GRASSE • SEPTEMBER 10TH - 13TH, 2006

37th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

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ISEO 2006

PROGRAM & BOOK OF ABSTRACTS

Grasse. French Riviera. *Land of naturals.*

37th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS ISEO 2006

Grasse – Opio
September 10-13, 2006

PROGRAM

BOOK OF ABSTRACTS

LIST OF PARTICIPANTS

Organization:

Club des Entrepreneurs du Pays de Grasse

PRODAROM

Association des Ingénieurs et Techniciens de la Parfumerie (AITP)

Edited by Daniel Joulain

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Conference venue

The conference and poster sessions will be held in the central building of the Club Med in Opio, Alpes-Maritimes, France. The after-dinner session of Monday, September 11th will be held in the Convention Center. The symposium will commence on Monday, September 11th at 9.00 and and close at 14.00, Wednesday, September 13th.

Registration

The registration desk will be located on the first floor of the building facing the main building on the other side of the Micocouliers court yard. On Sunday, it will be open from 16.00 to 20.00. On Monday, it will be open from 8.00 to 17.00, and on Tuesday and Wednesday from 8.30 to 14.00. Participants are kindly requested to wear their name badges at all times.

Incoming messages and message board

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

Conference website

The Internet homepage of the ISEO is constantly kept up-to-date: http://www.iseo-grasse.com

Oral presentations

Plenary lectures are limited to 45 minutes. Other oral presentations will not exceed 15, 20 or 30 minutes, including discussions. The time allotted to each presentation is shown in the program (page 5). The lecture hall is equipped with PC projection facilities only. To avoid technical problems, personal laptops cannot be utilized; a disc, CD-ROM or memory stick should be provided at least one hour prior to the beginning of the session. Precautions will be taken to avoid any unauthorized copying of data. Speakers should meet the chairperson and provide him/her with a short CV (3-5 lines maximum) 20 minutes prior to the beginning of their session. Speakers are invited to familiarize themselves with the microphone and laser pointer. Speakers should not use more time than is scheduled in the program. Due to technical constraints in the schedule of the conference auditorium, the session chairman will stop any lecture which runs overtime.

Posters

Posters will be located at the first floor of the central building, above the Palladio.
Posters **P-01** to **P-50** will be displayed during Poster Session 1 (Monday, 17.00-19.30), and should be installed as early as possible, from 8.30 on Monday morning. Guidelines and material to affix the posters properly will be available on site. Main authors must be present during the entire poster session. Posters must be removed by 19.30.
A similar procedure applies to **all other posters** (**P-51** and up), to be displayed during Poster Session 2, on Tuesday (17.15-19.30)

Miscellaneous recommendations regarding the presentations.

Cameras of any kind are strictly forbidden in the conference room. The same applies to tape recorders or the like. Any photography of posters may be done only after obtaining the permission of the main author. Cell phones must be switched off during the oral sessions. Smoking is not allowed in the conference and poster rooms, nor in the coffee break premises. Due to a tight schedule, delegates are kindly invited to be present in the conference room at least 5 minutes before the beginning of the sessions.

Exhibitors.

Exhibitors booths will be located at the first floor of the central building, above the Palladio.

Attire

Informal dressing is recommended throughout the symposium for all occasions, including dinners. Shirts are to be preferred to jackets. Shorts and swimming suits and bare feet are not allowed in the conference room, nor are "flip-flops".

Insurance / Liability

The organizers will not accept responsibility for any accommodation problem, nor for any accident, personal injury, loss or property damage sustained during the symposium.

37th International Symposium on Essential Oils

September 10-13, 2006, Grasse-Opio, France

Scientific Program

Monday September 11, 2006 – Palladio Grand Auditorium

9.00 Opening Session – Welcome addresses

9.30 Plenary Lectures 1 and 2 Chairperson: Prof. Dr. G. Buchbauer

PL-1 **R. Anton**, France
Advantages of the use of essential oils and questions of safety assessment.

10.00 PL-2 **L. Hagvall** and Ann-Therese Karlberg, Sweden
Air exposure turns common fragrance terpenes into strong allergens.

10.30 Coffee break

11.00 Plenary Lecture 3 Chairperson: Prof. Dr. Chl. Franz

PL-3 **T. B. Adams**, USA
Safety evaluation of essential oils.

11.30 L-01 **P. Garnon**, France
The new REACH policy on chemicals and natural products.

11.50 L-02 **H. Schilcher**, Germany
Pesticides in essential oils.

12.10 Lunch break

14.00 Plenary Lectures 4 and 5 Chairperson : Prof. J.-P. Reynier

PL-4 **J. F. Lalko**, USA.
The dermal sensitization potential of various essential oils in the Local Lymph Node Assay (LLNA).

14.30 PL-5 **G. Ellis**, Switzerland
Dermal sensitisation Quantitative Risk Assessment (QRA) for fragrance ingredients.

15.00 L-03 **C. Auriault** and H. Groux, France
The Immunosearch approach to *in vitro* alternative for animal model testing of chemicals and natural products used in cosmetics.

15.20 L-04 I. Renimel, F. Pellicier, F. Joly and **P. André**, France
Could essential oils be considered as true active cosmetic ingredients?

15.40 L-05 **D. Lemarquand** and D. Davenne, France.
Modulation of the synthesis of type I and type III human collagen by essential oils.

16.00 Open discussion: evaluation of potential risks and real benefits of essential oils.

16.30 Refreshments/coffee break

17.00 Poster session 1: P-01 to P-50
19.30 End of poster session 1

19.30 Dinner break

21.00 **After-dinner session – Convention Center**

Oral communications:
Essential oils from the South Hemisphere. Chairperson: Prof. C. Menut

L-06 **K. Patel**, S. Subramanian, A. Sadaquat and M. Frostin, Fiji Islands.
Biological studies of essential oils from some selected Fijian plants.

21.20 L-07 **D. N. Leach**, R. S. Spooner-Hart, J. L. Markham, P. G. Waterman and J. J. Brophy, Australia.
Endemic Australian essential oils with insecticidal and microbial bioactivity.

21.40 L-08 S. van Vuuren and **A. Viljoen**, South Africa.
Aromatic plants from South Africa and their constituents as a model to study phyto-synergy.

22.00 End of session

Tuesday September 12, 2006 – Palladio Grand Auditorium

9.00 Introductory remarks Chairperson: Prof. Dr. C. Bicchi

Plenary Lecture 6

9.05 PL-6 **K.-H. Kubeczka**, Germany
A historical overview on analytical techniques in the field of essential oils during the last 40 years.

9.50 L-09 R. Rubinovitz, K. Kunz and **J. Oelichmann**, Switzerland.
Applications of Near-IR spectroscopy as a quality control tool in the flavour and fragrance industry.

10.10 L-10 **L. Mondello**, Italy.
Comprehensive two-dimensional gas chromatography for the investigation of the volatile fraction of complex flavour and fragrance materials.

10.30 Coffee break

11.00 Session on authentication Chairperson: Dr. A. Chaintreau

L-11 **T. Cachet**, Belgium.
Criteria for the identification in nature of flavouring substances.

11.20 L-12 **F. Bensaid**, M. Lees, G. J. Martin, M. Sarraf , A. M. van Nederkassel, Y. Vander Heyden, D. A. MacKenzie and N. J. Walton, France/Belgium/England
Authentication of natural vanilla flavourings by isotopic analysis and vanillin fingerprinting LC-LC-MS analysis.

11.40 L-13 **G. Remaud**, F. Le Grand, G. George and S. Akoka, France
New faces of the SNIF-NMR method: application of its recent improvements to methyl salicylate.

12.00 L-14 A. Vainshelboim, K. Momoh, M. Hayes, C. Raats, K. Price and **K. Korotkov**, Russia/USA.
Investigation of essential oils and aroma ingredients using Dynamic GDV.

12.20 Lunch break

14.00 Workshop on allergen analysis Chairperson : Dr. P. Liddle

L-15 **A. Casilli**, D. Sciarrone, P. Dugo, G. Dugo and L. Mondello, Italy.
Multidimensional gas chromatography for allergens determination in perfume formulation.

14.15 L-16 C. Bicchi, **C. Cordero**, P. Rubiolo, D. Joulain, N. Barat and R. Laurent, Italy/France.
Identification, quantitation and method validation for the analysis of suspected allergens in fragrances by comprehensive GCxGC-FID and GCxGC-quadrupole MS.

14.30 L-17 **H. Casabianca**, Y. Pacaud, I. Chanel and G. Charvet, France
Full vaporisation of allergenic substances in cosmetic products using head-space and GC-MS quantification.

14.45 L-18 **A. Chaintreau**, D. Joulain, C. Debonneville and N. Barat, Switzerland/France.
On-line sample fractionation for the GC-MS determination of suspected allergens in natural extracts, cosmetics and detergents.

15.00 L-19 **G. Lösing** on behalf of IFRA Analytical Working Group, Belgium.
GC-MS Quantitation of suspected allergens. Method performance and data treatment strategies.

15.15 Open discussion: quantitative analysis of minor elements in essential oils and related complex mixtures, a challenge for analyticals chemists.

16.15 Refreshments/coffee break

Plenary Lecture 7 chairperson : Dr. J. Demyttenaere

16.45 PL-7 **K. H. C. Başer**, B. Demirci, E. Yüzbaşioğlu and M. Y. Dadandi, Turkey.
Essential oils of *Phlomis* species of Turkey;

17.15 Poster session 2: P-51 and up.
19.30 End of poster session 2

19.30 Dinner.

Wednesday September 13, 2006 – Palladio Grand Auditorium

8.45 Plenary Lecture 6 Chairperson: Dr. B. Lawrence

PL-8 **C. Sell**, United Kingdom
Commercial production of terpenoids.

9.30 PL-9 **B. Mompon**, France
Updating of extraction / purification technologies in the field of essential oils and related natural products.

10.00 L-20 **K. Allaf**, France
Instant Controlled Pressure Drop (DIC) as a process of extraction of volatile oils: the impact of the rate of pressure drop.

10.15 L-21 **M. Koşar**, Turkey.
Comparison of microwave-assisted hydrodistillation and hydrodistillation methods for the essential oils of *Foeniculum vulgare.*

10.30 Coffee break

General Chairperson: Prof. Dr. K.-H. Kubeczka

11.00 L-22 **Y. Asakawa**, Japan
Highly efficient production of fragrant compounds from crude drugs and liverwort constituents by microorganisms.

11.20 L-23 **A. Zada**, Israel
A convenient enzymatic resolution of racemic lavandulol: an important fragrance and pheromone component.

11.40 L-24 **J. Bernáth**, Hungary
Chemotaxonomic and production, biological evaluation and oil quality of *Foeniculum* accessions.

12.00 Concluding remarks.

12.30 Lunch

14.00 End of Symposium

Abstracts
of
Plenary Lectures

PL-1

Advantages of the use of essential oils and questions of safety assessment

Robert Anton*

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Essential oils which are very complex mixtures have been used for centuries worldwide for pleasure in relation with fragrance ingredients, but also for health benefits like antiseptic activities before the discovery of antibiotics, if we just take this example. In fact, their positive biological human effects are considerable (antifungal, anti-inflammatory, analgesic, antispasmodic, antisecretory ...).

It is generally admitted by the consumer that these flavouring compounds, these cosmetic products and even some drugs with essential oils are devoided of toxicity because they are considered as "natural substances".

As a matter of fact, their misuses recently observed give rise to a series of accidents and the national and European authorities are trying to elaborate risk assessment requirements.

Very few problems can occur when using essential oils as flavourings because of the small quantities taken orally. It is not the same for food supplements presented as well for maintaining health homeostasis or providing some physiological impacts at the interface of the drugs. In this last area, the marketing authorization of a new drug requires the elaboration of a complete pharmaceutical and pharmaco-toxico-clinical dossier in order to prove the quality, the efficacy and the safety of the final drug. For cosmetics, the main problems are linked with cutaneous sensitization and allergenic reactions and because of their lipophilic properties, essential oils could have a systemic bioavailability which may involve secondary potential toxic effects on the nervous system.

Thus the quality of these essential oils is of prime importance and their toxicological assessment increasingly must be aimed at in order to assume the safety of their use for the consumer and their future development on a scientific basis in the field of health *largo sensu* and of the art in cosmetics.

References.

J .C. Anton, B. Weniger and R. Anton, *Huiles essentielles*, in « Actifs et additifs en cosmétologie », 3e éd., Lavoisier, Paris (in press).
E. Teuscher, R. Anton and A. Lobstein, Plantes aromatiques, Lavoisier, Paris (2005).

(*) Professor Anton is a Member of the French Academy of Pharmacy and of the French Academy of Medicine

PL-2

Air exposure turns common fragrance terpenes into strong allergens

Lina Hagvall, Ann-Therese Karlberg

Dermatochemistry and Skin Allergy, Department of Chemistry, Göteborg University, SE 412 96 Göteborg, Sweden. E-mail: lina.hagvall@chem.gu.se

When considering the allergenic activity of a compound not only the possibility of bioactivation by skin metabolism but also air activation by autoxidation must be taken into account.

The common fragrance terpenes (*R*)-limonene and linalool easily autoxidize at air exposure. The introduction of oxygen enables the molecules to form antigens with skin proteins and thus cause skin sensitization. Since these fragrance compounds are used in a wide variety of consumer products we have found them to cause patch test reactions among consecutive dermatitis patients of the same frequency (2-3%) as the common allergens used for standard screening. In 60% of the cases a clear correlation with allergy to fragrances and perfumed products was observed.

Chemical investigations have identified primary (hydroperoxides) as well as secondary oxidation products (aldehydes, ketons, alcohols). Experimental sensitization studies using the Local Lymph Node Assay (LLNA) in mice revealed a significant increase in the sensitizing capacity of the oxidation mixtures compared to pure limonene and linalool. The primary oxidation products, the hydroperoxides, were shown to be the most potent sensitizers formed.

We have also compared air exposed lavender oil with its synthetic components, as well as a "synthetic" lavender oil, made of the three main components, linalyl acetate, linalool and caryophyllene, mixed together in the same proportions as in the natural oil. When exposed to air, the main constituents oxidized in lavender oil as well as in "synthetic" lavender oil, at approximately the same rates as the pure compounds. The same oxidation products could be isolated. The autoxidation was shown, using the LLNA, to influence the allergenic activity of both natural and "synthetic" lavender oil. The hydroperoxides were the strongest allergens of the oxidation products tested.

It is important to test the patient with the offending compounds for diagnosis of allergic contact dermtatitis. A negative diagnosis can be due to failure in testing with the correct substances. In the case of air activated compounds, testing should not be performed with the pure substances but rather with the oxidation mixture or with the most sensitizing oxidation products (the hydroperoxides).

Compounds, easily activated at air exposure, should be prevented from oxidative decomposition by proper handling and storage. More research is needed in this area.

PL-3

Safety evaluation of essential oils

Timothy B. Adams*

Scientific Secretary of the FEMA Panel Flavor & Extract Manufacturers' Association, 1621 I Street, N.W., Suite 225, Washington, D.C. 20006, USA.

Essential oils are chemical mixtures of volatile substances obtained primarily from botanical sources. Many essential oils are derived from plants that are also used as food. In the present paper, a science-based procedure for the safety evaluation of essential oils is discussed. The procedure involves the safety evaluation of the chemical constituents in the essential oil and the variability of those constituents in the commercial product. This procedure, which is not intended to be viewed as a rigid check list, begins with a description of the chemical composition of the commercial product, followed by a review of the data on the history of dietary use. Next, constituents of an essential oil are assigned to congeneric groups of structurally related substances (e.g., menthol and menthone). Each group is then assigned a toxic potential (Classes I, II, or III) based on a large database of toxicity data. In subsequent steps, the intake of each congeneric group is determined based for the consumption of the essential oil in flavorings. This intake level is then compared to data on the metabolic pathways and toxicity potential of the congeneric group. In some cases, additional toxicological and analytical data may be required for completion of the safety evaluation. Constituents of unknown chemical structure are also evaluated. Acceptable levels of unknowns are determined based on the intake of the essential oil as a component of food and the widely-accepted regulatory threshold for toxic and carcinogenic risk. The procedure concludes with an evaluation of the NFC in its entirety, also considering combined exposure to congeneric groups. The objective of this chemically-based procedure is that no significant portion of the essential oil should go unevaluated. In this manner, constituents with the greatest toxic potential will always be included in the evaluation. The first experiences with the use of this procedure are very promising. Future safety evaluations of larger numbers of essential oils will indicate the usefulness of the system, either in its present form or in a form modified on the basis of experience.

Note: This presentation is intended to explain the fundamental features of the safety evaluation of essential oils to the international industry in a form that is comprehensible by both those with and without scientific training.

* Dr. Adams is the Scientific Secretary to the FEMA Expert Panel.

PL-4

The dermal sensitization potential of various essential oils in the Local Lymph Node Assay (LLNA)

Jon F. Lalko

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Essential oils are commonly used fragrance ingredients derived by physical processes from odorous plant materials. These oils are utilized in the creation of fragrance compounds or may be appreciated for their individual character. In either case, they are routinely incorporated into various consumer products - soaps, shampoos, cosmetics, perfumes, detergents, etc. Essential oils are often complex mixtures in their own right, which may contain naturally occurring dermal sensitizers. The common use of these materials requires that accurate risk assessments be prepared to protect human health. The Research Institute for Fragrance Materials, Inc. (RIFM) routinely conducts dermal sensitization risk assessments of fragrance ingredients. Risk assessments for dermal sensitization proceed along the three tiers of general toxicology – hazard identification/quantification, exposure assessment and risk characterization. Recently, the Murine Local Lymph Node Assay (LLNA) has been increasingly used for hazard assessment. The LLNA is a validated alternative to traditional animal models for the identification of potential contact allergens.

There is little hazard data available, particularly in the LLNA, on the dermal sensitization potential of well-characterized samples of essential oils. We have conducted LLNAs on several essential oils with two aims. First was to utilize the LLNA to investigate the potential of individual essential oils to induce dermal sensitization and to determine the relative potency of those oils exhibiting a positive response. Second was to examine any difference in sensitization potential for the major components arising from their exposure in a mixture. Classically, a decrease in sensitization potential resulting from such exposure has been termed the 'quenching phenomenon' (*1*). To these ends, the oils were characterized by GC-MS and/or HPLC to determine their overall composition and allow for comparison of the results obtained with the oil to that of the individual components.

The results of our investigations to date show that, in the LLNA, the potency of essential oils with a predominant component (~≥ 70%) could be predicted based on the LLNA results of the component. Overall, no evidence of the 'quenching phenomenon' could be observed. For highly complex oils without a single major component, both positive and negative LLNA results could not be explained based on knowledge of the constituents. There is little information available on the utility of the LLNA to evaluate complex mixtures and several authors have cautioned against using animal assays for the safety assessment of mixtures without further research (*2*). As suggested, additional work is necessary to determine the relevance of LLNA data generated on mixtures to the human situation.

References.

(*1*) D. L. J. Opdyke, *Inhibition of sensitization reactions induced by certain aldehydes.* Food and Cosmet. Toxicol. 1976, **14**(3), 197-198.

(2) D. A. Basketter, K. E. Andersen, C. Liden, H. Van Loveren, A. Boman, I. Kimber, K. Alanko, E. Berggren, *Evaluation of the skin sensitizing potency of chemicals by using the existing methods and considerations of relevance for elicitation.* Contact Derm. 2005, **52**(1), 39-43.

PL-5

Dermal sensitisation Quantitative Risk Assessment (QRA) for fragrance ingredients.

G. Ellis[1], A. M. Api[2], D. A. Basketter[3], P. A. Cadby[4], M.-F. Cano[5], G. F. Gerberick[6], P. Griem[7], P. M. McNamee[8], C. A. Ryan[6] and B. Safford[3] (*)

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Some of the chemicals in common use today may have the potential to cause dermal sensitisation. However, the fact that a chemical is a skin sensitiser does not mean it cannot be formulated into consumer products at safe levels. This is also the case for fragrance ingredients, which include essential oils and their components. Based on advances in our understanding of a range of factors associated with the induction of dermal sensitisation, it is possible to conduct an exposure-based quantitative risk assessment (QRA) for induction of dermal sensitisation to determine safe levels of fragrance ingredients in different consumer product types.

The skin sensitisation QRA approach for fragrance ingredients follows the same four steps as used for general toxicology risk assessment:

- Hazard identification - This involves the use of experimental data to determine the skin sensitisation potential of the fragrance ingredient. Typically this would involve a murine Local Lymph Node Assay (LLNA), but may also involve the use of other assays such as the guinea pig maximization test or Buehler guinea pig test.
- Dose-response assessment or Hazard quantification - The dose response for induction of skin sensitisation is typically determined in the first instance using animal assays such as the LLNA. Human assays such as the Human Repeat Insult Patch Test (HRIPT) may also be conducted to provide confirmation of the no observed effect level (NOEL).
- Exposure assessment - Exposure to the fragrance ingredient is determined using habits and practice data for consumer product use and human parameters data.
- Risk characterization - The data from the previous steps are used to determine an acceptable exposure level to a fragrance ingredient against which the real life consumer exposure to that fragrance ingredient in a specific product type can be compared. The acceptability or unacceptability of real life exposures can then be determined accordingly.

In developing a quantitative risk assessment method for skin sensitisation of fragrance ingredients, based on the above recommended approach, some new terms have been adopted and will be presented. The new terms are "No Expected Sensitising Induction Level" (NESIL) and "Sensitisation Assessment Factors" (SAFs) that replace no observed effect level (NOEL) and uncertainty factors, respectively, in general toxicology risk assessment. These terms have been adopted to take into account unique elements of quantitative risk assessment for skin sensitisation.

This presentation provides an overview of the principles of exposure-based QRA as applied to fragrance ingredients and provides a practical example using a fragrance ingredient in different product types. The applicability of this approach specifically to essential oils and their components as used in fragrances will also be discussed.

(*) The authors are members of the QRA Expert Group convened by the COLIPA Toxicology Advisory Group and the Joint COLIPA/AISE/EFFA/IFRA Perfume Safety Group).

PL-6

A historical overview on analytical techniques in the field of essential oils during the last 40 years.

Karl-Heinz Kubeczka

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In the past, a great number of attempts have been made to improve the analytical techniques for the analysis of essential oils. Gas chromatography is the most efficient chromatographic technique for separating those complex mixtures due to the improved resolving power of the commercially available gc columns and the availability of highly sensitive detectors. Conventional gas chromatography using fused silica capillaries with different stationary phases, including chiral phases have been until recently the prime techniques for the analysis of essential oils. However, the time required for the separation of an essential oil takes one hour or even more and, therefore, several attempts have been made to shorten the time of analysis. The application of narrow bore capillaries, accelerated gas flow, high temperature ramps and fast data acquisition – requirements, which only can be realised with modern instruments – resulted in a substantial reduction of time required for the separation of oil with virtually no loss in resolution. This fast and ultra-fast GC separations yielded an outstanding reduction in time by factors of ten and even more, without affecting the resolution.

The problems encountered with peak overlapping or insufficient separation of components has been solved by pre-separation prior to GC analysis by conventional column chromatography, HPLC, or by two-dimensional gas chromatographic techniques. In the simplest case two GC capillaries with different selectivities are serially connected and the portion of unresolved components from the effluent of the first column is directed into second column, e.g. a capillary with a chiral coating. By means of this heart-cutting technique many separations of chiral oil constituents have been successfully performed in the past. However, the coincidental overlap of two or more components can be a serious problem for very complex samples. This can be overcome by the recently developed "comprehensive two-dimensional gas chromatography" (GCxGC). This method is able to subject the total sample to simultaneous two-column separation since it combines two directly coupled columns, which provide orthogonal separation of components on two capillaries with different selectivity. At present, however, there is only a limited number of publications in the field of essential oils analysis in which the striking potential of this method has been studied.

Furthermore, several online couplings of a gas chromatographic device with a spectrometer, the so-called hyphenated techniques will be discussed briefly, which enable the identification of the chromatographically separated components. The coupling of a gas chromatograph with a mass spectrometer is one of the most often used analytical techniques in the field of essential oils. Coupling of a GC with a Fourier transform infrared spectrometer (GC-FTIR) yields to some extend complementary results to GC-MS and proved to be especially useful in distinguishing isomers which cannot be distinguished by MS. Published GC-UV and GC-AES couplings have not gained much importance, due to the limited obtained information.

Finally the application of ^{13}C NMR spectroscopy for the analysis of unprocessed essential oils will be discussed.

PL-7

Essential oils of *Phlomis* species of Turkey

K. Hüsnü Can Başer[1], Betül Demirci[1], Ertuğrul Yüzbaşıoğlu[2], Mehmet Yaşar Dadandı[2]

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[2] Erciyes University, Faculty of Science and Letters, Department of Biology, 38039 Kayseri, Turkey.

The genus *Phlomis* L. (Lamiaceae) is represented by 34 species, 6 varieties and 10 natural hybrids as perennial herbs or shrubs in Turkey (*1*). Recently two new natural hybrids were added (*2,3*) and total taxon number reached 52, of which 34 are endemic (including hybrids) to Turkey.

The essential oils were obtained from aerial parts of *Phlomis* species growing in Turkey, namely; *P. monocephala, P. leucophracta, P. grandiflora* var. *grandiflora, P. russeliana, P. lycia, P. lunariifolia, P. amanica, P. longifolia* var. *bailanica, P. viscosa, P. bourgaei, P. chimerae, P. x vuralii, P. sieheana, P. physocalyx, P. angustissima, P. sintenisii, P. syriaca, P. kotschyana, P.bruguieri, P. brunneogaleata, P. linearis, P. armeniaca, P. oppositiflora, P. kurdica, P. nissolii, P. capitata, P. x bornmuelleri, P. x melitenense, P. pungens* var. *hirta, P. pungens* var. *pungens, P. pungens* var. *hispida, P. integrifolia, P. rigida, P. samia* constituting 34 taxa including 29 species, 3 varieties and 3 hybrids. The analyses were performed by using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems, simultaneously. The essential oil analysis of *Phlomis rigida, P. samia* and *P. linearis* have been published (*4,5*).

References.

(*1*) A. Huber-Morath, *Phlomis* L., in: *Flora of Turkey and East Aegean Islands* (P. H. Davis, Ed.). University Press, Edinburgh, **Vol. 7**, pp.102–126 (1982).
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PL-8

Commercial production of terpenoids

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Terpenoids are the most important group of secondary metabolites as far as the fragrance industry is concerned and their role as vitamins adds weight to their commercial importance. It is not surprising therefore that a great deal of effort has been expended in finding economic routes for synthesis of terpenoids on the industrial scale.

The two earliest commercial routes to the major terpenoid fragrance ingredients were developed almost simultaneously. One used β-pinene from turpentine as the starting material and the other used petrochemically derived methylbutenol and methoxypropene. These two basic approaches (albeit refined over time) have co-existed in economic balance ever since and more recent turpentine and petrochemical based routes have been added to the commercial equilibrium.

One clear driving force for process improvement and new route development is the desire to reduce environmental impact. However, full life cycle analysis of any of the routes is a very complex and arduous task and so it is difficult to evaluate their relative sustainability.

Menthol provides an interesting example of such an economic balance. Currently there are three major routes to crystalline menthol. One is extraction from mint, another uses an alternative renewable resource, turpentine, and the third relies on petrochemical feedstocks. A number of other syntheses, which start from natural feedstocks, have been forced out of production by competition from the three major routes but, as economic factors change, some of them could come back into use, even if only on a local basis.

Regulatory issues are having a major effect on the fragrance and flavour industry and terpenoid ingredients are no exception. Nine of the twenty six materials requiring labelling under the regulation of EC's 7th Amendment (*1*) are terpenoids or terpenoid derivatives and seven of these occur in a wide variety of essential oils. The vast majority of woody odorants, many of which are terpenoids or terpenoid derivatives, do not pass the OECD biodegradability test and may therefore become issues under REACH legislation (*2*).

In the past, many essential oils were used as sources of feedstocks for terpenoid synthesis and some still are. There are a number of possible sources of raw materials which are not fully exploited and these could have potential for fragrance ingredients of the future.

With all of these factors interacting in balance and with the degree of uncertainty around many of them, it is difficult to predict exactly what the future will hold. However, it does seem fairly certain that there will be plenty of scope for and need of research in terpenoid chemistry.

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For a comprehensive update, see: http://ec.europa.eu/environment/chemicals/reach.htm

PL-9

Updating of extraction / purification technologies in the field of essential oils and related natural products

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New technologies do not claim to overthrow traditional processes of aroma and essential oil preparation. But if stills are a reliable value, so are micro-waves and new solvents (fluorated solvents, supercritical CO_2) or are about to become so.

Among microwaves technologies : the VMHD (Vacuum Microwave Hydrodistillation) which is a production process of essential oils and aromatic waters. One of its advantages is the possibility to treat a fresh plant without water addition. Thanks to microwaves, the steam needed to convey the volatile aromatic fraction is generated from the sole water contained in the plant. Besides, applying vacuum reduces temperature treatment and so preserves the most fragile constituent integrity. It is therefore a fast process which uses little energy and the obtained extracts have an aromatic profile very close to treated vegetal material.

In the field of non-using organic solvent processes, supercritical CO_2 is now essential. It offers simple and efficient solutions to legal and safety restrictions. Thanks to pertinent parametering of temperature and pressure, it is possible to obtain either a standard essential oil aromatic fraction or an oleoresin fraction type from the same plant. Adjusting temperature and pressure during the same operation enables to obtain both a volatile aromatic fraction and a desaromatized oleoresin (e.g. rosemary).

Fluorinated solvents are a more recent alternative. On a regulatory level, they have the advantage of being non toxic and ozone layer friendly unlike chlorinated solvents. On a technical level, they behave like the supercritical CO_2 : they are lipophilic, they have a weak solubilising capacity with a great selectivity. A perfluorinated solvent like perfluorohexane enables to deterpenate some essential oils with a good recovery rate of functionalized aromatic constituents. With a standard hydrofluoroether (R_F–O-R_H), fractionating fats and obtaining an enriched unsaponifiable extract becomes possible. These solvents have applications in extraction and formulation.

In the field of purification, CPC now offers the same performances as preparative HPLC. But unlike the latter, it gives more operating flexibility by using liquid stationary phase only, therefore cheaper, and reducing solvent consumption. An industrial continuous process equipment is now available on the market.

Abstracts
of
Oral Communications

L-01

The new REACH policy on chemicals and natural products

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Perfumes and cosmetics ingredients are generally considered as chemical substances or preparations. As such, they were recently subject to different European regulations which aim to improve the safety of consumers and environment: biocides, cosmetics, detergents, chemical substances, etc.

As these rules are elaborated for defined synthetic chemicals, natural products are considered and included as ingredients. However, their own specifications generate many technical and economical difficulties to apply them, and carry out the required evaluation for completing authorization dossiers.

Whereas pharmaceutical and food regulations have been adapted to integrate such specifications, chemical regulations ignore them which, consequently, may generate a real threat for the survival of aromatic natural products as fine chemical ingredients.

Through the example of the recently implemented chemicals policy REACH (*1*), this paper intends to illustrate those technical and economical problems, and proposes solutions to maintain the production and uses of traditional and prestigious natural ingredients.

Reference.

(*1*) **R**egistration, **E**valuation and **A**uthorization of **CH**emicals.
For a comprehensive update, see: http://ec.europa.eu/environment/chemicals/reach.htm.

L-02

Pesticides in essential oils

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In view of their world-wide distribution in the plant kingdom and the atmosphere, their long half- life, their high degree of stability to meteorological and metabolic influences, and the fact that their **physical characteristics** are similar to those of essential oils, **pesticides**, especially **organochlorine pesticides**, let expect their presence in most essential oils.

The paucity and inaccuracy of the few available published analytical data are a consequence of the problem of suitable and reliable assay methods.

The mostly used analytical method is the modified DFG – method S 19 (*1*). After the cleanup steps on Biobeads SX 3, and if necessary with conc. sulphuric acid, the gas chromatographic determination will be carried out on two different columns with ECD or GC-MS. The DFG S 19 method is similar to our published method (*2*). Therefore, our results are comparable with the results of non-published data of other laboratories (*3,4*).

In addition to the pesticide analysis from 130 samples of 40 different essential oils in our own working group we got the results from 370 samples of 31 different essential oils from the two cooperating laboratories (*3,4*). We found organochlorine pesticides to be present in about 66 % of the examined samples. In about 58 % the levels exceeded those which are permitted by the German Residue Limits Ordinance (RHmV 1994).

The two laboratories found pesticides in about 60% of the examined essential oils, but only 30% were higher than the German Residue Limits. The mostly contaminated essential oils were those from *Mentha arvensis* var. *piperascens*, *Citrus aurantium* and *Citrus limon*, as well as *Cymbopogon* spp.

We propose own maximum limits for essentials oils, regarding the toxicological relevance. The following model calculation is proposed : for example a person of 60 kg with a daily intake of 1,5 g of essential oil with the relatively high concentration of 1 microgram/g lindane would be ingesting only 0,3% of the accepted daily intake (ADI) , which allows 8 micrograms per kg bodyweight.

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(*3*) Sebastian Kneipp Forschung, Labor für Rückstands- und Spurenanalytik, 86825- Bad Wörishofen, Germany.

(*4*) Phytolab GmbH & Co. KG, 91487- Vestenbergskreuth, Germany.

L-03

The Immunosearch approach to *in vitro* alternative for animal model testing of chemicals and natural products used in cosmetics.

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Today, we must understand more of the basic mechanisms of allergic and inflammatory reactions caused by perfume and cosmetic ingredients. Legislators will progressively require data as complex as those demanded for drug approvals. Regulatory pressures are growing, with the introduction of REACH and the 7th amendment to the European Cosmetic Directive. This will induce manufacturers to undertake more research and development designed to better characterise the components of essential oils and and to offer products with a minimum risk to induce skin sensitisation.

It is of paramount importance that we develop innovative *in vitro* test systems to replace current animal testing methods, which will be phased out by 2009. We should be sympathetic to this trend and even anticipate and participate in it..

The causes of noxious dermatological effects such as contact allergy and skin irritation must be differentiated. Currently there is no means to predict whether a given molecule will cause an unwanted effect. The primary objective of **Immunosearch** is to develop indicators of such changes to the physiological condition of dermal target tissues, by potential allergen and irritant agents. The powerful techniques of pharmacokinetic modeling and fast data analysis, coupled with recent advances in molecular biology, will be the techniques of choice.

Several industrial partners, including Robertet, Mane, SkinEthic, IrisPharma, together with government agencies are already setting up these procedures. Significant progress is being made by our young company, towards the definition of bio-markers which correlate with immuno-toxicity. Four complementary procedures are being used to quickly obtain predictive bio-markers for allergens and irritants. Already, **Immunosearch** techniques of analysis of gene expression by RT-PCR are well advanced employing our extensive experience of 800 genes of immunological and pathological interest. Our techniques are complimentary to those of P. Barbry at IPMC/CNRS-UNSA, which uses and stores statistical analytical data of gene pangenomic expression arrays. The data handling tools developed by INRIA, are assisting us. Finally all of this data handling and processing is then summarised by researchers at 13S/CNRS-UNSA.

Comparison, coordination and correlation of all the results of this work, with biological reality, is quickly undertaken by **Immunosearch**. It is absolutely imperative for the success of this project, that there is agreement between this *in vitro* testing (using cell-culture and reconstituted human skin) and current *in vivo* findings.

It is advantageous that all the participants in this consortium are from different fields of activity, yet reside on the same Provence-Alpes-Côte d'Azur (PACA) location, near to their industrial partners.

L-04

Could essential oils be considered as true active cosmetic ingredients?

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Essential oils have been used for a long time in different applications. They are mostly used in fragrances and perfumes. Aromatherapy is also well developed as an alternative medicine. But curiously, there are few applications in the field of cosmetology.

During the last years, some cosmetic companies have used essential oils for their activities on the human brain and consequently on the mind. The "Aromachology" approach combines psychic and physiological effects. Cosmetic industry is using this combined effect at present for its perfumes and make-up. But in the case of skin care products, the approach is more pharmaceutical-like, that is to say the activity of ingredients has been proved on skin biological targets. A new trend would be to enhance global effect of skin care products. Therefore, it is important to show that essential oils could be considered as true "cosmetic drugs" as well as holistic ingredients.The aim of this work is to illustrate by practical examples some properties of essential oils for skin care applications.

The first point deals with the field of slimming products. It has been shown that a specific composition of essential oils (EOC) can act as efficiently as some molecules usually used. *In vitro* and *in vivo* tests have been performed and they confirm the action of the specific composition. As far as cell culture of adipocytes are concerned, EOC works at the same level as Forskolin which is the best active molecule on the lipolysis. Ecography in vivo method has allowed us to see that EOC is as efficient as the best products on the market. These experiments prove the effectiveness of essentials oils and allow us to plan a new application of them.

The second point deals with the field of inflammation. It is more and more known that inflammatory mechanisms are very implicated in the ageing process. Inflammation occurs in sensitive skin problems and also in pigmentation problems. In vitro tests have shown that mediators, like Prostaglandin E2, Leukotrien B4 and Interleukine 8, can be modulated by specific essentials oils. These results validate the traditional use of these products and they enable us to anticipate a better implication of essential oils in the formulation of skin care cosmetic products.

An analytical assessment has to be done in order to reinforce the biological results. It could also be a way of identifying which molecules are responsible for the biological activities. But this work is still "in progress"...

L-05

Modulation of the synthesis of type I and type III human collagen by essential oils

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Companies marketing skincare products often claim that essential oils, when applied onto epiderm by cosmetic oils or creams, reduce wrinkles and improve skin tonicity. However, these claims have rarely been supported by reliable scientific studies.

This presentation is structured in 5 parts:

1. importance of the different types of collagen in skin aging;
2. description of the skin explants technique, protocol for application of essential oils, histological aspects (photos of the demonstrative data), image analysis and quantitative results;
3. origin and short analytical characterization of essential oils tested: lemon, grapefruit, rose, Roman chamomile, frankincense, myrrh, everlasting, black pepper, cypress, lemongrass, patchouli, angelica;
4. results on type I collagen;
5. results on type III collagen

In conclusion: our studies show highly significant variations of collagen levels through a rather short period (9 days). Increase of type I collagen has been established four times. No significant effect has been observed four times, and a negative effect twice.

Amongst the oils which are active on type III collagen, two have a huge effect. The homogeneity and the topography of the distribution of collagens are presented.

Reference

Biologie de la peau humaine. Publisher : Institut de la Santé et de Recherche Médicale.

L-06

Biological studies of essential oils from some selected Fijian plants.

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The increasing incidence and severity of infections and diseases have stimulated the search for natural drugs as a possible alternative to chemical substances. Essential oils have been accepted and recognized as having several therapeutic applications. The aim of the present study was to evaluate the antibacterial, antifungal and anti-malarial activity of essential oils from some Fijian plants: *Cinnamomum verum, Alpinia zerumbet, Coleus amboinicus* and *Cymbopogon coloratus*.

Microbiological activity of the essential oils of these 4 Fijian species was determined as these are used by native Fijians to scent coconut oils. Essential oils were obtained from the aerial parts of plants (also from flowers and rhizomes of *A. zerumbet*) by hydro distillation. The antibacterial and antifungal activities of essential oils were performed using the disc diffusion technique by placing 10 µL of the dilute essential oil (1 mg/10 µL). Disc moistened with 10 µg of Gentamicin was used as control. The results showed that *A. zerumbet* rhizome essential oils had good anti bacterial activity towards *Echerichia coli* and *Staphylococcus aureus*.

The essential oils were also tested for anti malarial activity using FBIT (Ferriprotoporphyrin IX Biomineralization Inhibition test) – a method developed by the members of a french group (UMR 152, Institut de Recherche pour le Développement/Université Paul Sabatier, Toulouse) based on the detection of solubilized β-haematin remaining after contact with drugs as a bioactive extract prevents its formation. *A. zerumbet* rhizome and *C. coloratus* leaf essential oils showed good anti malarial activity.

Results indicate that essential oils from some Fijian plants compare favorably and with some pathogens even better than the antibiotic or anti malarial drug. These results suggest a lot of applications of essential oils in different areas.

L-07

Endemic Australian essential oils with insecticidal and microbial bioactivity

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As part of a survey on biological activity of Australian essential oils, 324 oils selected from over 4000 in the "Brophy Collection", were screened for insecticidal activity against two-spotted mite and diamond back moth, as well as antimicrobial activity against *E. coli, S. areus, C. albicans* and *P. aeruginosa*. Sixteen oils from thirteen species (*Agonis parviceps, Crytptocaria cunninghamii, Crowae exalata, Melaleuca stypheloides, Rhodamnia whiteana, Leptospermum neglectum, Geijara parviflora, Backhousia, angustifolia, Achronychia acidula, Tasmania lanceolata, Tasmania glaucifolia, Eucalyptus cloeziana and Leptospermum morrisonii*) showed potent arthropod activity.

No activity was observed for any oil against *P. aeruginosa*, however fifteen oils showed significant antimicrobial activity against three of the test organisms (*Eucalytpus elata, Eucalyptus oblique, Eucalyptus mitchelliana, Melaleuca stipitata, Melaleuca argentea, Melaleuca nervosa, Melaleuca nesophyila, Melaleuca ericafolia, Melaleuca quinquenervia, Leptospermum petersonii, Leptospermum neglectum, Leptospermum oboratum, Myrtella retusa, Backhousia citriodora and Backhousia angustifolia*).

The high incidence of dual activity associated with oils that contained β-triketones led to a more focused survey of oils, principally from the family Myrtaceae. Oils rich in β-triketones were sourced from *Eucalyptus* (*E. cloeziana, E. bensonii, E. megacornuta, E. pilularis, E. macrohyncha, E. jensenii, E. baxteri* and *E. conjuncta*), *Leptospermum* (*L. scoparium* and *L. morrisonii*), *Backhousia angustifolia* and *Melalueca cajuputi ssp platyphyla*. β-triketones purified from these oils showed LD_{50} in the concentration range 0.04 - 0.36%. Data will be presented that led to the patent on natural insecticide "Qcide", based on the essential oil from an *E. cloeziana* chemotype with 85-95% tasmanone content.

The heartwood oil derived from *Eremophila mitchellii* has excellent termiticidal and termite repellant properties that have now ascribed to eremophilone and 2-hydroxydehydroeremophilone. Additional data on the chemistry, safety and efficacy of this oil will also be presented.

L-08

Aromatic plants from South Africa and their constituents as a model to study phyto-synergy

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Traditional healers rely not only on a single plant for therapeutic regimens but often combine various plant parts and even different species in the belief that efficacy may be enhanced. The extensive traditional use of aromatic plant in South Africa and their specific mode of preparation and administration prompted us to investigate various levels of possible pharmacological interaction. A number of *in-vitro* experimental procedures on indigenous South African medicinal plants have been undertaken which validate the role of synergism in phytotherapy.

1. Combination of different species:

Using time-kill methodology the synergistic interaction is demonstrated for the combined use of *Lippia javanica* (Verbenaceae) with *Artemisia afra* (Asteraceae) for the treatment of respiratory infections associated with *Klebsiella pneumoniae*. Similarly when *Salvia chamelaeagnea* and *Leonotis leonurus* (both Lamiaceae) are combined, synergistic actions were observed against Gram-positive bacteria while antagonism, synergism and/or additive actions were observed for the various ratios tested on the Gram-negative bacteria.

2. Interaction between various plant parts of the same species:

Antimicrobial studies on *Croton gratissimus* (Euphorbiaceae) show synergistic, additive, antagonistic or non-interactive action between plant parts depending on the specific ratio in which the plant parts are combined. Higher synergistic sensitivities have been noted for root/leaf combinations.

3. Interaction between volatile (essential oil) and non-volatile fractions:

Studies on *Tarchonanthus camphorates* (Asteraceae) and *Pelargonium* (Geraniaceae) species indicate that the antimicrobial activity of the non-volatile and volatile fractions singularly and in combination have different activity profiles and is pathogen specific.

4. Phytoconstituents:

The interaction on a molecular level is demonstrated for major oil constituents of *Osmitopsis asteriscoides* (Asteraceae), where camphor and 1,8-cineole in combination enhances antimicrobial efficacy. Conversely, the major volatile constituents of *Artemisia afra* in various combinations have no significant role on the antimicrobial activity of the plant.

5. Stereoselectivity:

Enantiomers and racemic mixtures of limonene displayed significantly different 5-lipoxygenase inhibitory activity suggesting stereoselectivity of the enzyme-catalysed reaction. Furthermore, the monoterpene 1,8-cineole appeared to cause partial potentiation of the anti-inflammatory activity displayed by limonene.

These examples detailing pharmacological interactions whether synergistically, antagonistically or on an additive level, play an important role in the understanding of traditional healing and advancing the phytotherapeutic application of medicinal aromatic plants.

L-09

Applications of Near-IR spectroscopy as a quality control tool in the flavour and fragrance industry

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The flavour and fragrance industry is dealing with a huge variety of raw materials from varied sources. It is common to use high volume and variety of materials with natural and geographic variations. There is the need to establish purity and potency controls. Because many other industries with high GMP requests, including food, cosmetic and pharmaceutical, rely on flavours and fragrances, there is a high demand for testing and control. The purity of every material entering and leaving facilities has to be tested and confirmed. Polarome International covers more than 1000 different ingredients with tens of thousands drums stored in three countries. Traditionally gas chromatography had been used for the analyses. However dealing with such a huge number of analyses an alternative method must be used.

Near-infrared spectroscopy has been proven to be an easy-to-use analytical technique. It requires virtually no sample preparation, it is universal and can be applied to solids and liquids, it is non-destructive, and multiple components can be analysed. NIR spectroscopy is an established method in pharmaceutical and nutraceutical industries for the verification of identity and in the food and feed industry for quantitative applications. Therefore it is obvious to apply NIR spectroscopy for essential oils, flavours and fragrances as well. Considering practical examples the potential of NIR spectroscopy for the quality control will be demonstrated.

One of the traditional techniques for identity control is Cluster Analysis, which is a well-established method. Normally essential oils are available from different sources, which often exhibit different compositions. For its applications it is important to differentiate between their origins. Practical examples will demonstrate the possibility to identify the geographic provenience, to distinguish between natural and synthetic samples, and to differentiate between chemically similar compounds. Even racemic forms can be identified.

Quantitative applications are always much more demanding than qualitative ones. There are many practical examples for quantitative applications. The ability of NIR spectroscopy will be demonstrated using the estimation of peroxide content. This is an important parameter to estimate, because it is an indicator of radicals and a potential for allergens. NIR spectroscopy is much more convenient than the standard laboratory method, which is a difficult multi-step titration. After calibration the peroxide content can be determined with the registration of just one spectrum, which is a matter of seconds.

In addition to identity control and quantitative analyses NIR spectroscopy can be implemented even more quickly with simple spectral comparison methods and the use of a ready-to-use spectral library.

The possibilities and benefits of NIR spectroscopy for the analyses of essential oils will clearly be demonstrated.

L-10

Comprehensive two-dimensional gas chromatography for the investigation of the volatile fraction of complex flavour and fragrance materials.

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Single column gas chromatography (GC) in combination with a flame ionization detector (FID) and/or a quadrupole mass spectrometer (qMS) is widely employed in the determination of complex matrix profiles. The latter, unfortunately, often can only be partially separated even on long capillaries. Inevitably, several monodimensional peaks are the result of two or more overlapping components hindering reliable identification and quantification. Consequently, a major objective in analytical chemistry concerns the continuous improvement and development of more powerful techniques.

Two-dimensional comprehensive gas chromatography (GCxGC) is a powerful multidimensional on-line technique for the analysis of very complex matrices providing a true comprehensive separation.

The present work, which can be considered the sum of distinct investigations, is based on the analysis of a variety of flavour and fragrance samples, ranging from medium to highly complex and with different chemical group compositions. The thorough separation/identification of all matrices was achieved through the exploitation of GC x GC peak capacity, the formation of group types on the 2D space plane and FID or MS hyphenation.

L-11

Criteria for the identification in nature of flavouring substances.

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From a regulatory and labelling perspective an important aspect related to the use of flavouring substances in food is the "nature-identical" status of a flavouring substance.

The International Organization of the Flavour Industry (IOFI) has therefore instituted a programme to evaluate the validity of identifications in nature of flavouring substances. This programme was put under the leadership of the IOFI "Working Group on Methods of Analysis" (WGMA), composed of industry and association scientists, for deciding on the validity of reported identifications.

The most important source of information of this programme is the scientific literature related to the identification of novel flavouring substances in various food products. However, over the period of time in which identification work has been undertaken, it has become apparent to the WGMA that a more thorough inspection of evidence is necessary in order to avoid mistaken identification.

The WGMA has discussed the problem and has agreed to a set of criteria to be met before a decision on a nature-identical status can be made (*1*). In the practice this means that any particular substance must have its identity confirmed by at least two methods, e.g. comparison of chromatographic and spectroscopic data with those of an authentic sample.

In this paper we will discuss the decision criteria and provide examples of recent decisions by the WGMA regarding acceptance or non-acceptance of reported identifications.

Reference.

(*1*) *Statement on the identification in nature of flavouring substances*, made by the Working Group on Methods of Analysis of the International Organization of the Flavour Industry (IOFI), Flavour Fragr. J. 2006, **21**, 185

L-12

Authentication of natural vanilla flavourings by isotopic analysis and vanillin fingerprinting LC-LC-MS analysis

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Vanilla is one of the most important aromatic flavour compounds used in food and cosmetics. The difference in price between naturally and chemically synthesised vanillin, combined with a "bio-trend" and demand for natural products by consumers has led the flavour industry to develop alternative sources of natural vanillin flavour based on different biotransformations of natural compounds.

A double analytical approach based on isotopic analysis and fingerprinting analysis by LC-LC-MS has been developed in order to distinguish between vanillin samples from different sources (natural ex-bean, synthetic, hemi-synthetic, ex-biotechnology from different micro-organisms). The isotopic part is based on a multi-element analysis using ^{13}C-IRMS and SNIF-NMR® techniques. The compositional part consists in the development of UV and MS trace enrichment fingerprint chromatograms of vanillin. The fingerprint chromatograms reveal trace components which are associated to the source of the vanillin.

The treatment of the isotopic data associated to representative samples of the main vanillin sources leads to a good discrimination of the different origins. An in-depth chemometric exploitation of the chromatographic data completes the isotope-based discrimination as the type of micro-organisms used for vanillin biotransformation can be differentiated from the trace fingerprints.

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L-13

New faces of the SNIF-NMR method: application of its recent improvements to methyl salicylate

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Quantitative deuterium NMR spectroscopy (^{2}H-NMR) allows the routine determination of site specific natural isotopic distribution. Known as the SNIF-NMR method, it measures significant variations in the isotopic distribution according to the origin of the molecule. Among several applications, the authentication of the origin of the products remains pertinent. This approach has been successfully applied on molecular probes from several essential oils (*1*) (*2*).

However, SNIF-NMR suffers from several drawbacks: (i) the long analytical time, (ii) need of internal reference, i.e. addition of exogenous compound in a precious sample and (iii) relatively large amount of purified molecule of interest. For these reasons, SNIF-NMR is still not a routine analysis that can be applied systematically within a quality control plan of raw materials.

We have shown recently (*3*) that the use of an electronic reference (ERETIC) can circumvent these above limitations. No chemical substance is added in the sample: no co-solubility, chemically stability, no peak overlap, no relaxation time restriction. Furthermore, with ERETIC as an independent reference, acquisition on samples can be performed in saturated conditions. The experimental duration can thus be dramatically reduced, without affecting the measurement accuracy (*4*).

As an illustration, we have applied this new approach (^{2}H-ERETIC-NMR) on the origin (natural vs. synthetic) of methylsalycilate as a tool for essential oils authentication. There is an interest in the identification of the origin of methylsalicylate since the natural extracts are 5-7 times more expensive than the synthetic product. Because the method allows a division by a factor of 4 of the experiment time, we were able to study about 20 samples of methylsalicylate, without saturated the NMR spectrometer schedule. On the basis of the isotopic data retrieved from the ^{2}H-ERETIC-NMR experiments the three main origins of methyl salicylate: synthetic, wintergreen oil and sweet birch bark oil are well characterised and separated (*5*).

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L-14

Investigation of essential oils and aroma ingredients using Dynamic GDV

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The perfumer, flavorist, and analytical chemist have struggled for years to assay the authenticity of essential oils and aroma ingredients. They investigate their origins, agricultural practices and extraction techniques; their synthetic, natural (conventional), or organic derivations; and their state of optical activity. Perfumers and flavorists may apply odor and taste successfully in distinguishing pairs of differing oils or aroma ingredients, but are not always accurate or analytically reliable. Chemists rely on single instrumental techniques such as gas chromatography (GC) and coupled techniques such as GC-MS (mass spectrometry), but they also cannot distinguish these differences. Advanced instrumentation and specialized techniques have been developed recently to distinguish accurately those differences but have a number of disadvantages (*1*). For example, high-resolution capillary GC cannot distinguish between optically active pairs of aroma ingredients, unless coupled with other specialized techniques, such as the use of chiral columns or the use of nuclear magnetic resonance (NMR) shift reagents. High-resolution MS and Site-Specific Natural Isotope Fractionation-Nuclear Magnetic Resonance (SNIF-NMR) techniques, which use isotopic analyses, have been used successfully to confirm the authenticity of essential oils and natural aroma ingredients. However, these techniques are costly and time-consuming.

A simple inexpensive technique that simultaneously and rapidly distinguishes between synthetic and natural materials or pairs of essential oils that are sourced, extracted or distilled differently, or exhibit different optical activity would be an important asset to the perfumer, flavorist, and chemist.

Essential oils are typically extracted from plants that proceed through different biological, physical, and chemical pathways, leaving subtle energetical traces that may be detected. A technique known as Dynamic Gas Discharge Visualization (GDV) has shown sensitivity in measuring and analyzing such intrinsic energetic differences of studied substrates. This technique elicits a corona glow during the interaction of a subject with a strong electromagnetic field (EMF). The corona (plasma) discharge, its image, and its changes can be captured, measured, and analyzed in real time using original computer software. This simple, rapid, and relatively inexpensive technique has been utilized as a supplement to traditional olfactory and advanced analytical techniques to differentiate between natural and synthetic essential oils (*2*). Additional applications may include distinguishing between natural oils and their nature identical counterparts that are synthetically composed to identically and chemically match those natural oils. Finally, it may be used to detect differences between organically and conventionally grown oils, optical isomers, and in oils extracted from plants in differing geographical regions.

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L-15

Multidimensional gas chromatography for allergens determination in perfume formulation

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Perfumes have been applied to human skin for thousands of years and are, today, characterized by a global social and economical importance. Hence, the improvement and development of analytical techniques is considered of the utmost importance by the perfume industries.

In recent years, the risk of contact allergy, induced by perfumery ingredients, has been the object of scientific debate. Under the current European legislation (7th Amendment of the Cosmetic Directive), the 26 most frequently recognized contact allergens identified by the Scientific Committee on Cosmetics and Non-Food Products Intended for Consumers (now "SCCP") must be labelled, by 11 September, 2004, on the final cosmetic product if specific quantities are exceeded. These limits are 10 mg/kg in a product intended to remain on the skin or 100 mg/kg in a product to be rinsed off of the skin, and as a consequence analytical methods should be developed to identify and quantify the potential allergens. Out of the 26, 24 are volatile and are amenable to GC analysis. Monodimensional gas chromatography-flame ionization (FID) and -mass spectrometry (MS) detection are commonly employed in the analysis of major and minor (comprehending suspected allergens) perfume components. Although the GC-MS approach, employed in the SIM mode, is a powerful tool for allergene quali-quantitative determination, the potential risk of false positives is rather high when complex matrices such as perfumes are analysed.

In the present work, the quantitation of potential allergens is achieved through a multidimensional (MD) GC-MS approach. In the latter, selected allergen-containing solute bands, are transferred from a first to a secondary column. In this way, the risk of coelution is dramatically decreased as long as primary column peak separation is sufficiently maintained. More reliable peak identification was achieved through a fast qMS detector, operated in the scan mode and connected to the secondary column outlet.

L-16

Identification, quantitation and method validation for the analysis of suspected allergens in fragrances by comprehensive GCxGC-FID and GCxGC-quadrupole MS

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One of the most important analytical tasks in which perfume industry has recently been involved is the quantitative determination of suspected allergens (SAs) limited by EU regulations in cosmetics. This study aims at developing a method to quantify SAs in fragrance comppounds and essential oils by comprehensive GCxGC-FID and GCxGC-quadrupole MS (GCxGC-qMS). The first part of this study dealt with quantitation of SAs by GCxGC-FID. Concentrations between 2 and 25 ppm (mg/L) of the analytes under investigation were spiked on a ring test perfume taken as a reference, while 1,4-dibromobenzene and 4,4'-dibromo-diphenyl were used as internal standards (*1*). Validation was carried out on the basis of the Eurachem protocols (*2*) through which the following performance parameters were determined: confirmation of identity, selectivity and specifity, limit of detection (LOD), limit of quantitation (LOQ), linearity (working and linear range), precision, accuracy and uncertainty. To fulfil the need for an unequivocal identification, the GCxGC system was also coupled with a qMS detector. This methodology applied to SA analysis has already been described (*3-5*). In the second part of this work, the effectiveness of a qMS operating at different scanning speed (1000 and 11111 amu/sec) was evaluated to identify (full scan mode acquisition) and quantify (SIM mode) SAs in fragrances compounds. In full scan mode, the mass range was reduced to 40-240 amu in order to increase the scan acquisition rate, while in SIM mode the influence of different dwell times (40, 10 and 5 ms) was tested. The number of scans for each single modulated chromatographic GCxGC peak and the total number of scans for the 2D peak together with half height peak width (referred to apex) of each SA in the standard mixture in both TIC and SIM modes were determined. Moreover, the match quality of the spectra obtained by GC-MS at 11111 amu/sec, GCxGC-MS at 1000 and 11111 amu/sec were compared. Identification (TIC) and quantitation (SIM) results showed that GCxGC-qMS with a limited mass range can be used sucessfully to analyse SAs in fragrance compounds.

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L-17

Full vaporisation of allergenic substances in cosmetic products using Head-Space and GC-MS quantification.

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We have developed a new approach using full vaporisation of samples in head space apparatus for complex matrices containing fats, surfactants and other additives in cosmetic/toilettry formulations (soap, toothpaste, shampoo, facial cream....). Headspace is used as an alternative to soxhlet or other SPE methods of sample preparation.

The first problem to be solved was the choice and the development of chromatographic conditions (phase polarity, temperature program), in order to obtain a good resolution between allergenic substances and main interferences (flavouring components found in fragrance materials). A polar column presented the best efficiency for such a problem.

Quantification was performed using proportionate standard addition (fortification with 5 different levels) with GC-MS SIM detection. The main disadvantage of quantification using head space sample preparation is the number of runs needed for standard addition. However, in many cases, standard addition offers a real advantage when matrix effects are observed in various samples.

Method validation was performed with regard to linearity, limit of detection (LOD), limit of quantitation (LOQ), reproducibility, accuracy and specificity.

Runs with GC-MS detection in SCAN mode were carried out to ensure the identification of components (Deconvolutive Mass Spectra software) and to adjust the sampling and standard addition levels, in respect to linearity evaluation obtained in the validation procedure.

Whatsoever, quantification of a great number of substances at such low levels in complex matrices remains a challenge for the analyst.

L-18

On-line sample fractionation for the GC-MS determination of suspected allergens in natural extracts, cosmetics and detergents

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The quantification of the 24 suspected allergens listed in the European Cosmetics Directive has given rise to an intense research activity in the recent years and several papers report their quantitation in fragrance concentrates and fine perfumery (*1*),(*2*),(*3*). In contrast, their determination in natural raw materials or in finished products remains an issue.

The existing sample preparation techniques compatible with a quantitative purpose will be briefly recalled, including the Automatic Liner EXchange (ALEX) presented at the previous ISEO. In the present work, this latter approach is extended to other systems capable of separating quantitatively volatiles from non-volatiles during the GC injection. This general approach of the on-line sample fractionation is applied to natural raw materials such as concretes, resinoids, absolute oils and fragranced finished products (shampoos, creams, detergents, etc.). The relative mean standard deviation and the relative mean squared error for all suspected allergens (except farnesol and ethyl salicylate) were below 32%. These figures look satisfactory as the method allows an important time-saving owing to automation, in contrast to conventional sample preparation techniques applied to the analysis of volatile compounds in cosmetics. Using this on-line sample fractionation, a single calibration curve can be used to quantify the suspected allergens in a variety of products independently from their matrix composition.

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L-19

GC-MS quantitation of suspected allergens. Method performance and data treatment strategies

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In 2003 the Analytical Working Group (AWG) of IFRA has published a method (*1*) to quantify 24 volatile compounds mentioned in the 7th amendment of the European Cosmetics Directive (*2*) in fragrance concentrates. This method was developed by members of IFRA/AWG and has been further improved for routine use with regard to GC and MS conditions. Special attention was put on the performance validation and strategies to overcome the risk of false positive or false negative results.

For the experimental work five different samples were prepared to challenge the method performance with respect to the complexity of fragrances, the risk of co elution and limit of quantification (LOQ). The results of this work are presented and are subject to an upcoming publication. It was proven through interlaboratory studies under practical conditions in quality control laboratories that the LOQ for all of the 24 substances is much higher than the limit of detection (LOD). Theoretical calculation of LOQ based on signal-to-noise ratios would be misleading, because they can not be achieved for such a complex matrix and the likelihood of co elutions.

The figures resulting from this study demonstrate realistic quantification limits based on eight laboratories of the IFRA members. It can be concluded that the complexity and the difficulties of proper quantification is more related to the nature of the constituents of a fragrance rather than just the number. Various strategies to minimise the risk of underestimation or false negative results with GC-MS SIM quantitation have been evaluated. The strategy using two different column polarities and three target ions with each successfully limits erroneous results compared to "two columns with one ion" and "one column three ions". However, it could be demonstrated that the possibility of false negative results still persists.

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L-20

Instant Controlled Pressure Drop (DIC) as a process of extraction of volatile oils: the impact of the rate of pressure drop.

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After having studied the use of the Instant Controlled Pressure Drop (DIC) technology as a process of extraction of volatile oils in the cases of some aromatic flowers and herbs as lavender and Indonesian ylang-ylang (*Cananga odorata*) flowers, we investigated the real impact of the rate of dropping the pressure on the process efficiency.

The DIC process is based on the thermo-mechanical effects induced by subjecting the raw material for a short time steam pressure (about 1.10^5 Pa to $6 .10^5$ Pa depending on the product), followed by an abrupt pressure drop towards vacuum (about 0.50 kPa.) (*1*). The fact of the use of abrupt pressure drop may provoke higher effect of autovaporization of volatile compounds, coupled to an instant cooling of the products allowing stopping thermal degradation, modification of the internal structure and eventually implies the rupture of cell walls, which enhances the internal diffusion.

In the present paper, we first describe the influence of process parameters, namely steam pressure (2.10^5-6.10^5 Pa), total processing time (30 sec - 20 min), and number of DIC cycles (1-9), on the oil yield and composition. The Instant Controlled Pressure Drop (DIC) (*1*) as a volatile oil extraction technique allows us to get a rapid, clean and environmentally friendly process: The DIC can be compared to the conventional technique of steam distillation (SD), but it is superior in terms of rapidity, oil yields and also oil quality. As an example, in the case of Indonesian ylang-ylang, the optimized total DIC processing time is 30 sec or 4 min versus 12 hours or 24 hours with steam distillation respectively; the oil yields are respectively 2.45% versus 2.4% or 2.74% versus 2.60%.

But we particularly studied the impact of the speed of pressure drop $\Delta P/\Delta t$. We increased this speed from 2.10^5 $Pa.s^{-1}$ up to 5.10^6 $Pa.s^{-1}$ with the same optimized processing parameters in terms of steam pressure, total processing time and number of DIC cycles. We proved systematically that the higher the pressure drop speed, the higher the total yields. In all cases, the total yield is doubled at the highest value of $\Delta P/\Delta t$; the characteristic of DIC as "instant" pressure drop may then be quantified.

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L-21

Comparison of microwave-assisted hydrodistillation and hydrodistillation methods for the essential oils of *Foeniculum vulgare*

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The fruits of *Foeniculum vulgare* Miller are used as a spice and for their different health effects such as appetizer, digestive, sedative and colic (*1*). Microwave-assisted hydrodistillation (MWHD) has been used as an alternative technique against the classical hydrodistillation (HD) in the last few years because of its shorter distillation time (*2-4*).

MWHD and HD were carried out for the extraction of volatile components in whole and ground fruits of *Foeniculum vulgare* Miller (fennel). Fruits were distilled using a microwave oven modified to fit a Clevenger apparatus. The effect of microwave energy on the yield and composition of the essential oil was investigated against the classical hydrodistillation. All the essential oils were analysed by GC-FID and GC-MS.

(*E*)-anethole was found as the main compound in the oils of both whole and ground materials (82.2-86.8%) using the two methods. Methylchavicol (4.0-4.9%) and limonene (2.2-4.9%) were also found in fennel oils obtained by HD and MWHD. The amounts of components characterized in the oils obtained from whole and ground fennel fruits were not affected significantly by microwave energy except for limonene.

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L-22

Highly efficient production of fragrant compounds from crude drugs and liverwort constituents by microorganisms

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Nootkatone **1**, one of the most important and expensive aromatic of grape fruit essential oil, decreases the somatic fat ratio and thus its demand is increasing in the cosmetic and fiber sectors. (+)-Valencene **2** was biotransformed by *Chloorella fusca* and fungi such as *Mucor* species, *Botryophaeria dothidea* (24 strains) and *Brotryodiplodia theobromae* (7 strains). Among them, *Chlorella fusca, Mucor* species, *B. dothidea* (PP8402, BD83911, BD830311) gave **1** in high yield (65-90%). However, the conversion ratio of valencene to nootkatone by *B. theobromae* is low. When **2** was treated by *A. niger*, seven metabolites, 11-hydroxy- **3** and a mixture of 11,12-dihydroxynootkatone (11*S*: **4**, 11*R*: **5**) with valenca-1,9-dien-11,12-diol **6**, 3-oxo-valenca-1,9-dien-11,12-diol **7**, valenca-1,9-dien-3β-11,12-triol **8** and eudesm-1-en-3 -one-11,12-diol **9**. On the other hand, 2α-nootkatol **10** with calcium antagonistic activity which was isolated from *Alpinia oxyphylla* was biotransformed by *A. niger* to give **4**, **5**, **6** and **9**. 2*β*-Nootkatol **11** was treated by the same *Aspergillus* to afford **4**, **5**, **8** and **9**. *Fusarium culmorum* converted valencene to 9β-hydroxynootkatone **12** along with **4** and **5**. Dihydro-**13** and tetrahydronootkatone **14** were biotransformed by *A. niger, A. cellulosae,* and *Mucor* species to give many oxygenated metabolites including acetonide and deisopropyl products. Biotransformation of aristolane-type sesquiterpene hydrocarbon (+)-aristolene **15** from the crude drug *Nardostachys chinensis* and of the 2,3-*seco*-aromadendrane-type sesquiterpene lactone plagiochilide **16** from the liverwort *Plagiochila fruticosa* by three microorganisms, *Chlorella fusca* var. *vacuolata*, *Mucor* species and *Aspergillus* was investigated. *C. fusca* and *Mucor* species introduced oxygen function into the cyclohexane ring of aristolene to give aristolen-2-one **17** possessing a characteristic citrus aroma and aristolen-8-one (=aristolone) **18** with inhibitory activity against melanin production while *A. niger* oxidized stereoselectively one of the 1,1-dimethyl group on cyclopropane ring of aristolanes and 2,3-*seco*-aromadendrane to give C-12 primary alcohol **19** and C-12 carboxylic acid **20**.
A. niger converted (+)- and (-)-cuparene- and herbertane-type sesquiterpene hydrocarbons into their cyclopentanones in high yield.

L-23

A convenient enzymatic resolution of racemic lavandulol: an important fragrance and pheromone component.

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Many natural products are chiral compounds. Optically pure enantiomers are required in perfumery as components or analytical standards. In some cases, the racemic mixtures are undesirable since the enantiomers may have different fragrances and odor thresholds. The direct synthesis of chiral compounds is usually long and complicated and, therefore, enzymatic separation of racemic mixtures is often the method of choice.

In this work we present the separation of racemic lavandulol, a primary terpenoid alcohol that can be found in lavender oil as the (*R*)-enantiomer. This compound is an important material in the fragrance industry and has been found recently to be a pheromone component of several pests. The odor quality and potency were analyzed lately (*1*) and it was found that the (*R*)-enantiomer is superior to the racemic mixture. We screened a large number of lipase enzymes for the separation of racemic lavandulol via transesterification with vinyl acetate in organic solvents (*2*). The enzymatic hydrolysis of several lavandulyl esters in a buffer solution has also been tested. The transesterification gave much better results and resulted in the formation of one enantiomer as the acetate and the second enantiomer was the free alcohol. The two products could be separated readily by column chromatography. We improved the resolution of racemic lavandulol by using succinic anhydride as the acylating agent. This method did not require the tedious chromatographic separation since one enantiomer was converted into a succinic half ester which can be separated by extraction with aqueous sodium carbonate.

The method is particularly suitable for the preparation of optically pure (*R*)-lavandulol with 98% *ee* in one resolution cycle (*3*).

The best results were obtained with *Porcine pancreas* lipase in hexane for vinyl acetate and in diethyl ether for succinic anhydride. The two enantiomers of lavandulol were obtained in good yield and with a high degree of optical purity. Racemic lavandulol and the enzymes are cheap and, therefore, this method is very convenient for the synthesis of the two lavandulol enantiomers.

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Chemotaxonomic and production, biological evaluation and oil quality of *Foeniculum* accessions

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Foeniculum vulgare Mill. and its intraspecific taxa were utilised as medicinal plants and spices, centuries back. The pleasant flavour and medical use of fruit and oils were known to the ancient Greeks and Romans, too. However, the genus *Foeniculum* shows a large diversity from both morphological and chemical point of view. Based on morpho-phenological characteristics two subspecies and three varieties are distinguished, having practical importance (*1*). The drugs of *Foeniculum vulgare* subsp. *capillaceum* var. *vulgare* (bitter fennel) and subsp. *capillaceum* var. *dulce* (sweet fennel), are used as fennel practically, and are authorised by most of the European and over European pharmacopoeias, including DAB 10, Ph.Helv. VII., Ph.Hg.VII., ÖAB, USP XXI, etc.. Furthermore the above mentioned subspecies and their drugs are involved into the European harmonisation processes, managed by ESCOP.

Between 1994 and 2002, 185 gene-bank accessions of different origin have been analysed by us in long term experiments under open field conditions. Both production biological and chemical characteristics of the accessions were described. In accordance to up to date quality requirement – in parallel with the theoretical approaches - populations producing small sized fruits with high essential oil content and methylchavicol ratio reduced to the minimum were selected (*2*).

According to the GC-MS analyses of the accessions the intraspecific chemical systematisation of *Foeniculum vulgare* genus was completed by us. Evaluating the amount and ratio of α-pinene, β-pinene, myrcene, limonene, fenchone, methylchavicol and anethole the existence of 8 different taxa on chemovarieties and 6 taxa on chemoform level were assumed.

As a result of practical efforts to get population with small fruit size three lines (SM1, SM2 and SM3) were selected. The 1000 seed mass of the lines was 4.38, 3.96 and 3.33 g, respectively, the essential oil content 6.1-7.95 per cent, with about 65-70% anethole and 2.-2.5 per cent methylchavicol ratio. The line SM1 went thorough the official cultivar test procedure in Germany and has bee registered under the name 'Foenipharm'.

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P-01

Characterization of volatile constituents of *Origanum onites* and studies on the antifungal activity against phytopathogens

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The genus *Origanum* (Lamiaceae) is represented by 22 species with 32 taxa, 21 of which are endemic to Turkey (*1*). *Origanum* species are widely used as medicines and food accents. Essential oils of several *Origanum* species have expectorant, antispasmodic, tonic, antiseptic, analgesic, antibacterial, antifungal, antioxidant and cytotoxic properties (*2,3*). *Origanum onites* L. is one species with many stems growing up to 65 cm tall and branches up to 13 cm long (*1*). This plant is commonly known as Izmir kekigi (Izmir oregano), Bilyali kekik or peynir kekigi. Essential oils obtained by hydrodistillation (HD) and microwave-assisted hydrodistillaton (MHD) of the *Origanum onites* aerial parts were analyzed by GC and GC-MS. Thirty-one constituents representing 98.6% of the water distilled oil and fifty-two constituents representing 99.6% of the microwave-distilled oil were identified by GC-MS. Carvacrol (76.8% HD, 79.2% MHD) and thymol (4.7% HD, 4.4% MHD) were characterized as major components of the essential oils. Essential oils were evaluated for antimalarial, antibacterial, and antifungal activities. Using a direct overlay bioautography assay, both essential oils demonstrated antifungal activity at 2 mM against the strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* (*4*). Neither essential oil showed antimalarial or antibacterial activity. Major essential oil components were then evaluated for antifungal activity and carvacrol demonstrated non-selective activity against the three *Colletotrichum* species. Antifungal compounds indicated in the bioautography assay were subsequent evaluated in a 96-well microdilution broth assay against *Phomopsis obscurans*, *P. viticola, Fusarium oxysporum, Colletotrichum* spp. and *Botrytis cinerea (5)*. No activity was observed against any of the three *Colletotrichum* species at 30 μM. However, at 0.3 μM, thymol demonstrated antifungal activity and produced 31.7% growth inhibition of *P. obscurans* at 120 hours, whereas carvacrol appeared inactive. Thymol and carvacrol at 30 μM showed 51.5% and 36.9% growth inhibition of *B. cinerea* at 72 hours.

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P-02

Antibacterial and antioxidant activity of *Thymbra spicata* L. essential oil obtained by hydro- and microwave distillation

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Thymbra spicata L. is an economically important member of the Lamiaceae family, widely growing in Turkey. It is one of the species collected and known as "kekik" (= thyme, oregano) due to its characteristic flavor and fragrance rich in carvacrol/thymol content (*1*) (*2*).

Developments in microwave distillation techniques withdraw attention for the isolation of essential oils (*3, 4*). In this study, the essential oil of the herbal parts of *Thymbra spicata* was obtained first by hydrodistillation and for comparison by microwave-assisted hydrodistillation. Although the oil yields were equal (2.4%, on moisture-free basis) when compared, the distillation time was much shorter in the microwave-assisted method, which was 60 min. in total. To reveal and correlate the chemical composition of essential oils both GC and GC-MS analyses were performed. In total thirty-two components were identified in the hydrodistilled essential oil, whereas fifty-two components in the microwave-assisted method. In both cases oxygenated monoterpenes constituted the majority with carvacrol (64.4 and 68.5%) and thymol (4.3 and 4.1%); the other major detected constituent was γ-terpinene (10.1 and 7.9%) for the hydrodistilled and microwave distilled essential oil, respectively.

Furthermore, the essential oils were tested against common food borne bacteria such as *Aeromonas hydrophila*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Yersinia enterocolitica*, and the anaerobic pathogen *Clostridium perfringens* using the *in vitro* micro-broth dilution method. When compared with standard antimicrobials strong (62.5-250 μg/mL) minimum inhibitory concentrations were observed. To correlate the antibacterial activity results with the antioxidant capacity of the *Thymbra* essential oils 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay (DPPH$^{\bullet}$), β-carotene-linoleic acid co-oxidation inhibition assay and finally hemoglobin-catalyzed peroxidation of linoleic acid methods were used. As a result, both essential oils showed the same level of antibacterial and antioxidant activity.

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P-03

Characterization of the essential oil of *Chaerophyllum libanoticum* and antimicrobial and antioxidant activities

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The Apiaceae family comprising about 300 genera and 3000 species worldwide is also widespread in Turkey. The genus *Chaerophyllum* L. is represented in the Flora of Turkey by fifteen species of which three are endemic (*1-3*). In this study, the plant material *Chaerophyllum libanoticum* Boiss. & Kotschy was collected from Osmaniye in Southern Turkey. The essential oil of *C. libanoticum* was obtained by hydrodistillation from crushed fruits in 1.5% yield. Consequently, the analysis was performed by using a gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems, simultaneously. Seventy three components were characterized representing 98.3% of the total oil. The main constituents were identified as monoterpenes, namely β-phellandrene (17.6%), limonene (15.9%), β-pinene (8.8%), and sabinene (8.5%), respectively. Furthermore, the essential oil was tested for its antimicrobial activity using a micro-dilution assay resulting in the inhibition of a number of common human pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and the yeast *Candida albicans*. The mininium inhibitory concentrations (MIC) varied between 125-250 µg/ml. The antioxidant capacity of the essential oil was evaluated using an *in vitro* radical scavenging activity test. The essential oil obtained from *C. libanoticum* was interacted with 1,1-diphenylpicrylhydrazyl (DPPH$^{\bullet}$) as a nitrogen centered stable radical. In addition, the effect on inhibition of lipid peroxidation of the essential oil was assayed using β-carotene bleaching and haemoglobin induced linoleic acid peroxidation methods.

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P-04

Micromorphology of trichomes, composition and antimicrobial activity of the essential oil of *Salvia wiedemannii* Boiss.

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Salvia wiedemannii an endemic plant of the Irano-Turanian phytogeographic region, is suffruticose, 15-30 cm long, corolla lilac-blue-coloured. They grows on limestone slopes, roadsides and fieldsides of central Anatolia and the flowering time is from May to July *(1)*. Plants were collected during the flowering period from Eskişehir (near Oglakcı village) province of Turkey. The aerial organs of *S. wiedemannii* bears numerous eglandular and glandular trichomes. Eglandular trichomes are simple, uni-multicellular with cuticular micropapillae. They consisted of elongated cells and with antrorse hairs. In the Lamiaceae, glandular trichomes, which are often microscopic and secrete various types of compounds, are generally classified as either capitate (clavate) or peltate (subsessile), based on morphological characteristics *(2)*. The glandular hairs in *S. wiedemannii* included peltate and capitate types. The peltate hairs, pale-yellow to colourless, consisted of a basal cell, a short unicellular stalk and a secretory head, usually composed of 4-8-celled and secrete an essential oil which accumulates in the large space formed at the tip of the head between the raised cuticle and the apical cell walls. The capitate hairs were quite simple in morphology. It composed of a short unicellular stalk, rarely bicellular stalk, and a head. Scanning electron microscopy (SEM) was used to determine the morphology of trichomes. Peltate types were extremely dense on the calyx and abaxial-adaxial surfaces of the leaves. However, short-stalked capitate types was rare and usually found on the corolla surfaces.

Many *Salvia* species are aromatic, rich in essential oils, and of potential economic interest besides their ornamental uses. *Salvia* species contain monoterpenes with antiseptic characteristics *(3)*. The aerial parts were subjected to microdistillation for the isolation of volatiles. The analysis was performed by using GC and GC-MS systems, simultaneously. The major components were characterized as 1,8-cineole (38.4 %), β-pinene (24.5%) and α-pinene (13.6%). Antimicrobial activity of the oil was tested against human pathogenic microorganisms using microdilution broth susceptibility assay *(4)*.

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P-05

Antimicrobial activity and composition of the essential oils of two *Sideritis* species from Turkey

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The genus *Sideritis* (Lamiaceae) is represented in Turkey by 46 species and altogether 55 taxa, 42 taxa being endemic (*1*). Some species of *Sideritis* are used as medicinal and aromatic plants. *Sideritis perfoliata* L. is known as "adacayi, dagcayi, Kandil cayı" in different regions of Turkey and are widely used as diuretic, in the treatment of coughs and gastrointestinal disorders (*2*). *S. trojana* Bornm. is an endemic species for Turkey and known as "kazdagi cayi" (*2*)(*3*).

The hydrodistilled essential oils of *Sideritis perfoliata* L. and *S. trojana* Bornm. were analyzed by GC and GC-MS. The main components in the oils were limonene (37.7%) and sabinene (18.8%) in *S. perfoliata* and β-pinene (18.4%) and α-pinene (13.2%) in *S. trojana*.

The essential oils were evaluated for their antimicrobial activity against various microorganisms. The oil of *Sideritis trojana* showed strong inhibitory effect against *Staphylococcus epidermidis* with a MIC value of 62.5 µg/ml. *S. perfoliata* oil, on the other hand, was less active (125 to 500 µg/ml) against the tested microorganisms except for *Candida. albicans*. The occurrence of higher content of oxygenated derivatives of mono and sesquiterpenes (20%) in the oil of *S. trojana* may be responsible for the better antimicrobial activity.

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P-06

Composition and antimicrobial activity of the essential oils of *Calamintha betulifolia* Boiss. & Bal.

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The water distilled essentials oil from the aerial parts of *Calamintha betulifolia* Boiss. & Bal. collected from three different localities in Turkey were analyzed by GC and GC-MS.
A : Icel; Gozne on July 11, 2003 (ESSE 14394)
B : Icel; Tarsus, Namrun, Camli Yayla, Guzeldere valley on July 12, 2003 (ESSE 14395)
C : Icel; Tarsus, Daripinari village, Guzeldere valley on July 12, 2003 (ESSE 14396)

Aerial parts were subjected to water distillation for 3h using a Clevenger-type apparatus to yield oils in the following percentages: 0.4%, 0.4% and 0.8% for A, B and C, respectively. Fifty six to seventy nine components representing 80.6-93.0 % of the oils were characterized. Pulegone (26-54 %) was found as the main constituent.

The antimicrobial effects of *C. betulifolia* essential oil are reported here for the first time. The antibacterial and anticandidal activities of the oils are presented. The essential oil of "C" strongly inhibited *Escherichia coli*, *Staphylococcus typhimurium*, *S. epidermidis*, *S. aureus* and *Candida albicans* with a MIC value of 0.015 to 0.062 while "A" and "B" oils were less active against these microorganisms. The oil of "C" showed strong inhibitory effect against *C. albicans* with a MIC value (0.062mg/ml) that was equal to Ketoconazole.

P-07

Composition and antimicrobial activity of the essential oil of *Tanacetum cadmeum* (Boiss.) Heywood subsp. *orientale* Grierson

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The genus *Tanacetum* (Asteraceae) is represented by 44 species and 59 taxa in the Flora of Turkey (*1*). *T. cadmeum* is represented by two subspecies, both of them being endemic in the Flora of Turkey: *T. cadmeum* (Boiss.) Heywood subsp. *cadmeum* and *T. cadmeum* (Boiss.) Heywood subsp. *orientale* Grierson. The present report deals with the composition and antimicrobial activity of the oil obtained from the aerial parts of *T. cadmeum* subsp. *orientale*. We have previously studied the oil compositions of *T. armenum* (DC.) Schultz Bip., *T. balsamita* L., *T. chiliophyllum* (Fisch. et Mey.) Schultz Bip. var. *chiliophyllum, T. haradjani* (Rech. fil.) Grierson, *T. argyrophyllum* (C. Koch) Tvzel. var. *argyrophyllum, T. argenteum* (Lam.) Willd. subsp. *canum* (C. Koch) Grierson var. *canum*, *T. praeteritum* (Horwood) Heywood subsp. *praeteritum* and *T. praeteritum* (Horwood) Heywood subsp. *massicyticum* Heywood (*2*) (*3*).

Plant material was collected on 16 June 2004 in Erzincan: Kemaliye province, at an altitude of 1600 m on a stony open space between Sirakonak village and Saricicek plateau, in Turkey. The air-dried aerial parts of plant material were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil yield (v/w) on moisture free basis was 0.5%. The essential oil was analyzed GC-MS. Ninety three constituents were characterized representing 97.8% of the oil. The oxygenated monoterpenes (68.1%) were found as the predominant group with 1,8-cineole (18.9%), terpinen-4-ol (14.8%) and borneol (9.8%) as major constituents. The monoterpene hydrocarbons (26.6%) occured in rather small amount than oxygenated monoterpenes. Their main representatives were characterized as *p*-cymene (15.7%), γ-terpinene (3.5%) and α-terpinene (2.1%).

Antibacterial and anticandidal activity of the oil was evaluated using the micro-dilution broth method. *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Salmonella typhimurium, Bacillus cereus, Staphylococcus epidermidis, Candida albicans* were used as the test microorganisms. No significant antimicrobial activity of the oil of *T. cadmeum* subsp. *orientale* towards to microorganisms tested except *S. epidermidis*.

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P-08

Composition and antimicrobial activity of the essential oil of *Centaurea aladagensis* Wagenitz

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In Turkey, the genus *Centaurea* is represented by 182 species including 113 endemics, distributed particularly in the Southwest, Central and East of the country (*1*) (*2*).

The air dried aerial parts of the plant were hydrodistilled for 3 h using a Clevenger-type apparatus to produce a small amount of essential oil which was trapped in *n*-hexane. The water-distilled oil of the aerial parts of *Centaurea aladagensis* Wagenitz, endemic in Turkey, was analysed by GC-MS. Hexadecanoic acid (39.3%), caryophyllene oxide (6.6%) and hexahydrofarnesyl acetone (4.3%) were found as main constituents in the oil. Furthermore the oil was tested against 7 human pathogenic microorganisms. The antimicrobial activities of the oils were evaluated by using microdilution broth method. The oil showed good inhibitory effects on *S. epidermidis*.

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P-09

Essential oil composition and biological activities of *Tanacetum densum* subspecies from Turkey

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Previous investigations on *Tanacetum densum* subspecies (ssp. *sivasicum,* ssp. eginense) yielded two new sesquiterpene lactones (sivasinolide, eginensolide), two new farnesol derivatives, a diterpene, an aromatic compound and two flavonoids besides known sesquiterpene lactones. Sivasinolide compound shows antibacterial activity; and some other sesquiterpene lactone constituents shows antibacterial and cytotoxic activity (*1*). Previously we reported the main essential oil composition of ssp. *sivasicum* as 1,8-cineole (21.1%), camphor (19.2%), borneol (5.8%) and ssp. *eginense* as camphor (30.9%), 1,8-cineole (12.4%), camphene (10.6%), α-pinene (7.0%), unknown compound (11.5%) (*2*).

In this study we are presenting the antibacterial, antifungal, cytotoxic, phytotoxic, antileishmanial activities and compositions of *T. densum* essential oils. Antibacterial activity tests were performed according to agar well diffusion method (*3*) and *Escherichia coli, Bacillus subtitis, Staphylococcus flexnari, S. aureus, Pseudomonas aeruginosa, S. typhi* microorganisms were used . Essential oils from ssp. *sivasicum* and ssp. *eginense* showed non-significant activity against test microorganisms. Antifungal activity tests were performed according to agar tube dilution method (*3*) and *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani, C. glabrata* were used as fungal microorganisms. Flower oil of ssp. *sivasicum* showed 80% inhibition in linear growth of *T. longifusus* and showed significant antifungal activity. None of the oils showed cytotoxic or antileishmanial activity which were performed according to the cited protocols (*4,5*). *In vitro* phytotoxicity tests were performed on *Lemna minor* according to a modified protocol of Mc. Laughlin *et. al.* and flower oils of ssp. *sivasicum,* ssp. eginense showed significant activity (100% growth regulation with 1000 μg/ml concentration).

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P-10

Antimicrobial activity of *Tanacetum cadmeum* ssp. *orientale* chemotypes from Turkey

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Tanacetum species are well known for their biological activity and the variation of their essential oil composition (*1*). Previous investigations on *T. cadmeum* ssp. *cadmeum* had shown that this plant contain eight sesquiterpene lactones, two coumarins and two flavonoids, which are known compounds (*2*). In our previous work we presented the variation in the essential oil composition of *T. cadmeum* ssp. *orientale* from two different locations (*3*). This report presents the antibacterial and antifungal activities of the essential oils of *T. cadmeum* ssp. *orientale* which show variation in their composition. The essential oils were tested against Gram negative microorganisms: *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae;* Gram positive: *Staphylococcus aureus*, *Enterococcus sp.*; and a fungal microorganism - *Candida albicans*. Disc diffusion method according to NCCLS was used (*4*). Both essential oils from Adana and Sivas locations showed high activity but Adana oil showed higher antimicrobial activity on all organisms and especially on *C. albicans*. Adana oils have *trans*-chrysanthenyl acetate, *cis*-linalooloxide, β-eudesmol and α-thujone as main components, whereas Sivas oils have camphor, borneol and α-thujone. Antimicrobial activity of the oils from Adana and Sivas locations showed differences within their essential oil compositions.

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P-11

In vitro antimicrobial synergism and antagonism of salicylaldehyde: the case of *Filipendula vulgaris* Moench essential oil

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Filipendula vulgaris (dropwort) has a long history of use in folk medicine and phytotherapy in many countries such as Serbia, Poland, Ukraine and Bulgaria, having the same ethnopharmacological use as *Filipendula ulmaria* (meadowsweet). The general plant usage, attributed to the higher content of tanins in the roots, extensively covers anti-inflammatory, antipyretic, analgesic and antirheumatic properties, but the best results were obtained while treating kidney problems, breathlessness, wheezing, sore throats and congestion.

Essential oil obtained by steam-distillation of *F. vulgaris* leaves was analyzed for the first time by means of GC and GC-MS analysis. The oil is characterized by a high amount of phenylpropanoid derived compounds, PHPD, (salicylaldehyde 68.6 %, α-asarone 5.9 %, methyl salicylate 2.4 % and benzaldehyde 2.3 %) and fatty acid derived compounds, FAD, (green leaf volatiles, formed by enzymatic degradation of unsaturated fatty acids, (*E*)-3-hexen-1-ol 6.0 %, (*E*)-2-hexenal 4.2 %) while the monoterpenoids (linalool 1.8 % and nerol trace amount) constituted only a minor fraction.

Disk diffusion method according to the NCCLS (*1*) was employed for the determination of *in vitro* antimicrobial activities of the essential oil, corresponding pure constituents and their mixtures (at a dose of 1.7 μg/disk) against a panel of laboratory control strains: Gram-positive: *Staphylococcus aureus*, Gram-negative: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and fungal organisms *Aspergillus niger* and *Candida albicans*. The essential oil remarkably inhibited the growth of all of the tested bacteria and fungi. It seems the antimicrobial nature of *F. vulgaris* essential oil can be attributed to the synergistic interactions of the compounds constituting the oil rather than to the presence of a single inhibitory agent. A synergy in salicylaldehyde/linalool mixtures was observed with a maximum interaction situated in the range between 60 : 40 and 80 : 20 (mol ratio). At this concentration range (at a dose of 1.7 μg/disk) no microbial growth was observed in the Petri dishes (one disk per dish) while the respective pure compounds, at the corresponding quantities, are shown to be dramatically less active. In addition, an antagonistic relationship between salicylaldehyde and methyl salicylate was established. The maximum (negative) interaction was shown to correspond approximately to the mixture at the 40 : 60 (methyl salicylate/salicylaldehyde) mol ratio resulting in the complete loss of activity at the investigated dose.

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P-12

The influence of storage on the composition of the essential oil of wild growing *Artemisia absinthium* from Serbia

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According to the Council Directive 88/388/EEC (*1*), on the approximation of the laws of the Member States relating to flavorings for use in foodstuff and source materials for their production, the addition of thujone containing plants was re-allowed in the European Union. For this reason, an increase in the industrial consumption of *Artemisia absinthium* L. (wormwood), Asteraceae, limited in the last century as the result of absinth prohibition, could be expected. Hence, the investigation of storage time influence on the chemical composition of the *A. absinthium* volatiles deserves attention.

During the storage of plant material under controlled conditions, a significant decrease of essential oil yield (isolated directly after drying (Aa1 oil) and after one year of storage (Aa2 oil)) was observed (the oil yield dropped from 0.29 to 0.08 (%, w/w)) and was accompanied by changes in the chemical composition.

In order to explain the quantitative changes within the terpene fractions, a closed model, based on the Rault's law, was established. The derived equations showed that the evaporation could be the only cause for some of the oil components quantity decrease. However, the results of this model, as well as the mere oil composition, indicate that processes such as isomerisation, oxidation, degradation or polymerization were also operational. All of these transformations were, most probably, temperature, humidity and light driven.

In the case of *cis*- and *trans*-linalool oxides, found only in Aa2 oil, suggested straightforward reaction pathways that could interconnect them with their potential precursors (*cis*-β-epoxyocimene, linalool or β-myrcene) are in agreement with the thermodynamical data. Higher quantity of 1,8-cineole in Aa2 then in Aa1 oil could be the consequence of *trans*-sabinol, *trans*-sabinene hydrate, linalool or *cis*-β-epoxyocimene (decrease in quantity detected) transformations. Inter-conversions of linalyl-, neryl- and geranyl esters could explain the increase of neryl 2-methylpropionate and geranyl 3-methylbutyrate content in Aa2 oil. During the storage, myrcene could have been, to some extend, transformed to α-thujene and α-pinene. Reactions of *trans*-sabinene hydrate and *trans*-sabinyl acetate could have had α-fenchene as a product. α–Thujone could have partially enolized to the thermodynamically more stable β-form.

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P-13

The presence of polyphenolic compounds in some volatile oil-containing plants and their biological activities.

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Three species of commonly known volatile plants were analysed: *Mentha piperita* (L.) Hudson, *Thymus serpyllum* L., *Thymus vulgaris* L. All these plants are described in European Pharmacopoeia V and should contain the following amount of volatile oils – *Menthae pipertiae folium* – 12 ml/kg, *Serpylli her*ba – 3 ml/kg, *Thymi herba* – 12 ml/kg. These three species are standardised for the presence of volatile oil while the other essensational plants also for the presence of polyphenolic compounds.

The qualitative and quantitative analysis of caffeetannins and flavonoid glycosides were made. Besides rosmarinic acid the following substances were detected in the samples: rosmarinic acid methyl ester, salvianolic acid K, 3'-(8"-caffeoyl)-rosmarinic acid and flavonoid glicosides: luteolin 7-*O*-glucuronide, eriocitrin, luteolin 7-*O*-rutinoside, diosmin, hesperidin, narirutin, isorhoifolin.

The main compounds in *Menthae piperitae folium* are eriocitrin (0.6-5.3 %) rosmarinic acid (0.1-0.8 %) and luteolin 7-*O*-rutinoside (0.1-0.7 %), in *Serpylli herba* luteolin 7-*O*-glucuronide (0.5-3.2 %), salvianolic acid K (0.5-3.0 %), rosmarinic acid (0.5-2.2 %) and 3'-(8"-caffeoyl)-rosmarinic acid (0.3-1.9 %) and in *Thymi herba* rosmarinic (0.5-3.0 %), 3'-(8"-caffeoyl)-rosmarinic (0.5-2.5 %) and salvianolic K (0.6-1.2 %) acids and luteolin 7-*O*-glucuronide (0.3-1.1 %). Using gradient HPLC and isocratic HPTLC analysis it was found that the amount of polyphenolic compounds is higher than the amount of volatile oil.

All compounds mentioned above were isolated by preparative column chromatography with sorbents: RP-18, LH-20, silica gel. The structures were established by chromatographic and spectroscopic methods in particular HPTLC, HPLC, LSI-MS, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{13}C HMQC.

Also antioxidant and anti free radicals activities of aquatic extracts of analysed species were shown. In *Mentha piperita folium* eritricitrin was the most active compound, in *Serpyllum herba* and *Thymi herba* – salvianolic K and rosamrinic acids. These activities can complete the synergic action of analysed medicinal plants based on the volatile oil effects.

P-14

Chemical constituents and antimicrobial properties of the essential oils of two *Alpinia* species from Sabah (Malaysia)

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Many plants from the Zingiberaceae family constitute important ingredients of spices, condiments and traditional medicine in the Malay Archipelago. They are also popular in the cut flower industry. Among the genera represented are *Alpinia*, *Amomum*, *Boesenbergia*, *Curcuma*, *Elettaria*, *Etlingera*, *Hedychium*, *Kaempferia* and *Zingiber*. Essential oils of plants from this family have been found to exhibit bioactivity ranging from anticancer to antimicrobial. Previous reports of analyses of their essential oils were mostly of oils extracted by steam distillation. In this paper, we wish to report on the essential oils obtained by hydro distillation from the rhizomes of *Alpinia galanga* Linn. and *Alpinia latilabris* Ridl. from Sabah (Malaysia) which were evaluated for antimicrobial action against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) using agar disc diffusion assay. *A. latilabris* showed the stronger inhibition against *S. aureus* while *A. galanga* was slightly more inhibiting than *S. aureus* toward *E. coli*. The minimum inhibitory concentration (MIC) for essential oil from *A. galanga* was 22 mg/ml against *S. aureus* and 11 mg/ml against *E. coli*. Meanwhile, the minimum inhibitory concentration (MIC) for essential oil from *A. latilabris* was found to be 70 mg/ml against *S. aureus* and 5 mg/ml against *E. coli*. The minimal bacterial concentration (MBC) was 10 µl/ml using the broth dilution method. GC analyses show the major component of the essential oil of *A. galanga* to be 1,8-cineole. Methyl cinnamate was the major compound detected in the essential oil of *A. latilabris*. GC-MS measurements corroborated these observations.

P-15

Toxicity testing of essential oils and hydrosols using a brine shrimp bioassay

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A brine shrimp (*Artemis salina*) bioassay was used to determine the preliminary toxicity of a range of essential oils. It is a rapid method for determining LD_{50} (lethal concentration for 50% mortality) of plant extracts, and brine shrimp dermal layers are comparable to mammalian skin for sensitivity prediction. Bioassay LD_{50} concentrations may serve as an alternative to direct application or gaseous contact, and therefore provide guidance for topical application of essential oils...

The brine shrimp is a crustacean belonging to the subclass Branchiopoda order Anostraca. It is found worldwide in saline water. The eggs, larvae and nauplii are relatively easy to maintain and test in bioassays and toxicology studies. Historically, the significance of the test lies mainly as an indicator of possible anti-cancer activity of compounds (an ideal LD_{50} being less than 40 ppm) or for use as an insecticide (an ideal LD_{50} being around 1 ppm).

The following oils have been tested: basil, bog myrtle, dragonhead, fennel, German chamomile, lavender, lemon balm, lemon myrtle, lemon tea tree, lovage peppermint, Roman chamomile, tea tree, thyme, and its main components (geranyl acetate, limonene, ocimene, α–pinene, β-pinene, α–terpinyl acetate). All oils were analysed by GC and GC-MS.

A multiwell plate consisting of 25 wells was used for each replication (x4) at concentrations between 10 – 1500 ppm. Each control replication comprised 4 cells of salt solution and 4 cells of salt solution plus 0.05% Tween 20. Dead and live shrimps were counted after 4, 6 and 8 hours. The logistic link function [Logit (p)= alpha + beta . $\log_{10}$(concentration)] was used to model the relationship between the probability of death and $\log_{10}$(concentration). ANOVA was then used to investigate differences between the slopes and LD50s between the test compounds (Genstat5, Release 4.1, Lawes Agricultural Trust, Rothamsted Experimental Station statistical package was used).

The average LD_{50} (ppm) for basil oil was 91; dragonhead, 40; fennel, 107; geranyl acetate, 95; German chamomile, 279; lavender, 479; lemon balm, 164; limonene, 872; lovage, 350; ocimene, 697; peppermint, 199; α–pinene, 494; β-pinene, 491; Roman chamomile, 192; α-terpinyl acetate, 350, and thyme, 124.

No oil apart from dragonhead had an LD_{50} of less than 50, or indeed, of 1ppm which is a recognised level for insect toxicity. There is a strong possibility that these oils would not be harmful in the natural environment and that they can be used at those concentrations in various products. However, by defining the toxicities and properties of individual components of an essential oil, it cannot be assumed that from this can be predicted the bioactivity of the whole oil. It is possible that within the mixture of chemicals some of them will show synergistic and/or antagonistic reactions.

Consistent results from the range of 15 oils, hydrosols and 6 monoterpenes indicate a moderate bioactivity of these compounds. The test can be easily applied to a wide range of oils to determine their particular LD_{50}s. These values can then be used as a general indication of product safety for various topical uses of essential oils.

P-16

Composition and antioxidative activities of the essential oil of cinnamon (*Cinnamomum zeylanicum* Blume) leaves from Sri Lanka

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The composition of the volatiles from leaves of *Cinnamomum zeylanicum* Blume from Sri Lanka was studied by GC/FID and GC-MS. The basic component of the oil was found to be eugenol (74.9%), followed by β-caryophyllene (4.1%), benzyl benzoate (3.0%), linalool (2.5%), eugenyl acetate (2.1%) and cinnamyl acetate (1.8%).

The scavenging of DPPH and OH• radicals by the cinnamon leaf oil is attributed to the hydrogen-donating capacity of the phenolic component eugenol, present in significant concentrations in the essential oil. *C. zeylanicum* leaf oil manifested higher antioxidant activity towards the DPPH radical than eugenol, BHT and BHA, confirmed by the lowest IC_{50} value. The inhibitory potential of the cinnamon leaf oil against OH• was displayed once again at a lower concentration of IC_{50} than that of eugenol and quercetin. The essential *C. zeylanicum* leaf oil demonstrated high chelative activity with respect to the Fe^{3+}, resulting in a prevention of hydroxyl radicals' initiation.

The essential *C. zeylanicum* leaf oil inhibited effectively the conjugated diene formation and the generation of secondary products from lipid peroxidation at concentrations equivalent to those of the standard BHT. Even more inhibited was the stage of the generation of secondary products of lipid autoxidation, i.e. cinnamon leaf oil could also be applied as an antioxidant at a more advanced stage of lipid oxidation.

The use of more than one method to determine the antioxidative properties of medicinal plant essential oils confirms the findings that antioxidative capacity detected by only a single method should be interpreted with some caution (*1*).

In addition, our study demonstrates that the essential leaf oil of *C. zeylanicum* from Sri Lanka possesses considerable antioxidant capacity and could readily be implemented as a natural preservative, thus reducing or avoiding losses due to oxidative processes.

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P-17

Antimicrobial activities and odor evaluations of phenyl ethanol and some of its derivatives

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In continuation of our research work in the field of systematic investigations of antimicrobial activities, odor evaluation and purity of aroma-active components (*1-3*), phenyl ethanol and some of its derivatives were analyzed.

As compounds (purity control using GC and GC-MS; olfactoric evaluations by professional perfumers or aroma chemists) were used: phenyl ethanol, phenylethyl formate, phenylethyl acetate, phenylethyl butyrate, phenylethyl isobutyrate, phenylethyl propionate, phenyl ethyl benzoate, phenylethyl salicylate, phenylethyl valerate, phenylethyl isovalerate, phenylethyl pivalate, phenylethyl cinnamate, phenylethyl methylether and phenylethyl phenyl acetate.The microorganisms for the antimicrobial testings (agar dilution and agar diffusion methode) were as follows: gram-(+)-bacteria *Staphilococcus aureus* and *Enterococcus faecalis*; gram-(-)-bacteria *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella sp.* and *Klebsiella pneumoniae* as well as the yeast *Candida albicans*. As reference compounds in the antimicrobial testings the phenolic monoterpene eugenol and the synthetic antibiotics Ciproxin®, Lidaprim® and tetracycline hydrochloride were used. The results of the antimicrobial testing for each single pure compound will be discussed and compared with those antimicrobial data obtained from those members of this group of phenylethyl substances.

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P-18

Chemical and morphological diversity of single oil glands of *Salvia fruticosa* Mill., Lamiaceae

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The glandular trichomes of *Salvia fruticosa* Mill. were analysed to look for intraindividual chemical variation. Single oil glands were punctured with a polydimethylsiloxane-coated fused silica fibre (PDMS) and the content of the gland was directly transferred to GC-MS or GC/FID (*1,2*).

The results show differences of the essential oil composition and appearance of essential oil gland types on different aerial parts. Green leaves and stamina bear sessile peltate oil glands, the calyx stalked oil glands. However, no glands were found on the corolla.

The differences in the composition of the essential oil compounds between the green leaves and the calyces are relatively small. The stamina, however, show a distinct pattern. The relative amount of β-pinene increases from 10 % in the old leaf to 37 % in the stamina. 1,8-cineole is present in the stamina with 17 %, a relatively low content compared to ca. 45 % in the other aerial parts. Glands on green leaves and calyces have almost no camphor while the glands on the stamina have a high content (23 %).

This is the first report on chemical composition of single essential oil glands of stamen.

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P-19

Antimicrobial effect of aroma compounds on airborne microbes using an airwasher.

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Many microbes are floating in the air, and sometimes they cause problems. Therefore, the development of an easy and safety way to decrease airborne microbes for the place where we treat foods or the place where people come together is worthwhile. There are some ways to clean the air in the room. As one of these tools, an airwasher which removes floating dusts and gives moisture into the room is praised. However, from the point of view or airborne microbes, it is estimated that spreading water into the room promotes an increase of airborne microbes.

In a previous study, we turned our attention to the antimicrobial activity of aroma compounds (1). Now we evaluated vapours of aroma compounds by using an airwasher. Citral, (E)-cinnamaldehyde, (-)-perillaldehyde, (-)-citronellal, eugenol, carvacrol, geraniol, terpineol (mixture of α-, β- and γ-), (-)-linalool, γ-terpinene and 1, 8-cineole were choosen as aroma compounds and tested for their influence on microbial count in the air by vaporizing with an air washer.

The air samples were collected at five points in the testing room whose air volume was 168 m^3. (-)-Perillaldehyde and terpineol showed high antibacterial activity. The average reduction of germs at each measuring point ranged from 28 % to 69 % for (-)-perillaldehyde, and from 46 % to 62 % for terpineol, respectively. On the other hand, the antimicrobial activity of eugenol was the lowest of these eleven compounds. The average reduction of germs ranged between 3 % and 19 %. When water without aroma compounds was sprayed, the colony forming units increased at each measuring point.

It is considered that this measurement is enough to estimate the antimicrobial activity of vapours of aroma compounds, and it seems that this large scale of evaluation is necessary and useful for practical applications. Therefore, these results suggest the utility of selected aroma compounds for the control of bacteria in the room. Furthermore, the toxicity upon inhalation of these chosen aroma compounds in the concentration which was used for this measurement is generally not recognized as being harmful. Thus, this convenient new safety method could contribute to improve environmental health.

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P-20

Antimicrobial activity *Cymbopogon citratus* essential oil.

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Different authors have been describing the antimicrobial activities of *Cymbopogon citratus* essential oil and of its major compound, citral. In the present work, the antimicrobial effect of this essential oil, popularly used as antifungal agent, was investigated. The lemongrass essential oil was obtained by hydrodistillation in a Clevenger-type apparatus and its analysis was performed in a HP 5890 gas chromatograph equipped with a FID detector and in an Agilent 5973N GC/MS system, both fitted with HP5 cappilary columns (30m X 0.25mm X 0.25μm). Oven temperature was programmed from 60 to 240°C/min, at 3°C/min. The constituents of the oil were identified by comparing their mass spectra with those in a spectral database (Wiley 6th ed) and by their retention indices (RI). The antimicrobial assay was carried out using the drop agar diffusion method. The microorganisms tested were the fungi *Cryptococcus neoformans*, *Fonsecaea pedrosoi*, *Trichophyton rubrum*, Candidas non-albicans, *Microsporum canis* and the bacteria *Escherichia coli Lactobacillus casei, Enterococcus faecalis* and *Staphylococcus aureus* methicilin-resistant. Microorganisms were spread over Petri dishes containing solid medium and, after 10 minutes, a 10 μL drop of the essential oil diluted 1:2 with Tween 80 was placed in the center of each plate. Reference antibiotics were: amphotericin B, methicillin and vancomycin. Plates were incubated at 37°C (incubation time depending on the m.o. tested), after which the diameter (mm) of the inhibition zone was measured. In addition, the growth inhibition activity of citral was determined using bioauthography methodology. After being purified from the essential oil, the citral minimal inhibitory concentrations (MIC) were determined using microdilution method, with MIC concentrations raging from 25 to 200 μg/ml depending on each microorganism tested.

These results provide experimental evidence suggesting the potential value of lemongrass oil and its major component citral (80%), for the treatment of human pathogenic fungi and bacteria.

P-21

Antimicrobial activity of *Lippia lacunosa* and *Lippia rotundifolia* essential oils.

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The genus *Lippia* (Verbenaceae) comprises *ca.* 200 species occurring mainly in Central and South America and also in some areas of Tropical Africa. One of the main diversity centers of the genus is located in the State of Minas Gerais, Brazil. As part of our continuing study on Brazilian *Lippia* species, *L. lacunosa* Mart. & Schauer and *L. rotundifolia* Cham. were selected for investigation. Both form a complex of very difficult taxonomic delimitation. Additionally, no previous studies concerning their chemistry or biological activity have been published. Fresh leaves and flowers of *L. lacunosa* and *L. rotundifolia,* cultivated, from original clones brought from Diamantina (MG - Brazil), were collected at the campus of Federal University of Juiz de Fora, Brazil. Their essential oils were obtained separately by hydrodistillation in a Clevenger-type apparatus for 2 hours. Essential oils analyses were performed in a HP 5890 gas chromatograph equipped with a FID detector and a HP5 fused silica capillary column (30mX 0.25mmX0.25μm), using H_2 as carrier gas. The oven temperature was programmed from 60 to 240°C at 3°C/min. The constituents of the oils were identified by comparing their mass spectra with those in a spectral database (Wiley 6th ed) and by their retention indices (RI). The antimicrobial assay was carried out using the drop agar diffusion method. The microorganisms tested were the fungi *Candida albicans* Serotype B (ATCC 36802), *C. albicans* (ATCC 2949) and the bacteria *Escherichia coli* and *S. aureus* MRSA (BMB9393). Microorganisms were spread over Petri dishes containing solid medium and, after 10 minutes, a μL drop of the essential oil diluted 1:1 with Tween 80 was placed in the center of each plate. Reference antibiotics were: amphotericin B, methicillin and vancomycin. Plates were incubated at 37°C (incubation time depending on the microorganism tested), after which the diameter (mm) of the inhibition zone was measured. The major components of the essential oils of flowers and leaves of *L. lacunosa* were myrcene (14.7% and 11.9%), myrcenone (45.2% and 64.2%), (*Z*)-ocimenone (5.7% and 5.2%), and (*E*)-ocimenone (14.7% and 4.1%), respectively; whereas in the essential oils of flowers and leaves of *L. rotundifolia* those were α-pinene (8.7% and 1.8%), myrcene (5.1% and 3.6%), limonene (26.0% and 7.9 %), *cis*-pinocamphone (4.5% and 3.1%) and myrtenal (22.3% and 16.7%), respectively. All the assayed essential oils were active against the microorganisms assayed (bacteria and fungi), with inhibition haloes ranging from 9 to 25 mm. Fractionation of *L. lacunosa* leaves essential oil by silica gel column chromatography afforded pure myrcene and myrcenone (99% purity by GC). Myrcenone was assayed by the drop test (diluted 1:2 in DMSO and then 1:2 in water) against the same microorganisms, with inhibition haloes of 8, 7, 10 and 10 mm, respectively.

P-22

Chemical and biological evaluation of essential oil of *Pentadiplandra brazzeana* (Baill.) from Cameroon

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Pentadiplandra brazzeana (Capparidaceae) is a spontaneous arborescent shrub or climber recorded from the west coast of Africa (Guinea) to Cameroon and Congo. This species is widely used in traditional medicine, especially for intestinal parasitic diseases and against Chlamydiae *(1)*. Several chemical investigations were performed on the non volatile constituents of *Pentadiplandra brazzeana*. The main ones concern a sweet protein obtained from the fruits *(2)*, brazzein, which constitutes a powerful natural sweetener. However, to our knowledge, there is no previous report on the essential oil of this botanical species.

The oil obtained by hydrodistillation of the roots of *P. brazzeana* collected in Cameroon (0.13% yield) presents an herbaceous and pungent odour in spite of sweet floral undernotes. The analyses by GC and GC-MS indicated the presence of two major compounds: benzyl isothiocyanate (78%) and benzyl cyanide (17%); this result is in agreement with a previous chemical examination of a solvent extract of the plant *(3)*; on another hand, we had previously identified these glucosinolate derivatives in high amounts in the essential oils from roots of *Rinorea subintegrifolia* (Violaceae) and from bark of *Drypetes gossweileri* (Euphorbiaceae) collected in Gabon *(4)*.

The free radical scavenging activitiy was tested using the 2, 2-diphenyl-1-picrylhydrazyl method: SC_{50} = 1.5 g/l. This result indicates an antiradical power comparable to the thymol one (0.3 g/l) but much less efficient than the synthetic phenol derivative, BHT (8 mg/l).
The potential antiinflammatory activity of the oil was evaluated comparatively to that of nordihydroguaiaretic acid (NDGA, taken as a reference) by testing its action against soybean lipoxygenase using linoleic acid as substrate: IC_{50} (oil) = 35ppm, IC_{50} (NDGA) = 0.23 ppm. The two main constituents tested individually were found to be less active than the whole oil.
A significant antibacterial activity was assessed against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as well as a high fungicidal activity against *Candida albicans*, *Trichophyton rubrum*, *Microsporum canis*, *Fusarium moniliforme*, *Aspergillus niger* and *Aspergillus flavus*.

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P-23

Piperitone suppresses the emergence of nitrofurantoin-resistance in Enterobacteria

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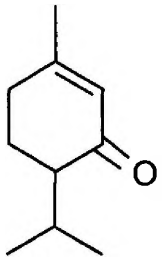
Essential oils from some aromatic plants have been shown to have antibacterial activities and are used in pharmaceutical products as antiseptic agents (1). In vitro experiments have shown that essential oils and some of their components decrease the minimum inhibitory concentration (MIC) of nitrofurantoin against different Enterobacteriaceae. The diluted essential oil of Mentha longifolia var. chorodictya and its main component piperitone **1**, enhance bactericidal activities of nitrofurantoin and furazolidone against the Enterobacteriaceae (2).

In this study, the effect of this compound on the rate of emergence of nitrofurantoin-resistance was investigated. Two nitrofurantoin sensitive clinical isolates (Klebsiella pneumonia, Enterobacter cloacae) were mutagenized by ethyl methanesulfonate; and plated on nitrofurantoin containing Luria-Bertani agar (70 and 140 µg/ml), in the presence or absence of piperitone (1 µl/ml). Also, the enhancement effect of piperitone on the antibacterial activity of nitrofurantoin was further studied against these resistant mutants. The emergence of nitrofurantoin-resistant variants among wild type strains was completely suppressed by piperitone. Moreover this compound enhanced the antibacterial activity of drug against all resistance mutants.



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P-24

Essential oils composition and antioxidant activity of *Eucalyptus camaldulensis* and *Eucalyptus gunnii* from Montenegro coastline

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Although native to Australia, eucalyptus trees with over 700 species widely grow in many parts of the world. Used for centuries as a traditional Aboriginal herbal remedy, eucalyptus leaves and their essential oils have many uses in everyday life due to their powerful antiseptic and anti-inflammatory properties. More than 100 species of *Eucaliptus* genus have been introduced in Montenegro cost at the beginning of the last century. However, no systematic study neither of chemistry nor biology of these species have been carried out until now.

In this study we have examined composition and antioxidant activity of the essential oils obtained from *Eucalyptus camaldulensis* Dehn. (syn. *E. rostrata* Schl.) and *Eucalyptus gunnii* Hook. F. collected from six localities of Montenegro coast. The essential oils were isolated from the dried leaves by hydrodistillation and analyzed by the means of GC/MS technique. Antioxidant activity was evaluated following the effect of essential oils on the Fe^{2+}/ascorbate induced lipid peroxidation (LP) and measuring their scavenging effect on DPPH radicals.

The differences between essential oils of *E. camaldulensis* and *E. gunnii* were reflected both in their content and composition. Higher oil yield was found in *E. gunnii* (1.7%) vs. *E. camaldulensis* (0.7%). The main compounds in *E. camaldulensis* essential oil are spathulenol, cryptone, *p*-cymene, 1-terpinen-4-ol (19,6%; 11,2%; 9,7%; 7,8% respectively) and in *E. gunnii* are 1,8-cineole, α-pinene, α-terpinyl acetate and α-terpineol (45,9%; 18,4%; 11,3%; 7,1% respectively).

The essential oil from *E. camaldulensis* inhibited the malondialdehyde formation in liposomes by 50% (EC_{50}) at the 0.26 μl ml^{-1} level and therefore acted more efficiently then the *E. gunnii* (EC_{50}=3.85 μl ml^{-1}). According to DPPH assay, similar ratio of scavenging capacity of these two species was obtained: EC_{50} (*E. camaldulensis*) = 0.40 μl ml^{-1}, EC_{50} (*E. gunnii*)= 3.80 μl ml^{-1}.

These results indicated that mono- and sesquiterpenes in *E. camaldulensis* are more powerful antioxidants than those in *E. gunni.* This fact was confirmed by the TLC-DPPH assay.

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Antioxidant activity assessment of Tunisian *Thymus capitatus* essential oils: the importance of the antioxidant activity evaluation methodology used.

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The use of natural antioxidants as food additives for inactivating free radicals receives major attention nowadays, not only for their scavenging properties, but also because they are non-synthetic products and favored by the consumers. Thyme essential oils have been previously reported to have antioxidant activity mainly mediated by the phenolic fraction of the oils (*1*,*2*). Tunisian thyme (*Thymus capitatus* Hoff. et Link.) is a perennial, herbaceous shrub belonging to the Lamiaceae family commonly used in Tunisia for culinary purposes.

In this work, the chemical composition of *Th. capitatus* essential oils, isolated by hydrodistillation from the aerial parts of plants collected during the vegetative, flowering and fructification phases was evaluated by GC and GC-MS. The antioxidant activity of the oils (100 to 1000mg/l) was assessed by measurement of metal chelating activity, of the reductive potential, free radical scavenging (DPPH) and by the TBARS assay. The antioxidant activity was compared to synthetic antioxidants BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene). Analysis of variance of the data set was performed by ANOVA procedures.

Carvacrol was the main component of all the essential oils attaining in average 73%, 74% and 66% in the vegetative, flowering and fructification phases, respectively. Although there was a general increase in the antioxidant activity with increasing oil concentration, maxima being obtained in the range of 500 and 1000mg/l, major differences were obtained according the methodology of antioxidant capacity evaluation. The essential oils as well as BHA and BHT showed no metal chelating activity. The essential oils isolated during the fructification phase gave the best results when the antioxidant activity was measured by the reductive potential, giving even slightly higher antioxidant capacity than BHA and BHT within the range of 500 and 1000mg/l. Much lower antioxidant activity was obtained with the vegetative phase oils and the flowering phase oils gave the poorest results with this methodology.

Flowering phase oils gave the best results when evaluated by DPPH method, within the range of BHA and higher than BHT at 750-1000mg/l. Fructification phase oils showed a similar antioxidant potential to BHT by this methodology and the vegetative phase oils showed a very low antioxidant activity.Vegetative, flowering and fructification phase oils gave similar antioxidant potential at 750-1000mg/l and equivalent to that of BHA and BHT when evaluated by the TBARS assay. The differences found with the different methodologies can be partly explained by the diverse relative amounts of minor compounds which could have a major impact in the final oil antioxidant effect of the oils. Further work is needed to fully understand the variables that can affect the evaluation of the antioxidant capacity by different methodologies.

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P-26

Composition of essential oil from organically grown sage (*Salvia officinalis* L.) and its properties

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Sage is a small, evergreen, perennial shrublet up to 0.8 m high, belonging to the Lamiaceae family. It has square stems bearing opposite pairs of gray, rough textured leaves and attractive purplish blue flowers. It is native to eastern Mediterranean region and southern Europe. Commercial cultivation takes place mainly in eastern Europe, Asia, USA and South Africa. The essential oil has been reported to have antiseptic, antispasmodic, and carminative properties.

Sage grown at Frontier organic farm in Iowa was harvested in October and fresh leaves were subjected to hydrodistillation to obtain the essential oil. Fresh leaves were also air dried and then hydrodistilled for essential oil. Sage leaves obtained from commercial sources were also hydrodistilled to obtain essential oils. The yield of oils ranged from 1 to 2% (dry weight). These essential oils and other commercial sage essential oil samples were analyzed by GC and GC-MS for composition. The major constituents in all the oil samples were α-thujone, β-thujone, camphor, 1,8-cineole, β-caryophyllene, α-humulene and viridiflorol. The essential oil from dried leaves had 1,8-cineole (5%), α-thujone (29%), β-thujone (15%), camphor (21%) and viridiflorol (4%); the essential oil from fresh leaves had 1,8-cineole (5%), α-thujone (24%), β-thujone (14%), camphor (24%) and viridiflorol (5%).

The total phenolic contents, chelating capacities, and radical-scavenging properties of the herb and the essential oil will be discussed.

P-27

Post Harvest Residual Elimination System (PHRES) in citrus essential oils

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Due to their extremely pleasant organoleptic characteristics, citrus essential oils have been used for many years in various industrial applications, from perfumery to cosmetics, from detergents to food (*1*).

A part of the citrus grown throughout the world is dedicated to processing into juice, the other part (which might also be the relevant one according to the type of citrus fruit) is destined for consumption as fresh fruit.

In the latter case, immediately after harvest, fruits are transported to "packing houses" where a number of treatments are carried out to select the best fruits and to preserve their characteristics for a prolonged time.

During this process the fruits are generally waxed and treated with an anti-mould agent before sorting. The best ones are packed and delivered to the market for sale as fresh fruit and the balance is usually transferred to the nearest citrus processing facility.

In citrus fruits, essential oil is contained in the flavedo that is located immediately under the epidermis; it is characterized by a green, yellow or orange colour and is interspersed with oil glands. Oil glands are characterized by very thin and fragile walls; within these walls, essential oil is contained with a certain positive pressure which facilitates oil recovery by abrasion of the flavedo layer.

If fruits that have treated "treated" in the "packing houses" are subsequently processed there is no way of avoiding the fact that the anti-mould agents will finish in the oil; moreover, and from a more general point of view, all oils are often contaminated by phytosanitary substances.

What we have developed is an innovative technological process, exclusively physical, that is able to remove some of these contaminants from citrus oils without altering in any way the organoleptic and chemical characteristics of the oils themselves.

Various samples of citrus essential oils have been subjected to this technology, that we have named "PHRES", and analysed by SPT, GC-NPD, HPLC, GC-FID and GC-MS (*2*) to verify the complete elimination of these contaminants and at the same time to confirm that the chemical characteristics remain unaltered after treatment.

The system has been developed and tuned for selective elimination of post-harvest contaminants like Thiabendazole and Imazalyl. For different phytosanitary substances, some tests have been done and others will be performed attempting to enlarge the spectrum of chemicals that is possible to selectively remove from citrus oils.

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P-28

Biological activity related to chemical composition of the essential oils from *Helychrysum*, *Juniperus*, *Rosmarinus*, and *Lavandula* genus growing wild in Sardinia.

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The application of natural extracts in the pharmaceutical, cosmetic, food and agriculture fields, is nowadays of great interest. The aromatic characteristics and the biological activity of these extracts depend on their chemical composition, which is related to the species and cultivar, but also to the plant part, the pedoclimatic characteristics, etc.

In this investigation we studied the antimicrobial and antifungal activity of the essential oils (EO) extracted from four aromatic plants growing wild in Sardinia.

The influence of the altitude on the composition of the EO from *Helichrysum italicum* ssp. *microphyllum*, was studied. Two chemotypes were identified; Type A, was characterized by nerol and its esters, and Type B, by *ar*-curcumene, *γ*-curcumene, and rosifoliol. The activity of the oils was evaluated on plant pathogens fungi. Type B oil showed a moderate action on *Phytophthora capsici,* and *Septoria tritici*, and a good activity on *Pythium ultimum,* and *Sclerotium rolfsii.*

The activity of the EO from ripe and unripe berries and leaves of *Juniperus oxycedrus L. ssp. oxycedrus*, *Juniperus phoenicea ssp. turbinata* and *Juniperus communis ssp. communis* was studied against *C. albicans, S. aureus, E. coli,* and *Pseudomonas aeruginosa.* The major compounds were *α*-pinene, *β*-pinene, *δ*-3-carene, sabinene, myrcene, *β*-phellandrene, limonene, and germacrene-D. All EO from *J. phoenicea ssp. turbinata* and the essential oil from the leaves of *J. oxycedrus ssp. oxycedrus* exhibited activity against *C. albicans* and *S. aureus.*

α-pinene, borneol, camphene, camphor, verbenone, and bornyl acetate were the major compounds of the EO of *Rosmarinus officinalis* L. collected at different latitude and longitude. The antimicrobial tests against *S. aureus*, *S. epidermidis*, *E. coli, P. aeruginosa*, *C. albicans*, and the antifungal tests against *B. cinerea*, *F. oxysporum lycopersici*, *F. graminearum*, *F. culmorum*, and *R. solani* showed weak activity. On the other hand it was observed an inductive effect on fungal growth, especially towards *F. graminearum*.

The EO from stems/leaves and flowers of *Lavandula stoechas* L. ssp. *stoechas* in different phenological stages was analyzed. They were characterized by fenchone and camphor. The EO tested were effective on the inactivation of *R. solani, F. oxysporum*, and less effective against *A. flavus*.

P-29

Antioxidant and antimicrobial activity of the essential oil of *Hypericum perforatum* L.

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Hypericum perforatum L. (Hypericaceae) is a well known medicinal plant and has been used as a natural remedy to treat a variety of complaints since ancient times. Recently, the plant is used against mild viral infections and depression. The healing properties of St. John's wort are due to the effects of detaily explored naphtodiantrone (hypericin, pseudohypericin) and phloroglucinole derivatives (hyperforin), flavonoide glycosides, tannins and procyanidins *(1)*. Beside phenolic compounds St. John's wort also consist essential oil. The content and chemical composition of essential oil depend on the origin of plant material. However, biological activities of H. perforatum essential oil are not examined in details so far. With respect to this, the antioxidant and antimicrobial effects of the essential oil of St. John's wort (mountain Golija, Serbia and montenegro) are investiagted.

Essential oil was obtained from the air-dried aerial parts of *H. perforatum* by hydrodistillation. Chemical composition of essential oil was evaluated by GC-MS. Antibacterial activity was determined by modified antibiogram test on 19 multiresistant strains bacteria, all originating from hospitalized patients. Evaluation of antioxidant activity included free radical scavenging activity towards 2,2-diphenyl-1-pycrylhydrazile (DPPH) radicals, together with inhibition of Fe^{2+}/ascorbate induced lipid peroxidation (LP) in liposomes. Compounds responsible for DPPH-scavenging activity were determined by DPPH-TLC assay.

The main compounds of essential oil were monoterpene hydrocarbons α- and β-pinene (32.7% and 11.65%) and sabinene (10.85%). Essential oil of *Hypericum perforatum* expressed strong free radical scavenging activity (IC_{50} = 4.31 µl/ml) and high inhibition of LP (IC_{50} = 2.27 µl/ml). In both cases dose dependant activity was observed. Mono- and sesquiterpene hydrocarbons were responsible for DPPH-scavenging activity. Investigated essential oil also exhibited strong antibacterial activity on *Streptococcus agalaciae*, *S. Pyogenes*, *S. Pneumoniae*, *Staphylococcus aureus*, *Pasteurella multocida* and *Rhodococcus equi*. Weak inhibitory effect was expressed on *Streptococus viridans*. On strains of *Pseudomonas aeruginosa* and *E. coli* essential oil showed no activity.

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P-30

Composition, chemical variability and antimicrobial activity of the twig oil of *Abies alba* Miller from Corsica

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The genus *Abies*, which is regarded to be complex in comparison with other genera of the family *Pinaceae*, was established by Miller in 1754. It comprise 39 species and 23 and all the taxa are native to the Northern Hemisphere and are widely distributed across both Eastern and Western parts of the world. The Silver fir (*Abies alba* Miller) is currently one of the most important conifer in many Eastern Europe mountain forests (France, Germany, Poland, etc.). In Corsica, the South-Western limit of its surface of distribution, *A. alba* constitutes, on wet mountainous terrain, pure fir forests and mixed forests with other conifers such as *Pinus nigra* ssp. *laricio*.(*1,2*)

The composition of the essential oil of twigs of individual trees of *Abies alba* Miller from Corsica was investigated by CC, GC (RI), GC-MS and ^{13}C NMR. In order to carry out a detailed analysis, two oil samples, selected on the basis of their very different chromatographic profiles, were repeatedly fractionated over SiO_2. In total 65 constituents were identified and they represented 98.1% and 95.4% of the total amount of the oils, respectively. Both oils were characterized by very high content of monoterpene hydrocarbons (90.8 and 85.0%) accompanied by much smaller amounts of oxygenated monoterpenes (5.1 and 6.3%) and sesquiterpenes (1.8 and 3.2%).

Fifty three oil samples were analysed and the results were submitted to chemometric analysis (K-mean's clustering and Principal Component Analysis). Two groups were distinguished within the essential oils. Samples belonging to the first group (64% of the samples) were characterised by a very high content of limonene (mean value 46.1%). Conversely, the mean composition of the samples of the second group (36% of the samples) was dominated by camphene, α-pinene and limonene.

The antimicrobial activity of both oils was assayed against different bacteria strains.

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P-31

Chemical composition and antimicrobial activity of *Santolina corsica* essential oil

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Santolina corsica Jordan et Fourr. is an endemic species to Corsica and Sardinia. It is an under-shrub, 30 cm high, with persistent leaves and yellow flowers. It grows preferably on the rocky and sunny places. In Corsica, *S. corsica* is found in the centre of the island (around the city of Corte) while in Sardinia it grows on Monte Albo (North-west part of the island).

A detailed analysis of *Santolina corsica* essential oil was carried out by combination of GC/RI, GC-MS and ^{13}C-NMR spectroscopy. After fractionation by column chromatography, 49 components were identified, accounting for 87.9% of the total amount of the oil. The chemical composition was dominated by monoterpene hydrocarbons, myrcene (34.6%), β-phellandrene (13.7%) and santolinatriene (13.5%). Beside the main compounds, we noted the occurrence of irregular mono and sesquiterpenes belonging to three families: santolinane (santolinatriene, lyratol, lyratal, lyratyl acetate and isolyratol), artemisane (yomogi alcohol, artemisia ketone, artemisia alcohol) and lavandulane (lavandulol and sesquilavandulane aldehydes).

Antibacterial activated was tested against six bacteria strains: *Staphylococcus aureus*, *Listeria innocua*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, and *Campylobacter jejuni*. *Santolina corsica* essential oil had a significant inhibitory effect on the growth of *S. aureus* and *C. Jejuni*. For both bacteria, the most significant antibacterial activity was noticed from the polar fraction, while the non polar fraction showed weak activity.

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Identification of skin contact chemical allergens by direct gas chromatography-human sense coupling.

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Our abilities to taste and smell are exquisitely sensitive to trace concentrations of many compounds. These senses allow us to tell whether the beverages we taste and the foods we eat are really fresh and satisfying. Generally, fragrances are complex chemical mixtures and their commercial exploitation is becoming an increasingly important aspect of everyday life.

The increasing use of fragrances in consumer products and the decreasing air exchange rate of buildings have focused attention on the toxicological properties of fragrance compounds, including components of essential oils (*1,2*). An increasing number of people are claiming that exposure to certain fragrances, including perfumes and scented products, adversely impacts their health.

In the past decades, many detection techniques have been hyphenated to gas chromatography. While relatively less attention has been paid to GC-human sense in which the human nose is involved, no device has been reported so far in which skin plays the role of the detector. A similar lack of attention has restricted the use of GC in tandem with other off-line detection techniques such as immunoassay or NMR.

We describe the use of a splitting interface for the coupling of GC to both the ODO-II olfactory detector and to other off-line or independent detectors such as human skin, respiratory system and other allergen sensitive systems or to off-line analytical techniques.

By way of example, a case study is described that employed human sense to investigate patients with verified allergic to organic products in an industrial environment. Such examples are of importance in establishing occupational exposure limitations for sensitive individuals and for the isolation of hazardous materials.

Importantly, the use of GC separation techniques makes it possible to identify low concentrations of potent analytes with specific physiological or immunological activity, such as contact allergens or degradants that may be present in complex mixtures, using a modification of the known gas chromatography-olfaction-mass spectrometry apparatus (*3*).

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Bioraffinery of blackcurrant buds: chemical composition of essential oil and antioxidant activity of by-products of hydrodistillation

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Blackcurrant (*Ribes nigrum* L.) was the most extensively grown bush fruit in Asia, Australia and Europe, especially in Lithuania. It was an important raw material for the food and cosmetic industries, respectively due to the characteristic colour and typical flavour of its berries and of its dormant buds used as perfume enhancer. If the litterature reported that the essential oil of blackcurrant buds emitted a strong terpenic odor characterized by a "catty note"(*1,2*), the composition and properties of blackcurrant buds grown in Lithuania seemed to have not been investigated.

The first aim of this work was to assess chemical composition and relative compounds odour identification of blackcurrant buds essential oil obtained from six cultivars grown in Lithuania. The second aim was to assess Radical Scavenging Capacity (RSC) of the by-products of hydrodistillation.

Dormant buds of blackcurrant were harvested from cuttings in experimental field in Lithuania during December 2004. The buds were hydrodistillated and the extracts were analyzed by GC-FID, GC-MS and GC-O. By-products of hydrodistillation were process water and solid wastes (buds). The buds were dried before extraction with acetone while process water was divided in two parts and either spray-dried nor freeze-dried. All the three kind of extracts were tested for their RSC by using $ABTS^{\bullet+}$ decolorisation and $DPPH^{\bullet}$ scavenging methods.

The major compounds of blackcurrant buds essential oil were sabinene, δ-3-carene, β-pinene, β-phellandrene, terpinolene and β-caryophyllene while key odor compounds were α-thujene, α-pinene, ..terpinolene, δ-cadinene, α-humulene and β-caryophyllene. RSC of acetone, spray-dried and freeze-dried extracts were respectively from 10.6 to 20.9 %, 73.5 to 82.1 % and 57.2 to 83.4% in $DPPH^{\bullet}$ reaction system and from 2.4 to 5.1 %, 44.7 to 73.7 % and from 31.4 to 88.22 %. in $ABTS^{\bullet+}$ reaction system .

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Biological properties of some Moroccan essential oils

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The anti-inflammatory properties, toxicity and anti-microbial effect of *Artemisia herba alba*, *Cinnamomum cassia*, *Eucalyptus camaldulensis*, *Eugenia caryophyllus* thumb, *Lavandula hybrida abrialis* and *Ormenis mixta* essential oils have been studied in order to use them in aromatherapy with total safety.

The study in-vitro of the anti-inflammatory activity revealed that the essential oils of *Artemisia herba alba, Eugenia caryophyllus* thumb, *Ormenis mixta* and *Eucalyptus camaldulensis* have no anti-inflammatory activity, while essential oils of *Cinnamomum cassia* and *Lavandula hybrida abrialis* are slightly active.

The study of anti-bacterial activity revealed that essential oils of *Artemisia herba alba* and *Cinnamomum cassia* inhibited, in the same way, *E.coli*, whereas for *S. aureus*, the CMI of *Artemisia herba* Alba essential oil was 3 times higher than the one of *Cinnamomum cassia* essential oil. This means that *S. aureus* was more sensitive to *Cinnamomum* oil *cassia* than *Artemisia herba alba* oil.

The study in vivo of toxicity revealed that *Artemisia herba* Alba and *Cinnamomum cassia* oils have very low toxicity, the LD was respectively 1412 mg/kg et 1622 mg/kg.

The histological analysis of the mice livers revealed that the cell vacuolisation is the result of toxic effect of certain essential oil compounds.

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Essential oil composition and antimicrobial potential of three *Zanthoxylum* species against sexually transmitted pathogens.

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Zanthoxylum species has a folklore history in the treatment of sickle-cell anemia and malaria. The chemical profile of the essential oils from three *Zanthoxylum* species and the evaluation results when tested against sexually transmitted pathogens is hereby reported.
Hydrodistilled essential oils of leaves and barks of the three species were analyzed for their chemical compositions using Gas chromatography (GC) and Gas chromatography- Mass Spectrometry (GC-MS). Evaluation of their antimicrobial potentials using the cup plate dilution method was also investigated.

The essential oil percentage yields from the leaves and bark of *Zanthoxylum rubescens*, *Zanthoxylum macrophylla* and *Zanthoxylum leprieurii* were 0.05-0.09% (w/w). Seventeen to twenty-eight compounds were identified representing 99.9–76.3% of the oil compositions. Sesquiterpenoid compounds – (*E*)-nerolidol (44.4-70.2% respectively) and *β*-caryophyllene (22.1%) dominated the chemical profile of the leaf and bark of *Z. rubescens*. Monoterpenoid was the major class of compounds found in the leaf oil of *Z. macrophylla* with linalool accounting for 80.5% of the oil composition, while linalool (28.9%), *(E)*-nerolidol (12.4%) and caryophyllene oxide (7.6%) were the major compounds identified in the bark oil. *Z. leprieurii* leaf oil had limonene - another monoterpenoid as the main constituent (94.9%). The bark oil of *Z. leprieurii* was rather dominated by sesquiterpenoids with elemol (5.72%), guaiol (5.80%), humulenol (16%), *β*-bisabolene (10.4%) and (*E*)-nerolidol (23%), as major constituents.
The essential oils were tested against seven bacteria strains including sexually transmitted bacteria. The leaf and bark oils of *Z. leprieurii* was most effective in inhibiting the growth of the organisms. The antimicrobial activity of the oil of *Z. leprieurii* unlocks new usage of the plant in ethnopharmacology and possible new drug formulation.

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Comparative antibacterial and antifungal activity of a new type of essential oil from the Australian medicinal plant *Eremophila longifolia* (F. Muell)

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Here we report on characterisation of a pleasant smelling steam distilled essential oil from the traditional Australian medicinal plant *Eremophila longifolia* (F. Muell) (Scrophulariaceae) by GC-MS. During collection of this species variant populations exibiting unusual morphological variations and an essential oil fraction containing a large proportion of (-)-menthone, α-terpineol and limonene were discovered growing in a region of Western NSW (Mutawintji National Park). The morphological variations and essential oil compositions reported here have not been previously described in any of the botanical or phytochemical literature pertaining to this species (*1,2*). Antibacterial MIC values of the oil were determined for *Escherichia coli* and *Staphylococcus aureus* using an agar dilution method (*3*). MIC values for these species were also obtained for various blends of *E. longifolia* and Lemon myrtle oils (*Backhousia citriodora aeth.*). The antifungal and antibacterial activity of citral rich lemon myrtle oil is well characterised. Citral however is known to produce a sensitisation reaction when applied directly to the skin (*3*). This reaction has been found to be absent when citral or citral rich oils are combined with oils containing α-terpineol. Currently there are efforts to find α-terpineol containing oils that may be blended with lemon myrtle oil without significantly reducing activity (*3*). Growth inhibition equivalent to 100% lemon myrtle oil was observed with a 1:4 ratio blend (MIC: 0.075% $^{v}/_{v}$ for *S. aureus* and 0.150% $^{v}/_{v}$ for *E. coli*). We have also compared the antibacterial and antifungal activity of this oil against a panel of human pathogens commonly occurring in surface infections with the activities of other essential oils from native Australian plants both commercially available and novel. The new oil has definite commercial potential either neat or in combination as a 'natural' topical treatment for common fungal skin infections.

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Antibacterial and anticancer activity of leaf essential oil of *Croton malambo*

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The composition of the essential oil obtained from leaves of *Croton malambo* was studied by means of GC and GC-MS. Methyleugenol, γ-bisabolene, *iso*elemicin and γ-curcumene were identified as the major components whilst smaller amounts of δ-cadinol, caryophyllene, α-phellandrene, globulol and bisabolol were also detected. In previous work we had reported the chemical composition of the oil obtained from the barks of *C. malambo* (*1*). Here we report the chemical composition of the oil from the leaves obtained by hydrodistillation. The components in this oil were identified by calculating their Kovats index in relation to homologous series of *n*-alkanes (C_8-C_{22}) under the same conditions and by comparing their mass spectra with those reported in the computer database (*2*).

The medicinal properties antibacterial and anticancer attributed to this plant for the local inhabitants prompted us to study the chemical composition of the oil from leaves of *C. malambo.* The antibacterial activities of the oil from leaf were assessed against *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginose, Escherichia coli* and *Candida tropicalis.* A significant antibacterial activity was determined with the agar diffusion method. The anticancer activities of *C. malambo* essential oil were tested against human breast cancer cell line MCF-7, on prostate cancer cell line PC3 and normal human fibroblasts. The results indicated a moderate activity on MCF-7 cancer cell line.

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Effects of inhalation of carvone on autonomic nervous system (ANS) parameters and subjective evaluation in humans during sympathetic activation by the Cold Pressor Test (CPT)

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Various studies definitely evidenced that exposure to odorants affected physiological and psychological responses in humans not only in resting state but also detected modulating properties on the reaction to autonomic activation (*1*). The present study investigated the reactions of human subjects to a short stimulation of ANS during inhalative application of an single fragrant substance in respect to chirality and concentration effects. Carvone was chosen for this experiment due to its well-investigated actions on animals as well as humans. For instance, (*R*)-(-)-carvone exhibited sedative and relaxing properties on locomotion activity in mice whereas (*S*)-(+)-carvone displayed both sedating and activating effects (*2*). On human subjects in rest, inhalation of (*R*)-(-)-carvone provoked an increased pulse rate, diastolic blood pressure and subjective restlessness. Then again, (*S*)-(+)-carvone raised levels of systolic and diastolic blood pressure (*3*). Twelve healthy human subjects were repeatedly exposed to a CPT in a crossover-designed study. During the experimental sessions, three different concentrations of (*R*)-(-)- as well as (*S*)-(+)-carvone were administered by inhalation and compared to a blank control. The determined ANS parameters were heart rate, electrodermal activity, systolic and diastolic blood pressure. Subjects had to rate subjective condition on a questionnaire before and after experimental session as well as directly after CPT.

Results revealed that on physiological level (*R*)-(-)-carvone increased the sympathetic response to CPT except for systolic blood pressure which was attenuated. However, reaction to (*S*)-(+)-carvone inhalation did not follow a consistent scheme: Low concentrations acted as slight sedatives, whereas medium concentrations diminished relaxation and high ones could not be attributed to an unambiguous effect at all. Concerning effects on subjectively evaluated condition, (*R*)-(-)-carvone amended mood ratings significantly and proportionally to the inhaled amount of substance. Then again, (*S*)-(+)-carvone did not demonstrate a distinct effect: In low concentrations, it revealed an activating effect on self-ratings such as attentiveness. In medium concentrations, diminished physiological relaxation was supported by decreased ratings of well-being and an increase in fatigue. High concentrations of (*S*)-(+)-carvone, however, could not be unequivocally interpreted but resulted in a form of psychological exhaustion towards the end of the experimental session. This response is apparently connected to the low acceptance for the (*S*)-(+)-carvone odour. In conclusion, the present study evidenced that odour effects depend not only on chirality which is crucial for hedonic qualities but also on applied concentration.

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Chemical composition and antimicrobial activity of leaf essential oil of *Croton huberi* from Venezuela

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The chemical composition of the essential oils obtained from fresh and dried leaves of *Croton huberi* S, was determined by GC and GC-MS (*1*). The oils were obtained by hydrodistillation in 0.05% and 0.1% yield (v/w) from fresh and dried leaves respectively. The analysis of the essential oils obtained from fresh leaves resulted in the identification of thirty-four components out of thirty-seven detected in the complex mix. This oil was characterised by a high content of sesquiterpenes (62.5%), with γ-eudesmol (18.7%) as the major constituent, followed by germacrene-D (11.7%), β-eudesmol (7.6%), caryophyllene-oxide (6.9%), kaurene (5.3%), humulene (4.3%), cedrol (4.6%) and isoelimicine (3.0%). In the oil obtained from the dried leaves, twenty-two components were characterised from a total of 29 compounds detected. This oil presented percentages of sesquiterpenes similar to those of the fresh material where γ-eudesmol (20.2%) is the main, followed by α-bisabolol (18.1%) and calacorene (9.2%). As part of an ongoing research project were we studied the pharmacology profile of some *Croton* species used in our traditional medicine (*2*), the antibacterial activity of the oils was assayed in vitro against five microorganisms using the disk susceptibility test. The results showed that the oil had antibacterial activity against *Staphylococus aureus*, *Escherichia coli*, and against the fungi *Candida albicans*.

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Thujone-less *Salvia* species cultivated in Liguria (Italy).

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The genus *Salvia* (tribe *Mentheae*), constituted by 900 species, is a tropical and sub-tropical genus and it is one of the most representative genus of Lamiaceae (*1,2,3*).

An investigation on the essential oils of several extra-european Salvia species cultivated in Liguria (Italy) are reported here. The study was carried out on *Salvia x jamensis* J.Compton cv. *'La Luna'* and *Salvia x jamensis* J. Compton cv *'La Siesta'* (Mexican species), *Salvia disermas* L., *Salvia somalensis* Vatke and *Salvia repens* Burch. ex Benth. Fresh plant samples were analysed in order to avoid any sort of alteration in their essential oils due to the drying process.

These species were collected in two different years (2004 and 2005). Only one study is reported in literature for *S. somalensis* and it was carried on dried plant samples, collected at the Hanbury Botanical Gardens of La Mortola, Ventimiglia (Italy) (*4*).

The essential oils were hydrodistilled by Clevenger apparatus and analysed by GC-MS, in order to evaluate differences in the main constituents of these species. Futhermore it was considered the influence of different harvesting periods and in different cultivars of the same species. In addition a comparison between the essential oil and exudate composition of the two varieties of *Salvia x jamensis* J.Compton was carried out in order to characterise the persistent smell expecially when *Salvia x jamensis* J. Compton cv. *'La Luna'* was handled.

As the distribution of terpenoids in *Salvia* is considered of taxonomic interest at subfamilial, phytogeographical and infrageneric level, the aim of this work is to get a contribution to the knowledge of some African and American species cultivated in Italy (*5,6*). All the analysed essential oils from fresh cultivated plant samples did not contain thujones as common feature.

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Chemical composition of essential oils from aerial parts of *Zosimia radians* and flowers, leaves and stems of *Zosimia absinthifolia* from Iran.

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The genus *Zosimia* is represented in Iran by two species: *Z. radians* Boiss. and Hohen. and *Z. absinthifolia* (Vent.) Link, which *Z. radians* is endemic plant (*1*). The composition of the oil of dried fruits of *Z.absinthifolia* from Turkey has been reported. Octyl acetate (38.1%) and octyl hexanoate (31.9%) were the main constituents (*2*).

The water distilled essential oils from aerial parts of *Z. radians* and flowers, leaves and stems of *Z. absinthifolia* were analyzed by GC and GC-MS.

The oil of *Z. radians* was characterized by higher amount of octyl acetate (58.8%). In the flower oil of *Z. absinthifolia,* octyl acetate (58.4%) and octanol (25.0%) were also the predominating compounds. The oil obtained from leaf and stem of the plant were rich in germacrene-D (25.5% and 18.5%), β-caryophyllene(18.1% and 10.5%), and bicyclo-germacrene (11.2% and 7.6%) respectively.

The main component of the stem oil was *cis*-chrysanthenyl acetate (15.5%). The flower oil of *Z. absinthifolia* consisted mainly of aliphatic compounds, while in leaf and stem oils of the plant sesquiterpenes predominated over monoterpenes.

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Chemical composition and antimicrobial activity of the essential oil of *Tetrataenium nephrophyllum* (Apiaceae) from Iran

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The aerial parts of *Tetrataenium nephrophyllum* were collected from West Azarbaijan province, Takab, Iran and its essential oil was obtained by hydrodistillation and analyzed by GC and GC-MS *(1-2)*. Forty components accounting for 97.9% of the total oil were identified. Germacrene-D (38.5%), 2-ethyl hexyl acetate (11.2%), *n*-octyl 2-methyl butanoate (9.2%) and geranyl isovalerate (8.3%) were the major constituents. Sesquiterpene hydrocarbons (51.3%) and aliphatic esters (40.4%) were found to be the main group of compounds. Antimicrobial activity of the essential oil *(3)* of *T. nephrophyllum* was performed against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) as well as three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*). The results of the bioassays showed that the oil exhibited moderate to high antimicrobial activity.

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Hydrodistillation-headspace solvent microextraction (HD-HSME): an efficient method for the analysis of the seed essential oil of *Foeniculum vulgare* Mill.

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Foeniculum vulgare Mill. from Umbelliferae family commonly known as fennel is a native perennial herb in Iran (*1*). It is widely cultivated in central Europe and Mediterranean region and through out the temperate regions of the world for its aromatic fruits, that are used as medicinal and spice herb (*2*).

Very recently, we have introduced hydrodistillation-headspace solvent microextraction (HD-HSME) as a simple and rapid method for essential oil analysis of *Lavandula angustifolia* Mill. (*3*). The method consisted of the extrusion of a microdrop from the needle tip of a gas chromatographic syringe that was inserted into the headspace above the hydrodistilled plant sample. After extraction for an optimized time, the microdrop was retracted into the syringe and injected directly into a GC injection port.

As the next step, we were interested to study the applicability of this new method for essential oil extraction of aromatic seeds. Here, the application of HD-HSME for preconcentration and analysis of the seed essential oil of *Foeniculum vulgare* is reported.

The effect of different parameters such as sample mass, extraction time, microdrop volume, and choice of the solvent on the extraction efficiency was studied and all were optimized.

n-heptadecane was found to be the solvent of choice for efficient extraction of essential oil constituents.

The repeatability of the method performed under optimized conditions (i.e. sample weight, 0.7 g; extraction time, 2.5 min; drop volume, 1 μl) was studied by analyzing the samples in triplicate. It was proved that RSD values for the main compounds were less than 10%.

The results were compared to hydrodistillation as the reference method. 14 compounds were identified by GC and GC-MS and the major components were (*E*)-anethole (70.4%), fenchone (9.3%) and estragole (8.8%). The results were in good agreement with those obtained by hydrodistillation method.

In conclusion, HD-HSME technique is a straightforward, rapid and inexpensive method for preconcentration and analysis of the essential oil from aromatic plants and their seeds. The method is green and only a few microliter of the solvent is used. Although the applicability of this method to other plant materials have to be proved, we believe that HD-HSME could also be used for rapid and quantitative determination of volatile compounds in foods, cosmetics, medicines and perfumes.

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P-44

Effects of feed, enzyme supplements and gut digesta on the anti-Clostridial activity of essential oils and condensed tannins

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Clostridium perfringens is the causative agent of the poultry disease, necrotic enteritis, a major disease of commercial poultry flocks, resulting in significant financial losses to the industry, as well as affecting animal health and welfare. Infection with *C. perfringens*, along with other dietary factors, results in the formation of necrotic lesions in the gut, reduced performance and in severe cases, death of the bird. Traditional treatments have involved the prophylactic use of in-feed antibiotics, but with their banning from the EU, as well as the reduced use of anticoccidial drugs, commercial poultry flocks are again at risk from this highly infectious disease. Non-antibiotic antimicrobials are needed to alleviate this problem.

Plant secondary products, particularly essential oils and condensed tannins, have long been recognised for their antimicrobial properties, but despite anecdotal reports, they have not been thoroughly tested for their potential use in the poultry industry. We have therefore investigated the use of some of these compounds as potential agents to reduce the risk of *C. perfringens* colonisation in chickens. Lemon myrtle (*Backhousia citradora*), tea tree (*Melaleuca alternifolia*) and thyme (*Thymus vulgaris*) essential oils, as well as grape seed condensed tannin (GSCD) were tested in a fermentation assay and shown to be effective *in vitro* at minimum inhibitory concentrations (MIC) of 0.02% v/v (essential oils) and 0.5% w/v (condensed tannin) respectively. However, although effective *in vitro*, these agents must be shown to be effective in the environment of the gastrointestinal tract, where the interaction between proteins, carbohydrates, lipids, feed additives and potential antimicrobial agents may significantly reduce their effectiveness. It is therefore important to determine what effect feed, feed additives and normal gut contents may have on this anti-clostridial activity.

Using a fermentation assay *in vitro*, we tested the effect of the above essential oils and GSCD on growth of *C. perfringens*, in the presence of normal chicken feed, commercial enzyme supplements and chicken gut digesta.

The results show that the presence of normal feed constituents increased the fermentative activity of *C. perfringens* 8 fold, and the MIC's of essential oils and GSCD by up to 10 fold. The exogenous addition of commercial enzyme preparations, xylanase, avizyme, or protease, which are often used as feed supplements to enhance digestive activity in the gut, had no effect on the MIC's of essential oils or condensed tannins, but did increase the fermentative activity of *C. perfringens* 5 fold in the absence of feed. The addition of normal gut digesta also increased the MICs and reduced the effectiveness of the essential oils and GSCD.

These results demonstrate that interactions between gut contents and essential oils or condensed tannins will increase their MICs as antimicrobial agents and MIC values based on *in vitro* assays alone will result in a significant overestimation of their effectiveness; higher concentrations of essential oils or GSCD will be needed to maintain effectiveness *in vivo*. Nevertheless, these compounds may still be cost effective alternative to in-feed antibiotics, provided a delivery system eg encapsulation, can be devised to reduce feed-associated inactivation of the agents.

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Volatile Constituents of *Perovskia abrotanoides* Kanel. from Iran

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Perovskia abrotanoides is a species of Lamiaceae herb which growing in many regions of Iran. Chemical composition of the essential isolated by hydrodistillation from the aerial parts of *P. abrotanoides* (labiataea) which harvested from Kashan, Province of Markazi was characterized by means of capillary gas chromatography coupled mass spectroscopy (GC-MS) using a HP 6890-5973 GC-MS system with programming temperature 60°C (3 min to 220 °C (10 min) with rate of 5°C/min. With he as the carrier gas. Mass spectra were recorded at 70 eV. Characrization of oil constituents performed using Wiley 275 mass spectra library of GC-MS system and authentic spectra (*1*). Identification was accomplished by comparison of retention Index (RI) with authentic sample (*1*). Percentages of components were calculated using a 14A-Shimadzu gas chromatograph as like as GC-MS system condition. Among thirty four compounds of the oil identified that representing 95.5% of the oil the main constituents were 1,8-cineole (RI=1026) (12.3%), limonene (RI=1029) (14.2%), δ-3 carene (9.1%), α-phellandrene (RI=1002) (7.6%), camphene (RI=647) (6.0%), β-pinene (RI=973) (6.5%) camphor (RI=1136) (5.4%), α-pinene (RI= 932) (17.8%) and sabinene (RI= 970) (6.5%). Other constituents were tricyclene (0.1%), α-thujene (0.3%), myrcene (1.8%), *p*-cymene (0.4%), (*Z*)-β-ocimene (0.3%), γ-terpinene (0.5%), terpinolene (0.5%), pinocarveol (1.2%), pinocarvone (0.1%), borneol (0.3%), 1-terpinen-4-ol (0.1%), α-terpineol (0.4%), verbenone (0.1%), *trans*-carveol (0.1%), bornyl acetate (0.4%), α-terpenyl acetate (0.2%), α-copaene (0.1%), α-gurjunene (0.6%), β-caryophyllene (0.5%), β-gurjunene (0.3%), α-himachalene (1.2%), *allo*-aromadendrene (0.1%), β-selinene (0.2%), vidiflorene (0.1%) and δ-cadinene (0.2%). The oil of some *Perovskia* species were subjected of study. The oil of *P. atriplicifolia* and *P. abrotanoides* from Afghanistan contained α-pinene, β-pinene, camphene, α-terpinene, 1, 8-cineole, camphor, borneol, menthole, bornyl acetate and β-caryophyllene as the main constituents (*2*). In another reports, β-thujene (45.9%), sabinene (26.6%), α-pinene (12.1%) and 1,8-cineole (45.9%) were identified as the major compounds in *P. atriplicifolia* (*3*). As seen, the major componenets of the oil of *P. abrotanoides* from Iran are similar to other species which reported. Some of compounds which characterized in the oil of *P. abrotanoides* have biological effect. For example, 1,8-cineole and camphor have antiseptic effect; α-pinene and β-pinene have antibacterial effect.

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P-46

Essential oil composition of some *Dracocephalum* species

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We have investigated the essential oil composition of four *Dracocephalum* (dragonhead) species: *D. moldavica* L., *D. ruyschiana* L., *D. grandiflorum* L., *D. renati* Emberg.

The *Dracocephalum* genus belonging to the Lamiaceae family (subfam. Nepetoidae) contains some 70 species. *D. ruyschiana* is spontaneous in Hungary, it is a protected ad endangered species. *D. moldavica* is a cultivated medicinal plant. *D. renati* is native in North Africa; *D. grandiflorum* is spontaneous in the North-Eastern territory of Siberia.

The cultivated plant material was hydro-distilled using the apparatus official in the 7th edition of the Hungarian Pharmacopoeia. GC analysis was performed on a Fisons 8000 gas chromatograph equipped with flame ionisation detector; 30 m x 0.25 I.D. mm capillary column with enantioselective Rt-β-DEXm stationary phase (film thickness 0.25μm); injector at 210°C, detector at 240°C; column temperature: 8°C min^{-1} from 60 to 230°C, then 230°C for 5 min. GC-MS apparatus: Finnigan GC with 30m x 0.25 I.D. mm capillary column (MDN-5S stationary phase, film thickness 0.25 μm); injector at 200°C; temperature program: 60°C for 3 min., 8°Cmin^{-1} from 60 to 200°C, 200°C for 2 min., 10°C min^{-1} from 200 to 250°C, finally 15 min. at 250°C. The identity of constituents was confirmed using data reported in the literature ad by comparison with the mass spectra of the reference compounds.

The essential oil of *D. moldavica* (0.4%) contains mainly oxygenated monoterpenes: neral and geranial, nerol and geraniol, neryl acetate and geranyl acetate. We have identified methylchavicol, linalool, *β*-caryophyllene, thymol and carvacrol, too.

In the essential oil of *D. ruyschiana* (0.23%) the predominant compounds are oxygenated bicycled monoterpenes as camphor ad *iso*-pinocamphone. Other identified constituents are *β*-caryophyllene, *β*-cubebene, ledol (sesquiterpenes); furthermore *β*-pinene, myrcene, limonene, *p*-cymene ad methylchavicol.

Sesquiterpene hydrocarbons as aromadendrene, *β*-caryophyllene, β-cubebene, *β*-bourbonene; caryophyllene oxide ad minor constituents as *β*-asarone ad methylchavicol were found in the essential oil of *D. grandiflorum* (0.08%).

The main constituents of the essential oil of *D. renati* (0.5%) are monoterpenes: a compound not yet identified and the limonene. Carvone, neral, geranial, linalool, linalyl acetate, *β*-caryophyllene and bicyclo-vetivenol (a sesquiterpene alcohol) were also identified in the oil. The essential oil composition of D. ruyschiana and *D. renati* is reported for the first time.

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P-47

Content of essential oil in *Anethum graveolens* L. accessions in Czech gene bank

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Dill (*Anethum graveolens* L.) belongs to one of the most favourite vegetables and/or spices in Czech Republic but in contrast to this situation there is no any official standard declaring its quality and essential oil content in the Czech Republic. This genus is represented by 22 accessions in collection of medicinal, aromatical and culinary plants of Czech Gene bank in Olomouc. This set is consisted from 6 cultivars and 15 landraces originally from Czechoslovakia, Netherland, Germany and Soviet Union and a screening of essential oil content in three developmental stages of plants was the aim of this work.

Dill plants were analysed at the three following stages: green tops before flowering (stage 1 – it is used to vegetable salads, dressings and cream sauces), tops with seeds in milk ripeness (stage 2 – it is used into the vinegar stock for pickled gherkins etc.) and seeds in full ripeness (stage 3 - standard). The essential oil content was measured gravimetrically by steam distillation according to the Czech Pharmacopoeia (*1*).

Results (average values of two resp. three parallel analyses of fresh plant material recalculated later on to the dry matter) show that the content of essential oil is continuously increased has a clear decreasing tendency during the plant ripening. In the stage of green tops before flowering there was the average essential oil content established at 0.79 % (0.45 - 1.35 %) and the highest value was reached in 'Gribovskij' variety from Sovet Union. In the stage 2 (tops with seeds in milk ripeness) there was the highest essential oil content stated in one landrace accession originally from Czechoslovakia (2.75 %) and the average was evaluated to 2.21 %. The highest amount of essential oil was found in full ripened seeds – 4.55 % in average and 5.30 % in the best evaluated sample – landrace accession from Germany.

According to our results, the accessions with low essential oil content at the beginning of experiment mostly had low essential oil content also in the other developmental stages. But on the other hand there were also some exceptions - for example the cultivar 'Gribovskij', which reached the highest amount of essential oil at the beginning of evaluated period, was absolutely ordinary at the later stages.

This study presents only the preliminary experiment and more detail evaluation (influence of the year, dependence of essential oil content on climatic conditions and so on) of *Anethum graveolens* accessions collected in Czech Gene bank will come next.

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Constituents of the volatile oil of *Eremostachys adenantha* Jaub & Spach. from Iran

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Eremostachys is one of the genuses in Lamiaceae family. It is represented by fifteen species in the flora of Iran which five of them are endemic species. The extract of *Eremostachys laciniata* showed antioxidant activity. We can not find any report on the chemical composition of the essential oils of this genus. *Eremostachys adenantha* jaub & Spach. is one of the endemic species, which grows wildly in south of Iran. Aerial parts of the plant were collected from Shiraz in Fars provenance at the time of flowering. The aerial parts were air-dried at ambient temperature in the shade and hydrodistilled by using a Clevenger-type apparatus for 4 hours. The essential oils were analyzed by GC and GC-MS. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley library or with the published mass spectra. The main components of the oil were dodecanal (12.6%), hexadecanoic acid (9.8%) and 6,10,14-trimethyl-2-pentadecanone (8.9%). The oil consists mainly of fatty acids and aliphatic compounds. Monoterpenes and sesquiterpenes were present in the oil as minor compounds.

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Composition of the essential oils of *Calamintha tauricola* P. H. Davis

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Calamintha Miller (Lamiaceae) is represented in Turkey by 9 species and altogether 13 taxa, 6 being endemic. The rate of endemism in Turkey is over 45% (*1*),(*2*). *Calamintha* species are locally called as ''Güzel Nane, Dağ Nanesi, Miskotu,, Dağ Miskotu, Yabani Oğulotu'' and used as herbal tea (*3-5*).

In the present study, aerial parts of the endemic *Calamintha tauricola* P. H. Davis collected from the following regions of the Icel province were water distilled to yield oils which were analyzed by GC and GC-MS the diterpene manool (33.9-75.4 %) was found as the main constituent.

A : Icel; Mut-Gülnar on July 10, 2003 (ESSE 14391)

B : Icel; Silifke-Uzuncaburc on July 11, 2003 (ESSE 14392)

Aerial parts were subjected to water distillation for 3h using a Clevenger-type apparatus to yield oils in the following percentages: 0.2% and < 0.1% for A and B, respectively. Manool was identified by comparison of its mass spectrum and retention time with genuine manool.

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P-50

Composition of the essential oils of five Serbian *Equisetum* species

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Sterile stems of *Equisetum arvense* L. (Equisetaceae, subgenus *Equisetum,* sect. *Heterophyadica*) are used as medicines in various countries, constituting "Equiseti herba" of European Pharmacopedias (DAB 10, Ph. Helv. VII, OAB 90, Ph. Pol. III, Ph. Ross 9 and Ph. Hung.).

Usually and easily mistaken to be horsetail, the other *Equisetum* species of the subgenus, have the same relevance and utilization in the diet of Balkan people as the true *E. arvense* and are known as potential adulterations of Equiseti herba. All *Equisetum* species, especially the hybrids, are extremely variable in their morphology and their identification by morphological characters can be particularly difficult. The HPLC method for the detection of a possible adulteration was published earlier based on phenolics patterns in methanol extracts (*1*).

In this study we undertook a GC-MS analysis of the essential oils of five species of the subgenus *Equisetum*: *E. arvense* L. (sect. *Heterophyadica*), *E. sylvaticum* L. (sect. *Heterophyadica*), *E. fluviatile* L., (sect. *Equisetum*), *E. palustre* L. (sect. *Equisetum*), and *E. telmateia* Ehrhart. (sect. *Equisetum*).

The volatile constituents obtained by hydro-distillation of the aerial sterile stems of the investigated *Equisetum* species differ only quantitative. The yield the semi-solid essential oils was very low and ranged from 0.002-0.0035 %, w/w. The oils were characterized by the presence of the diterpene phytol and a great number of carotenoid degradation metabolites, among which hexahydrofarnesyl acetone, (*E,E*)-farnesyl acetone, *(Z)*-geranyl acetone, (*E*)-*β*-ionone, (*E*)-α-ionone, 3,4-didehydro-γ-ionol, and 5,6-epoxy-β-ionone represent major contributors.

β-Ionol being the second most abundant constituent of *E. sylvaticum* oil, whereas only detected in trace amounts in the other oils, can serve as a drastic example of the oil constituent quantitative variation.

Hexadecanoic acid was the major component in *E. telmateia* (36.4%), *E. fluviatile* (20.4%), and *E. sylvaticum* (12.4%) oils, the second runner up in *E. palustre* (14.9%) oil, while being present in *E. arvense* with only 2.3%. It seems worthy to note that the summation of phytol and palmitic acid reaches around 39% in *E. telmateia*, *E. palustre* and *E. fluviatile*, and 15% in *E. sylvaticum* and *E. arvense* oils. Phytol and hexahydrofarnesyl acetone add up to 20% in all investigated species but in *E. sylvaticum* where it takes a value of around 7%. These data could provide evidence for plant classification and contribute to achieving better quality control of crude drug materials and can be used additionally to distinguish hybrids from their sympatric parents.

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P-51

The essential oil composition of *Salvia vermifolia* from Turkey

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The genus *Salvia* L. (Lamiaceae), also called Sage, includes about 900 species found throughout the temperate and warmer parts of the world. Some of them have been used as medicinal plants since earliest times.

In the Turkish flora this genus is represented by eighty eight species 51% of which are endemic*(1,2)*. Some species of these plants are used also in Turkish folk medicine *(3)*.

In the present study, the composition of the essential oil of *Salvia vermifolia* Hedge & Hub.-Mor., an endemic Salvia species, was studied. No literature data could be found on the essential oil of this plant. The flowering tops of *S. vermifolia* were collected in June 2005 from wild populations in Central Turkey.

The essential oil was isolated by hydrodistillation for 3 hours in a Clevenger type apparatus. The oil yield of the plant was 0.30 % (v/w).

The oil sample was analysed by GC-MS method on two columns with different polarities.

The oil contained at least 35 compounds.The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature.

The essential oil of *S. vermifolia* was found rich in sesquiterpenes. The major constituent of the oil was calarene (10.94%).

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Comprehensive two-dimensional Gas Chromatography-Olfactometry: an approach for odour fingerprint acquisition of fragrant complex matrices

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Perfumes have been intimately associated with human history and are represented by complex mixtures of odorant materials which blending is a good example of product engineering. Today, perfumers work with a total of several thousands ingredients, synthetically manufactured, as well as natural fragrances. The successful blending of fragrant ingredients into ever more complex perfume compositions is a real challenge, likewise can be the obtention of detailed analytical information. For decades monodimensional gas chromatography (GC) and GC-Mass Spectrometry (GC-MS) are being commonly employed in perfume industries, as well as GC-Olfactometry (GC-O). However taking in consideration that perfume raw materials are characterized by a variety of components belonging to several chemical classes, extensive co-elutions may occur leading to inaccurate identification of odor-active compounds. As is well-known, numerous compounds although being present at trace-level concentrations, can still exert an important impact either olfactively or economically. Since a satisfactory chromatographic separation is required and can be hardly achieved by single capillary column analysis, comprehensive two-dimensional gas chromatography (GCxGC) presents to be the most appropriate choice to fulfill the request for enhanced separation, and hence better sensitivity. This orthogonal two-column separation, with complete sample transfer executed by means of a modulator able to trap, refocus and release fractions of the GC effluent from the first column (1D), and periodically introduce it into the second column (2D), enables the separation performance from each system to be preserved. Offering thereby increased signal to noise ratio, very high resolution and enhanced detection sensitivity. The hyphenation of the latter technique to olfactometry (GCxGC-O), makes a bidimensional separation and simultaneous olfactive characterization of components in a complex matrix possible. This novel technique associates the resolution power of GCxGC with the selectivity and sensibility of the human olfactory system.

The purpose of this research is the application of GCxGC-O as a screening procedure for establishing the odor fingerprint of fragrant matrices by means of a complete qualitative characterization of this complex sample.

P-53

Gas chromatographic elucidation of the volatile fraction of *Teucrium flavum*, a Sicilian endemic plant

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The chemical characterization of the volatile fraction of Teucrium oils is restricted to a low number of studies. With regards to the non-volatile composition, research related to the neo-clerodane glucoside and diterpenoid content has been reported.

The genus *Teucrium* belongs to the Lamiaceae family. Almost 50 species are found in Europe and they are distributed in the Mediterranean area. The present work is focussed on the elucidation and monitoring of the essential oil profile of Sicilian *Teucrium flavum* L.. Leaves, flowers and fruits were considered during the vegetative (December), pre-flowering (February), flowering (April) and post-flowering (June) stages.

Although *Teucrium* species present a large number of secretory structures, they contain only a small quantity of essential oil. The latter was obtained by using microwave assisted hydrodistillation (MAHD), as this approach essentially maintains the oil chemical profile. Quali/ quantitative analysis was carried out by means of GC-qMS and GC-FID.

It has been reported, in previous limited studies carried out on this sample-type, that *Teucrium flavum* oil is generally characterized by monoterpene hydrocarbons such as α-pinene, β-pinene and limonene. Greek oils, in particular, are characterized by sesquiterpene hydrocarbons such as germacrene D and (*E*)-caryophyllene.

In the present study, it was observed that the leaf-derived product showed changes in its chemical composition during the entire season, although in all samples β-bisabolene remained one of the main constituents. Generally, hydrocarbon and oxygenated monoterpenes characterized all leaf, flower and fruit samples.

The derived analytical information is of interest both with regards to seasonal variations of this specific essential oil profile and for the taxonomic classification of flora located in the south of Italy.

P-54

Automated fast solid phase microextraction-gas chromatography with analyte cryo-focussing for the headspace analysis of essential oils

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Amongst the primary objectives to be considered in the development of any GC method are both a rapid sample preparation process and the separation of the most critical components in the minimum time. As a consequence, there has been an ever-present interest within the chromatographic community for the introduction of faster analytical techniques.

A valuable tool for rapid headspace sample extraction has proved to be solid phase microextraction (SPME). This sample preparation method exploits the high sorption power of a fused silica fiber coated with a specific absorbent/adsorbent in contact with the matrix analytes. Furthermore, the automation of the entire SPME procedure produces a series of unquestionable advantages: lower probability of sample contamination, less time-costs and higher analytical precision.

The primary aim, relative to any fast GC technique, is to maintain (compared to traditional GC) sufficient resolving power for the separation between the compounds of interest. In respect to this aspect, the micro-bore column approach is a very effective modality of increasing analysis speed.

Although the use and validity of these columns was demonstrated many years ago, their routine use in fast GC applications is only quite recent. The reason behind this delay is merely technical and was due to the lack of suitable GC systems. Modern GC instruments are now capable of supplying the extreme experimental conditions that micro-bore columns necessitate: high inlet pressures, highly controlled split flows, rapid oven temperature heating/cooling and fast electronics for detection.

The present research is based on the rapid automated extraction of headspace compounds relative to an essential oil sample by using solid phase microextraction and the subsequent fast GC separation of the isolated compounds on a 0.1 mm ID capillary. The injected sample band was focussed by using a cryo-trap positioned at the head of the micro-bore column. With respect to a conventional method, a great reduction of analytical time-costs was observed.

P-55

Determination of the quality of Italian bitter orange essential oils

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Bitter orange essential oil is obtained by cold-pressing fresh peels from fruits of *Citrus aurantium*, L., and its production is mainly in Mediterranean countries.

Bitter orange oils extracted during the productive season 2005-2006 were taken as sample. The qualitative and quantitative composition of the volatile fraction, carried out by conventional gas chromatographic (GC) equipped with FID, as also GC hyphenated to quadrupole mass spectrometer (qMS). Compounds where detected and out of these 64 were identified by interactive mass spectra library matching. The oils were also analysed by means of enantioselective GC (e-GC) on distinct chiral stationary phases. Furthermore the non-volatile fraction of the bitter orange oils were analysed by means of reversed phase high performance liquid chromatography (HPLC) with the aim to determine their oxygen heterocyclic compounds, such as coumarins, psoralens and polymethoxyflavones. The odour profile of the oils were characterized by means of direct olfactive analysis. Physical-chemical analysis were also carried out.

The aim of the present work is to characterize, chemically, physically and olfactively, different bitter orange essential oils. Moreover a comparison was established, not only between each analysed oil, but also relating experimental obtained data to the ones reported in literature.

P-56

Characterization of the volatile and non-volatile fractions of genuine bergamot essential oils

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Bergamot essential oil is obtained by pressing peels from the unripe fruit of *Citrus bergamia, Risso* and is considered to be of high economic importance with an annual production of *ca.* 200 t.

As a consequence of the utmost interest and high market demand, attention has been paid to this oil leading to several studies on its the rich volatile fraction (representing 93-99% of the oil).

As well-known bergamot is still grown almost exclusively in the Italian province of Calabria, where the soil and climatic conditions are very favourable for its cultivation. Furthermore the non-volatile fraction of this oil, consisting of oxygenated heterocyclic compounds, presents to be higher than other citrus oils, and the qualitative and quantitative composition of residue plays an important role in the control of quality and genuineness of the bergamot essential oil.

For this reason the enatioselective evalutation of the linalool and linalyl acetate ratios are commonly carried out along with the qualitative and quantitative gas chromatographic analyses of mono- and sesquiterpenes.

The present research reports the quantitative data obtained by means of GC analyses of eight genuine industrial cold pressed bergamot essential oils cultivated in Calabria under identical environmental conditions, extracted during the productive season 2005-2006. The work attends to establish useful standard parameters for the quality determination of the genuine bergamot oil. The oil composition was analysed by GC-FID and GC-MS, both carried out on an Equity 5MS column (Supelco, Bellefonte, U.S.A.). Moreover each oil was subjected to enantioselective analysis by using a diethyltertbutylsilylbetaCDX stationary phase. Reversed phase HPLC has been used for the determination of the non-volatile residue composition. The odour profile of each oil has also been characterized.

P-57

FFNSC (Flavour & Fragrance Natural & Synthetic Compounds) GC-MS library: how to bring innovation in GC-MS peak assignment

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Gas chromatography-Mass Spectrometry has become in the last years the most powerful technique applied to the identification of unknown compounds in even complex matrices. Several drawbacks arise from the use of such technique, though. First of all, when dealing with classes of compounds having similar structures (e.g. sesquiterpenes in essential oils), fragments generated by the ionization process are very similar, thus leading to the acquisition of nearly identical spectra for different compounds. The GC-MS libraries available on the market cannot always face up to such a case, since mistaken peak assignment occurs very easily. In addition, commonly used GC-MS libraries list thousands of compounds whose spectra quality is neither reliable nor experimental conditions of the acquisition procedure are well defined. All these reasons have increased the need for a new GC-MS library, denominated "FFNSC" (Flavour and Fragrance Natural and Synthetic Compounds), built-up with spectra gathered from essential oils and pure standard compounds.

The innovative feature of the FFNSC library lies in the "LRI filter" option provided by the software (GC-MS solution ver. 2.5). This consists of setting an LRI range within the unknown compound LRI value has to fall: this tool allows to shorten the list of library matches given by the searching process, thus, to get closer to the best search result.

Furthermore, many other parameters can be set in the interactive windows of the software, such as the degree of similarity between the target and the library spectra. All these features make often possible to obtain in a single step search process the real identity of the unknown compound.

The FFNSC library contains around 1500 spectra, each of them provided with CAS registered information and LRI value. The Linear Retention Indices have been calculated under accurate experimental conditions, acquiring, before the real sample, a mix of C7 to C30 *n*-alkanes onto an SLB-5MS capillary column.

P-58

Analysis of volatile and heavy compounds in the absolute oil of mimosa (*Acacia dealbata*).

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Nowadays, natural products are still important in perfume compositions (*1*). One of the reasons is that many of the most famous fragrances are actually quite ancient, and their formulations have to stay unchanged. In perfumery, essential oils are the most commonly used natural extracts, but some plants do not produce interesting materials when hydrodistilled. In these cases, the preparation of solvent extracts (concrete or absolute) is an interesting alternative for the extraction of odorant compounds. An absolute is made from the concentration of the ethanol soluble part of the concrete, which is the non-polar solvent extract of a plant.

Mimosa extracts have contributed to the growth and hegemony of Grasse in the domain of perfumery. Mimosa absolute smells typically like the live flowers and is recognized as a great blender and as a "smoothing agent" as well as an effective fixative in high-grade perfumes. These raw materials have to be controlled to proof their authenticity, to prevent adulterations and to detect some potentially toxic or allergenic compounds (*2*). The analysis of mimosa extracts is also interesting because some sources suggest an antimicrobial activity for these extracts (*3*).

In the course of our studies on the chemical composition of absolutes, we applied modern analytical techniques in order to 1) develop a rapid method that allows quality control of mimosa absolutes (SPME/GC-MS), 2) identify the compounds responsible for the typical mimosa odor by studying the volatile and semi-volatile compounds by GC/Olfactometry (GC/O) and 3) characterize the non-volatile compounds of this absolute by preparative chromatography/structural analysis in order to discover new molecules.

Mimosa absolute appeared to be very complex with a large proportion of semi-volatile compounds. Many linear saturated fatty acids have been identified as well as different ester derivatives. The main components of the absolute were found to be pentacyclic triterpenoids and were volatile enough to be observed by GC-MS. Two of them had already been described in the leaves of *Acacia dealbata (4)*.

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P-59

Analytical investigations on natural 2-methylbutanol and its derivatives.

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Many foodstuffs naturally contain 2-methylbutyric acid and its simple esters, particularly, ethyl 2-methylbutyrate. These compounds have fruity notes and are widely used in food flavours. They are produced from 2-methylbutanol coming from fusel oils mainly by chemical synthesis or biotechnology approach (by yeast like *Saccharomyces cerevisiae* or bacteria like *Gluconobacter roseus* or *oxidans*).

Enantioselective gas chromatography and $^{13}C/^{12}C$ isotope ratio analysis are well known suitable tools to confirm the origin of the alcohol.

The aim of this study is to examine whether a sophisticated approach with the determination of the enantiomeric excess and a multi-element isotopic analysis (IRMS $^{13}C/^{12}C$, D/H, $^{18}O/^{16}O$) allows to discriminate the types of oxidation and esterification processes.

A large range of samples of 2-methylbutyric acid and ethyl 2-methyl butyrate were prepared, purified and analyzed. The biological oxidation of (*S*)-(-)-2-methylbutanol into (*S*)-(+)-2-methylbutyric acid has been carried out by *Saccharomyces cerevisiae*; the chemical oxidation involved a $KMnO_4$ treatment of the alcohol. Enzymatic esterification of (*S*)-(+)-2-methylbutyric acid to (*S*)-(+)-ethyl 2-methylbutyrate was done by *Candida antarctica* lipase. The chemical esterification was achieved by acid catalysed reaction of the acid with ethanol. We also prepared some esters by a physical process involving soft thermic transformation without catalyst.

Two different chiral columns were employed to determine all the enantiomeric ratios.

The combination of gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) and gas chromatography-pyrolysis-isotope ratio mass spectrometry (GC-P-IRMS) was applied. The measurement of $^{18}O/^{16}O$ ratios was done with an elemental analyser (EA).

The ability to differentiate the methods of oxidation or esterification by the analytical approach is presented and discussed.

P-60

Steam distillation of essential oil - comparison of methods with addition of decaline and xylene

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Steam distillation belongs to one of the most often used methods of essential oil extraction. This method is very old and relatively easy for application and it provides sufficient exact results. According to regional customs and also differences in physical characteristic of essential oils several distillation devices is presented in literature and there are also huge differences in particular distillation process. Comparison of steam distillation with addition of decaline (Pharmacopoeia Bohemoslovenica IV 1987) and/or xylene (Czech Pharmacopoeia 1997) was the aim of this work.

For methods testing 15 parallel samples of yarrow drug (*Achillea millefolium* L. s.l.) were used. Plant material was pre-treated by drying in room temperature and grinding to sieve mesh size 2 mm. 30 g of this mass was putted into cupping-glass with distil water and boiled under regressive cooler for 3 hours. In case of method with decaline, after this boiling the cupping-glass with plant material was changed for another one with distilled water and 0.1 ml of decaline ($C_{10}H_{18}$) and boiled for another hour. In case of xylene (C_8H_{10}) it was added directly into distillation apparatus (0.1 ml) to the place where essential oil is accumulated (it stays floating on the water surface) and another 0.2 ml of it was also added into cupping-glass after end of plant material boiling and boiled the same way as decaline.

The essential oil content was (depending on yarrow genus and ecotype) established between 0.17 – 0.61% of dry mass and the average essential oil contend was got at 0.50% by both methods. The difference between types of analysis made 0 – 9.1% and the average difference between them was established 5.52%. No statistically significant differences (Anowa, one-factor analysis of variance at the significance level $\alpha = 0.05$) were found between these methods.

Nevertheless during qualitative analysing of essential oils by GC, an unpleasant fact was discovered: whereas decaline gives two GC peaks which do not interfer with the essential oil components, xylene is eluted as 4 peaks, which give interferences with some compounds coming from the plants! Even in using four different temperature programs, GC was not able to achieve a good separation, which makes this solvent unsuitable for this purpose. As a result, for the analysis of essential oil content, the method using decaline should be unambiguously recommended.

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P-61

The influence of chemicals on the regulation of camphor and thujone biosynthesis in sage (*Salvia officinalis* L.)

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Essential oil variation is well known in aromatic plants. The degree of variation, especially in wild growing populations, depends on the genetic and environmental conditions. In particular, many Labiatae species are known to possess the ability of genetical polymorphisms that affects the composition of the essential oil dramatically, resulting in chemical polymorphisms (chemotypes) within closely related individuals or populations. The environmental influence is the other main reason for chemical variation, explaining chemical differences even within the same clones and resulting in characteristic flavour and fragrances of many essential oil bearing species for a specific growing habitat.

One of these well exploited aromatic species is sage (*Salvia officinalis* L.), where the characteristic compounds α- and β-thujone and camphor are mainly responsible for the characteristic aroma. This species was used to investigate the effect of different chemicals on the variation of essential oil compounds under controlled conditions in growing chambers. The ontogenetic phytochemical variation alone was used as background matrix and genetically ident sage plants in vegetative growth were forced by 20 different chemicals to change growth and the production of typical sage essential oil compounds. The chemicals applied include plant growth regulators, herbicides, antibiotics and others.

With some of the chemicals, changes in the accumulation rates of the following main defined groups could be observed: 'thujone' (with α-thujone, β-thujone, α-thujene and others) 'camphor' (including camphor, borneol, camphene and others) sesquiterpene hydrocarbons (with β-caryophyllene, and α-humulene) and sesqui- and diterpene alcohols (viridiflorol and manool). Gibberellinic acid for example diminishes the 'thujone' production and enhances the 'camphor'-line, whereas daminozide seems to have a suppressing effect on camphor biosynthesis. However, the changes within the 'camphor' group were not always homogenously and consistent, in contrary to the 'thujone'-group. Fosmidomycin, a DOXP-inhibitor, completely blocks the monoterpenoid biosynthesis, indicating that all monoterpenes in sage derive from the plastidial DOXP-pathway.

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Isolation of essential oil from root of Chinese Ginseng using hydrodistillation and hydrodistillation with ultrasonic technique

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Ginseng *(Panax ginseng)* is specified as life root in traditional Far East medicine, where it is known and used from over 5000 years. Ginseng is long-term herb plant from *Araliaceae* family. It is recognized as preventive and supporting agent, used in improving of psychical and physical efficiency of organism, strengthening in tiredness and weakness periods, lack of concentration and during convalescence. Furthermore, the root of ginseng shows ageing counteraction, blood pressure regulation and anti-stress, anti-oxidative and antiviral activity. The raw material used in therapeutics is ginseng root, which obtains his best properties after 4-5 years of vegetation.

Essential oil for researches was obtained from crushed vegetable raw material using two methods: hydrodistillation and hydrodistillation with ultrasonic technique. Using GC and GC-MS method, above 30 volatile compounds were identified: sesquiterpene hydrocarbons – 48%, oxygenated sesquiterpene – 20,9%, monoterpene and oxygenated monoterpene – 0,7%, other – 30,4%.

Using hydrodistillation method essential oil was obtained with the yield 0,1%. In the hydrodistillation with ultrasonic technique the yield increased about 25% – 30%.

P-63

Composition of the essential oil of *Eryngium planum* L.

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The genus *Eryngium* L. belongs to the Apiaceae family and, with about 250 species, has a cosmopolitan distribution. It is native to the tropical regions of Asia and occurs in most parts of Europe as well. This genus is rich in several compounds of therapeutic value. Native species of *Eryngium* are used in folk medicine.

Eryngium planum L. (Flat sea holly) is a rare and endangered herbaceous perennial species of native flora with restricted distribution in Poland. It is European evergreen eryngo with twisted spiny dark green leaves. Both the flowers and the stems are blue.

The composition of the essential oil of *E. planum* growing wild in Poland was investigated. The aerial plant parts were collected at the full flowering stage, near Torun, central Poland. The essential oils was obtained by hydrodistillation of the air-dried parts of plant with the yield: flowers 0.29% and leaves 0.10%, respectively. Because of very complex composition, the essential oil was subjected to repeated flash chromatography and components were identified by GC, GC-MS and ^{1}H-NMR.

The main components of the flower and leave oil of *E. planum* was *trans*-chrysanthenyl acetate – 43.2% and 9.2%, respectively. α-Pinene, myrcene and camphor were other important monoterpenes in both oils while filifolone, α- and β-thujone were present only in leaf oil. Sesquiterpene hydrocarbons consisted of about 20% of flower oil and 40% of leave oil, being β-elemene, β-caryophyllene, germacrene D and B as well as bicyclogermacrene the main ones, but they occurred in very different amount in each oil.

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DNA-based authentication of raw and processed plant materials

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DNA analysis has become routine technique to identify raw materials of food and feed. Especially the discussion about traceability of genetically modified organisms (GMO's) in the whole production chain from raw material to finalized product sped up research in processed food (*1,2*). Therefore especially DNA analysis of processed food has received much attention (*3*) although DNA degradation during processing steps may hamper the analysis.

Peppermint became a study object of pathway engineering and essential oil yield improvements by genetic engineering where different technologies were used to alter the composition and to increase the content of the essential oil (*4*). This first GMO in essential oil production will bring the methodology of DNA-based authentication into the focus of the essential oil industry because it is not verified yet that an essential oil does not contain DNA.

This presentation will summarise the approaches to identify raw and processed materials and will give an outlook to possible applications in quality control in relation to essential oils.

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P-65

New synthetic odoriferous compounds with *p*-menthane system

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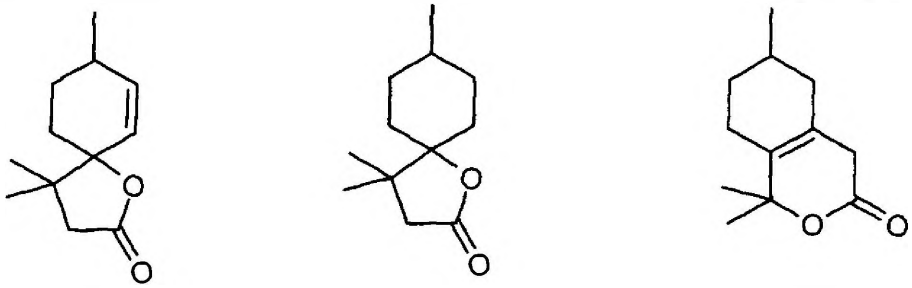
Many *p*-menthane derivatives have been identified in essential oils. The (+) and (-) isomers of mintlactone and isomintlactone are representatives of this group of natural compounds. They have been identified in the essential oils of several *Mentha* species (*1*) and in the oils of the wood *Bursera graveolens* (*2*). A quite large number of natural and synthetic compounds with *p*-menthane system have found an application as the components of many cosmetic products and as the flavoring additives in food products.

Being interested in the synthesis of terpenoid lactones as potential insect feeding deterrents, we have noticed in the course of the syntheses carried out that many intermediate compounds with *p*-menthane system as well as final lactones possess very interesting odours. We present herein the synthesis and odour characteristics of lactones **1**, **2** and **3**.



1 **2** **3**

Enantiomeric pair of lactone **1** and racemic lactone **2** were obtained in five-step syntheses from (+)- or (-)-pulegone (*3*). In the first step, pulegones were reduced ($NaBH_4$) to the corresponding pulegols, which were transformed *via* Claisen rearrangement into γ,δ-unsaturated esters. The acids obtained from hydrolysis of these esters were subjected to the iodolactonization with I_2, KI in basic conditions. Pure (+) and (-) enantiomers of lactone **1** were the products of dehydrohalogenation of the corresponding isomers of iodolactones. Lactone **2** was obtained from both isomers of iodolactones as a product of their reduction with *n*-Bu_3SnH. Enantiomers of lactone **2** were obtained in four-step synthesis from (+) or (-) isopulegols. The key step of this synthesis was the Wadsworth-Emmons reaction of isomeric isopulegones with ethyl diethylphosphonoacetate. The α,β-unsaturated esters were hydrolyzed to acids which were heated (60°C) with 30% of H_2SO_4. The odour characteristics of final lactones and intermediate esters will be presented.

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P-66

Volatile constituents of *Bupleurum falcatum* L. and *Pimpinella affinis* Ledeb., two Umbelliferae herbs growing wild in Iran

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The genus *Bupleurum* is represented in the flora of Iran by fourteen species, out of which three are endemic. *Bupleurum falcatum* is one of the most widely used components in traditional oriental medicines. *B. falcatum*, or its important principle saikosaponins, is known to have numerous pharmacological activities, including anti-inflammatory, antipyretic and antitussive actions.

The Iranian flora comprises twenty species of *Pimpinella*, among which six are endemic. Some species are used as flavouring agents and for medicinal purposes , e.g. anise, *Pimpinella anisum* (L.) is an eastern coast of Mediterranean to Asia Minor region.

Water distilled volatile oils from aerial parts of *Bupleurum falcatum* L. and *Pimpinella affinis* Ledeb (syn: *Pimpinella multiradiata* (Boiss.) Korov.), two Umbelliferae species, were analyzed by GC and GC-MS.

The aerial parts of two Umbelliferae species were collected during the flowering stage, from Talaghan area, North of Tehran, Iran, both in June 2004.

Eighteen compounds were identified in the oil of *B. falcatum* representing 90.0% of the total oil with α-pinene (29.4%) and spathulenol (27.7%) as the major constituents, followed by lavandulyl acetate (6.7%) and caryophyllene oxide (6.1%). Thus, the oil of *B. falcatum* consisted of three monoterpene hydrocarbons (32.1%), two oxygenated monoterpenes (7.9%), seven sesquiterpene hydrocarbons (7.0%), four oxygenated sesquiterpenes (37.1%) and two nonterpenoid compounds (5.9%). The major constituent of the fruit oil of *B. gibraltaricum*, was α-pinene (42.7%) (*1*).

Twenty-one components in the oil of *P. affinis* , which represent about 99.4% of the total oil were identified. The major components of this oil were *trans*-α-bergamotene (56.2%) and (*E*)-γ-bisabolene (12.3%). The oil of *P. affinis* was characterized by large amount of sesquiterpenes (94.6%) and the monoterpene fraction of the oil was relatively small, representing only 4.8% of the total oil. In our previous investigation, the oil obtained from *P. aurea* was rich in also *trans*-α-bergamotene (72.8%) (*2*).

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P-67

Chemical composition of the essential oil from flowers, stems and leaves of *Astragalus schahrudensis* Bge. from Iran

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The genus *Astragalus* (Papilionaceae) contains about 800 species of perennial and annual in Iran, and most of them are endemic (*1*,*2*). Only one investigation has been carried out on the chemical composition of the essential oils of the genus *Astragalus*, and deals only with the roots of *A. membranaceus* Bge. (*3*).

The present analytical study is part of a program aimed at the enhancement and development of research activities on medicinal and aromatic plants in Iran, and determination a new chemical components. The aim of our study is to compare the yield and to identify the constituents of the essential oils of flower, stem and leaf of *A. schahrudensis Bge.* growing wild at flowering stage in North-East of Iran.

The yellowish colored oils were obtained by hydrodistillation, using a Clevenger-type apparatus for 3 hours, from flower, stem and leaf in 0.13%, 0.08% and 0.1% yield (w/w), respectively, and analyzed by GC and GC-MS. Seventeen compounds representing 96.6% of flower oil of *A. schahrudensis* were identified; among them germacrene D (47.6%) and germacrene B (17.8%) were the major ones. The stem oil of the plant was characterized by higher amount of β-selinene (29.4%), δ-guaiene (21.7%), α-guaiene (13.4%) and α-selinene(10.9%), among the fourteen detected components, comprising 94.6% of the total oil.

Eighteen compounds representing 97.3% of the leaf oil of the plant were identified. Among them, α-pinene (33.8%), bornyl acetate (14.2%), limonene (12.2%) and *endo*-fenchyl acetate(10.0%) were the major ones.

The flower and stem oils *A. schahrudensis* consisted mainly of sesquiterpenes, while in leaf oil of the plant monoterpenes predominated over sesquiterpenes.

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P-68

Enantiomeric ratios of selected chiral compounds in the essential oils from some *Achillea* species.

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The *Achillea* species are known to exist in polyploid forms. These forms differ from each other also in the composition of volatile oils. The enantiomers of camphor, borneol and α-pinene were analysed in nine *Achillea* species. The examined materials were collected in Poland during blooming. The herbs, previously air-dried, were distilled with water in a Deryng apparatus, according to the method of obtaining and measuring volatile oils in Polish Pharmacopoeia. Enantioselective gas chromatograph studies were performed using a Hewlett-Packard gas chromatograph and two commercial chiral columns – beta DEX 120 and gamma DEX 225. Large variations in the enantiomeric composition of investigated compounds were observed between samples. In samples of *A. grandifolia* and *A. millefolium* only (-) enantiomer of camphor was found while in *A.salicifolia* the (+)-camphor dominates. In all samples except *A. Salicifolia,* (-)-borneol is dominating enantiomer. (+)-α-pinene prevails in *A. salicifolia* sample while its optical antipode dominates in *A. pannonica* sample. Also *A. crithmofolia* in diploid and tetraploid forms were examined showing interesting quantitative differences in selected chiral compounds between these two species.

Chiral gas chromatography together with traditional GC analysis and chromosome data may give deeper information about plants and can help to establish relationships between them.

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Phytochemical evaluation of supercritical extracts obtained from *Curcuma domestica*

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Curcuma domestica Val. (syn. *C. longa* L.) is an Indian spice plant. The main ingredients of the rhizome are the essential oil (2.5-7.2%) ad the curcuminoid pigments (1.8-5.5%). Its cholagog, choleretic *(2),* and antihepatotoxic effects are known *(1)*, but its antihyperlipidaemic and anti-inflamatory *(2)* activities are also remarkable.

The first aim of our work was to study the volatile compounds of supercritical extracts (SFE fractions) and compare them with the composition of essential oil obtained by steam distillation.

The supercritical extraction was carried out in instrument ISCO2-10 using fluid carbon dioxide. The process was made at constant temperature (60°C), on various pressures (100, 125, 150, 200, 300, 400 bar) for 60 and 90 minutes respectively.

For identifying of volatile constituens we used a GC-MS method: Agilent 6890N GC with 30m x 0.25mm I.D. capillary column, HP-5MS stationary phase, (film thickness 0.25 μm); injector at 280°C; 5973N Mass Selective Detector. Temperature program: 60°C for 3 min., 8°Cmin^{-1} from 60 to 200°C, 200°C for 2 min., 10°C min^{-1} from 200 to 250°C, finally 15 min. at 250°C. The percentages of compounds were determined by GC-FID: Fisons 8000 gas chromatograph equipped with flame ionisation detector; 30 m x 0.25 mm I.D. capillary column with enantioselective Rt-β-DEXm stationary phase (film thickness 0.25μm); injector at 210°C, detector at 240°C; column temperature: 8°C min^{-1} from 60 to 230°C, then 230°C for 5 min.

We established that the qualitative composition of essential oil ad SFE fractions was not diverse: ar-curcumene, α-α''-dicumyl, *turmerone, *ar-turmerone, *curlone and atlantone were detected in the samples. The SFE-03 fraction (125 bar, 40°C, 60min.) was the richest in the three main sesquiterpene keton compounds* (20.4, 35.1, 22.6%). Curcuminoids were not present in SFE fractions, or in traces only.

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P-70

Essential oils of marjoram (*Origanum majorana* L.) and summer savory (*Satureja hortensis* L.) distilled in pilot plant scale

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Marjoram (*Origanum majorana* L.) and summer savory (*Satureja hortensis* L.) belong to the family of mint plants (Lamiaceae). Marjoram and savory are used as spices and condiments. Their essential oils are known to contain high amounts of components with antibacterial, sedative and antioxidative properties and are employed in food industry as flavouring and in perfumery for their spicy herbaceous notes. To determine application possibilities of essential oils in new market fields e.g. use in fodder for animals, their chemical compositions and the oil yields have to be examined by distillation in pilot plant scale.

As main components of the essential oil of marjoram monoterpenes with the basic structure of sabinene *cis*-sabinene hydrate, *trans*-sabinene hydrate, *cis*-sabinene hydrate acetate and sabinene were identified in literature. These components are known to be responsible for the typical odour and flavour of marjoram. Other components found in the oil are mainly artefacts. It should be noted, that in acidic solution sabinene hydrate rearranges to 1-terpinen-4-ol and small amounts of α-, γ-terpinene and *p*-cymene during the distillation process. Two chemotypes were postulated for marjoram in literature: the sabinene hydrate/1-terpinen-4-ol-type and the carvacrol/thymol-type.

The essential oil of summer savory contains mainly carvacrol, *p*-cymene and γ-terpinene. Few data are available in literature regarding the chemical composition of summer savory.

Essential oils of marjoram (sort "Marcelka", CZ) and of summer savory (sort "Aromata", D), both grown 2005 in one habitat in the southeast of Lower Austria, were analysed. The plant material was prepared for distillation in case of marjoram with or without stems, in case of summer savory wilted or dried. The essential oils were obtained using a hundred litres distillation plant of the type Herba-tec TWE 250-2000, which in average processes about 10 to 15 kilograms of fresh plant material per batch.

In the end of the distillation process samples were taken to investigate the relative amounts of main compounds of the different essential oils. Samples were subjected to gas chromatographic investigation (GC-MS and GC/FID). Their compositions were determined by comparing the relative retention times of standards and mass spectra from data library of oil components (NIST, WILEY). The results provide the possibility to determine the relative amounts of main compounds of essential oils of marjoram and summer savory distilled in pilot plant scale.

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Investigation of chemical composition of essential oils of five different genotypes of *Origanum vulgare* L. distilled in pilot plant scale over three years

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The quality of essential oils and by means of that their possible economic use in cosmetics, pharmaceuticals, functional food and feed strongly depends on the scale of distillation process used, climatic and weather conditions of the years examined and in addition genetic conditions. It is well known, that the distribution of chemical components differs appreciably between e.g. the Origanum species even within the same taxon. To ensure the use of essential oils in other economic fields than the well known fields of cosmetics and perfumery, their quality through their chemical composition and the oil yield have to be examined over many years by distillation in pilot plant scale.

Essential oils of five different genotypes of oregano, all grown 2004, 2005 and 2006 by organic farming in one habitat in the northeast of Styria (Austria), were analysed. The essential oils were obtained using a hundred litres distillation plant of the type Herba-tec TWE 250-2000, which in average processes about 10 to 15 kilograms of fresh plant material per batch and a ten litres distillation plant of the type UMWEX 100-1000 with a maximum of 1 kilogram per batch. Because of the small oil yield in the summer of 2004 only distillation with UMWEX was carried out. In 2005 and 2006 both distillation methods were used.

In the end of the distillation process samples were taken to investigate the relative amounts of main compounds of the different genotypes. Samples were subjected to gas chromatographic investigation (GC-MS and GC/FID). Their compositions were determined by comparing the relative retention times of standards and mass spectra from data library of oil components (NIST, WILEY).

The results provide the possibility to determine the genotype with the highest relative amount of the main compound(s) of the years examined and differences in the chemical composition between the two distillation methods. The oils were found to be rich in carvacrol and they contain minor amounts of the two monoterpene hydrocarbons γ-terpinene and *p*-cymene, the biosynthetic precursors of thymol and carvacrol (*1*).

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P-72

Chemical composition of the essential oil of *Achillea grandifolia* Friv. from flowering tops and leaves

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White yarrow (*Achillea grandifolia* Friv.) is a perennial, pubescent herbaceous plant which belongs to the *Asteraceae* family and grows spontaneously in south-eastern Europe, especially in Balkans, Bulgaria and Greece. Stems can be up to 1 m high, leaves are deeply pinnatified, 10-12 cm long and corymbs with many capitula are creamy-white. While essential oils from different species of yarrows have been widely investigated, in literature only few data were found about the chemical composition of the essential oils of *Achillea grandifolia* Friv. (*1*). Moreover, no data were found about the comparison of the chemical composition of essential oils extracted from flowering tops or from leaves and stalks.

Cultivated samples of *A. grandifolia* were collected in blooming state (in the same flowering conditions) on July 2004, in an experimental field in Wrocław (Poland). The specimens were identified by the Garden of Medicinal Plants Wrocław Medical University and deposited in the Department of Farmacognosy Wrocław Medical University. Samples were hydrodistilled with Clevenger-type (HD) and simultaneous micro distillation-extraction (SMDE) apparatus, with lighter (n-hexane, L-SMDE) and heavier (dichloromethane, H-SMDE) solvents than water. The yields were 0.35 % ± 0.03 and 0.12 % ± 0.02 (v/w, volume/dry weight) for the HD of flowers and leaves respectively. The essential oils were analyzed by GC/FID and GC-MS (*2*) and a total of 62 components were detected. The major compound both in flowering tops and stalk and leaves essential oil were 1,8-cineole, camphor, and borneol. 1,8-cineole and borneol amount were higher in stalk and leaves. No azulenic compounds were found. From a qualitative point of view, no relevant differences between HD and SMDE were observed, while statistically significant differences on quantitative relative percentage were noticed. No significant difference between L-SMDE and solvents H-SMDE has been observed.

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P- 73

Automating retention time updating for accurate and ongoing peak identification in complex flavour and fragrance chromatographic separations

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Qualitative and quantitative analysis with chromatographic systems relies on the ability to predict the time at which any given compound will elute from a column in order to confirm its identity. This is particularly important in the flavour and fragrance industry where complex mixtures containing large numbers of often closely eluting components are separated and compared to reference standards on an ongoing basis. As a GC column's condition changes, be it due to age or thermal damage, the eluting components retention times will change and this has to be managed in order to continue achieving reproducible results.

There are several approaches to continuously managing peak retention time changes that occur in routine chromatographic separations: one such instrument centric approach, the so-called Retention Time Locking method (*1*), relies on changes to the column carrier gas flow rate to be made after each analysis, such that the component retention times are brought back to their original preset values for the following run.

Another novel, and somewhat more universal approach, is termed Column Ageing. This technique is an actual reflection of the column/compound interaction and does not rely on the automated adjustment of carrier gas flow rate or any other physical constant on the actual instrument. Instead, this approach uses automatic updating of compound retention times within the analytical method after each run so that subsequent chromatograms are analysed using a peak identification table that reflects the most current expected retention times on that particular column at that time. Column Ageing also differs from the established Reference Peak identification technique in that retention time updates are calculated on an ongoing basis rather than for each injection based only on one or more identified reference peaks with preset retention time windows.

Column Ageing can therefore be used with any manufacturers GC as this technique does not rely on instrument specific software to constantly modify carrier gas flow rates and instead deals with updating retention time changes in real time on an ongoing basis. This reduces the requirement for operator intervention during post-run data processing. The current work describes the Galaxie chromatography data system Column Ageing Monitor feature that automates the retention time updating of an analytical GC method and presents example data showing how this technique can be successfully applied to improve peak identification in complex flavour and fragrance analyses.

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Chemical composition of esssential oils of three *Artemisia* species growing wild in Iran: *A. kermanensis*, *A. kopetdaghensis* and *A. haussknechtii*.

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The genus *Artemisia* is one of the largest and widely distributed genera of the family Asteraceae.Thirty-four species of this genus are found in Iran, among which two are endemic: *A. melanolepis* Boiss. and *A. kermanensis* Podl. The genus *Artemisia* has always been of great botanical and pharmaceutical interest, and used in the liqueur-making industry, in addition of considerable attention of the antimalarial activity of artemisinin that is present in the aerial parts of *A. anuua*. This plant is valued for its essential oil and sometimes used in fragrances, and in perfumery and cosmetic products.The large genus *Artemisia* has been studied chemically by many researchers and the presence of acetylenic compounds and terpenoids, especially sesquiterpene lactones were reported.

Although numerous reports appear in the literature on the volatile oil of different species of *Artemisia* no studies have been reported on those of *A. kermanensis* Podl., *A. kopetdaghensis* Krasch., M. Pop&Lincz. Ex Poljak. and *A. haussknechtii* Boiss. So we decided to examine these oils.

The water distilled essential oils from aerial parts of three *Artemisia* species: *A. kermanensis*, which is endemic to Iran, *A. kopetdaghensis* and *A. haussknechtii* has been analyzed by a combination of GC and GC-MS. The oil of *A. kermanensis* and *A. kopetdaghensis* were rich in davanone (21.4% and 47.9%, respectively). The main components of the oil of *A. kermanensis* were 1,8-cineole (16.0%) and chrysanthenone (14.8%), whereas geranial (6.5%) and ethyl nerolate (6.3%) were the other main components of *A. kopetdaghensis*.

In the oil of *A. haussknechtii*, 1,8-cineole (16.5%), camphor (14.5%) and artemisia ketone (10.5%) were found to be the major constituents.

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Chemical composition of the essential oil from aerial parts of *Stachys palustris* L. growing wild in Southern Italy

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Stachys. is a subcosmopolitan genus of herbs and shrubs that comprises more than 270 species (*1*) and is one of the largest genera of the Lamiaceae, distributed in temperate and tropical regions of the world with the exception of Australasia. The taxonomy of the genus is complicated as there is a wide range of variability among some species; however, the chemistry of volatile compounds has been proven particularly helpful in assessing taxonomic relationships of several genera in Labiatae (*2*). For this reason we have analyzed the essential oil of *Stachys palustris* L. *S. palustris* (common name in Italy *erba strega* or *scabbiosa*) is a common herbaceous creeping perennial of marshy ground, in flower from July to September, with rather pale purplish flowers in spikes, growing up to about 90 cm (*3*). *S. palustris* is considered a wholesome and nutritious food; the edible parts of the plant are leaves, roots and seeds. Tubers are consumed raw or cooked, and they have a pleasant mild nutty flavour. The tubers, harvested in autumn, can be dried and ground into a powder that is used in making bread. The young shoots can be used as an asparagus substitute, as they have a pleasant taste despite the disagreeable smell (*4*) In folk medicine *S. palustris* flowered aerial parts harvested in spring or summer, when just coming into flower, are known as antiseptic, antispasmodic, emetic, emmenagogue, expectorant, haemostatic, nervine, sedative, tonic, vulnerary. The plant has been highly valued for its wound-healing activity, being effective against both internal and external bleeding, and is also used in the treatment of gout, cramps and pains in the joints (*5*). The active principles of the plant are tannins, resins, flavonoids, heterocyclic alkaloids. For this study aerial parts of *S. palustris* were collected at the full flowering in Southern Italy in June 2005. The air-dried samples were crushed and then subjected to hydrodistillation for 3 hours using *n*-hexane as a solvent, according to the standard procedure described in the *European Pharmacopoeia*, to give oil in a yield of 0.21%. The oil was analyzed by GC and GC-MS. Totally 92 components were identified representing 93.6% of the total oil that contained as most abundant compounds carbonylic compounds with a prevalence of ketones and among them hexahydrofarnesylacetone (7.4%) predominated. Fatty acids and their esters, with a prevalence of hexadecanoic acid (6.8%), were the other abundant components of the oil. Among sesquiterpenes predominate (*E*)-caryophyllene (3.6%) and caryophyllene oxide (7.8%).

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Composition of the fruit essential oils of *Tordylium trachycarpum* (Boiss.) Al-Eisawi et Jury and *Tordylium hasselquistiae* DC. growing in Turkey

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The genus *Tordylium* L. *(Syn.: Hasselquistia* L., *Condylocarpus* Hoffm., *Ainsworthia* Boiss., *Synelcosciadium* Boiss.), is represented by 15 species in Turkey (*1*) (*2*). This is the first report on the oil compositions of *T. trachycarpum* (Boiss.) Al-Eisawi et Jury and *T. hasselquistiae* DC. We have previously studied the essential oil compositions of *T. apulum* L., *T. pustulosum* Boiss., *T. ketenoglui* H. Duman et A. Duran, *T. pestalozzae* Boiss. and *T. lanatum* (Boiss.) Boiss. (*3-5*).

Fruits of *T. trachycarpum* were collected on April, 2004 along Adana-Antakya highway. Fruits of *T. hasselquistiae* were collected on April, 2004 in Hatay province, on a way between Belen and Kici, in area enclosed by olive trees. The dried crushed fruits of *T. trachycarpum* and *T. hasselquistiae* were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil yields (v/w) on moisture free basis were 1.85% and 0.5%, respectively. The oils were analyzed using an Agilent 6890N Network GC System with 5973 Network Mass Selective Detector. An HP-Innowax FSC column (60 m x 0.25 mm *i.d.*, with 0.25 μm film thickness) was used for separation of components in the oils.

Fifty three compounds were characterized, representing 99.5 % of the oil and sixty one compounds were characterized, representing 98.8 % of the oil of *T. trachycarpum* and *T. hasselquistiae,* respectively. The main constituents were octyl octanoate (79.9 %), octanol (11.0 %) and octanoic acid (2.9 %) in *T. Trachycarpum,* and octyl hexanoate (72.7 %), octyl octanoate (12.7 %) and octanol (3.3 %) in the oil of *T. hasselquistiae.*

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Volatile constituents of endemic *Artemisia gorgonum* (Asteraceae) from Cape Verde Islands

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The Asteraceae is one of the largest species of plants, and more than 28000 substances have been identified in chemical studies on this family (*1*).

The genus *Artemisia*, usually represented by small herbs and shrubs, is one of the largest and most widely distributed genera of the Asteraceae (or Compositae) family. Members of this genus have botanical and pharmaceutical interest due to their characteristic scent or taste and are used in the liqueur-making industry (*2*). In food industry, the *A. annua* leaves are employed in the culture media formulation for *Aspergillum* growth, which is used in wine production. The essential oil of *A. annua* is present in some alcoholic beverages as flavoring agent, i.e.vermouth. In the cosmetic industry, the essential oil is utilized in the perfume and soap formulation due to its pleasant, refreshing, and slightly balsamic odor (*3*).

Numerous studies have been reported on the chemical analysis of the essential oil of *Artemisia* species, and on their biological activities. *Artemisia absinthium* has been used as an antipyretic, antiseptic, antihelmintic, tonic, and diuretic and for the treatment of stomachache in Turkish folk medicine. *Artemisia santonicum* has been used as an antihelmintic substance and in the treatment of diabetes (*2*). The essential oil of *A. annua* is used as a repellent, as a bactericide, and as an antioxidant (*3*).

Artemisia gorgonum has been used in Capverdian folk medicine to treat symptoms associated with fever and malaria. In this study we report on the first chemical study of *Artemisia gorgonum* by simultaneous extraction-distillation (SDE). The extract thus obtained was analyzed by GC-MS and the main compounds presented were camphor (46%), α-phellandrene (15%), linalyl valerate (11%), camphene (4%), geraniol (1%), piperitone (2%).

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Chemoenzymatic synthesis of chiral dimethylbicyclo[3.1.0]hexane derivatives with olfactory properties

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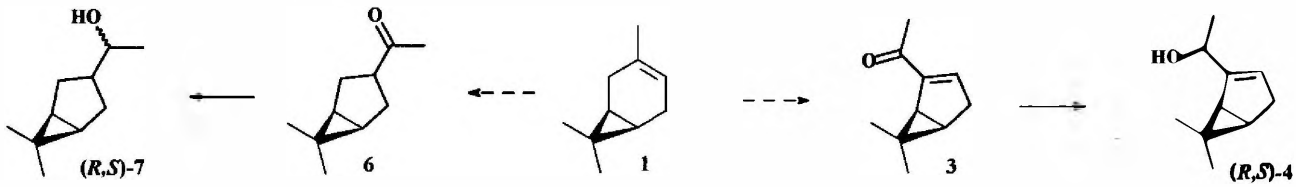
In search for the new chiral odorants we present here chemoenzymatic synthesis of bicyclo[3.1.0]hexane derivatives. The key-compounds are two secondary alcohols: 1-(6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)ethanol **4** (*1*) and 1-(6,6-dimethylbicyclo[3.1.0]hex-*trans*-3-yl)ethanol **7** (*2*), obtained in three step synthesis from monoterpene hydrocarbon (+)-3-carene **1**, inexpensive, readily available major constituent of turpentine from some species of pine (in Poland from *Pinus sylvestris* L.).

Ozonolysis of **1** followed by intermolecular aldol condensation of ketoaldehyde afforded bicyclic enon **3** (*3*), which was reduced with lithiumaluminium hydride to the alcohol **4**. Reaction of **1** with *N*-bromosuccinimide gave bromohydrine, which after treating with silver oxide transformed to the bicyclic acetyl derivative **6** (*4*). Reduction of ketone **6** with $LiAlH_4$ led to the desired alcohol **7**.



Both alcohols (*R,S*)-**4** and (*R,S*)-**7** were subjected to the biocatalytic transesterification using lipases from *Pseudomonas sp.*, *Aspargillus sp.* and *Candida sp.* genus. After screening, appropiate genus of lipase was selected and influence of solvent and temperature was investigated. In both cases bioconversion to the one form of acetate was observed followed by separation of pure diastereoisomers.

Odour characteristic of newly obtained diastereoisomers, synthetic and biosynthetic details of the applied procedure will be presented with emphasis on odour – structure relationship

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Comparative analysis of the volatile constituents of in vitro and ex vitro plants of *Petiveria alliacea* L.

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Petiveria alliacea L. (Phytolaccaceae) is an herbaceous perennial herb that reaches up to 1.5m height and is characterized by erect branches, alternating leaves and very small white flowers. It occurs in tropical and sub-tropical regions, and is commonly found in Brazil in the Amazon region. Although the species is traditionally used in popular medicine due to several pharmacological properties, the continuous use of the root powder brings about neurological effects such as super-excitation, insomnia and hallucinations, followed by convulsions, paralysis and death. Previous phytochemical investigations have reported the presence of sulphur containing substances on the flowers of this plant, which are responsible for the characteristic garlic aroma of the species. In the present work tissue culture protocol for *P. alliacea* was established for comparison of the phytochemical profile between *in vitro* and *ex vitro* plants. *In vitro* propagation was achieved through the culture of nodal segments on MS medium. Rooting of shoots induced in half strength MS supplemented with IAA 0,6 μM. Rooted shoots were transferred to green house and morphological abnormalities were not observed in the plants. The volatile oils were obtained by Simultaneous Distillation and Extraction (SDE) from *ex vitro* comminuted fresh leaves and roots and from fresh leaves and roots of the *in vitro* cultivated plantlets, for four hours. The solvent used was dichloromethane and for GC quantification 0,32μl of tridecane (0, 24μg) were added as internal standard. Analyses were performed in a HP 5890 gas chromagraph equipped with a FID detector and a HP5 fused silica capillary column (30m X 0.25mm X 0.25μm), using H_2 (1.0mL/min) as carrier gas. The injector temperature was kept at 250°C and the oven temperature was programmed from 60 to 240°C at 3°C/min. Pure oils (0.03μL) were injected in split mode (100:1). The GC-MS analyses were recorded using an Agilent 5973N GC-MS system, using the same column and chromatographic conditions, but helium as the carrier gas. The constituents of the oils were identified by comparing their mass spectra with those in a spectral database (Wiley 6th ed) and by their retention indices (RI). A standard solution of n-alkanes (C_7-C_{26}) was used to obtain the retention indices.

Forty two different compounds were identified in the essential oils of *in vitro* and *ex vitro* structures of *P. alliaceae,* many of them were present only in trace amounts (below 0.1%). The comparative analysis of the essential oils from both leaves and roots showed differences between them. However, benzaldehyde and a series of unsaturated long chain fatty acid methyl esters were present in all analyzed samples. Also, sulphur containing substances such as bis-phenylmethyl)-disulphide, isothiazol (1,2-thiazol), 2-thiopropane, dimethyl sulphide, ethylene disulphide and 2,3-dimetyltiirane were identified in both leaves and roots of *in vitro* and *ex vitro* plants. This is in accordance with the fact that not only the flowers of this plant smell like garlic, but also all the plant parts do. Sesquiterpenes were found mainly as trace components of *ex vitro* roots essential oil.

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Identification of the odour components of *C. aurantifolia* Swingle and *C. limonia* Osbeck (lime) cold-pressed peel oils growing in Vietnam

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Lime is one of the principal sour citrus fruits in the world, and the two varieties, Mexican or Key lime (*Citrus aurantifolia* Swingle), and Tahiti or Persian lime (*Citrus latifolia* Tanaka), are well known. In Vietnam, lime is one of the major commercial fruits and traditionally cultivated and harvested year round, throughout the country. This fruit is popularly used in daily meal, as a folk medicine, deodourant, and in food and beverages industry. Widely available lime fruits in the market are *C. aurantifolia* Swingle and *C. limonia* Obsbeck (or "Rangpur" lime). Although lime essential oils have been studied worldwide, there are few reports on the chemical composition of lime essential oils in Vietnam (*1*), (*2*).

GC and GC-MS are used to detect and identify the volatile components of two Vietnamese lime cold-pressed peel oils, cultivated in Bentre and Dalat provinces in Vietnam. The chemical composition of lime oil contains mainly the terpenes (monoterpenes and sesquiterpenes), aldehydes, alcohols, ketones, acids and esters. As in other citrus oils, monoterpenes are major compounds in these lime oils, including limonene, α-pinene, β-pinene, myrcene and γ-terpinene, accounting for more than 69.6% in *C. aurantifolia* Swingle and 88.1% in *C. limonia* Osbeck. γ-Terpinene, which has been mentioned as important compound and its quantity may contribute to the aroma of cold-pressed lime oil (*3*), is detected in these Vietnamese lime oils in high proportion. The content of neral and geranial in lime oils from Bentre are much higher than those from Dalat. A comparison of the volatile constituents between the two varieties will be presented. This study is partly contributing to the future development of Vietnamese citrus industry.

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P-81

Essential oil composition of leaf and peel of *Citrus maxima* from Iran

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Water-distilled essential oil of leaves and the cold-press oil of peel of *Citrus maxima* were analyzed by means of GC-MS. Identification of the constituents was based on comparison of their mass spectra and retention indices with those obtained from authentic samples and Wiley library spectra. The quantification of the components was performed by GC-FID by internam normalization. Fifteen components representing 99.7% of the oil of leaves were characterized with β-pinene (RI= 979, 2.5%), geranial (RI= 1267, 4.4%), limonene (RI=1029, 13.3%) and neral (RI=1238, 2.0%) as the major constituents. Other components were : α-pinene (1.6%), myrcene (1.5%), (*E*)-β-ocimene (1.3%), γ-terpinene (1.6%), citronellal (2.2%), linalyl formate (7.3%), linalyl acetate (0.6%), *iso*-3-thujyl acetate (5.4%), *neo*-3-thujyl acetate (5.3%), (*E*)-β-caryophyllene (3.6%), β-bisabolene (1.3%).The oil of peel of *Citrus maxima* consisted of eighteen components representing 98.5% of the oil. Limonene (RI=1029, 57.7%), γ-terpinene (RI=1060, 11.7%), β-pinene (RI=979, 10.7%) and myrcene (RI= 991, 3.3%) were found as the main constituents in the peel oil. Other identified constituents in this oil were : α-thujene (0.6%), α-pinene (2.7%), (*E*)-β-ocimene (0.2%), terpinolene (1.1%), α- terpineol (0.6%), linalyl formate (0.6%), neral (1.4%), linalyl acetate (0.8%), geranial (1.9%), *iso*-3-thujyl acetate (1.8%), *neo*-3-thujyl acetate (0.6%), (*E*)-β-caryophyllene (0.5%), α-farnesene (0.9%) and β-bisabolene (1.4%). Oils of *Citrus maxima* from other areas in the world had been studied previously. Limonene (94%) was the main component in the shaddock oil from Malaysia (*1*). The oil of shaddock peel from China contains terpenic compounds (93.9%) (*2*). Limonene, terpinolene were found as the main constituents in the Vietnamese shaddock (*3*) and limonene (93.2%) was the major one in the peel oil of Indian shaddock (*4*). In contrast with the other oils, the Iranian oil contains β-pinene and geranial as the main constituents. This is the first report on the analysis of the essential oil of peel and leaves of *Citrus maxima* from Iran.

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P-82

Composition of the essential oil of three *Nepeta* species from Iran

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The genus Nepeta (Lamiaceae) includes aromatic and medicinal plants comprising about 280 species in the world. Many of them are used in folk medicine as a fortifier, disinfectant, bacteriostatic as well as against eczema-type disorders. *N. kotschyi* Boiss., *N. oxyodonta* Boiss. and *N. glomerulosa* Boiss. were collected from Fars province at the time of flowering. The aerial parts were air-dried at ambient temperature in the shade and hydrodistilled by using a Clevenger-type apparatus for 4 hours. The essential oils were analyzed by GC and GC-MS, and their constituents were identified by comparison of retention indices with those reported in the literature and by comparison of their mass spectra with the Wiley library or with the published mass spectra. The main components of the oil of *N. kotschyi* were 4*aα*, 7*α*, 7*aα*-nepetalactone (31.6%), 2,3,4,5-tetramethyl-1,4-hexadiene (10.0%), (*E*)-*β*-farnesene (8.8%) and caryophyllene oxide (5.6%). The main compounds of the oil of *N. glomerulosa* were caryophyllene oxide and 1,8-cineol (17.8%). Caryophyllene oxide was the major compound of the oil of *N. oxyodonta*.

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Composition of the essential oil of three endemic *Stachys* species from Iran

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Stachys is one of the important genuses in Lamiaceae family. It is represented by thirty-one species in the flora of Iran which eighteen of them are endemic species (*1*). Plants of this genus have been reported to be used to treat genital tumors, inflammatory tumors and cancerous ulcers in folk medicine (*2*). Methanol extract of tuber of *S. sieboldii* presented anti-anoxia action in mice and hydroalcoholic extract of aerial parts of *S. inflata* showed potent anti-inflammatory activity in rats. *Stachys acerosa* Boiss., *Stachys aucheri* Benth. and *Stachys benthamiana* Boiss. are three endemic species of Iran. Aerial parts of these plants were collected from Fars provenance at the time of flowering. The aerial parts were air-dried at ambient temperature in the shade and hydrodistilled by using a Clevenger-type apparatus for 4 hours. The essential oils were analyzed by GC and GC-MS, and their constituents were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley library or with the published mass spectra (*3*). The main components of the oil of *S. acerosa* were caryophyllene oxide, intermedeol, 7-*epi*-α-selinene and linalool. The oil of *S. aucheri* consists mainly of viridiflorol, *cis*-chrysanthenyl acetate and spathulenol. Caryophyllene oxide, 1-octen-3-ol, linalool and hexadecanoic acid were the major compounds of the oil of *S. benthamiana*.

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P-84

Essential oils of ornamental oregano cultivars growing in Czech Republic

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Origanum vulgare L. (oregano) is perennial species, family *Lamiacea*. The morphological variation within the genus result in the distinction of 10 sections consisting of 42 species or 49 taxa (species, subspecies and varieties). Several species in this complexed taxonomy genus are used as spices around the Mediterranean basin.

Phytopharmaceutical products are based on the flowering tops of oregano and designed for oral use. Topically they are traditionally used: 1. as an adjunct in the emollient and atipiruriginous treatment of skin disorders, as a trophic protective agent for cracks, bruises, frostbite, and insect bites; 2. as antalgic lozenges and collutoria for diseases of the oral cavity, pharynx, or both; 3. to relieve nasal congestion in the common cold.

Origanum vulgare L. is extremely variabile in appearance and in chemical composition. It produces an essential oil generally rich in thymol, or carvacrol, or both (*1*), linalol and 1-terpinen-4-ol (*2*). Oregano is important ornamental plant, e.g. cultivars 'Album', 'Aureum', 'Compactum', 'Gold Splash', 'Thumbles Variety' and 'Variegated'. Origanum essential oils are characterized by a number of main components which are implicated in the various plant odours.

The six cultivars (72 plants) where evaluated from the aesthetic point of view and the volume of essential oils. Average amount of essential oils content is 3.15 ml.1000 g^{-1} of dried plant material (*3*). Essential oils obtained by steam distillation (*4*) were analysed by gas chromatography-mass spectrometry (GC-MS) HP-5MS (5% Phenylmethylsiloxane, 30 m x 0.25 mm i.d., film thickness 0.25 μm). The following constituents were identified in the essential oil: α-terpinene, *p*-cymene, ocimene, γ-terpinene, (±)-linalool, (-)-1-terpinen-4-ol, carvacrol methylether, thymol and carvacrol.

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Supercritical extraction of biological compounds from Italian coriander seeds

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Coriander is an annual Apiaceae herb widely applied in food industry to flavour several commercial foods as liqueurs, teas, meat products and pickles. On the other hand, their seeds are also used in pharmaceutical industry as a drug against gastrointestinal problems, rheumatism and pain in the joints (*1, 2*).

Supercritical fluid extraction (SFE) of the volatile oil from Italian coriander seeds (L'Ortolano, Cesena, Italy) was carried out using different conditions of pressure (90, 100 and 150 bar), temperature (40 and 50°C), mean particle size (0.4, 0.6 and 0.8 mm) and CO_2 flow rate (6, 6.5, 7, 8, 10 and 14 L/min) in order to assess the influence of these parameters on the volatile oil composition and yield. The best SFE conditions were found to be 90 bar, 40 °C, 10L/min and 0.6 mm. Hydrodistillation (HD) was also performed in a Clevenger apparatus, using seeds with the same mean particle size.

The volatile oils were analysed by GC and GC-MS, being linalool (66.5-78.8%), γ-terpinene (4.0-7.2%), α-pinene (1.0-4.0%), camphor (2.9-3.4%), limonene (1.4-3.4%), myrcene (0.5-3.3%), geranyl acetate (0.9-3.5%) and geraniol (0.9-3.1%) their main components. The comparision with the essential oil (HD) showed a decrease in the monoterpene hydrocarbon fraction and an increase of the oxygen containing monoterpene content in SFE volatile oil.

For each SFE experimental run, samples were collected during the extraction to study the change of the composition with the time. The profile of the relative percentage of the components was different according to the extraction conditions used.

Furthermore, the antioxidant activity of the extract, obtained using the best supercritical conditions, is discussed. On the other hand, the effect of increasing the pressure up to 250 bar on this activity is also analysed.

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Isolation of 2-ethenyl-3-methyl-phenol and its derivatives occurring as natural products in *Juniperus* of the Southwestern United States and Northern Mexico

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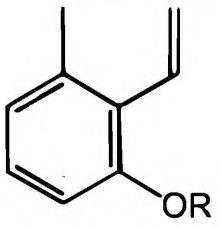
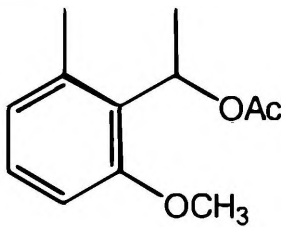
The composition of the leaf essential oils of the one seeded, serrate leaf *junipers* of the Southwestern United States and Northern Mexico was first reported in 1981 (*1*). A re-examination of the leaf essential oils of these *junipers* has yielded 2-ethenyl-3-methyl-phenol **1**, 2-ethenyl-3-methyl-anisole **2**, and 2-(1'-acetoxyethyl)-3-methyl-anisole **3**.



1 : R = H
2 : R = CH_3



3

The structural relationship of **1** to **2** was confirmed by its methylation to **2**. Although both have been previously reported as synthetic products, the current study indicates **1** and **2** along with **3** to be the first examples of their occurrence as natural products. The reported ^{1}H and ^{13}C NMR data for **1** are consistent with the structure (*2*). However, the report lacks specific chemical shift assignments. The reported ^{1}H NMR data for **2** do not correspond with its structure (*3*). To rectify these deficiencies and to establish the structure of **3**, extensive NMR spectral data including ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and NOE were acquired. The results of the analysis of NMR data in support of the structures of **1**, **2**, and **3** will be presented along with a survey of the occurrence of these compounds in the *junipers*.

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P-87

New organonitrogen and organosulphur compounds in watercress species

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In order to answer the constant request of consumers for new odor and taste, flavor and fragrance companies are continually in the search of new molecules. Natural extracts such as essential oils, concretes, absolutes are an inexhaustible source of these molecules.

To identify such molecules, one our main objectives is the study of natural matrices. Continuous progress in the development of analytical techniques, in particular GC-MS and GC/Olfactometry (GC/O), allow the diminution of detection thresholds and the identification of compounds showing a low perception threshold and thus an considerable olfactory impact.

We particularly focus on organonitrogen and organosulphur compounds because these molecules are known for their frequent low perception thresholds. Their presence in a matrix, even if in sub-ppb quantities, can account for a significant effect on the organoleptic properties of the matrix (*1-3*).

The chosen matrices are two solvent extracts (concrete and absolute) obtained from two different cress species. Both species belong to the *Brassicae* order and produce glucosinolates which are one of the main precursors of sulfur- and nitrogen-containing molecules in plants: watercress (*Nasturtium officinale* R.BR.) and Indian cress (*Tropaeolum majus* L.) (*4*).

The extracts were studied by analytical and sensorial means (GC-MS, GC-FPD and GC/O) to identify odorant compounds. Compounds having an olfactory impact and their homologues were subsequently synthesized and submitted to static and dynamic organoleptic tests.

We have thus discovered a new class of odorant molecules containing both nitrogen and sulfur heteroatoms that has never been used in flavors and fragrances before. Laboratory synthesis, natural origin, organoleptic properties and possible applications will be presented.

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P-88

Essential oil from leaf-buds of service tree, *Sorbus domestica* L.

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Service tree (*Sorbus domestica* L., Rosaceae family) is a rare tree of gardens and forests. The fruit of service tree is nutritive and contains biological active compounds *(1,2)*. In early spring leaf-buds of service tree are large, sticky and have characteristic fragrance. This material contains many waxes and semi volatile compounds which can diminish the rapidity of essential oil distillation and its yield.

Buds were collected in early spring (March). Essential oil was isolated from fresh leaf-buds by simultaneous superheated steam distillation-solvent extraction (modified Likens Nickerson method, home made apparatus) for 3 h. Superheated steam (*ca.*105°C) was generated by heating 20% NaCl aqueous solution to boiling *(3)*. The yield was 0.03% w/w. Isolated oil was analysed by using GC and GC-MS. The essential oil mainly contains phenylpropane derivatives and related compounds (benzaldehyde, benzyl alcohol, 2-phenylethanol, 4-hydroxy-3-methylacetophenone), sesquiterpene compounds (α-longipinene, longicyclene, longifolene, longiborneol) and aliphatic compounds (6-methyl-3,5-heptadiene-2-one, (*E*)-2-decenal, 2,4-decadienal, octadecenal, pentanoic, hexanoic, heptanoic and octanoic acids). Benzaldehyde is a characteristic compound found in many species of the *Rosaceae* family in form of glycoconjugated compounds such as amygdalin, prunasin.

The main compounds of the oil were benzaldehyde (56%), longicyclene (11.5%), α-longipinene (4.8%), 6-methyl-3,5-heptadiene-2-one (3.6%), longifolene (2.4%), pentanoic acid (1.9%), longiborneol (1.7%). Hexanoic, heptanoic and octanoic acids, benzyl alcohol, 2-phenylethanol, (*E*)-2-decenal, 2,4-decadienal, octadecenal, 4-methyl-5,6-dihydro-2*H*-pyran-2-on, 2,3-dihydrobenzofuran, 4-hydroxy-3-methylacetophenone and other compounds that were identified in smaller amount. Leaf-buds essential oil of service tree has not been yet investigated.

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P-89

Enzymatic glucosylation in the synthesis of natural glucosides of volatile compounds

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Alkyl(C_4–C_9)-glycosides, glycoconjugates of phenylpropane derivatives and terpenes belong to naturally occurring glycosides of volatile compounds. They are present in aromatic plants (*1*) mostly in the form of *β-O*-glycosides. They can be considered as flavour precursors, since their enzymatic/acidic hydrolysis or pyrolysis liberates volatiles. These glycosides are involved in the flower fragrance formation, in the release of aroma compounds of fruits or spicy materials and in the aroma formation of tea and vine. It was demonstrated that glycosidically bound volatiles are gradually changed to fragrant materials by skin microflora (*2*), suggesting that they might be useful as fragrant materials with alternative lasting effects.

As a part of Croatian grant No. 0011010, *β*-D-glucopyranosides of pentan-1-ol, (±)-pentan-2-ol, hexan-1-ol, octan-1-ol, benzyl alcohol, 2-phenylethanol, (±)-2-phenyl-propan-1-ol, 3-phenyl-propan-1-ol, geraniol and nerol were synthesized by reverse hydrolysis of the respective alcohols using almond *β*-glucosidase. These alcohols were also used in transglucosylation reaction with the same enzyme starting from cellobiose. The reactions were carried out in acetonitrile with aqueous acetate buffer (vol. ratio 9:1). Exclusively *β*-anomers were formed, and the obtained yields for both methods were comparable.

The methodology of monitoring the reaction course by GC-MS (after product acetylation) was developed on the model reaction of enzymatic condensation with 1-pentanol and (±)-pentan-2-ol. The products were characterized by GC-MS analysis of prepared tetraacetyl glucosides. Fragment ion characteristics of the aglucone moiety are present in all mass spectra, along with the fragments obtained from acetylated glucose, similar as in other papers (*3*) (*4*). Acetylated glucosides are separable on HP-101 column (even diastereomeric tetracetyl *β*-glucosides of enantiomeric alcohols). *β*-Glucosidase did not favour exclusively enantioselection, since both alcohols of the racemic mixture were glucosylated. The results of this work could be useful for preparative purposes as well as for analytical determination of these glucosides in various plants.

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P-90

Essential oil production by hairy root cultures: the pros and cons of an *in vitro* technology

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In vitro essential oil production is only known for a limited number of plant cell cultures. In most cases, these cultures were unable to produce the same compounds as found in the essential oil from the *in vivo* plant, or the production was rather low. As an example, the yield of the essential oils from cell suspension cultures of *Cryptomeria japonica*, *Pimpinella anisum*, *Foeniculum vulgare*, *Coleonema album*, *Artemisia dracunculus* and *Achillea millefolium* were *ca.* 100 to 1000 times less than those obtained *in vivo* (*1*). In general, the composition of the oils from these undifferentiated cultures was quite different from that of the parent plant oils, and in some cases the production of quite unusual products was achieved. Several procedures have been tested in attempts to surmount cell suspension culture problems, and hairy root cultures appeared to be useful to increase the essential oil production by plant cell cultures. Hairy roots are autonomous roots obtained by transformation with *Agrobacterium rhizogenes*. This phenomenon is due to the transfer and expression of the T-DNA from the bacterial Ri-plasmid into the nucleus of the plant. Transformed roots are fast growing, sometimes 10 times faster than the cell suspension cultures; they are laterally highly branched and show a massive biomass increase over relatively short periods of time, in the absence of exogenous phytohormones, because the Ri T-DNA regulates the balance of endogenous hormones. Our study has focused mainly on the essential oil production by hairy roots of different species, e.g. such cultures of *Achillea millefolium* (Asteraceae) maintained during 6 years and those of *Pimpinella anisum*, *Anethum graveolens* and *Levisticum officinale* (Apiaceae) maintained already for 10, 9 and 6 years, respectively, with a fortnight or a three-weeks interval subculture (*1*). These hairy roots have been evaluated for growth, either in batch or bioreactor systems, for essential oil production under control and stress conditions, for interrelationships with biotic and abiotic factors, andas for the production of regenerants.

Hairy roots growth was measured by the dissimilation method and by fresh and dry weight determination. In all experiments, the essential oils from the hairy roots and from the parent plant roots were analysed by GC and GC-MS. The yarrow, anise, dill and lovage hairy roots have shown a high biosynthetic stability. The essential oil yield obtained with the transgenic roots showed major differences compared to that obtained with the corresponding cell suspension cultures and was, in some cases, equal to or higher than that obtained with the parent plant. This capacity was strictly correlated with the differentiated state of the cultures, the level of production being severely impaired or lost when the hairy root phenotype was lost. Other factors, *e.g.* the type and/or age of the inoculums, the gap between subcultures, the combinations of nutritional and environmental stress factors, including different medium composition, photoperiod conditions, cultivation in a two-phase system and bio-transformation, also affected biomass growth and essential oil production. The successful obtention of hairy roots together with the production of an essential oil with a more or less different profile from that of the roots of the parent plant highlights the potential for essential oil production by this technology and widens the knowledge needed to manipulate, in a controlled way, their biochemical capabilities.

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P-91

Biotransformation of β-pinene, myrtenol, nopol and nopol benzyl ether by *Aspergillus niger* TBUYN-2

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In the continuing study on microbial biotransformation of terpenoids (*1*,*2*), the biotransformation of (-)- and (+)-β-pinene (**1** and **2**), (-)-myrtenol **3**, (-)-nopol **4** and (-)-nopol benzyl ether **5** by *Aspergillus niger* TBUYN-2 was carried out . *A. niger* was cultivated rotatory (100 rpm) at 30°C for 3 days in the 500ml Erlenmyer flask containing 200ml of Czapek medium (*p*H 7.0). After the full growth of microorganisms, each 100mg of substrate was added to the culture broth and biotransformed for 7 days under the same condition. After filtration of the cultured broth the aqueous layer was extracted with ether. The ether extract was applied to silicagel CC and the metabolites were isolated. The stereostructue of metabolites were established by means of X-ray crystal analysis and spectroscopic techniques (MS, IR and NMR). Compounds **1** and **2** were transformed via (-)-**6** and (+)-α-terpineol to the corresponding (-)-**8** and (+)-oleuropeyl alcohol by *A. niger*. Compounds **3** and **4** were biotransformed with the cleavage of the 4-membered ring to give **8** and (-)-7-hydroxymethyl-*p*-menthen-8-ol **9**, respectively. However, in case of **5**, the 4 membered ring was not cleaved. Compound **5** was hydroxylated at 3 positions to give 4-oxonopol-2',4'-dihydroxybenzyl ether **9** as the main product and its hydroxylated compound, 4-oxonopol **10**. In the case of **5**, hydroxylation was carried out 3 positions to give **9**. Compound **9** showed a strong antioxidant activity. Based on the above results, we proposed the new metabolic pathway for the formation of **8** by the cleavage of the 4 membered ring of **3**. The cleavage reaction of 4 membered ring is found in cases of **3** and **4**, which commonly have a C=C double bond and a 4-membered ring.

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P-92

Headspace Solid Phase Microextraction (HS-SPME), Headspace Solid Phase Dynamic Extraction (HS-SPDE) and Headspace Sorptive Extraction (HSSE) applied to the analysis of the volatile fraction and of aroma active components in herbs

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In the context of a research project in which the aroma profile of spicy plants is investigated, the suitability of headspace solid phase dynamic extraction (HS-SPDE), headspace solid phase microextraction (HS-SPME) and headspace sorptive extraction (HSSE) as easy to handle preparation techniques – that accumulates aroma components on a specific layer in a syringe (SPDE), on a fiber (SPME) and on a magnetic stir bar (HSSE) – should be determined.

The main difference of this sampling method is the amount of PDMS polymer. The volume of PDMS coated on the SPDE needle wall is about 4.5 µL in comparison to about 0.6 µL coating on a 100 µm SPME fiber. The coated bars have a volume of PDMS from 126 µL.

For the analysis predried plant samples of Oregano (*Origanum vulgare* ssp.*hirtum*) and basil (*Ocimum basilicum* var. *basilicum*) were obtained from Dr. Junghanns GmbH, Groß Schierstedt, Germany. Five different samples of oregano with essential oil content between 2.95% and 3.2% and five different samples of basil with essential oil content between 0.55% and 0.6% were analysed.

The methods for HS-SPDE, HS-SPME and HSSE are precise with variation coefficients in the range 0.69% - 1.29% (HS-SPDE), 0.83% - 2.95% (HS-SPME) and 1.62% - 2.68% (HSSE). The related conditions were used for herb sample analyses with recoveries between 73.18% - 99.37% (HS-SPDE), 61.45% - 89.77% (HS-SPME) and 58.85% - 83.28% (HSSE).

This lecture reports the comparison of all three techniques to analyse the volatile aroma active components in herbs. Because of the different surface area and film thickness of PDMS the appropriability to develop profiles of volatiles of basil and organo accessions has to be verified.

It could be shown that HS-SPDE and HSSE have higher detection limits for minor components. Up to 37 compounds could be verified with the HSSE technique for basil-samples and 33 compounds for oregano samples - but only 25 (basil samples) and 15 (oregano samples) compounds were detected with the HS-SPME technique. This matter of fact is especially important for establishing aroma profiles due to the sensory affectivity of secondary components.

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A non-equilibrium Solid-Phase Micro-Extraction application for analysing chemical variation of oil glands on leaves of African *Vitex* L. from herbarium specimens

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Vitex L. (Lamiaceae) is a taxonomically complex genus comprising approximately 250 tropical, subtropical and a few temperate taxa. In Africa the genus is represented by c. 87 species included in two subgenera, *Vitex* and *Homskioldiopsis*. Grex *pilosae* is a group of 26 species, 10 of them with oil glands (*V. amboniensis*, *V. angolensis*, *V. bogalensis*, *V. chrysocarpa*, *V. cuspidata*, *V. ferruginea*, *V. marquesii*, *V. mombassae*, *V. payos*, *V. welwitschii*).

In the present work we used the non-equilibrium solid phase micro-extraction (SPME) method in herbarium specimens to obtain the essential oils and to explore the chemotaxonomic value of their compounds.

The SPME was tested for the rapid extraction of essential oil from single oil glands of *Vitex* species. The content of 4-6 oil glands was sampled with a polydimethylsiloxane-coated fused silica fibre and directly injected in the GC-FID. The oil glands used were collected from the middle portion of the first expanded leaves in flowering and fruiting stages. Eighty-five components were detected by Fast-GC and GC-MS. The compounds of the essential oils of the ten species analysed are mainly sesquiterpenes, with prevalence on sesquiterpene hydrocarbons, such as α-copaene, β-caryophyllene, γ-muurolene, germacrene-D and γ-cadinene.

P-94

Fast GCMS-analysis of essential oils using narrow bore columns

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In the past fast GC and GCMS using narrow bore columns have become a powerful tool to increase analysis efficiency in different fields (*1-4*). This aproach reduces analysis time drastically by mainly maintaining the resolution (*3,4*). Columns with reduced inner diameter and phase ratios of 250 or larger have minimum values for the height equivalent of a theoretical plate (HETPmin) which aproach the inner diameter of the columns. By using these columns the instrument hardware has to fulfill some needs. To run the columns at optimum separation efficiency for different temperatures the GC part should be able to maintain the mean linear velocity of the carrier gas. Other parameters like pressure range linear temperature ramp and rapid cooling contribute also to the efficiency of the system. Regarding the detector part the system must be able to follow sharp increases of signals as the peak widths at half height (FWHM) in fast GC with narrow bore columns of 0.1 mm inner diameter are expected to be about or even below 0.5 s (*5*). For a quadrupole GCMS system this means it should provide both a high number of scans per seconds to ensure reliable quantitative work and a high scannig speed in order to have a good quality spectrum.

The approach was adapted in this work to rather high concentrated essential oils (10 %). The columns used for the fast essential oil analysis was a 5% phenyl phase with 10 m , 0.1 mm , 0.1 µm and a Wax phase with 10m, 0.1mm, 0.2 µm. The essential oils were diluted in ethanol and then injected into the split/splitlesss injector. As narrow bore column have considerably lower sample capacity compared to standard columns the split ratio had to be increased to between 500 and 800:1 which is about 6 times higher than compared to the standard method. The essential oils of lavender and and geranium show TIC data with all peaks resolved which were observed in the standard method. The analysis time was about 5 minutes where temperature ramp rates of 50 °C/min were used with a mean linear velocity of the carrier gas of 80 cm/s. As the peak widths is also about 6 times smaller compared to standard GCMS (30 m, 0.25 mm, 0.25µm) the peak heights correspondingly are larger resulting in no loss of sensitivity for small peaks in the fast method despite the larger split ratio used. The speed gain compared to standard GCMS is about a factor of 7 with virtually no loss of resolution. The peak widths (FWHM) were about 0.6 s and every 0.04 s a scan was taken which corresponds to 25 spectra per second acquisition rate. The mass range selected was 40 to 385 amu resulting in a scanning speed of 10000 amu/s which is possible due to a rather small interscan delay time of about 6 ms. The spectrum quality was very high and there was no difference in similarity indices observed when compared to standard analysis (up to 98% similarity with the wiley library).

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P-95

Automated difficult matrix introduction (DMI) for screening of washing powder with GC-MS/sniffing

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Complex matrices are encountered in numerous application areas of gas chromatography. Difficult matrices occur for example in environmental analysis, food characterisation or in the analysis of home and personal care products. For such complex and difficult matrices the technique DMI (difficult matrix introduction) is a powerful analytical tool.

In the DMI technique a small aliquot of the sample is put into a small glass vial which is automatically inserted into the injector. The injector is then heated to a temperature just high enough to transfer the compounds of interest from the sample onto the chromatographic column. Only the vaporized compounds are transported from the injector onto the GC-column where they are refocused at the low starting temperature of the GC program. Because the non-volatile matrix species remain in the vial and the injector liner, the GC-system no column contamination occurs.

One of the advantages of the DMI method is that only minute sample quantities are required. For inhomogeneous samples such as washing powders, however, this could also be a potential drawback. To determine the reproducibility of DMI analysis for such samples a washing powder was analysed multiple times.

In the present contribution the DMI technique is applied for screening of washing powders. Also in this study it was necessary to identify the fragrances in perfumed cosmetic products and this is done just in a single run during the normal screening because the DMI-GC-MS is also coupled to a sniffing port ('PHASER').

P-96

New approaches for the management of tomato spotted wilt on tomatoes with plant essential oils and particle films

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The thrips-vectored *Tomato spotted wilt virus* is a limiting factor in tomato production in the southern USA. Because insecticides do not effectively control primary infection by thrips immigrating into crop fields, we are investigating alternatives that would be effective and environmentally non-disruptive. We conducted a field trial to determine the effects of three plant essential oils, geranium, lemongrass and tea tree, as natural plant derived chemical repellent to thrips, and kaolin based particle films on the incidence of tomato spotted wilt and population dynamics of thrips. Plant essential oils were applied at 250 ppm twice per week and were compared with a grower standard insecticide treatment (Spintor rotated weekly with Baythroid, and Endosulfan) and a control. All treatments were applied with and without kaolin (25 lbs/acre/week), in a 5x2 factorial design. When combined with kaolin, the three plant essential oils controlled adult thrips and the incidence of tomato spotted wilt as well as the grower standard treatment. Kaolin significantly increased yield. When applied with kaolin, the plant essential oils produced yields similar to the grower standard. Kaolin may reduce the volatility of the oils, thus increasing their repellency to thrips. These findings indicate that naturally occurring products, such as plant essential oils and particle films, could be used successfully to reduce insecticide use on tomatoes.

P-97

Volatile constituents of *Teucrium persicum* Boiss. and *Thymus caucasicus* Willd. ex Ronniger subsp. *grossheimii* (Ronniger) Jalsas., two Lamiaceae herbs growing in Iran.

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The genus *Teucrium* comprises *ca.* 340 species, 12 are described in the flora of Iran, among which three are endemic: *T. melissoides* Boiss. and Haussku. ex Boiss. and *T. Persicum* Boiss (*1*). Some species of *Teucrium* are reported to possess pharmacological properties. *T. polium L.* subsp. *valentinum* (Schreber) Borga and *T. gnaphalodes* are two medicinal plants endemic to the Iberian Peninsula. The genus *Thymus* includes about 350 species worldwide and is distributed mainly in temperate Eurasia. In Iran, 14 species are present, among which 10 are endemic. *Thymus* species have also several folkloric uses. The essential oils from the aerial parts of two Lamiaceae species: *T. persicum* and *T. caucasicus* obtained by hydrodistilation were analyzed by GC and GC/MS, and their compositions were compared.

epi-α-cadinol (23.2%)and α-pinene (17.3%) were the main components among the 31 constituents characterized in the oil of *T. persicum* representing 95.5% of the total components detected.

Seventeen compounds were identified in the oil of *T. caucasicus* representing 99.6% of the total oil with thymol (34.2%), methylchavicol (25.1%) and γ-terpinene (12.7%) as the major constituents.

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P-98

The effect of drying on the chemical composition of the essential oil of *Ocimum basilicum*

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Common basil (*Ocimum basilicum* L), member of the *Lamiaceae* family, is widely used as condiments in food, cosmetics, liqueurs, medicines, and perfumes. Basil is a pleasant smelling perennial shrub which grows in several regions all over the world. It is commonly known that the presence of the essential oils and their composition determine the specific aroma of plants and the flavour of the condiments (*1*).

There are usually considerable variations in the contents of the major components within this species (*2*) and the variation in the essential oils can also depend of different factor as genetic, chemotype, geographical origin, soil type, fertilisation, stress, water precipitation, harvest treatment and post harvest treatment as drying (*3*).

The major constituents of the essential oils obtained by hydrodistillation of the aerial parts of fresh basil are linalool (48,7%), eugenol (27,4%), *trans*-α-bergamotene (5,4%) and τ-cadinol (3,4%). During the drying process, linalool content of the oil increase to 80% and eugenol decrease to less than 1%.

The effect of drying on the relative abundance of other flavour volatiles of the plant is evaluated and no valuable variation is notice.

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P-99

Composition and chemical variability of leaf and fruit oils from *Pistacia lentiscus* L. growing wild in Algeria

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Pistacia lentiscus L. is widely distributed all around the Mediterranean basin. In Algeria *P. lentiscus* grows wild in the coastal areas and it is also present in the sub-Saharan regions, up to 1500 m altitude. It is a brush that plays a particularly important role in the ecosystem of the Mediterranean maquis. It is a rustic, drought resistant, evergreen species, with a good capacity to resprout after cutting or after a fire and it has a good potential for use in restoration of degraded arid areas.

The essential oil obtained from the aerial parts of *P. lentiscus* is used in the perfumery, food and pharmaceutical industries. For instance, it is used as flavouring in alcoholic beverages and chewing gums. It also possess appreciable antifungal, antibacterial and antimicrobial activities.

The chemical composition of 17 samples of leaf oil and nine samples of fruit oil from *Pistacia lentiscus* L. growing wild in Algeria was investigated by GC (retention indices), GC-MS and ^{13}C NMR. Leaf and fruit oils were dominated by monoterpene hydrocarbons. α-Pinene (L 20.0-34.2%; F 37.9-51.5%), myrcene (L 23.0 -33.1%, F 27.0-69.7%) or limonene (L 25.5-43.8%) were the major components. However, one sample displayed an appreciable content of sesquiterpenes (69.8%).

Statistical analysis of our results combined with literature data confirmed the chemical variability of the leaf oil of *P. lentiscus* all around the Mediterranean basin. Six groups were distinguished between the individuals. The samples of the most important cluster are characterized by an appreciable content of α-pinene, always accompanied by another monoterpene such as myrcene, sabinene, β-pinene, limonene, 1-terpinen-4-ol, or α-phellandrene. The samples of the four other groups are dominated by limonene, 1-terpinen-4-ol, myrcene, Δ-3-carene, respectively. The last group is quite heterogeneous. In this group were found samples that possess two or three major components with comparable contents including one or more sesquiterpenes.

Two compositions of the fruit oil were observed: the first one was dominated by myrcene and the second one by α-pinene.

P-100

Valorisation of a new grade of Siam benzoin gum

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Siam benzoin gum is a balsam obtained from *Styrax tonkinensis* Craib of the Styracaceae species, produced in Laos. It is very used in the flavour and fragrance industry owing to its sweet-balsamic odour with a distinct note of vanilla. The gum is a pathological product resulting from incisions made through the bark. The resin is sorted and graded according to the size of the pieces from #1 (largest pieces) to #5 (dust and siftings) (*1*). The product remaining after sorting, which contains a mixture of bark and gum, can represent more than 10% in weight of the total harvested benzoin gum. To the best of our knowledge, this Siam benzoin gum-harvesting by-product has not yet been exploited and its chemical composition has never been reported.

The composition of Siam benzoin gum has already been studied; it is mainly composed of aromatic acids and esters (*2*). Recently, we reported on the chemical composition of Siam benzoin gum volatile extracts by direct GC and SPME-GC analysis of oil and crushed benzoin gum (*3*). This work was completed by the study of two grades (#3 and #5) of Siam benzoin gum using various headspace sampling methods which led to the identification of 42 volatile and semi-volatile compounds in Siam benzoin gums by GC-RI and GC-MS techniques (*4*). In addition, we reported on the use of electronic noses as a new tool for an easy distinction between different grades of Siam benzoin gum (*5*).

In the scope of our research on benzoin gums, the aim of this study is to determine whether the harvesting by-product can be considered as a new grade of Siam benzoin gum. In this purpose, we investigated the chemical composition of the crude by-product and its extracts, which were compared to the gum and the extracts of the trade.

The headspace fractions were analysed by GC-FID and GC-MS using two sampling methods: Headspace-SPME and Static-Headspace. Afterwards, the chemical composition of the resinoid was established by GC-MS and HPLC. Two different extracts obtained from the raw material and from a sorted one containing barks only were studied to determine the influence of barks on the chemical composition. Finally, both chemical composition and olfactory properties were compared to those of Siam benzoin gum.

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Composition of the essential oil of *Babingtonia leratii* from New Caledonia

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Babingtonia leratii (Schltr.) A.R. Bean (*Myrtaceae*), previously known as *Baeckea ericoides* Schl., is an endemic plant of New Caledonia. The white flowered shrub can reach a height of 2 m and is widespread over the whole western coast of the island, where it grows spontaneously, serving as a substitute after the deforestation of the Niaouli woods.

The plant, locally called "fausse bruyère", is used in traditional medicine for the treatment of inflammation and fevers, especially rheumatisms, and was also found to have moderate antiprotozoal activity. To the best of our knowledge, no data has been previously reported about the composition of its essential oil.

In this study, the essential oil from the flowering branches of *B. leratii* growing wild was investigated. The plant material was collected in January 2006, in the region Poingam situated in the northern part of the island, where the plant is growing abundantly over hundreds of hectares. It is estimated that the production potential of the plant in this region is about 150 - 300 kg essential oil per annum. The dried plant material was subjected to hydrodistillation using a Clevenger type apparatus. The oil yield was 0.7% (ml/Kg).

The analysis of the oil was performed via GC and coupled GC-MS. Identification of the compounds was performed using MS library search in combination with retention indices.

More than 20 compounds were identified, representing over 90% of the total oil content. The main compounds were: α-pinene (28%), 1,8-cineol (7%), (*E*)-nerolidol (21%), spathulenol (3.4%) and *iso*spathulenol (2.3%). It is assumed that various esters of valerianic, isovalerianic and tiglic acid, identified in relatively high concentrations, contribute to the specific odour of the oil. In the rich sesquiterpene fraction, two unknown alcohols making up more than 6% of the oil were detected. Further studies will try to identify the chemical structure of these compounds.

The enantiomeric distribution of the main chiral monoterpene hydrocarbons was also determined. The ratios were found to be as follows:

- (*R*)-(+)-α-pinene (65.9 %) : (*S*)-(–)-α-pinene (34.1%)
- (*S*)-(+)-camphene (27.4%): (*R*)-(–)-camphene (72.6%)
- (*R*)-(+)-β-pinene (10.7) : (*S*)-(–)-β-pinene (89.3%)
- (*R*)-(+)-limonene (14.2%): (S)-(–)-limonene (85.8%)

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Comparative study of substitute solvents for resinoids obtention

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Due to the future european directive REACH application, aromatic industry searched after safe and environmentally friendly organic solvents in remplacement of the widely used hexan or to its recent substitute, cyclohexan. In the framework of the Green Chemistry concept, supercritical carbon dioxide appeared to be the genuine green solvent. But due to its operating conditions, its use needed costly pilot plants. Alternative options could be to use liquid green solvents which could be either petrochemical solvents recognized environmental friendly and not classified as VOC by international regulations nor agrisolvents or biosolvents obtained from agriculture by-products. As a part of the AROMATIC Program, the extractive potentialities of isoamyl acetate and of two hydrofluoroethers (methoxynonafluorobutane and ethoxynonafluorobutane) respectively obtained from agricultural by-products and recently introduced in cosmetic industry due to their safety, were compared to those of hexan and cyclohexan for the obtention of resinoids from various medieval aromatic plants. Extractions were performed by maceration of dry plants at ambiant temperature. Before analysis, absolutes were obtained by extraction with ethanol of resinoids followed by a cold filtration in order to remove fats, waxes and dyes. Complementary hydrodistillations were performed on the same plants in order to obtain the corresponding essential oils. All aromatic extracts obtained were analyzed by GC-MS-Olfactometry in order to identify their key flavor compounds. Two plants, *Helichrysum italicum* and *Galium odoratum*, were particularly investigated in a first approach as they are used respectively as alimentary condiment in roman cuisine and aromatic base of the Luxemburg national drink (Maitrank). The first results showed that both hydrofluorothers gave similar extraction yields as hexan and a solvent recovery by distillation up to 80% while isoamyl acetate extracts need a complete elimination of the solvent in order to remove the banana off-note.

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Comparison of methods of isolation of volatile substances from complex matrix

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For several years, feed additives have been offered on the market for ecological as well as conventional animal feeding. Based on secondary plant components that do not involve the risk of antibiotic efficiency promoter and partly contribute their advantages.

Due to the all-year-round availability and mainly becauce of its standardizability against whole-crop processing, essential oils as carriers of the antimicrobial/antioxidative potential are of special importance. On this basis, various product groups are beening established on the market which contain various carriers either in liquid or free-flowing form.

The added and effective concentrations of essential oils (e.g. origanum oil) are within the range of 10 g/t to 50 g/t finished feed. The content of the single active compounds (e.g. carvacrol) is lower depending on the percent distribution of the essential oil.

Feed enriched by active agents is prepared advantageously by using highly concentrated additives (for free-flowing products silicic acid is frequently used as a carrier for the essential oils, for liquid products plant oils are used) which are added to the single feed components, such as cereal flours and oil-seed press cake.

The analytical detection of the fixed essential active compounds up to the trough-ready food undoubtedly is a significant criterion for product safety and retraceability for manufacturers of feed additives as well as for users (breeders). For the isolation of volatile substances from such a matrix the following methods were tested:

- watersteam distillation (WD);
- solvent extraction with Soxhlet (Sox);
- simultaneous distillation/extraction (SDE).

The recovery rates were determined with feed samples spiked with test substances (limonene, eugenol, carvacrol). The tested methods were comparatively evaluated under the following aspects:

- efficiency of the isolation (recovery of volatile compounds);
- reproducibility;
- influences of matrix components;
- manageability (handling).

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Composition of the essential oil of *Tanacetum turcomanicum* (Krasch.)Tzvel., from Iran

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The genus *Tanacetum* is represented in the flora of Iran by twenty-six species including twelve endemics (*1*) (*2*).

Plant belonging to the genus *Tanacetum* are reputed to have excellent medicinal values, and a large number of sesquiterpenoids and sesquiterpene lactones, which are typical constituents of these drugs, were isolated from *Tanacetum* species (*3*).

The aerial parts of *T. turcomanicum* were collected from Fariman in Khorasan Province, located in North-East of Iran during the flowering period in August 2004.

Air-dried aerial parts of the *T. turcomanicum* were grounded and subjected to hydrodistillation, for 4 hours, using a Clevenger-Type apparatus to produce yellowish oil in 0.4% (w/w) yield based on dry weight.

The oil was analysed by GC and GC-MS. Identification of the constituents of each oil was made by comparison of their mass spectra and retention indices (RI) with those given in the literature and those authentic samples (*4*). Thirty-four components, representing 86.55% of the total oil, were identified of which trans-chrysanthenyl acetate (19.7%), *trans*-thujone and chrysanthenone (24.68%), camphor (7.28) were the main compounds.

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Compostition of the essential oil of *Haplophyllum furfuraceum* from Iran

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Eighteen species of the genus *Haplophyllum* (Rutaceae) are found in Iran, among which nine are endemic. Previous chemical investigations on different species of *Haplophyllum* have shown the presence of cumarins, alkaloids, flavonoids and lignans. The oils of the genus *Haplophyllum* have been the subject of only a few studies. Previously we reported the essential oils composition of *H. tuberculatum* (*1*) and *H. robustum* (*2*). In the first oil limonene (27.3%) and α-pinene (21.9%) and in the latter oil sabinene (30.5%), β-pinene (18.2%) and limonene (12.1%) were the major constituents.

Water distilled essential oil from aerial parts of *H. furfuraceum* Bge. ex Boiss. (syn : *H. khorassanicum* Rech. f. Aell. 8 Esfand.), which is endemic to Iran, was analyzed by GC and GC-MS.

Aerial parts of the plant were collected from Kashmar, Province of Khorassan, in June 2003, during the flowering stage. The yield of the oil was 0.2% (w/w).

In *H. furfuracem* oil, 34 components, which represented about 98.1% of the total composition, were identified. The oil of *H. furfuraceum* consisted of nine monoterpene (25.9%), two sesquiterpene hydrocarbons (34.9%) and two aliphatic esters (1.6%). Elemol (11.7%) and β-eudesmol (10.1%) were the major components in this oil, followed by 1.8-cineole (9.3%). α-pinene (8.5%), β-pinene (7.7%), caryophyllene oxide (5.9%) and *p*-cymene (5.2%).

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Chemical composition of the leaf essential oil of a *Coleus* species from Gabon

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Coleus species (Labiatae) (syn. *Solenostemon, Plectranthus*) are herbs up to 6 feet high, with pubescent stems and variegated leaves with purple dominant over green *(1)*. The infusion of leaves of the species grown in Gabon are used to treat indigestion, but also by the young mothers to promote lactation.

Two samples of leaves (one with a dark purple colour and the other one with a prevailing green colour) were collected in March 2006 near Franceville, Gabon (*). The plant material was air-dried at room temperature for three days then subjected to hydrodistillation for 4 hours using a Clevenger apparatus. The oil yields (v/w) on moisture free basis were 1.7 % and 1.3 % respectively.

The oil samples, which are fluid with a faint yellow colour, present an herbaceous and terpenic odour. The analyses by GC and GC-MS indicated very similar compositions, characterized by a major component (*E*)-epoxyocimene (76% and 82% respectively) accompanied by the (*Z*)-isomer (1.4% and 0.4%) and other acyclic monoterpenes (ocimenes, myrcene and their oxygenated derivatives). Sesquiterpenes represent only 5-10% of the whole oils.

Many studies concerning the composition of essential oils of several *Coleus* species have been reported in the literature. Nevertheless our results differ completely from these previous reports, which mention generally mono- or bicyclic terpenic structures in their volatiles extracts. On another hand, high contents of (*Z*)-epoxyocimene (>50%) have already been found in *Artemisia absinthium* essential oil *(2)* and 2,3-epoxymyrcene was the major constituent (70 %) of the essential oil of a sample of *Lippia multiflora* collected in Central African Republic *(3)*.

The free radical scavenging activity of the oils was tested using the 2,2-diphenyl-1-picrylhydrazyl method and the potential anti-inflammatory activity was evaluated by testing their action against soybean lipoxygenase using linoleic acid as substrate. Only a small antiradical activity (SC_{50} = 1,7 g/L and 1,9 g/L respectively) could be observed.

(*) The formal identification of the botanical species is in progress.

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Changes in essential oil composition in the needles of Scots pine under anthropogenic stress

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Climatic and anthropogenic factors, such as air and soil pollution, also temperature and rainfall extremes lead to biochemical responses in a tree. Trees, especially coniferous are very sensitive to the stress. Changes in the amounts of secondary metabolites may be early indicators of invisible injuries.

Essential oil qualitative and quantitative analyses were performed for the needles of Scots pine (*Pinus sylvestris* L.) growing in the impact zone of the nitrogen fertilizer factory (JSC "Achema", Lithuania). Former studies in that area revealed ammonia aerial concentration and nitrogen deposition gradients; also differences in the soil and pine stand parameters were documented. Four pine stands along 25 km transect according to the prevailing wind were selected. Current-year and one-year-old needles of 8 pines in each site were sampled in July 2005. Volatile components of needles were extracted by simultaneous hydrodistillation-extraction and analysed by gas chromatography (GC equipped with FID and a polar capillary column HP-FFAP, 30 m × 0.25 mm i.d.) and gas chromatography-mass spectrometry (GC/MS equipped with a nonpolar capillary column CP-Sil8CB, 50 m × 0.32 mm i.d.). Qualitative analysis was based on a comparison of retention times and indexes and mass spectra with corresponding data in the literature (*1*) and computer mass spectra libraries.

Over 70 components were identified in the essential oils obtained in the current-year ans one-year old needles. Main constituents were: α-pinene, δ-carene, (*E*)-caryophyllene, δ-cadinene, *epi*-α- and α-muurolol, *epi*-α- and α-cadinol. Some of the components were only in the minor amounts. There were observed significant differences in the amounts of essential oil components between current-year and one-year-old needles. For the indication of ammonia pollution current-year needles were more informative than one-year-old ones. In the site closest to the factory the lowest percentage concentrations of monoterpenes and oxygenated monoterpenes also the highest concentrations of sesquiterpenes and oxygenated sesquiterpenes were documented. In conclusion, changes in the amounts of the essential oil components differing in the lengths of the chain may modify susceptibility of the pine stands to the biotic factors.

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P-108

Essential oil composition and variability of *Glechoma hederacea* L. growing in Lithuania

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The overground parts of *Glechoma hederacea* L. (ground ivy) collected during flowering are used for healing different diseases and as a spice [1-3]. The *G. hederacea* in Lithuania has a strong odour. Two species of *Glechoma* L., i.e. *G. hederacea* and *G. hirsuta,* grow wild.

The essential oils of wild ground ivy collected in seven localities of Lithuania and were analysed by GC and GC-MS. More than half of the oils were rich in sesquiterpene hydrocarbons (56.5-7.9 %). Germacrene D (15.6-8.8 %) was the dominating compound. The other main constituents were γ-elemene (9.7-16.0 %), β-elemene (8.7-12.9 %), phytol (2.8-15.6 %), (*Z*)-β-ocimene (2.2-8.5 %), 1,8-cineole (2.2-5.4 %), β-ylangene (2.7-4.1 %) and germacrene B (2.2-.9 %). Forty three identified compounds made up 89.1-96.2 %. About the half of investigated essential oils of *Glechoma* L, formed compounds with germacrane and elemane carbon skeletons.The data allowed to conclude that the main direction of volatile compounds biosynthesis was formation of sesquiterpenoids.

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Polyacetylene distribution can be observed and mapped in living plant tissue applying micro-Raman spectroscopy

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Polyacetylenes are known to occur in numerous plants, especially in various species related to the Compositae and Umbelliferae families (*1*). Falcarinol and panaxydol are among the most bioactive polyacetylenes and hence they are very important in relation to anti-cancer effects and other pharmacological properties. In particular the cytotoxic properties of falcarinol have recently been extensively investigated in relation to the health promoting properties of various food materials as this substance is also a major polyacetylene constituent in carrot and ginseng roots as well as other vegetable or medicinal plants (*2,3*).

This study presents that Raman spectroscopy is a powerful and sensitive tool to determine the distribution of different polyacetylenes in the intact plant tissue at cellular dimensions. The obtained spectra taken *in situ* from the fresh sample show strong characteristic polyacetylene key bands in the wavenumber range between 2100 and 2300 cm^{-1} which are due to the triple bonds in the molecule. It has been found that the number of triple bonds as well as the substituents influence the frequency of the polyacetylene -C≡C- stretching modes. Thus, the spectral position of -C≡C- vibrations and pattern of Raman bands usually provide important information to recognize the type of substitution and to support the identification of polyacetylenes. Generally, for compounds containing a -C≡C-C≡C- grouping, the vibrational modes are described as asymmetric and symmetric -C≡C-C≡C- stretching, and accordingly they are IR and Raman active, respectively.

The presented results show also the special advantage of micro-Raman spectroscopy in the investigation of polyacetylenes in various plant species. The distribution of polyacetylenes which occur mainly in the essential oil cavities or ducts can be analysed at different spatial resolutions in cellular dimensions. Beside the described qualitative measurements also quantitative analyses of polyacetylenes can be performed, if calibration equations for the individual analytes have been successfully established before and if the samples are homogeneous enough.

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P-110

Supercritical fluid extraction of the volatile oil from tea tree (*Melaleuca alternifolia*) leaves

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Tea tree (*Melaleuca alternifolia*) is indigenous in Australia and New Zeeland (*1*). The essential oil obtained from this plant is mainly made of monoterpenes and is used in the therapeutical and cosmetics industries (*2*) due to its antimicrobial, fungicide, antiseptical, antibiotic, anti-inflammatory, antiviral, and insecticidal activities (*1*)(*3*). The main monoterpene present in the essential oil is 1-terpinen-4-ol. This compound is used in the prevention and treatment of skin parasites such as mites and fungus.

The conventional method to obtain extract from tea tree leaves is steam distillation. There is no report about the supercritical fluid extraction of monoterpenes from this plant focusing the global yield and extract composition.

In this work, the influence of extraction pressure and temperature on the tea tree oil composition obtained by supercritical carbon dioxide extraction (SFE) was verified using an experimental design with central point. The selected temperatures were 40 and 60 °C and pressures of 80 and 120 bar. At these conditions the CO_2 density ranged from 0.25 to 0.75 g/mL. The powder of dried tea tree leaves cultivated in Brazil, with particle diameter from 150 to 707 μm, was used in the experiments. The extracts were collected in ethyl acetate to minimize the lost of the more volatile terpenes. The monoterpenes profile of the SFE extracts were compared to the Australian commercial essential oil. The amounts of 1,8 cineole and 1-terpinen-4-ol were quantified in the extracts.

There was no significant effect of pressure and temperature on the extract composition. The supercritical fluid extracts profiles were different from the Australian commercial essential oil probably due to the raw material adaptation in Brazil or to differences in the species used to obtain the extracts.

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Distribution of volatile compounds in *Artemisia cana.*

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Artemisia oils are obtained by steam distillation of the whole over-ground parts of wild growing and cultivated herbs such as *Artemisia herba-alba* in Morocco, *A. alba* in Tunisia, *A. mendozana* in Argentina, *A. annua* in Yugoslavia and *A. vulgaris* in the South of France, Morocco, Germany, Hungary, India, China and Japan. They are light yellow to green liquids with an herbaceous, balsamic and fresh-camphoraceous odor. Some *Artemisia* species serve as a source of locally distilled and locally used essential oils for the cosmetic and toilet industry. *A. herba-alba* oil is used in fairly large amounts worldwide in fine fragrances (e.g., for chypre notes) (*1,2*). The aim of the present study was to determine the content and the chemical composition of essential oils extracted from different aerial parts of *A. cana* (flowers, leaves and stalks) growing wild in Western Canada. *A. cana* was harvested just before the flower-buds open when the essential oil content is at its maximum. Flowers, leaves and thin stalks were dried at ambient temperature and comminuted using a hammer mill. The oils were extracted by hydrodistillation using a Clevenger-type apparatus until total recovery of oil. Analyses were performed by GC-MS using two columns of different polarities, HP-5MS column (5% phenyl 95% polydimethylsiloxane) and DB-Wax column (polyethylene glycol). The identification of single components was performed by comparison of GC retention indices, mass spectra, and co-injection with authentic standards. The major components identified were 1,8-cineole and camphor in the oils. The chemical composition of different plant parts was very similar: α-pinene (3.6% flowers, 2.0 leaves, 2.3 stalks); camphene (7.0% flowers, 3.7% leaves, 7.6% stalks); 1,8-cineole (15.1% flowers, 17.8% leaves, 11.0% stalks); camphor (52.6% flowers, 52.8% leaves, 55.2% stalks); borneol (2.8% flowers, 4.2% leaves, 3.8% stalks) and 1-terpinen-4-ol (1.7% flowers, 1.8% leaves, 1.1% stalks). Up to 2.3% of oil was obtained after 8 hours of distillation of the aerial parts including only thin stalks, the wooden-type stalks were separated. It was found that stalks contained insignificant amounts of essential oil. The oil contents in the flowers, leaves and stalks were 2.8%, 2.4% and 0.1% respectively.

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An improved microwave Clevenger apparatus for distillation of essential oils from orange peel

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Microwave Clevenger or microwave accelerated distillation (MAD) is a combination of microwave heating and distillation, performed at atmospheric pressure without added any solvent or water (*1*).

Isolation and concentration of volatile compounds are performed by a single stage. MAD extraction of orange essential oil was studied using fresh orange peel from Valencia late cultivar oranges as the raw material. MAD has been compared with a conventional technique, which used a Clevenger apparatus with hydro-distillation (HD). MAD and HD were compared. In term of extraction time, yields, chemical composition and quality of the essential oil, efficiency and costs of the process.

Extraction of essential oils from orange peels with MAD was better in terms of energy saving, extraction time (30 min versus 3 h), oxygenated fraction (11.7% versus 7.9%), product yield (0.42% versus 0.39%) and product quality. Orange peels treated by MAD and HD were observed by scanning electronic microscopy (SEM). Micrographs provide evidence of more rapid opening of essential oil glands treated by MAD, in contrast to conventional hydro-distillation.

Reference.

(*1*) US Patent Application No. 2004/0187340 (Priority: January 7, 2004).

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Development of an analytical methodology to determine the phenylurea pesticide residue in chamomille.

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The aromatic species are susceptible to insect and pathogen attacks and pesticides use is a common agronomic practice around the world for plant protection. Although the pesticides can help plants to complete its development, it can also leave residues that can affect the environment and consumers. A recent evaluation of pesticide residues in different spices and medicinal plants (*1*) has shown the predominance of malathion in most of analyzed samples and the detected concentration exceeded the maximum permissible levels (MPLs). This study also showed that, specifically for chamomille, residues levels of lindane, aldrin, dieldrin, DDT, chlordane and endrin exceeded the MPLs. The pesticide linuron is widely used for weed control on chamomille production, but no studies so far have been carried out to evaluate the level of the residue which can remain in this aromatic plant species. Because linuron belongs the phenylurea group, initially was thought the residue levels could be determined only by high performance liquid chromatography (HPLC) and not by gas chromatography because of its low thermal stability. The main disadvantage of the HPLC technique is the separation efficiency and sensitivity that is poorer than a gas chromatography. A new technique using gas chromatography with nitrogen-phosphorus detection direct method for methoxyurea herbicide determination in potato samples has been recently developed (*2*). This technique qwas tested to monitor linuron residues in chamomille, which would increase the quality and safety of the product. Samples were collected from South Brazil farms that is considered the main region of chamomille production in the country. The herbicide residues were extracted from the samples through liquid-liquid extraction with acetonitrile, followed by gas cromatography using a NPD detector. Samples were previosuly fortified using 5, 10, 20 and 30 ppm of linuron to determine the detection limit. The results showed that the linuron could be only detecetd on samples treated with concentration higher than 10 ppm. Comparing to the detection limit obtained for potato samples which is much lower (*2*), the obtained detection limit observed on chamomille samples was not satisfactory. The limitation of the anlytical methodology applied to the aromatic plant is probably due to the nature of the plant tissue. Potatos samples accumulate predominantly starch in the tuber, while chamomille tissues present many other molecules, includind terpenoids, that might interfere on the detection of linuron. Different sample preparation strategies are necessary to allow detection of linuron in chamomille and maybe other aromatic species.

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P-114

Identification of freelingyne in the heartwood of *Myoporum crassifolium* from New Caledonia

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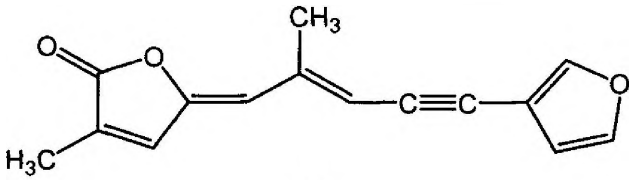
Out of the 15 known *Myoporum* species (Myoporaceae), two are indigenous in New Caledonia, namely *M. crassifolium* Forst. and *M. tenuifolium* Forst. The former also occurs in Vanuatu. Early attempts to extract an essential oil from the inner wood of *M. crassifolium* were made at the end of the nineteenth century, and a 4% yield of essential oil and suggested uses in perfumery were reported as early as 1913 (*1*). However, the composition of this oil was reported only very recently, and was found to be made of more than 90% sesquiterpene derivatives, including (-)-*epi*-bisabolol as the major element (*2*). Solvent extraction of the heartwood is a preferred method for the high yielding recovery of sesquiterpenoids, including those which may not be recovered by hydrodistillation for a variety of reasons. Thus, upon extraction with 96% ethanol, a crude resinoid was obtained in 9.2% yield. Standard fractionation steps allowed to isolate pure freelingyne **1** as bright yellow needles (F. 162°C). It was fully identified by HRMS, UV, IR, ^{13}C- and ^{1}H-NMR. The NMR data are strictly identical to those assigned to unambiguously synthetized (*Z*)-freelingyne (*3*).



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Quantitation of freelingyne in the resinoid was carried out by HPLC/UV, which allowed to determine that its concentration in the heartwood of *M. crassifolium* is *ca.* 0.076%. It is noteworthy that freelingyne is not detected by conventional GC in the essential oil, although sesquiterpene congeners with similar molecular weights such as the crassifoliones are found in significant concentration in it. Although freelingyne has been identified previously in the heartwood of *Eremophila freelingii,* an other member of the Myoporaceae genus (*4*), this is the first report of this unusual acyclic furanosesquiterpenoid in a *Myoporum* species (*5*).

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Comprehensive two-dimensional GC (GCxGC)/FID and -qMS quantitative analysis of sandalwood essential oils

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When analyzing sandalwood essential oils, it is generally recognized that full resolution of all sesquiterpene primary alcohols cannot be achieved satisfactorily in a single GC run, whatever the stationnary phase. This drawback is particularly severe when quantitation of individual components is needed to fulfill regulatory requirements. In theory, quantitation of a given compound present in a complex mixture does not require full GC separation when mass spectrometry with selected ion monitoring (SIM) is used as a detector. However, even when using de-convolution techniques, this method has severe limitations when compounds with isobaric ions are co-eluted or closely eluted, as in the case of many terpenoids. A two-dimensional GCxGC separation offers a greater opportunity to resolve the target characteristic components into single component peaks and gives better insight into the number of true single component peaks that are present in the one-dimensional chromatogram of essential oils (*1*). In the present study, we report the first quantitative analysis of (*E,E*)-α-farnesol, a suspected allergen in cosmetics, in essential oils from various *Santalum* species: *S. album* from India, *S. spicatum* from Australia and *S. austrocaledonicum* from New Caledonia and Vanuatu. Whereas previous work has reported the semi-quantitation only of individual components of *S. spicatum* essential oil by GCxGC-FID with internal normalization (*2*) we report herein how comprehensive GCxGC can achieve full separation of (*E,E*)-α-farnesol in several sandalwood essential oils, and its quantitation by internal standardization, using either a flame ionization detector or a quadrupole mass spectrometer in SIM mode. We confirm that the (*E,E*)-farnesol content is less than 500ppm in the essential oil of *S. album*, whereas it is very high in *S. spicatum* (*ca.* 7%, and up to 20%) (*3*). In contrast, the low levels of farnesol (below 10000ppm) (*4*) make essential oils of *Santalum austrocaledonicum* good candidates as natural raw materials for compounding allergen-less fragrances.

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P-116

Heliotropin, heliotrope odor and Tahitian vanilla flavor: the end of a saga?

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"It remains doubtful whether this aromatic aldehyde occurs in the beans (fruit) of various vanilla species, also whether the odor of heliotrope flowers is due to piperonal.". Since its first publication in 1949 (*1*), this statement has not motivated yet any complete investigation, and, conversely, there has been a continuing controversy on the alleged occurence of heliotropin (or piperonal) in cured vanilla beans from Tahiti (*2*), even until recently (*3*).

In this paper, we report that:

1) heliotropin is not detected in the flower fragrance of heliotrope (*Heliotropum arborescens*), using dynamic headspace SBSE sampling, followed by thermal desorption and analysis by conventional GC-MS. Main constituents in the fragrance given off by helioptrope flowers are anisaldehyde and benzaldehyde, which confirms previous findings (*4*).

2) heliotropin is not detected in laboratory-made extracts from authentic Tahitian vanilla bean (*Vanilla tahitensis*), in using both GC-MS in SIM mode and LC-MS/MS. This confirms and conforts previous observations first published in 1978 (*5*), and later (*6*). It has been known for almost a century that the characteristic elements in the flavor of Tahitian vanilla are 4-methoxyaromatic compounds (*7*), including *p*-anisaldehyde as the the main aroma donator.

We suggest that heliotropin was named after the discovery of piperonal by degradation of piperine, the odor of which was reminiscent of the heliotrope flower. Later, its identification in vanilla extracts was the result of either a wrong interpretation of analytical data, or, much more probably, adulterations of said extracts.

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