* (Fosberg & Sachet 1985).

During the last 2 centuries, sandalwood has been heavily logged at the point it has disappeared from some islands and became quite rare on the others (Dening 1980).

Recently, a project to safeguard and multiply this natural resource in the field of forestry was initiated by the cooperation of the Territory of French Polynesia (Rural Development Service, SDR) and the International Cooperation Center in Agronomic Research for the Development (CIRAD). Concurrently, chemical analysis of wood samples gathered from 4 islands of marquesan archipelago have been done at the chemistry laboratory of the French Polynesia University (UPF).

Concrete from heartwood of 45 trees spared on 4 islands from Marquesas archipelago were analyzed by GC/MS method in order to estimate the quality of marquesan sandalwood and to put in relief eventual composition variability amongst sandal from different populations or islands.

Thus, multivariate analysis on the composition of the concrete volatile part put in evidence notable chemotype differences, depending on the geographical distance between islands but also inside the same island. Concentrations of main compounds (Alpha *et al.* 1996, 1997) were investigated and can be illustrated by variations of α- and β-santalol proportion between 15 and 60% and these of (*Z*)-nuciferol from 1 to 17% of the concrete.

So, before further genetic research, SDR initiated a program to develop sandalwood nurseries and plantations in order to conserve specificity of each sandalwood provenance. Following this rule, separated plantations from different chemotypes are done on each island.

Moreover, on islands where the sandalwood resource is very scarce, it might not be possible to multiply and replant local provenance. In this case, sandalwood from the closest chemotype will be provided to the island inhabitants.

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P. 115. *Origanum virens* L. essential oil preservation by encapsulation in starch

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Flavour encapsulation protects less-stable flavour components against oxidative and photochemical degradation. Stability of encapsulated flavours depends on the flavour constituents and on the encapsulating agent and process (Anandaraman and Reineccius, 1986).

Encapsulation flavour in starch seems to be an efficient method to preserve aroma compound. According Boutboul *et al*. (2002) aroma-starch interactions mainly result from an adsorption phenomenon involving hydrogen bonds and not from inclusion complexes.

The spherical aggregates produced by spray dryer of starch granules dispersions contain spaces in the form of interconnecting cavities that provide extensive porosity that can be used to protect aroma compounds. This kind of encapsulation is suitable to preserve aromas to be incorporated in foods intending to be subjected to processing at high temperatures.

More than 90% of the encapsulated flavouring on the market is produced by spray drying (Nussinovitch, 1997).

The aim of the present study is the encapsulation of the aroma of *Origanum virens* in a rice starch matrix produced by spray drying.

Starch spherical aggregates are produced by spray drying the dispersion of starch granules in water containing different bonding agents.

Essential oil is produced by steam distillation conducted for 60 minutes at atmospheric pressure. Essential oil was sprayed at different concentrations in the spherical aggregates of starch.

Retention and stability of the main aroma compounds were analysed by GC and GC-MS.

Main compounds of studied *Origanum* were: carvacrol (about 68 %), *p*-cymene (about 4.5 %) and myrcene (about 2.3 %). From the bonding agents tested, carboxymethylcelulose (CMC) produces a smaller size and a higher number of spheres being a good retention medium for essential oil compounds. From the preliminary results it is possible to conclude that the encapsulated essential oil in the starch matrix has a good stability.

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P. 116. Aroma volatiles of rhizomes and roots of cultivated *Rhodiola rosea* L. from Norway

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In a 3-year project, 5 different clones of rose root (*Rhodiola rosea* L.) collected from coastal and mountain regions of Norway have been cultivated at the Apelsvoll Research Centre, Division Kise[b]. Rhizomes and roots of female and male plants have been analysed by headspace solid-phase microextraction procedure (HS-SPME)[1,2,3] and gas chromatography coupled with mass spectrometry. As reported earlier, terpenic and alipathic volatiles were detected as the main aroma compounds contributing to the characteristic rose-like fragrance of the underground parts of *R. rosea*[1,2]. (*E*)-Pinocarveol (21.8 %) was detected as the main terpenic volatile in the roots of 3-year old plants, as well as cumic alcohol and myrtenol, whereas geraniol, cinnamaldehyde and cinnamyl alcohol showed highest concentrations in the tuberous rhizomes. These results were confirmed by recent HS-SPME analyses (May 2002) of fresh samples of cultivated rose root from Mid-Norway[4] with (*E*)-pinocarveol and myrtenol as the main compounds in the roots (22.6 % and 19.6 %, respectively). The volatiles of rhizomes were dominated by geraniol (42.1 %) and decanol (36.9 %). Although the drying process (48 h at 35°C) resulted in significantly reduced concentrations, the relative distribution of aroma volatiles was almost unaffected.

Only slight differences where observed when comparing 3-year old female and male plants from Apelsvoll Research Centre analysed by HS-SPME. Higher amounts of phenyl propanoids (cinnamyl alcohol, cinamaldehyde), oxygen-containing terpenes (cumic alcohol, cuminaldehyde) and alipathic volatiles (octanol and decanol) were detected in rhizomes and roots of *R. rosea*, whereas higher concentrations of characteristic volatiles such as myrtenol, geraniol and phenylethyl alcohol were observed in female plants.

The essential oil analysis of steam-distilled samples from dried plant material of 2-year old clones (2001) from Apelsvoll Research Centre showed that rhizomes consisted mainly of oxygen-containing terpenes such as geraniol (51.9 %), myrtenol (10.4 %) and the phenyl propanoid cinnamyl alcohol (9.1 %). Straight-chain aliphatic alcohols and acids occurred in minor amounts (10.8 %). These results will be supplemented by further investigations of male and female rose root plants in the summer of 2002, to gain further information about rose root essential oil as a potential source for the production of fragrance and perfume essences.

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P. 117. Analysis of essential oils and flavonoids of *Dittrichia viscosa* subsp. *revoluta* wild grown and micropropagated plants

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Dittrichia viscosa W. Greuter (Compositae) is a sub shrub widespread in Europe, especially on Mediterranean areas (Valdés, 1987). *D. viscosa* subsp. *revoluta* (Hoffmanns & Link) P. Silva & Tutinis is an endemic species of Portugal, only growing in the south of Portugal (Pinto da Silva and Tutin, 1973). This sub shrub can be found in waste places and it has been used for years in folk medicine topically as an anti-scabies, anti-inflammatory, and wound-healing agent (Font Quer, 1973). Previous studies reported that this plant has also an anti-ulcerogenic effect, related to its flavonolic components (Alarcon de la Lastra *et al*., 1993), as well as an anti-inflammatory effect due to the terpene composition of the essential oils (Mañez *et al*., 1999).

The present contribution reports on the main components of the essential oils from field-grown plants (FP), *in vitro* shoot cultures (InV) and micropropagated plants (MP), obtained from the same clone, determined by capillary GC and GC-MS. This work also describes *in vitro* vegetative propagation of *D. viscosa* subsp. *revoluta*, an endemic Portuguese species, from mature field-grown plants.

The yield of essential oil from *D. viscosa* subsp. *revoluta* isolated from the original field-grown plant (FP) was 0.18% (v/wt). Yields lower than 0.05% (v/wt) were obtained from InV and MP of the same clone. The oils were shown to be a complex mixture of several components. Only 24 constituents were identified, representing 39.9, 42.0 and 49.5% of the total oils from FP, InV and MP samples, respectively. The major constituents of the essential oils were 1,8-cineole (11.0, 5.1 and 5.1%), δ-cadinene (5.0, 7.0 and 6.6%); *E*-nerolidol (4.4, 9.7 and 11.9%); T-cadinol (7.1, 14.4 and 17.4%) and α-cadinol (6.7, 8.4 and 9.4 %), for FP, InV and MP, respectively. Other constituents were found in minor amounts or even at trace levels. The flavonoid fraction was mainly constituted by quercetin with concentrations ranging from 0.3 mg/100 mg FW, in FP, to 48.5 mg/100 mg FW, in InV samples.

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P. 118. Essential oil of *Johrenia ramosissima* Mozaffarian from Iran

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Five species of the genus *Johrenia* are found in Iran, all of them endemic. The aerial parts of *J. ramosisima* Mozaffarian, a new endemic species in Iran, were collected from Marzan Abad-Chalous in the Province of Mazandaran in the north region of Iran, in July 1999. The dried aerial parts were hydrodistilled, for 3h, using a Clevenger-type apparatus to yield 0.1% w/w of oil. The oil was analysed by GC/MS. Thirty-five compounds, representing 89% of the total oil, were identified, with bornyl acetate (14.8%), *trans*-pinocarveol (12.5%) and α-campholenol (9.5%) as main constituents.

P. 119. Steam distillation of *Thymbra spicata* oil

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Thymbra spicata is a thyme-like plant widely grown and used in Turkey. It is usually between 10-50 cm long and it has a black-green colour. Flowers are pink-purple. Leaves have 10-15 mm length and 2-3 mm width. It has various aroma compounds in it. Generally carvacrol is the main compound but thymol chemotypes also exist. Oil of *Thymbra spicata* is used for many purposes such as flavoring of all kinds of food products, as antioxidant and antimicrobial for foods, preparation of some liqueur, for scenting of the soaps and lotions, medical purposes, as an antiseptic, antiplasmatic and antimicrobial.

The spice essential oils have for long been used in seasoning formulations for their obvious advantages over ground spices (Heath and Pharm, 1978). Essential oils are generally uniform in flavor quality. They are sterile and free from all extraneous contaminants. They can readily be incorporate into liquid concentrates and emulsions.

Steam distillation is a special method used to separate high-boiling mixtures or to separate a material from a non-volatile impurity. In this process, temperature of volatilization is lowered by the injection of live or open steam into the charge (Pratt, 1967). Steam distillation is used to separate mixtures at a temperature lower than the normal boiling points of their constituents. In this way, it is similar to vacuum distillation and finds its main use in separating or purifying heat sensitive materials. The principle advantage of using steam is that the volatiles are easily condensed in water (Teranishi *et al.*, 1977). A further advantage of this process is that the steam displaces atmospheric oxygen and hence protects the material from oxidation (Krell, 1963).

The objective of the study is to study the effect of steam flow rate and particle size on yield and composition of essential oil obtained from *Thymbra spicata*.

For all conditions, during the first period of the distillation there is a rapid increase in the amount of oil, but then it remained almost constant. For both flow rates, size was found to be an effective parameter on yield. The higher oil yield was reached with higher flow rate. The composition of oil did not vary with time, size and flow rate.

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P. 120. Antifungal activity of *Thymus* oils on species of Dermatophytes

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Dermatophytoses are common superficial infections that can be found all over the world. Industrialized drugs used against these affections by official academic medicine are of high economical cost, have undesired secondary effects and fungi strains show each time greater resistance towards them.

Previous research of our team demonstrated the antifungal activity and some of the mechanism of action of some essential oils (EO) (Pina-Vaz *et al.* 2001) against *Candida* species. The aim of this study is to evaluate the antifungal activity of the EO of *Thymus vulgaris*, *T. zygis* subsp. *zygis* and *T. mastichina* subsp. *mastichina* (*Thymus* oils), used in folk medicine, in order to support its application as therapeutical agents in the treatment of dermatophytoses.

The composition of the oils was investigated by gas-chromatography and gas-chromatography/mass spectroscopy (Cavaleiro *et al.* 2001). The Minimal Inhibitory Concentration (MIC), determined according to the NCCLS protocol, M 38-P, was used to evaluate the antifungal activity against dermatophytes strains (*Microsporum canis*, *Trichophyton rubrum* and *Epidermophyton floccosum*). The antifungal activity of their major components was also evaluated. *T. vulgaris* and *T. zygis* oils showed similar antifungal activity, higher than *T. mastichina.* This is probably due to their chemical composition, (*T. vulgaris* mainly composed by carvacrol and *p*-cymene; *T. zygis* mainly composed by thymol and *p*-cymene; *T. mastichina* mainly composed by 1,8-cineole).

The MIC values were determined against three dermatophytes. Results are shown in the table.

	MIC µl/ml (v/v)		
EO/Compound	*Epidermophyton floccosum*	*Trichophyton rubrum*	*Microsporum canis*
Thymus vulgaris	0.078	0.078-0.156	0.078-0.156
Thymus zygis	0.078	0.078	0.078
Thymus mastichina	0.625	0.625	0.625-1.250
1,8-Cineole	5.0	2.5-5.0	5.0
p-Cymene	0.020-0.039	0.020-0.039	0.039
Carvacrol	0.078	0.078	0.039
Thymol	0.156	0.156	0.078

This study showed the antifungal activity of these EO on dermatophytes, particularly *T. vulgaris* and *T. zygis*, which may contribute to the scientific basis for supporting future therapeutical trials on cutaneous mycoses due to dermatophytes.

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P. 121. Hairy root cultures of *Levisticum officinale*: growth and essential oil production in a bioreactor system

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Levisticum officinale (lovage) is an Apiaceae herbaceous plant known for its aromatic properties. Lovage hairy root cultures, established as described by Santos *et al.* (2000) and maintained either in SH (Schenk and Hildebrandt 1972) or B5 (Gamborg et al. 1968) liquid medium, were inoculated in bubble-column bioreactors containing 1 litre of the same media. Each bioreactor was inoculated with 0.5g fresh weight of hairy roots (corresponding to about 24mg and 15mg dry weight, for SH and B5, respectively), and the experiments were performed in the dark at 24ºC. The hairy roots were harvested after 7, 14, 21, 28 and 35 days, and their growth determined in terms of fresh and dry weight. The essential oils from the hairy root samples were isolated by distillation-extraction and analysed by GC and GC-MS.

Hairy roots grown in SH medium attained a maximum fresh weight of 59g.L^{-1} (4g.L^{-1} d.w.), at the end of 28 days, while hairy roots grown in B5 medium showed a maximum fresh weight of 23g.L^{-1} (1g.L^{-1} d.w.), at the end of 35 days.

Falcarinol was the dominant component of the essential oils from both the SH- and the B5-grown hairy root cultures (10-53% and 10-72%, respectively), but differences were found as to the other major components. *trans*-β-Farnesene (2-20%), Z-ligustilide (9-17%), *n*-octanal (2-9%), γ-elemene (2-9%), Z-3-butylidenephthalide (1-6%) and pentylcyclohexadiene (traces-5%) were present in large relative amounts in the oils from the SH-grown hairy roots. Apart from falcarinol, the essential oils from the B5-grown hairy root cultures were dominated by Z-ligustilide (4-13%), *n*-octanal (2-11%) and limonene (traces-6%).

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P. 122. Biotransformation of terpenes – preliminary studies of microbial activity

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Terpene hydrocarbons can be considered as industrial waste due to their low flavour intensity and their toxicity to the environment. Special biological systems are able to convert terpenes into defined, valuable aroma and fragrance compounds with potential use for the food, cosmetics and pharmaceutical industries. Some of the microorganisms, especially the higher fungi of the ascomycetes and basidiomycetes, are known to have a marked capacity for the biotransformation and bioconversion of terpene precursors.

No terpenoids are produced biotechnologically on an industrial scale despite their often unique organoleptic properties. The main reasons lie in the chemical nature of the substrates and the target molecules: the low water solubility, high volatility and cytotoxicity of terpenes and terpenoids impede processing by conventional bioprocesses. Moreover, due to their microbial metabolic versatility one precursor molecule is very often converted into a wide variety of derivatives, partly representing useless or not readily separable by-products.

For this reason, to achieve an economically viable utilization of terpene resources, the main goal of this interdisciplinary research with different research institutes and industry is to develop an innovative bioprocess capable of reducing or even overcoming the aforementioned constraints. Diverse process features are being investigated, such as activity-controlled substrate feeding and in situ product removal strategies, to obtain long-term optimal growth conditions and thus maximum productivity.

The bio dry weight cannot be used to determine the activity of submerged fungi, either under normal growing conditions or under transformation conditions, due to the formation of pellets. A bioelectrochemical sensor was applied to determine the activity of basidiomycetes. During transformation the microorganisms show different activity depending on the substrate fed. The oxygen uptake rates under different conditions were measured and they can be used as an indicator of fungal activity.

Various fermentations have been performed to optimize the gassing technique and the feeding strategy in order to fulfil the demands of transforming fungi and to optimize the quality of the processed essential oil to be used in the food and cosmetics industry.

P. 123. Fast analysis of natural products by SPME-GC/MS

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Fragrances and flavours are important to natural raw materials based industries. Quantitative limitations and cultivation factors of plant and other natural resources that are difficult to control as well as consumers' preference for natural products and ingredients are driving forces for developing new production methods.

In such cases the qualitative and quantitative analysis of the ingredients of natural products is very important as well from the side of the residual material limitations as from the taste and flavour of food products.

Often there are used complex methods for such purposes. Sampling and preparation and other handling steps are very time consuming. Search of alternative methods is very important especially in industry. There fast results of analysis are required. Additional aspects are that the quantitative analysis of single compounds in complex mixtures as usually done under such condition is not the solution of the problem mentioned.

In such unusual application SPME is an easier practicable method, which gives mainly a profile of the component mixture in natural products together with the information about the relative composition of the product components. Further advantages of SPME are their easy handling, no limit of volume, fast results, long-run investigation and anywhere you can use it. One more advantage of using SPME is the high sensitivity of this method (De la Calle Garcia *et al.*, 1997; Weber *et al.*, 1999). In these cases the variety recognition is the main application field in industrial use.

An automation of considerably investigation is leading to reproducible results.

SPME-GC/MS is applicable to investigate natural products. Furthermore the multiple extraction by SPME can be used for quantitative determination.

Here methodical aspects are shown together with examples from various natural products analysis.

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P. 124. Rapid determination of volatile components in phytopharmaceuticals and cosmetics by headspace SPME-GC analysis

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Since 1993 solid phase microextraction (SPME) is available as a commercial product, mainly used for environment and food determinations [Pawlizyn 1999, Schulz 2000]. Only few applications were published in the pharmaceutical field of research. There is a very high demand for analysing active compounds in various phytopharmaceutical products. Therefore the aim of this study was to develop rapid and solvent free applications in order to analyse the volatile components in selected drugs (thyme, camomile, peppermint and marjoram) and related pharmaceutical preparations. Saving of time in sample preparation results in a considerable reduced analysing time especially in case of series determinations.

The results of SPME experiments performed for the plant species mentioned above were determined and compared at different conditions. The influence of the following parameters was studied: quantity of the sample, sample preparation (powdered/not powdered; addition of water, variation of sodium chloride content), temperature of the sample (35° C, 45° C, 55° C, 65° C) and extraction time (5, 10, 15, 20, 25, 30, 40, 60 minutes). Different SPME-fibres were tested at these parameters (fibre coatings: PDMS, PDMS/DVB, polyacrylate, CAR/PDMS, CW/DVB, DVB/CAR/ PDMS).

In order to obtain high reproducibility with respect to equilibration time, temperature as well as homogeneity of the sample, an automated headspace-SPME device was used. This instrument provided also a high sample through-put which is especially important in context with quality control and support of breeding experiments. Generally headspace technique was used in the study which guaranteed extended life-time of the SPME fibres and improved adsorption conditions; this prohibits the adsorption of undesired plant compounds such as sugar components. The valuable plant substances were determined both qualitatively and quantitatively. An internal standard method was applied for quantification of selected volatile substances.

The results of SPME experiments received under different conditions were compared with those obtained by usual GC analysis of the isolated essential oils. The correlation between the SPME methods and the usual GC assays is described by a statistical approximation.

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P. 125. Composition and chemical variation during daytime of constituents of the essential oil of *Pogostemon patchouli* Pellet leaves

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The patchouli (*Pogostemon patchouli* Pellet) is used in herbal medicine as an aphrodisiac, antidepressant, and antiseptic. Patchouli essential oil is used in aromatherapy to treat skin complaints. It is also employed for headaches and fever. Its essential oil is constituted of benzoic aldehyde, eugenol, cinnamic aldehyde and sesquiterpene patchoulol.

To verify the composition and the chemical variation of its essential oil along 24 hours, leaves of patchouli of medicinal collection plants of the Department of Vegetable Production/Horticulture of UNESP-Botucatu – SP, were collected every three hours during one day (of the 06.00 to 03:00 h), between November, 25 and 26, 2000. The leaves had its oil isolated by hydrodistillation, in Clevenger apparatus during three hours with two repetitions. The chemical composition analysis of the extracted essential oils was carried out by gas chromatography/mass spectroscopy (CG-MS, Shimadzu, QP-5000). The chemical representatives identification was made through the comparative analysis of the mass of spectra of the oil components with the database of the system GC-Em (nist 62.lib), literature and retention index.

The chemical composition of the essential oils was not affected by the schedule of the harvest. The five more abundant components were: a major sesquiterpene (59.44 to 65.22%) not identified yet, α-bulnesene (5.37 to 7.66%), α-guaiene (3.80 to 5.73%), δ-patchoulene (2.54 to 3.36%) and another sesquiterpene (5.58 to 7.44%) also not identified, with retention time quite near to the main component. In bibliographical rising was detected divergences in relation to the majority component (Massada, 1976) and to patchoulol (Betts, 1994), an alcohol sesquiterpene. In our oil samples, it was not detected the presence of eugenol and patchoulol.

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P. 126. Influence of the organic manuring on the content and composition of ginger (*Zingiber officinale* Roscoe) essential oil

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Native to Asia, ginger (*Zingiber officinale* Roscoe) is grown throughout the tropics. Ginger is a well-investigated plant, and its therapeutic benefits are largely due to its volatile oil and oleoresin content. The powdered rhizomes are used in food and to flavour jams, sweets, and ginger ale, and as ingredients for curry. The spices, as the ginger, present variations in their chemical composition due to several factors such as origin area, of the natural hybridisation and of the agriculture-climatic variations (Bartley & Fley, 1994). The objective of this work was to verify the influence of the organic manuring of bird manure in the composition of the essential oil of the ginger.

The experiment was carried out in Department of Vegetable Production - Horticulture of University of Agronomic Sciences of UNESP in Botucatu-SP and in the laboratory of phytochemistry of IAC - Campinas-SP. The experimental area was of 240 m^2 and the used spacing was of 0,70 x 0,40 m, being 45 plants/plot, and adopted at random in blocks with 4 repetitions. The used treatments were 0, 1, 2, 4, 8 Kg/m^2 of chicken manure. The rhizomes used in the planting they were Hawaiian type that varied from 20 to 30 grams. The application of the fertiliser was accomplished together in December 2, 2000 and the planting in the 4 of that same month. The harvest was on July 1, 2001. The picked rhizomes were dried at to 30 ºC. For the extraction of the essential oil the hydrodistillation method was used, in Clevenger apparatus. The time of distillation was of 1,5 hours. The analysis of the chemical composition of the extracted essential oils was led in gas chromatography/mass spectroscopy (GC-BAD, Shimadzu, QP-5000). The chemical identification was made through the comparative analysis of the mass spectra of the oil components with the database of the system GC-IN (Nist 62.lib) and literature.

The results showed the presence of 34 identified components. Sixty percent of the composition of the oil was formed for α-zingiberene, neral, geranial, β-phellandrene, camphene and β-sesquiphelladrene in all treatments. The treatment T2 showed higher content of the oil. The organic manuring (chicken manure) didn't show significant influence on the composition of ginger essential oil.

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P. 127. Composition and biological activity of the essential oil from *Aloysia sellowii*

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Aloysia (Verbenaceae) is a genus that comprises about 100 species widespread all over the world, mainly in the South and Central America contries. *Aloysia sellowii* (Briq.) [= *Lippia affinis* (Briq.) Moldenke] (Pascual, 2001), know as "garupá", "cidrozinho" or "erva de sepultura" is a large aromatic shrub up to 2-4 meters in height, found throughout the greater part of Southern of Brazil (Rio Grande do Sul), Uruguay, Paraguay and Argentina. In Rio Grande do Sul it is traditionally used by local people as diuretic, stomachic agent and as a remedy for colds, grippe and bronchitis. In our continuing research on the essential oil of Rio Grande do Sul aromatic plants, we have investigated the essential oil of *A. sellowii* and its antibacterial activity.

The essential oils were obtained by hydrodistillation in 1.2-1.4 % yield of the aerial parts from two samples (A and B) of *Aloysia sollowii* from different collections sites of South of Brazil (Rio Grande do Sul), and were analysed by GC, GC/MS, and chiral phase gas chromatography (CPGC). Its constituents were identified by GC/MS using an electron-impact ionisation technique and by co-chromatography with authentic samples.

Fifteen compounds, constituting 87 % of the oil from Santa Maria (sample A), and nineteen compounds, constituting 80 % of the oil from Livramento (sample B), were identified. The major constituent of the sample A was 1,8-cineole (43 %), which is not present in sample B. The oil from sample B was richer in sesquiterpenoids than the oil from sample A, and was characterised by a high content of sabinene (12.6%) and *β-Z*-santalol (12.9%), which are not present in sample A. In addition, the configurations of the chiral monoterpene constituents were analysed on a dual column system, using two 25 m fused silica columns coated with modified cyclodextrins (2-6-Me-3-Pe-β-CD and 6-Me-2,3-Pe-β-CD) as the chiral stationary phase. Of the chiral monoterpenes identified in the oils of *A. sellowi*, the main constituents were: (-)-sabinene (100% ee), (+)-*β*-pinene (98% ee), (+)-*α*-pinene (93% ee), (+)-terpinen-4-ol (66% ee), (+)-limonene (91% ee) and (+)-linalol (76% ee).

The essential oils were screened for biological activity using the brine shrimps (*Artemia salina*) lethality test. Antibacterial activity was determined by the disc diffusion method and by the bioautography method (Homans and Fuchs, 1970). The essential oils of *A. selowii* (samples A and B) displayed high toxicity (LC_{50} = 2.85 μg/mL) in the brine shrimp bioassay. The essential oil of sample A was more active against the tested Gram-positive and Gram-negative bacteria than the oil of sample B. The result suggest that the higher activity of the oil of sample A could be associated with the significant contribution of 1,8-cineole, which is known to posses antimicrobial activity (Sivropoulou, 1997).

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P. 128. Volatile constituents from *Helietta longifoliata*

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Helietta longifoliata Britt (Rutaceae), locally called "canela-de-veado", belongs to the Rutaceae, and is a plant that grows in South America (Southern Brazil, Uruguay, Paraguay and Argentine). It has been used in Brazilian folk medicine as a natural remedy, for the treatment of various diseases (Cruz, 1985).

The aerial parts of the plant (leaves) were submitted for 4 hours to hydrodistillation, using a modified Clevenger-type apparatus to yield 0.70% of yellowish oil {n_d^{25}: 1.51, $[\alpha]_D^{25}$: + 22.8 (in $CHCl_3$, c = 0.01)}. The essential oil was analysed by GC using a Varian 3800 Gas Chromatograph equipped with a capilary fused silica column (25 m x 0.25 mm) coated with SE-54. The GC conditions used were: carrier gas He (1 ml min^{-1}); on column injector 200 ^oC; FID 280 ^oC; column temperature 50 ^oC to 250 ^oC at 4 ^oC/min. GC-MS analyses were performed on a HP 5973-6890 GC-MSD system operating in the EI mode at 70 eV, equipped with a HP-5 cross-linked capillary column (30m x 0.25mm). Column and the injector temperatures were as above. The identification of the components was based on comparison of their mass spectra with those of a NBS Librarie (Massada, 1995) and/or published by Adams (Adams, 1995).

The oil was mainly composed by sesquiterpenes (*ca* 80%) and a lower amount of monoterpenes (*ca* 17,5%). Twenty five constituents were identified representing *ca* of 97,5% of the oil, limonene (17.50%), germacrene-D (16.60%), elemol (11.81%), bicyclogermacrene (11.67%), guaiol (11.53), and *α-epi*-bisabolol (7.24%) being the most abundant components.

The oil was bioactive against some Gram-positive and Gram-negative bacteria, as revealed by bioautography. The microorganisms used in the antibacterial assay *Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus* (Gram-positive bacteria), *Klebsiela pneumoniae, Salmonella setubal* and *Escherichia coli* (Gram-negative bacteria), have been maintained at the Chemistry Department of Santa Maria - RS, Brazil. For the antibacterial assays, 10 μl of a solution corresponding to 100, 50, 25, 12.5, 6.25, 3.12 and 1.06 μg of the oil was applied to pre-coated TLC plates. The inoculum was prepared by culturing each organism in tryptone soya agar (TSA, Oxoid) at 37 ^oC to a turbidity equivalent to Mc Farland 0.5 standard (1.5x 10^8 CFU/mL). One microliter of each diluted inoculum (10^4-10^6 CFU) was applied onto Mueller Agar (MHA-DIFCO) plates. Amoxicillin (0.2 μg) was used as positive control.

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P. 129. Characteristics of the essential oil from fennel flowers in two stages of development

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Foeniculum vulgare var. *dulcis*, a native plant original of Mediterranean, has medicinal uses as antispasmodic, appetite stimulant, stomachic, diuretic, anti-inflammatory, anti-diarrheic, against colics and as a lactation promoter. Several components of the essential oil from this plant have important applications, namely, fenchone is used as counterirritant; limonene is used as solvent, manuf resins, wetting and dispersing agent; trans anethole is used for flavoring agent in perfumary, in soap, pharmaceutic aid (flavor); metyl chavicol or estragole is used in perfumarie and as flavor in foods and liquers; α-Pinene, used in manufacture of comphor, insecticides, solventes, perfume bases.

The present work had as objective to identify the chemical composition of the essential oil of *Foeniculum vulgare* var. *dulcis* in two stages of development. The study was carried in the Lageado Experimental Farm (Agronomical Sciences College) of the Department of Plant Production, Horticulture Section, São Paulo State University, Campus of Botucatu, Brazil. The statistical design was complete randomized block with 2 treatments and 9 replications. Flowers buds and inflorescences in anthesis phase were collected. The essential oil was isolated by hydrodistillation from fresh in Clevenger apparatus; 100g of fresh plants parts were used in each extraction. The extraction proceeded for three hours. The analysis of essential oil was conducted by GC/MS. The means of yield (Tukey Test 5%) were 0,96% for inflorescences in anthesis phase and 0,39% for flowers buds. Statistical differences were observed only in myrcene and *trans*-anethole in essential oil of flowers buds.

Table 1. Relative amount of main components from the essential oil of the flower buds and inflorescences in anthesis from fennel

	Flower buds	Inflorescences in anthesis
trans-Anethole	66.75	59.77
Limonene	20.78	18.36
Fenchone	3.07	6.79
Metyl chavicol	2.47	2.23
α-pinene	1.56	2.12
γ- terpinene	1.05	tr
Myrcene		1.18

P. 130. Yield of essential oil in green seeds of fennel

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The fennel (*Foeniculum vulgare* Mill.), it is an annual herbaceous plant, whose seeds are very used in the homemade medicine. The fennel seeds produce a yellow-clear aromatic essential oil, used in the production of several liquors drinks and on perfumery. They act as carminative and stimulant. An experiment was carried out at farm (Agronomical Sciences College) of the Department of Plant Production - Horticulture of São Paulo State University - Campus of Botucatu-Brazil. The statistical design used was complete randomized block with 3 replications and 8 treatments (collected of epoch). The seeds were sowings in summer of the 2001 (January 2001) and picked every 14 days, starting from fifteen days after the anthesis until the complete maturation during one year. The parameters evaluated in this work were essential oils yield of green seeds and chemical major compound of the essential oil of fennel. The essential oil was determined through hydrodistillation, in Clevenger apparatus; 50g of green seeds were used in each extraction. The extraction proceeded for three hours. For the Tukey Test (5%) there was statistical difference for the composts and the 5th, 4th, 6th and 3rd harvest epochs respectively, what corresponded between 186 to 228 days after sowing and presented the best results for fresh mass of green seeds. With relationship to the yield of the essential oil, for the Tukey Test (5%), there was not statistical difference among 3th, 4th, 5th, 6th and 8th harvest (Spring) in relationship the others. However the generality means of compounds majorities were: *trans*-anethole (74%), fenchone (15%), limonene (5%), methyl chavicol (3%), α-pinene (1%), γ-terpinene (0.9%) .

Table 1. Means of fresh matter of green seeds of fennel and yield of oil, in function of the collected epochs (treatments). UNESP- FCA/Botucatu – S.P, 2001.

Treatments (collected epoch)	Days after sowing	Seasons of Year	Fresh matter (g)	Yield of oil (ml)
1	158	Spring	308.47 D	0.0 B
2	172	Spring	532.67 CD	0.0 B
3	186	Spring	1018.40 BC	1.88 A
4	200	Spring	1338.13 AB	1.84 A
5	214	Spring	1615.63 A	1.83 A
6	228	Spring	1229.53 AB	1.68 A
7	242	Spring	430.97 D	0.45 B
8	256	Summer	197.33 D	1.53 A

Means followed by same letter in the column and in the line they don't differ significantly, at the level of 5% of probability for the Tukey Test.

P. 131. Supercritical fluid extraction of *Thymbra spicata*

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Supercritical fluid extraction (SFE) is a separation method that exploits the unique properties of gases above their critical points to extract soluble components from a raw material (Simandi *et al.*, 1998). SFE is an interesting technique for the extraction of flavor and fragrance compounds because relatively rapid reactions can be carried out under wild conditions and at low temperature and there is no need to evaporate organic solvents (Ozek *et al.*, 1988). The combined liquid-like solvating capabilities and gas-like transport properties of supercritical fluids make them particularly suitable for the extraction of diffusion-controlled matrices such as plant tissues (Scalia *et al.*, 1999).

Carbon dioxide is the most commonly used solvent as a supercritical fluid because of its low critical temperature and pressure (T_c=31.1 °C; P_c=72.8 atm), its non-toxic and non-flammable properties and its availability in high purity with low cost. It does not react with the food constituents. In addition, it is easily removable from the extract following decompression. Due to its relatively low critical temperature, thermal sample decomposition is reduced, supercritical CO_2 has good solvent properties for extraction of non-polar compounds such as hydrocarbons (Scalia *et al.*, 1999).

Thymbra spicata is a thyme-like plant widely grown and used in Turkey. It has various aroma compounds in it and carvacrol is the main compound.

The objective of this study is to study the effects of temperature, pressure and time on yield and composition of the oil in supercritical CO_2 extraction.

The yield decreased with increasing temperature and remained constant at high values. It was seen that when pressure was increased, yield increased. Monoterpenes were preferably extracted at the beginning of the process. The decrease in monoterpenes for higher levels of temperature, pressure and time may be due to the enhanced leaching of harder to extract substances (sesquiterpenes and oxygenated compounds). Both temperature and pressure positively affected *p*-cymene content in the extract. Sesquiterpene content of essential oil was found to be almost constant for shorter extraction times. However, for long time of extractions, its concentration increased as time increased. In addition, it was seen that sesquiterpene content increased with increasing pressure.

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P. 132. Synergistic activity of components of *Thymus* oils on *Candida* species

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The raising of fungal infections with the consequent increase of resistance to antifungals urged the search of therapeutic alternatives. Previous research of our team demonstrated antifungal activity of the essential oils (EO) from different species of *Thymus*, in which carvacrol, thymol, *p*-cymene and 1,8-cineole are often the major components. The objective of this study is to evaluate the antifungal activity of each one of these components and the potential synergic effects of their combinations.

For this purpose, thymol/carvacrol, thymol/*p*-cymene, thymol/1,8-cineole, carvacrol/*p*-cymene, carvacrol/1,8-cineole and *p*-cymene/1,8-cineole combinations were studied by the checkerboard method for two clinical isolates of *Candida albicans* and *C. krusei*. The MIC of each component was compared with the MIC obtained with the combination and the fractional inhibitory index (FIX) was calculated. FIX<0.5 means synergism; FIX >0.5 and <4.0 means indifferent interaction; FIX>4.0 means antagonism.

C. albicans and *C. krusei* showed similar behaviour. No antagonism between the components were observed and the most synergic combinations were thymol/1,8-cineole and thymol/*p*-cymene, with FIX=0.125 (corresponding to a decrease of the MIC around 3 dilutions).

This study shows the advantages of the combination of some essential oils or some of their components by the MIC decreasing, and eventually side effects, important issues in future clinical trials on mucocutaneal candidosis.

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P. 133. The effect of eleven individual essential oil constituents on the phytopatogenic bacterium *Clavibacter michiganensis* subsp. *sepedonicus*

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In the present study, the antibacterial activity of eleven monoterpenoids against the phytopathogenic bacteria *Clavibacter michiganensis* sbsp. *sepedonicus* was examined. These terpenoids are α-pinene, limonene, 1,8-cineol, α-thujone, fenchon, pulegone, geraniol, carvacrol, α-terpineol, linalool and linalyl-acetate. They are common essential oil constituents of widely distributed Mediterranean ecosystems aromatic plants of *Labiateae* family, such as sage, lavender, rosemary and oregano. The target phytopathogen *C. michiganensis* subsp. *sepedonicus*, is a Gram positive bacterium, which is responsible for the bacterial ring rot of potato.

After determining the optimum growth conditions for the tested bacterial strain in the lab, two methods were applied to examine the antibacterial activity of the compounds in question. With respect to the cell structure (integrity of bacterial membranes), the sensitization of bacteria to lysis effects, after incubation with the tested compounds, was examined. With respect to the cell function (respiratory chain), the inhibitory activity of the terpenoids was tested on the respiration of bacterial cells.

Most of the tested compounds did not remarkably inhibit growth and function of *C. michiganensis* subsp. *sepedonicus* cells compared to their action against other microorganisms. Only carvacrol and geraniol showed a potent antibacterial activity. These oxygenated compounds strongly influenced bacterial respiration and caused enhanced lysis of bacterial cells. The two terpenoids could be further tested as of their probable use as biological antibacterial agents against this pathogen in the field.

P. 134. Brazilian savannah: alive pharmacy in danger

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Brazil is the 5th country in territorial dimension, the 10th world economy and hold one of the biggest patrimony as far as planet biodiversity is concern. Although the country has this three important indicators Brazil also host strong socio-economic inequality. Beside the importance of the Amazon, the central region of Brazil is characterised by a Biome typical of region of Savannah with an immense natural resource. The vegetation of this region has a diverse use such as food source, medicinal and oil scent potential. The last 30 years this potential has been dramatically reduce by the agriculture in which to grow on the region need to take the natural vegetation losing important species.

Another aspect of this development model is related to the fact that the small land owners who used to live, know and depend on the resources were forced to leave their land and pushed to live on peripheral home in bigger cities. This fact makes them to loose the connection with the land source of food and health. The objective of this paper is to search for solution helping them to revert this situation and work with the low-income population in the surroundings of Brasilia the capital of Brazil. This study aim at recovering the traditional knowledge of needy people on medicine plants and, from that point, offer alternative health treatment to the community which lives in the periphery of the capital. The research consists on a survey of the best known medicine plants in the region of the Brazilian Savannah, identify them, and make their cultivation viable, within a public area where the community has the possibility of getting together and valuing the cultivation of alternative plants. To the survey of the plant species, as well as their therapeutic use, the research-action method was used in weekly meetings with the community, in which the information passed through the participants was noted down and registered. All together, around 50 species were catalogued, from which 30 were chosen for cultivation. The cultivation and production work of the seedling and phytotherapeutic, has been developed by researchers from the University of Brasilia and by the community involved. The soil fertilisation, which is of low quality, is being done under the Stonemeal (1) technique rules, which consists of the use of dust of determined rocks rich in macro and micro nutrients (Ca, Mg, P, V, Mo, among others). To this material, organic fertiliser is added, which has the role of breaking the present clay in the material, and therefore, accelerating the offer of micronutrients. This double fertilisation has the advantage of supplying a production free of agrotoxic, besides encouraging the community to consume better quality products. The results obtained so far demonstrate that the population's traditional knowledge, together with the use of alternative treatments, may be converted into a social insertion mechanism and the preservation of the region's biodiversity.

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P. 135. Constituents of flowers from *Solidago microglossa* DC

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The genus *Soligado* (*Asteraceae*) comprises hundred twenty species some of them possessing several therapeutic properties (*S. virgaurea*, *S. gingantea*, *S. canadensis*). *Solidago microglossa* DC is a South American species, that in Brazil replaces *Arnica montana* L. for treatment of inflammatory diseases. Clerodane and labdane diterpene substances are present in roots of *S. microglossa* and the epigeal part shows antifugal diterpenes (Vila *et al*., 2002), while some flavonoids were detected in leaves (Correia, 1998).

The main aim of this work is to isolate, identify and characterise biological active compounds from the ether extract of *S. microglossa* flowers. The extract was chromatographed by TLC, using toluene-ethyl acetate (7:3) as eluting system, and three spots were detected after spraying the plate with an acid anisaldehyde solution. The less polar constituent was re-chromatographed by preparative TLC using hexane-ethylacetate(8:2) as eluting system, affording a pure substance with the following data: *m.p.* 128-130ºC; *m/e* = M^+ 316 (2%), 192 (10%), 111 (20%), 82 (100%); *^{1}H-NMR* in $CDCl_3$, δ 7.37 (s); 7.27 s; 6.28 (s); 5.71 (s); (CH); four methyl groups at 2.01 (s); 1.19 (s); 1.14 (s); 1.00 (s). These values suggest a diterpene compound that could be responsible for the reported anti-inflammatory activity (Singh, 1999; Demetzos *et al*., 2001). An attempt to find out sesquiterpene lactones (STL), in *S. microglossa* has failed despite such compounds are abundant in *Arnica montana* L. which have been related to the anti-inflammatory properties of this species.

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P. 136. Constituents of the bark oil of *Cedrelopsis grevei* H. Baillon from Madagascar

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Ptaeroxylaceae family, is present in southern Africa and Madagascar and is constituted by three genus: *Ptaeroxylon*, *Bottegoa* and *Cedrelopsis. Cedrelopsis grevei* H. Baillon is an endemic species from Madagascar. This tree, of the vernacular name Katafa or Katrafay (Katra: bitter and fay: juice), measures up to 15 meters high, has grey branches and smelling bark (cedar wood-like odour). This species is abundant in the dry dense forests or in the bush (South and West parts of the island), from the sea level to 900 meters of altitude.

As part of our on-going work on the characterisation of essential oils from Madagascar (1), we investigated the bark oil of *Cedrelopsis grevei*. A commercial sample of *C. grevei* from Madagascar was repeatedly chromatographed and the combined analysis of all the fractions by GC-RI, GC-MS and ^{13}C-NMR let to the identification of 116 components. The major constituents were (E)-caryophyllene (9.3%), α-copaene (7.7%), α-selinene (5.8%), δ-cadinene (4.9%), β-selinene (4.5%), α-humulene (3.3%) β-bisabolene (2.8%) and ishwarane (1.5%). The analysis of five other commercial samples confirmed the pre-eminence of sesquiterpenes and exhibited a quantitative chemical variability of the main components ishwarane (17.4-1.0%), (E)-caryophyllene (12.5-1.3%), α-copaene (11.0-4.9%), β-elemene (9.6-0.2%) and α-selinene (9.4-1.1%).

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P. 137. Essential oil of *Santolina corsica* Jord. and Fourr. from Corsica

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Santolina corsica (*Asteraceae*) is an endemic species growing wild in altitude in the centre of Corsica and in Sardinia [1]. *Santolina* species are known for the pharmacological activity of their extracts containing bioactive compounds such as coumarins [2], or acetylenic compounds [3].

Continuing our research on essential oil bearing plants growing wild in Corsica we report here on the chemical composition of the essential oil from the aerial parts of *Santolina corsica* (plant collected in full blossom). Identification of the individual components was carried out by (i) the GC-retention indices (RI) on apolar and polar columns; (ii) comparison of the mass spectra with those of reference compounds; (iii) ^{13}C-NMR spectroscopy according to an experimental procedure and a computerised method developed in our laboratory. In this procedure, the components are identified by comparison of the carbon chemical shift values in the mixture spectrum with those of reference spectra compiled in a computerised data bank [4].

After fractionation by column chromatography, 48 components accounting for 85.1% of the amount of the oil were identified. The main constituents were β-phellandrene (18.0%), β-myrcene (13.7%), santolinatriene (11.9%) and β-pinene (5,7%). The sample is characterised by the presence of 12 irregular monoterpenes. The most important are those bearing the santolinane skeleton: santolinatriene, santolina alcohol (0.2%), (Z)-lyratol (4.4%), (E)-lyratal (0.5%), lyratyl acetate (0.5%) and lyratyl propionate (0.1%). Other irregular compounds are artemisane monoterpenes: artemisia ketone and artemisia alcohol (1.4% and 0.2%, respectively), yomogi alcool (2.7%). Finally, we identify lavandulol (0.2%). The simultaneous occurence of the three irregular skeletons was never reported in an essential oil of *Santolina* species, while this profile is commonly encountered in *Artemisia* oils [5]. The composition of our sample differs drastically from that of *S. corsica* oil from Sardinia where camphor, artemisia ketone, borneol and aromadendrene were reported as main components [1].

1. Poli F., Bonsignore L., Loy G., Sachetti G., Ballero M. (1997) *Journal of Ethnopharmacology* 56: 201-208.
2. Maqua M.P., Vines A.C.G., Caballero E., Grande M.C., Medarde M., Bellido I.S. (1988) *Phytochemistry* 27: 3664-3667.
3 Christensen L.P. (1992), *Phytochemistry* 31: 7-49.
4 Tomi F., Bradesi P., Bighelli A. Casanova J. (1995) *J. Magn. Reson. Anal*. 1: 25-34,.
5 Stojanovic G., Palic R., Mitrovic J., Djokovic D. (2000), *J. Essent. Oil Res.* 12: 621-624.

P. 138. Essential oil composition from *Melaleuca quinquenervia* of New Caledonia

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Melaleuca quinquenervia (Cavanilles) S. T. Blake (Myrtaceae), common name Niaouli, is an indigenous species of New Caledonia Island.

Essential oil of Niaouli is produced in secretory glands located in the leaves. The essential oils were extracted by hydrodistillation.

In this study, 135 samples collected from 7 harvesting locations in New Caledonia were analysed by GC/MS and Principal Component Analysis (PCA – statistic method). The chemical composition of the essential oil of *M. quinquenervia* allowed the definition of six chemotypes. The oil yield is in ranged between 0% and 3%. The industrial mean of niaouli oil yield was 0.7%. The industrial essential oil selection criterion was the 1,8-cineol content above a concentration of 60% (yes is 60% it's a typing mistake).

The different characteristics of *M. quinquenervia* preserve by in vitro culture.

P. 139. Examination of antimicrobial activity of different medicinal plant extracts by impedimetry

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In food processing there is an increasing consumer demand on food products with the quality characteristics of fresh products, and foods containing only natural compounds. The extracts of medicinal or aromatic plants are one of the potential biopreservatives (Smid and Gorris 1999). There are various methods that are used to investigate antimicrobial activity of essential oils. One of the most successful method of the rapid and automated microbiological techniques is based on electrical measurements. Microbial metabolism usually results in an increase in both conductance and capacitance causing a decrease in impedance of the culture media. Inhibitory activity of essential oils and supercritical fluid extracts of *Origanum vulgare* ssp. *hirtum* (Link) Ietswaart, *Satureja montana* L. and their main components was tested by impedimetry on a number of food-borne bacteria and fungi.

Greek oregano (*Origanum vulgare* ssp. *hirtum*, epithet of Department of Medicinal and Aromatic Plants) and winter savoury (*Satureja montana* "Bokroska", variety of Department of Medicinal and Aromatic Plants) grown at the Research Station of Department of Medicinal and Aromatic Plants of SZIE, Soroksár were used. *Essential oils* were produced by hydrodistillation in Clevenger apparatus according to VII. Phg. *Supercritical fluid extracts* (SCFE) were obtained by Isco SFX 2-10 laboratory-scale apparatus. Each sample was analysed by using Shimadzu GC-14B equipped with a flame ionisation detector.

Inhibitory activities were investigated by using a RABIT type automated impedimeter from Don Whitley Scientific (Shipley, U.K.). Stationary growth phase cultures of the test organisms were included in triplicates into Don Whitley impedimetric broth (for bacteria, direct technique) and yeast-extract peptone glucose broth (for fungi, indirect technique (Owens *et al*. 1989) and the conductance of the cultures were recorded automatically for 48 hours at 30°C without or after addition of essential oils or SCFEs in concentrations of 0.1 % and 0.05% (v/v). Viable cell counts of the cultures were estimated initially, the impedimetric detection time (TTD) were automatically determined by the instrument.

In comparison of herb extracts the highest antimicrobial activity was detected in case of *Origanum vulgare* subsp. *hirtum.* Results of GC/MS analyses show that the main component of herb extracts investigated is carvacrol. The results obtained demonstrated that using carvacrol as an antimicrobial agent had stronger antimicrobial activity then herb extracts.

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Smid EJ, GM Gorris (1999) *Handbook of food protection*, New York, Marcel Dekker

P. 140. Composition and antifungal activity of the essential oil from aerial parts and rhizomes of *Valeriana dioscoridis*

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Valeriana genus (Valerianaceae) comprises about 200 species and is represented in Europe by 20 species. All the members of the genus have a characteristic foetid odour.

Valeriana species are widely used in Western phytotherapy for the preparation of phytomedicines with mild sedative and antispasmodic properties (Wichtl, 1994; Blumenthal, 1998). The best known species is *V. officinalis*, whose oil composition has been the subject of considerable study. As part of on going study on the essential oils of *Valeriana* sp., in this work we report on the qualitative and quantitative analyses of the volatile constituents produced by the aerial parts and rhizomes of *Valeriana dioscoridis,* one of the several valerian species mentioned by Dioscorid.

Valeriana dioscoridis Sibth. & Sm. grows in the Balkan Peninsula, in rock-crevices, rocky woods and damp grassland. This species has a very short rhizome, with a cluster of fusiform tubers and stem solitary, slightly hairy, 25-75 cm high.

The oil analyses were performed by GC/MS and the identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and mass spectra with those obtained from authentic samples and/or the NIST/NBS Wiley libraries.

The main components of the oil from the aerial parts and the oil from the rhizome were patchouli alcohol (14.3%, 16.3% respectively) and α-pinene (7.1%, 9.0% respectively). There were no substantial qualitative differences between these two oils.

The antifungal activities of *V. dioscoridis* rhizome oil and patchouli alcohol were analysed by modified microdilution technique (Daouk *et al.*, 1995). The results showed that the oil from rhizomes and the main component exhibited variable degrees of antifungal activity against the strains tested. The essential oil tested showed higher antifungal properties than patchouli alcohol. MICs of the oil were 1.0-10.0 μl/ml, while MFCs were 1.5-15.0 μl/ml. The most resistant fungal strains were *Aspergillus flavus*, *Trichoderma viride*, *Penicillium ochrochloron* and *Penicillium funiculosum*, while *Cladosporium cladosporioides*, *Fusarium tricinctum* and *Phomopsis helianthi* and dermatomycetes were the most sensitive ones.

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P. 141. Chemical composition of the essential oils from *Thymus samius* and its related taxa *Thymus atticus* and *Thymus parnassicus*

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The genus *Thymus* L. (Lamiaceae) includes more than 350 taxa. *Thymus* species have been used for more than 2,000 years as medicinal herbs and many of them are still in use. *Thymus samius* is an endemic plant known only from the island of Samos (E. Greece). Morphologically, it resembles *Th. atticus* but has narrowly spathulate, pubescent leaves. It may be of hybrid origin, with *Th. cilicicus*, *Th. zygiformis* and *Th. parnassicus* as possible parents (Baden, 1991).

The aim of the present study was to compare the constituents of the essential oil from *Th. samius* with those from *Th. atticus* and *Th. parnassicus* growing in various localities of C. Greece. All samples collected during the flowering period. The dried aerial parts were subjected to hydrodistillation for 3h and the obtained oils were analysed by GC/MS. The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and mass spectra with those obtained from authentic samples and/or the NIST/NBS Wiley libraries.

The major components of *Th. samius* oil were germacrene-D (26.40%) and β-bisabolene (22.73%), two sesquiterpenes that arise from the precursor *E,Z*-farnesyl cation in the mevalonate pathway, as well as (*E*)-caryophyllene (15.48%) a sesquiterpene that arise from *E,E*-farnesyl cation.

The oils from all samples of *Th. parnassicus* oils were qualitatively similar but display some quantitative differences. Those from Mt. Pateras and Mt. Gerania samples were rich in (*E*)-caryophyllene (13.10%, 10.02% respectively). In the oil from Mt. Parnitha sample the main components were (*E*)-caryophyllene (8.48%), linalyl acetate (8.24%) and γ-terpinene (8.04%).

Th. atticus oil from Mt. Parnitha contained the monoterpenes 1,8-cineole (11.81%) and β-myrcene (7.32%), and the sesquiterpenes (*E*)-caryophyllene oxide (9.18%) and (*E*)-caryophyllene (6.66%) as main constituents. The oil from the same species grown on Mt. Parnassos was dominated by the sesquiterpenes (*E*)-nerolidol (17.49%), (*E*)-caryophyllene (15.84%), germacrene D (11.50%) and by the acyclic monoterpene β-myrcene (9.45%).

Another related taxon *Th. cilicicus* has been studied from Turkey (Akgul *et al.*, 1999). The oil from Beysehir region was found to contain α-terpineol (16.4%), camphor (9.7%), 1,8-cineole (7.8%) and α-pinene (6.9%); the sample from Silifke region had as main components α-pinene (16.7%), 1,8-cineole (10.4%), isoborneol (9.2%), *cis*-verbenol (8.2%), camphor (6.4%) and *trans*-verbenol (6.2%); the sample from Ermenek region revealed α-terpineol (33.44%) as the major component and camphor, citronellol and linalool (ca. 6-8% each). All samples of *Th. cilicicus* are rich in monoterpenes, whereas *Th. samius* and its related taxa *Th. atticus* and *Th. parnassicus* are rich in sesquiterpenes with the exception of *Th. atticus* sample from Mt. Parnitha. None of the studied oils belong to the most abundant phenol-rich chemotype.

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P. 142. Antimicrobial properties and geographical variation in essential oil composition of fever tea - *Lippia javanica* (Verbenaceae)

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Lippia javanica is a widely spread woody shrub and the major traditional use is reflected in its vernacular name; fever tea and 'koorsbossie'. An infusion of the leaves is also used as a decongestant for colds and coughs. Infusions may also be used topically to treat scabies and lice. This study shows that the essential oil chemistry varies dramatically both within and between natural plant populations and thus has a direct impact on variations of antimicrobial activity. The aerial parts were collected from individual plants in five natural populations. The hydrodistilled essential oils were analysed by GC-MS and a cluster analysis was performed on the essential oil dataset. From sixteen samples (representing 5 natural populations), five chemotypes were identified;

a myrcenone-rich type (36 – 62%),
a carvone-rich type (61 – 73%),
a piperitenone-rich type (32 – 48%),
an ipsenone rich-type (42 – 61%) and
a linalool-rich type (>65%)

The myrcenone and linalool chemotypes have been mentioned in the literature but the carvone, ipsenone and piperitenone chemotypes have not previously been reported. The oil showed minimal activity against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus*, and no apparent activity against *Pseudomonas aeruginosa* in a disk diffusion assay. The oil did however show activity against *Candida albicans* and *Cryptococcus neoformans*, in the same assay. Time kill studies were performed on three microbial respiratory isolates to document the scientific rational of using *Lippia* for respiratory complaints in traditional herbal medicine. *Klepsiella pneumonia, Cryptococcus neoformans* and *Bacillus cereus* showed reduction in microbial populations with the strongest bacteriostatic effect observed for *Klepsiella pneumonia.*

P. 143. The antimicrobial activity and death kinetics of the essential oil and chemical components of the South African endemic, *Osmitopsis asteriscoides*

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The essential oil composition and anti-microbial activity of *Osmitopsis asteriscoides*, a medicinal plant used in traditional herbal preparations in South African is investigated. A preliminary screening was done using the disc diffusion method on nine bacterial and four fungal isolates. Time kill studies indicated a more detailed bacteriostatic progression with the antimicrobial determination of *Candida albicans, Pseudomonas aeroginosa* and *Staphylococcus aureus*. A strong fungicidal activity against *Candida albicans* was found at concentrations ranging from 0.5 to 2 %. Sixty-six compounds were identified by GC-MS in the hydro-distilled essential oil, which contained (-)-camphor and 1,8-cineole as the main constituents. The high concentration of these two terpenoids is presented as a possible explanation for the traditional use of *Osmitopsis asteriscoides* for treating microbe-related illnesses. A comparative antimicrobial time kill study of *Candida albicans* with 1,8-cineole and camphor standards as well as in combination were compared with the pure *Osmitopsis asteriscoides* essential oil. The time-kill method is presented as a superior assay to determine antimicrobial properties of essential oil and its constituents.

P. 144. Modelling of the lemongrass essential oil supercritical extraction process

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The extraction process of the lemongrass essential oil with supercritical CO_2 is studied in this work. We used plants of the experimental cultivated stands from Southern Brazil and the HP7680 apparatus to extract the essential oils. The oils were extracted from leaves and the following process parameters were used in this study: 40ºC to 80ºC, 100 bar, and 2mL/min (Table 1). The analysis was performed by GC and GC/MS.

Table 1 – Extraction Experimental Data (P = 100 bar)

	% mass concentration (g/g)				
Time	5 min	10 min	15 min	20 min	30 min
Temperature					
40°C	1.08	1.22	1.23	1.23	1.23
50°C	0.73	0.84	0.85	0.85	0.85
60°C	0.56	0.69	0.71	0.72	0.72
70°C	0.70	0.83	0.86	0.86	0.86
80°C	0.69	0.79	0.80	0.81	0.81

The aim of this work is simulate the lemongrass oil extraction process at supercritical condition. To perform this objective a mass transfer model is necessary and we guess the procedure proposed by Reverchon (1996). In this model two parameters are adjusted: the diffusivity of the solute in the solid matrix and the partition coefficient. Therefore the behaviour of these parameters is analysed in the function of the temperature and we present a correlation for the diffusivity and partition coefficient adjusted parameters. The extract yield curve is presented, comparing the experimental results with the model. The differences obtained between calculated and experimental data to solute concentrations profile, confirm that this model is efficient to represent the supercritical extraction of the lemongrass essential oil.

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P. 145. African wormwood – essential oil composition, geographical variation and antimicrobial properties of a coveted traditional herbal remedy

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Artemisia afra (Jacq. Wild) also known as African wormwood, "umhlonyane"(Xhosa, Zulu), "lengana" (Sotho, Tswana) and Wildeals (Afrikaans) is an aromatic shrub belonging to the family of Asteraceace. It is widespread in South Africa extending from the mountainous regions of the South Western Cape, along the eastern coast to the Northern Province. Due to the popular use of *A. afra*, herbal tinctures have been prepared for commercial distribution. As the chemotypic variation remains unrecorded it has been impossible to standardise extracts containing *A. afra*. The aerial parts of 17 samples from four natural populations were hydrodistilled and the essential oil analysed by GC-MS and tested for antimicrobial property on a number of bacteria and fungi. The essential oil composition varied quantitatively and qualitatively within and between populations. With the aid of cluster analysis several chemotypes have been identified based on the presence and quantity of the following compounds; α-thujone (5.55-77.65%), β-thujone (1.37-57.73%), camphor (1.00-48.99%), 1,8 cineole (2.31-50.09%), Artemisia ketone (14.47-27.97%), Artemisia alcohol (9.31-27.76%) and santolinyl acetate (4.14-24.32%). The essential oil was active against all organisms except *P. aeurigosa* and *E. facaelis*. The oil exhibited more antifungal properties than antibacterial effects. The pure standards (thujone, cineole, camphor etc) showed no antimicrobial activity and it was concluded that the oil could exert antimicrobial properties be working in a synergistic way. *Artemisia afra* varies within and between natural populations and standardising of commercial products will be problematic without cloning of a favourable chemotype.

P. 146. Synthetic odoriferous lactones

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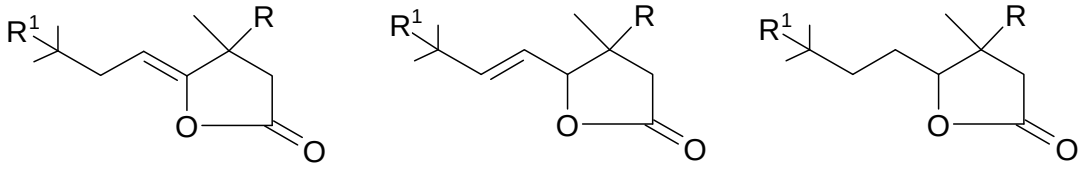
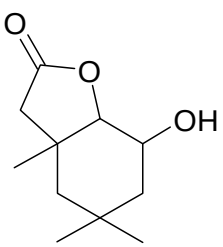
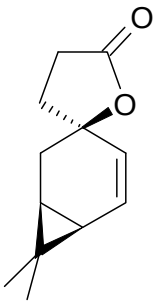
Compounds with lactone moiety are widely spread in nature. They are components of many essential oils, usually responsible for their odoriferous properties (Ohlof, 1994). Lactones are also present in fruits, vegetables and other foods (Maga, 1976). We have also observed that some of terpenoid lactones synthesized by us possess very interesting odours. Recently we have published the synthesis and odour characteristics of some terpenoid lactones with limonene system (Paruch *et al*, 2000).

Now we present the synthesis of further mono-, bi- and tricyclic odoriferous terpenoid lactones. The structures of some of them are given below.







R^1, R = Me or H

Lactones were obtained in four or five step syntheses from simple or natural starting materials *via* the iodolactonization of γ,δ-unsaturated acids. The saturated lactones were synthesized from iodolactones by their reductive (nBu_3SnH) dehalogenation. The unsaturated ones were obtained by dehydrohalogenation of iodolactones with DBU.

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Paruch E., Ciunik Z., Nawrot J., Wawrzeńczyk C. (2000) *J. Agric. Food. Chem.*: 48, 4973-4977

P. 147. Developments in SPME for fast analysis of herb components for quality control and variety recognition

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In quality control fast results of analysis are required. Traditional Methods are often time consuming. A lot of several activities has to be done. In some cases automation is hardly applicable. Therefore our main intention was to develop a fast, easy and representative method for analysing volatile compounds in natural sources. The use of headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography offers a lot of advantages not only in the analysis of herbs or other essential oil containing materials (De la Calle Garcia *et al*., 1997; Weber *et al*., 1999)

For quantification of maceration extracts a useful method was developed. Influences of extraction process will be discussed on thyme and chamomille maceration extracts. These are examples for liquid samples analysed by HS-SPME.

A more interesting challenge is the analysis of solid essential oil containing samples. Differences to liquid samples in handling and extraction force the development of a new handling procedure. The results to be presented show the efficiency of the new developed method. Quantitative analyses were performed on solid thyme and chamomille samples as well as on flavoured candles. In addition, several varieties of thyme were investigated for variety recognition using the new method.

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P. 148. Composition of *Achillea filipendulina* essential oil

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Achillea filllipendulina is a deciduous perennial grown for its attractive summer and autumn flowers for the dried flower market. It is a member of the Yarrow family and is also known as Cloth of Gold. *Achillea fillipendulina* has flat heads of bright yellow flowers which last all summer, feathery foliage and grows to heights of 1.2m (4ft). The composition of *Achillea filipendulina* has previously been reported in the literature (Maffei *et a*l, 1994).

The flowering aerial parts of *Achillea fillipendulina* were collected from a farm in Kent. The aerial parts were extracted by hydro-distillation using a Clevenger – type apparatus to produce 0.07% oil yield.

The essential oil was analysed by combined GCMS using a DB-1 column (30m x 0.32mm x 1µm) and a BP20 wax column (25m x 0.32mm x 0.25µm). Temperature programmed retention indices of the compounds were determined relatively to the retention times of a series of *n*-alkanes. The constituents of the oil were identified by GCMS comparison with reference libraries (NIST98) and comparison of retention indices and GCMS of those reported in the literature (Adams, 1989; Adams, 1995; Jennings *et al.*, 1980). Quantification was determined by peak area normalisation without consideration of calibration factors.

Over thirty compounds were identified and the main constituent was 1,8-cineole (45.8%). The remaining oil was made up of a number of major and minor fractions. The major fractions included monoterpene alcohols (13.8%), monoterpene hydrocarbons (15.4%), monoterpene esters (10.2%) and sesquiterpenes (8.9%). The minor fractions included monoterpene ketones (1.7%), other oxygenated monoterpenes (0.4%) and sesquiterpene oxides (1.7%) and sesquiterpene alcohols (2.1%).

The composition of this oil was significantly different to that reported by Maffei *et al*, where the principal component was found to be artemisia acetate.

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P. 149. Composition of methyl esters in European *Humulus lupulus* essential oils

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Methyl esters are extracted from the essential oil of *Humulus lupulus* (hop) and used as downstream aroma and flavour products in brewing and the flavour and fragrance industry. Methyl esters have principally been extracted from the Target hop variety. This research attempts to determine the methyl ester profile of twelve other European hop varieties by extracted ion GCMS of the whole essential oils.

Twelve varieties of *Humulus lupulus* and the Target variety were obtained from various parts of Europe and the essential oils were extracted by hydro-distillation using a Clevenger-type apparatus. The methyl esters were extracted from target hop oil using liquid-liquid extraction and column chromatography.

The essential oils were analysed by combined GCMS using a DB-1 column (30m x 0.25mm x 1µm). The two principal methyl ester ions 74 and 87 amu were extracted from the total ion chromatogram and the temperature programmed retention indices of the methyl esters were determined relatively to the retention times of a series of n-alkanes. The methyl esters were identified by GCMS comparison with reference libraries (NIST98) and comparison of retention indices of those reported in the literature (Adams, 1989; Adams, 1995; Jennings *et al.*, 1980). Quantification was determined by peak area normalisation without any consideration of calibration factors.

The extracted ion technique was verified by matching the extracted Target methyl ester profile to the extracted ion methyl ester profile from the target whole oil. The methyl ester profiles of the twelve hop oil varieties were grouped into four categories. The first category consists of the Target hop variety and eight of the twelve other hop oil varieties which all contain the major constituents methyl 4-decenoate and methyl 4,8-decadienoate. The second category consists of one variety, which contains the major constituent methyl heptanoate. The third category consists of one variety, which contains the major constituent methyl 9,12-octadecadienoate. The fourth category consists of two varieties which contain the major constituents methyl tetradecanoate, methyl hexadecanoate, methyl 9,12-octadecadienoate and methyl 13, 16-octadectrienoate.

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P. 150. Optical purity of (R)-(-)-1-octen-3-ol in the aroma of various species of edible mushrooms

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Mushrooms have long been used as a food or a food flavouring material because of their unique and subtle flavour. The characteristic flavour of certain mushrooms species have been well documented [Wąsowicz, 1974; Fischer & Grosch, 1987; Mau, 1992 and 1997; Venkateshwarlu *et al.*, 1999]. It is established that the main odorant of mushrooms is 1-octen-3-ol. It occures in mushrooms in predominant minus optical form [Chambers *et al.*, 1998].

The characteristic enantiomeric ratio of 1-octen -3-ol in various species of edible mushrooms was the subject of the investigation. The microdistillation-extraction apparatus of Likens-Nickerson was used for aroma isolation. The volatiles were separated on usual GC capillary column HP-5 PHMS, and on chiral GC column RtβDex s.a. connected to HP-6890 gas chromatograph. The following species were analysed: *Agaricus bisporus (*two varieties), *Pleurotus ostreatus, Hericium erinaceum, Xerocomus badius* and *Lentinula edodes* known as *Shii-take.* The samples of *Agaricus bisporus, Pleurotus ostreatus* and *Hericium erinaceum* were grown on few kind of growing media.

Despite significant differences stated in the flavour profiles of investigated mushrooms, in all of the species 1-octen-3-ol was one of the most abundant compounds: at the highest concentration in *Agaricus bisporus* (about 60% of total volatiles). In all of the species the predominant form of this compound there was (R)-(-)-1-octen-3-ol. Its optical purity was the highest in *Agaricus bisporus* (over 98.9%) in both varieties, but also very high in the other mushrooms: *Pleurotus ostreatus* (over 97.2%), *Hericium erinaceum* (over 96.8%), *Xerocomus badius* (over 88.9%) and *Shii-take* (over 95.5%)

The data obtained allow to suggest high optical purity of (R)-(-)-1-octen-3-ol in the edible mushroom aroma independently from the species. It can serve as the criterion of aroma authenticity of mushroom flavouring substances on the market. Stereochemistry has to be considered when studying xenobiotics, agrochemicals, food additives, flavour and fragrances [Maier *et al.*, 2001]. Optical purity of some odorants can be useful in aroma authenticity control system [Mosandl, 1995]. Several mushroom-like flavouring substances were controlled in this work. In all of them the racemic ratio of 1-octen-3-ol was stated, thus mean not natural origin of flavour.

It was also found that SPME (solid phase microextraction) used instead of distillation can be beneficial in the control system because of its convenience and speed. The enantiomeric ratios measured by SPME – with polydimethylsiloxane fiber- were just the same as with distillation.

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