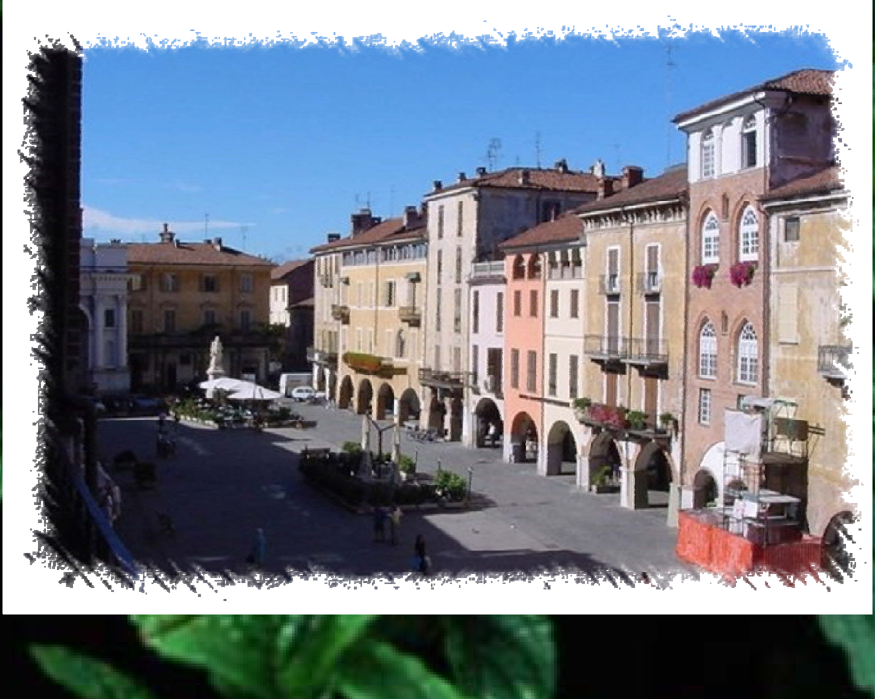
40th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

ISEO 2009

Programme and Book of Abstracts



Savigliano (Italy), September 6-9, 2009

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LAVAZZA

40th International Symposium on Essential Oils

PROGRAMME AND BOOK OF ABSTRACTS

September 6-9, 2009
Savigliano, Italy

Organization:

Faculty of Pharmacy
University of Turin

Editors

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University of Turin
Dipartimento di Scienza e Tecnologia del Farmaco
Via Pietro Giuria 9
I-10125 Turin, Italy

General Informations

GENERAL INFORMATIONS

CONFERENCE VENUE

The conference will be organized at the University of Turin - Polo Universitario di Cuneo
Ex Convento di S. Monica
Via Garibaldi n.6
I-12038 Savigliano (CN) – Italy
Phone: +39 011 670 8339

The opening Plenary will be held in Teatro Milanollo
Piazza Turletti, 7
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Phone: +39 0172 21439

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Savigliano (IT), September 6-9, 2009
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REGISTRATION

The Registration Desk will be opened on Sunday, September 6, from 15:30 to 18:30, on Monday Sept. 7, and Tuesday Sept. 8, from 8:00 to 18:30, and on Wednesday Sept. 9 from 8:00 to 12:30.
Participants and their accompanying will receive their badges and conference materials at the desk.
For safety reason, participants are kindly requested to wear their name badges during all events of the meeting.

ASSISTANTS AND CONFERENCE SECRETARIAT

If you need any help during the conference you can find the staff at the registration desk. Conference assistants will be recognizable by their pink badge, and organizing committee by their yellow badge. They will help you in all practical aspects of conference participation.

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.

LUNCH AND REFRESHMENT

Refreshments are included in the registration fee and will be served in the cloisters' garden of the ex Convento Santa Monica. The Symposium organization will not be involved with lunches, but an agreement with several Savigliano bars, coffees and restaurants at a special price for participants provided with the symposium badge will be established. Further information will be available in the Symposium folder.

CONFERENCE WEBSITE

The Internet homepage of the ISEO is constantly kept up-to-date: http://www.iseo2009.unito.it

INSTRUCTION FOR SPEAKERS

The plenary lectures are foreseen in 45 minutes. The time scheduled for Oral presentations is 20 minutes, including discussions. The times allotted to each presentation are shown in the programme (pages 7-10). The lecture room will be equipped with PC projection facilities only. Power point software will be provided. The use of MAC file format is discouraged as well as zip disks. The PCs are provided with windows XP together Office 2003 with patch for Vista; the file format preferred for the presentation is ppt for Office 2003. Pdf files can also be handled.
To avoid technical problems, personal laptops cannot be utilized; a pen drive or CD-ROM should be provided, properly closed to technician during one of the breaks well in advance before your presentation, for checking it and loading it to the PC in the lecture room. Speakers should meet the chairperson and provide him/her 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.

POSTERS

Posters will be located under the arcades of the cloister of the ex Convento Santa Monica, at the ground floor.

Posters for the 40th ISEO should be left on the poster boards during a one day period.
Period 1: Monday (Poster Session A), the posters will be displayed from 8.30 a.m. on Monday morning and they must be removed by 6.30 p.m. of the same day
Period 2: Tuesday (Poster Session B), the poster will be displayed from 8.30 a.m. on Tuesday morning and must be removed by 6.30 p.m. of the same day
The presenting authors, preferably, or one of the co-authors must be present during the entire poster session.

EXHIBITORS

Exhibitors booths will be located at the ground floor under the arcades of the cloister of the ex Convento Santa Monica.

MISCELLANEOUS RECOMMENDATIONS

Due the tight schedule, delegates are kindly invited to be present in the conference room at least 5 minutes before the beginning of the sessions.
Informal dressing is recommended throughout the symposium for all occasions, including dinners.
The organizers will not accept responsibility for any accommodation problem, nor for any accident, personal injury, loss or property damage sustained during the symposium.

40th International Symposium on Essential Oils
September 6-9,2009, Savigliano, Italy

Scientific Programme

Sunday September 6, 2009

16.00 – Registration (ex Convento Santa Monica, Via Garibaldi 6)

18.30 - Opening Concert (Teatro Milanollo, Piazza Turletti 7)

20.00 - Get together party

Monday September 7, 2009

9.00 - 9.30	Opening Cerimony (Teatro Milanollo)
9.30 - 10.15	PL-1 - Opening plenary lecture ***Essential oil research: past, present and future*** **Franz Chlodwig** *Institute for Applied Botany and Pharmacognosy, University of Vienna, Austria*
10.15 - 10.40	Coffee Break (ex Convento Santa Monica, Via Garibaldi 6)
10.40 - 11.25	PL-2 ***Plant-insect interaction and volatile emission of terpenoids*** **Boland Wilhelm** *Max Planck Institute for Chemical Ecology, Jena, Germany*
11.25 - 12.15	OR-1 - Oral Communications ***Fragrance contact allergy: chemical reactivity of terpenic allylic hydroperoxides toward amino acids in iron systems*** **Kao D., Gimenez-Arnau E., Chaintreau A., Lepoittevin J. P.** *Université de Strasbourg-CNRS, Strasbourg, France* OR-2 ***Tahitian liverworts – rich source of terpenoids and other volatile components*** **Ludwiczuk A., Komala I., Pham A., Bianchini J. P., Raharivelomanana P., Asakawa Y.** *Tokushima Bunri University, Tokushima, Japan*
12.30 - 14.00	Lunch
14.00 – 14.45	PL-3 ***High Impact Odourants in Essential Oils*** **Clery Robin** *Givaudan Schweiz AG, Dübendorf, Switzerland*

14.45 – 15.45
OR-3
Structure-odor relationships of Santalane and Zizaane derivatives
Baldovini N., Delasalle C., Filippi J. J., Meierhenrich U. J., Brevard H., Lavoine S., Pierrat J. P.
Université de Nice-Sophia Antipolis, Nice, France

OR-4
Metabolomics and Genomics: combining tools for chemotyping and genotyping
Maffei M. E., Gnavi G., Bertea C. M.
University of Turin, Turin, Italy

OR-5
Evaluation of angiogenic activity of Lamiaceae essential oils by using the in vivo chorioallantoic membrane (CAM) assay
Demirci F., Demirci B., Koparal A. T., Baser K. H. C.
Anadolu University, Eskişehir, Turkey

15.45 – 16.10 Coffee Break

16.10 – 18.00 ***Poster session A***

Tuesday September 8, 2009

8.45 - 9.30
PL-4 - Plenary lecture
***Microbes at work in perfumery: The microbial community of Vetiver root and its involvement in essential oil biogenesis*.**
Alifano Pietro
University of Salento, Lecce, Italy

9.30 - 10.30
OR-6 - Oral Communications
Higher Resolution Comprehensive Two-dimensional Gas Chromatography applied to the Analysis of Essential Oils
Mondello L., Dugo G.
University of Messina, Messina, Italy

OR-7
^{13}C NMR: a tool for essential oil analysis
Tomi F.
Université de Corse-CNRS, Ajaccio, France

OR-8
Appling chemometric methods to identify volatile aroma active compounds of oregano
Bansleben A. C., Schellenberg I., Einax J.W., Schaefer K., Ulrich D., Bansleben D.
Institute of Bioanalytical Sciences (IBAS); Bernburg, Germany

10.30 - 10.55. Coffee Break

10.55 - 12.30 YS-1 - Young Scientist Session
Polymorphism of "katrafay", a complex essential oil from Madagascar
Rakotobe M., Menut C., Danthu P.
Université d'Antananarivo, Antananarivo, Madagascar

YS-2
Chemical characterisation and antimicrobial capacity of essential oils from Eremophila longifolia (F. Muell) (Myoporaceae): an indigenous Australian medicinal plant with high geographic chemovariabiliy
Sadgrove N., Smith. J., Alter D., Watson K., Tucker D., Jones G. L.
University of New England, Armidale, Australia

YS-3
A thorough analysis of the chemical composition of treated bergamot oil (Citrus bergamia)
Costa R., Dugo P., Dugo G., Mondello L.
University of Messina, Messina, Italy

YS-4
New cyclodextrin derivatives to improve selectivity, specificity and speed of essential oil ES-GC analyses
Cagliero C., Bicchi C., Cordero C., Sgorbini B., Cravotto G., Rubiolo P., Liberto E.
University of Turin, Turin, Italy

YS-5
Antimicrobial activity of essential oils: the possibilities of TLC-bioautography
Horvat G., Jambor N., Vegh A., Boszormenyi A., Lemberkovics E., Hethelyi E., Kovacs K., Kocsis B.
University of Pécs, Pécs, Hungary

12.30 - 14.00 Lunch

14.00 - 14.45 PL-5 - Plenary lecture
State of the art and outlook for the bio-production of terpene essential oil constituent.
Schalk Michel
Firmenich SA, Geneva, Switzerland

14.45 - 15.25 OR-9 - Oral Communications
Microbial transformation of isopinocampheol and caryophyllene oxide as terpenoids with four carbon ring
Noma Y., Hashimoto T., Uehara S., Asakawa Y.
Faculty of Human Life Sciences, Tokushima, Japan

OR-10
Essential oils and their major constituents as alternative antifungal agents: a South African perspective
Combrinck S., Regnier T., Du Plooy W. G.
Tshwane University of Technology, Pretoria, South Africa

15.25 – 15.50	Coffee Break
15.50 - 17.30	***Poster session B***
17.30	Meeting time
20.00	Symposium dinner

Wednesday September 9, 2009

8.45-10.25	REACH-1 - REACH session ***Perspective of the fragrance industry*** **Arregui C.** *EFFA, Brussels, Belgium* REACH-2 ***Toxicological Challenges for Essential Oils in REACH*** **Ellis G.** *Givaudan, Vernier, Switzerland* REACH-3 ***REACH-Driven developments in analysis and physico-chemistry*** **Chaintreau A., Bégnaud F., Debonneville C., Keller U.** *Firmenich SA, Geneva, Switzerland*
10.25 - 10.50	Coffee Break
10.50 – 11.30	OR-11 - Oral Communications ***Unattended odors influence facial attractiveness*** **Stappen I., Hoferl M., Heuberger E.** *University of Vienna, Vienna, Austria* OR-12 ***Fragrances and Flavours of Zululand*** **Swanepoel K. M.** *University of Zululand, Kwadlangezwa, South Africa*
11.30 - 12.15	PL-6 - Plenary lecture ***Metabolomics: what is new?*** **Verpoorte R.** *Institute of Biology, Leiden, ,The Netherlands*
12.15 – 12.30	Closing remarks

Plenary Lectures

PL-01

Essential oil research: past, present and future

Franz C.
Institute for Applied Botany and Pharmacognosy, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria

Essential oil research of the last 40 years was strongly influenced by the development of respective methods. Thin-layer and gas chromatography made the complex composition accessible showing for the first time the large inter- and infraspecific variation and the enormous diversity of compounds with often much more than 100 different substances within one essential oil. These tools enable – together with spectrometric methods – the structure elucidation of thousands of essential oil compounds and the phytochemical characterisation of many essential oil bearing plants filling libraries with this encyclopaedic data. At the same time, genetic and physiological studies resulted in a more and more detailed knowledge on the biosynthesis of the different oils and substances.

This was followed by in-vitro tests of essential oils and single compounds concerning their effect, especially the antimicrobial and antioxidant activity. The more effects were found the more risk assessment and toxicological studies have been the consequence.

Today more sophisticated and quantitatively exact methods are introduced to fulfil all requirements concerning risks and traceability, e.g. the authentification of the starting material, sometimes forgetting the fact that essential oils are of biological origin with the respective variation.

The challenge in future will be in finding new applications for essential oils e.g. in animals or for technical or hemisynthetical purposes and to produce plants with very few or single desired substances only, either on the classical way or by genetic modification.

PL-02

Induced Volatiles of *Medicago truncatula*: molecular diversity and mechanistic aspects of a multiproduct sesquiterpene synthase from *Medicago truncatula*

Boland W., Garms S.
Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, D 07745 Jena, Germany.
Email: Boland@ice.mpg.de

All tens of thousands sesquiterpenoids known today are based on 300 hydrocarbon skeletons, formed by so called sesquiterpene cyclases, utilizing farnesyl diphosphate (FDP) as substrate. The structural diversity is created by generating an environment that binds the flexible isoprenoid substrate in a proper orientation and conformation to enforce specific trajectories for C-C-bond formation. Beside high fidelity enzymes producing only a single product also promiscuous enzymes are known that can generate up to 52 different products [1]. The biosynthetic promiscuity of individual enzymes contributes to the enormous molecular diversity of the plant's defence against herbivores and pathogens. Modes of induction [2] and aspects of their circadian emission will be exemplified with volatiles of *M. truncatula* [3]. To understand to which extent the enzymes control the reaction pathways, we analyzed the stereochemical course of product formation of the multiproduct sesquiterpene synthase MtTPS5 from *Medicago truncatula* [4]. The incubation of the recombinant MtTPS5 with FDP provides 18 sesquiterpene hydrocarbons and 10 sesquiterpene alcohols with different carbon skeletons such as Germacrane or Cubebane. The stereochemical analysis of 17 enzyme products showed that only one enantiomer of each product was present. Products derived from a common cationic precursor share the same configuration of all their stereocentres. Together with a conformational analysis of the transition states we could show that the starting conformation of FDP is under stringent control of the enzyme, predefining the configurations of the stereocentres. Labeling experiments conducted in D_2O and mass spectrometric analysis, revealed information about the formation of the key intermediate (*E*,*Z*)-germacradienyl cation. The strong incorporation of deuterium atoms (up to 95%) in products derived from the (*E*,*Z*)-germacradienyl cation show that the cationic intermediate is generated via protonation of the neutral intermediate Germacrene D.

References

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[4] S. Garms; M. Maffei; S. Bossi; B. Schulze; M. Leitner; A. Mithöfer; W. Boland, *Planta* 2008, 227, 453–464.

PL-03

High Impact Odourants In Essential Oils

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There are various reasons, beyond mere curiosity, why there is continuing interest in determining what gives an essential oil its characteristic odour. This has given rise to the development of various analytical methods to determine the “key olfactory components” or “character-impact” compounds in essential oils and flavour extracts. Each different approach addresses a slightly different question and produces a subtly different answer. There can be no simple generalization about the key odourants in essentials oils, the odour of some essential oils is due to a large amount of a single compound while trace components are crucial to the odour of others and in most cases the true odour is the manifestation of a complex mixture of compounds. Here we will consider the different analytical approaches used and the range of molecules that have been identified as contributing to the odour of essential oils focusing on those with low odour thresholds.

The main aim in discovering what gives essential oils their characteristic odour is usually the need to produce an olfactorily acceptable alternative. This could be a fully formulated odour match perhaps avoiding the use of certain undesirable components or it could be a single chemical that can be used to imitate the odour of the oil. The high price or scarcity of the natural is often the driver behind this work, but it can also simply be the search for new aroma compounds that impart the recognizable character of the natural. It applies equally in both flavour and fragrance research and over time has led to the discovery of some important materials used in the flavour and fragrance industry such as the thio-esters in galbanum [1], oxane from passion fruit [2], grapefruit thiol [3], 3-hexylpyridine from orange [4] and continues to date with the discovery of Yuzuol, (6Z,8E)-undeca-6,8,10-trien-4-ol [5] and (Z)-4-hepten-2-yl salicylate [6,7].

The principal analytical technique used in the determination of odourants in essential oils is GC-olfactometry which is as old as the science of gas chromatography itself since early experiments in GC involved smelling the effluent from the column to detect the analytes. Adaptations were made to improve odour perception [8] and GC-sniffing equipment evolved quickly. Today a sniff port is often combined with other specific detectors by means of a splitting device to identify or characterize the analytes on the same system – mass spectrometry (GC-MS-O) provides a means of identification while an NPD or FPD detector can indicate the presence of N- or S- compounds in association with the odour response.

Several techniques to assess the comparative importance of the odours have been developed and those involving sequential dilutions of the sample and the detection or not of the analyte have generally been more successful than asking even expert panelists to rank odour intensity. CHARM analysis [9] and Aroma Extract Dilution Analysis (AEDA) [10] have become the methods of choice. Various units such as ‘flavour dilution factor’, ‘odour value’ ‘aroma value’, ‘odour activity value’ and ‘odour contribution coefficient’ have been derived to describe the combination of the potency of the particular compound and its relative abundance in the sample. From all of these the AEDA method has proved most useful and another level of accurate quantification has been achieved by combining it with Stable Isotope Dilution Analysis to determine the precise quantity in the original extract and thus the relative importance of trace odourants as demonstrated recently in the case of guava [11]. SIDA can also be used to determine the amount being smelled by GC-O and thus to estimate the detection threshold values. However, determining thresholds by means of GC-O is somewhat complex and is not a substitute for olfactometry. A recent method described by Benzo [12] uses a reference compound, limonene, with a published ODT value to calibrate the GC-O. Estimating ODTs by GC-O has both advantages and disadvantages compared to measurements obtained by testing the pure material using an olfactometer and assessment panel. By GC-O the compound is presented pure and free of trace contaminants, provided there are no co-elutions, but only for a short period of time and at varying concentration across the peak, whereas an olfactometer delivers the odour continuously at constant concentration and with a conveniently short cycle time between different concentrations.

The odour detection threshold of a compound is a remarkably simple concept, yet the different methods for its determination lead to remarkably variable values in the literature often quoted in different units and the different units are not always readily interconvertible. This is well known to anyone who works in this field and the subtleties of recognition thresholds vs detection thresholds, odour thresholds vs flavour thresholds and measurements made in air or water are all too familiar and yet all too infrequently described with precision in many papers. Various compilations of data from different sources have been made [13,14,15] but even with these data it is difficult to make direct comparisons between literature data. For this reason it is rarely valid to simply measure the relative percentage of an odourant in an essential oil and assume that because this is greater than a literature value for its odour threshold that it will contribute to the odour of the oil. The relative polarity and vapour pressure of an ingredient compared to the matrix as well as the complexity of the sense of smell have a role to play as all perfumers know. The fixative effect of certain materials versus the boosting effect of others is at the heart of a balanced perfume creation, and the same effects exist within the composition of essential oils. For the same reasons it is not always adequate to define a 'high impact' material on the basis of its odour threshold alone, nonetheless anything with an ODT in air that is below 0.1ng/L could be considered a very powerful odourant. In the context of essentials oils 'high impact odourant' implies one which noticeably alters the odour of the oil at a concentration that is not easily detectable using current instrumentation. Thus to identify these materials a significant amount of enrichment or fractionation is required and it is these 'low ODT' materials that will now be considered in more detail.

Galbanum oil from *Ferula galbaniflua* has a very powerful and distinctive odour and was an early subject for investigation. In 1967 Naves [16] identified the 1,3,5-undecatrienes as high impact odourants and the (3E,5Z) isomer was subsequently found to be the most powerful with an ODT in the region of 0.2ng/L. Bramwell [17] identified 2-sec-butyl-3-methoxy pyrazine and 2-isobutyl-3-methoxy pyrazine from galbanum the former having a ODT in water of 0.001ppb which puts it among the stronger smelling of the pyrazines and pyridines. A series of powerful thio-esters were also found in galbanum by Burrell in 1971 [1]. The undecatrienes have subsequently been found in several different oils, angelica root, lavender, and celery seed but always at low levels <0.1%. The oil of another species *Ferula asafoetida* is extremely pungent and contains unusually high amounts of 1-propenyl sec-butyl disulphide (>40%) and 1-(1-methylthiopropyl) 1-propenyl disulphide (>3%) [18]. Other pungent sulphides are common in the Alliaceae and Brassicae but these do not yield an essential oil as such.

Buchu leaf (*Agathosma betulina*) is another powerful oil which has been well studied. Lamparsky [19] identified both isomers of p-menthane-8-thiol-3-one and Kaiser [20] went on to identify the corresponding acetates and methyl esters. This ketone has become an important fragrance material in the form of "Corps de Cassis" and its odour threshold is among the lower ODTs in air measured by Givaudan at 0.17ng/L in a similar range to 'grapefruit thiol', 1-*p*-menthene-8-thiol which was determined by Demole [3] to be not only an important odourant of grapefruit juice but one which "….*practically settles the longstanding problem of synthetically reproducing the full bodied flavour of fresh grapefruit juice.*" It has subsequently been found in the peel oil of several grapefruit varieties, in the resin of *Gardenia resinoides* and at ppb levels in headspace samples of certain conifers; *Cryptomeria japonica, Tsuga heterophylla, Pinus paraviflora, Abies chinensis, Abies alba* and *Torreya nucifera* [21] but is otherwise not very abundant in nature. Other sulphur compounds are more abundant such as the 4-methyl-4-mercapto-2-pentanone found in basil oil and more than 20 flower headspace samples [21]. It also occurs in Sauvignon wines at trace levels along with 3-mercaptohexanol and 3-mercaptohexyl acetate, which impart the cassis character and are more usually associated with tropical fruits having been reported in passion fruit [22] and guava [23,11]. Other sulphur compounds occurring in foods have been reviewed by Boelens [24].

In addition to the extremely powerful 2-*sec*butyl-3-methoxy pyrazine other nitrogenous compounds, notably pyrazines and pyridines also have low threshold values and have been identified as trace components in a range of essential oils including orange [4], black pepper [25], vetiver [26] and ambrette seed [27] where they contribute subtly but importantly to the odour of the natural oil.

Geosmin is probably the most well known example of a high impact material with an ODT of 2x10^{-12}g/L and its source as bacterial and fungal volatile and a contaminant in water and wine is widely published but it is rarely reported in essential oils and is known in only 3 flower scents of the Cactaceae and a single rare Moraceae *Dorstenia turnerifolia* [28]. Dehydrogeosmin is both more frequent and more abundant in these species and is also well represented in many other Cactaceae.

Sesquiterpenes are very abundant in essential oils but not often noted for their low odour thresholds but the guaiene type ketone ‘rotundone’ has a very low threshold and is now becoming recognized as a key odourant in several oils including nargamotha (*Cyperus scariosus*), patchouli and black pepper [29].

Finally we should consider those materials that do not have such low ODTs but which impart the characteristic odour to the majority of oils on account of their abundance. Materials such as 1,8-cineole, thymol, linalool, eugenol, indole, geraniol all have ODTs in air of 2ng/L or less while the frequently more abundant terpene hydrocarbons limonene, pinenes, and caryophyllene have ODTs in air of 200-1000ng/L. So a few percent of the former in an oil will influence the odour and will dominate the odour when present over 10%.

In summary, there is a wide range of materials that contribute the “character impact” to essential oils whether present in a few percent or at sub-ppb levels and there are still more high impact molecules to be discovered. However these alone cannot singly represent the complex odour of natural oils which are difficult to reconstruct even with careful consideration of trace analytes and odour activity values and the interactions and synergies between components – and herein lies the element of alchemy that still conceals the reasons why certain oils smell the way they do.

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PL-04

Microbes at work in perfumery: The microbial community of Vetiver root and its involvement in essential oil biogenesis

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Background: The Vetiver plant and the essential oil

Vetiveria zizanioides (L.) Nash (Vetiver) is a perennial graminaceous plant growing wild, half wild or cultivated in many tropical and subtropical areas [1]. It is cultivated for its unique ability among grasses to produce in the root an essential oil, a complex mixture of sesquiterpene alcohols and hydrocarbons, which are mostly used as a basic material for perfumery and cosmetics. Because of this complexity, the oil is difficult to reproduce with synthetic aromatic chemical formulations. Moreover, differences in the quality of the oil may depend on genetic, environmental and technological factors [1, 2]. The biological activity of the Vetiver oil is also important. Termicidal, insecticidal, antimicrobial and antioxidant activities of Vetiver oil have been described [3, 4]. Finally, Vetiver, which also has a feed value [5], has been extensively used for land protection purposes as a barrier against erosion and for the restoration of contaminated lands [6].

The Vetiver oil is produced in secretory cells localized in the first cortical layer outside the endodermis of mature roots [1, 7]. Thus, while most essential oils are extracted from aerial tissues of dicotyledonous plants, the Vetiver oil is distilled from the roots of this monocotyledonous plant. The terpene oils in aerial tissues are often found as a complex mixture of different terpene compounds, including monoterpenes and sesquiterpenes, arising from complex interactions between the action of the cytosolic (mevalonate) and plastidic (2-C-methylerythritol-4-phosphate) pathways, and the oils accumulate as extracellular exudates or in specialized glands (lactifiers) or oil bodies (associated with trichomes) [8]. Much less is known about the biosynthesis, regulation and localization of terpenes synthesized in roots.

The microbial community of the Vetiver root

The idea that the Vetiver root bacteria may be involved in essential oil biogenesis was stimulated by light and transmission electron microscopy studies demonstrating the presence of bacteria in the cortical parenchymatous essential oil-producing Vetiver cells and in the lysigen lacunae in close association with the essential oil. In addition, axenic Vetiver cultured *in vitro* produced only trace amounts of oil with a strikingly different composition compared to the oils from *in vivo* Vetiver plants [9, 10, 11, 12].

In a recent paper, a number of bacterial species were isolated from surface-sterilized roots of 12 month-old Vetiver plants [12]. Among cultivated microorganisms, a total of 10 taxa were represented including 4 strains belonging to Pseudomonadaceae (VET-3, VET-4, VET-5 and VET-8), 4 to Enterobacteriaceae (VET-2, VET-7, VET-37 and VET-40), one to Aeromonadaceae (VET-1) and one to Micrococcaceae (VET-35). Some of them, *Pseudomonas* sp. VET-3, VET-4, VET-5 and VET-8, were taxonomically related to *Pseudomonas* spp. previously found as root-associated in a variety of plants belonging to *Magnoliophyta* division, *Brassica napus* (oilseed rape), *Arabidopsis thaliana* and others. Other isolates, VET-7 and VET-37, occupied taxonomical positions very close to *Enterobacter (Pantoea) agglomerans* and *Enterobacter ludwigii*, respectively. The phylogenetic analysis collocated VET-2 in the *Serratia liquefaciens* complex, while both phylogenetic and biochemical data demonstrated that VET-40 was a non-pigmented *Serratia marcescens* strain, and assigned both VET-7 and VET-37 to the genus

Enterobacter. Biochemical data and 16S rRNA sequence analysis identified VET-1 as *Aeromonas caviae*. Lastly, phylogenetic data collocated VET-35 close to *Arthrobacter nitroguajacolicus.*

The complexity of the Vetiver root-associated microbial community was also analyzed by using culture-independent methods [12]. In addition to most of cultivated species, this approach led to detection of: i.) uncultured α-proteobacteria related to *Afipia* genospecies 14 (Bradyrhizobiaceae); ii.) β-Proteobacteria belonging to the genus *Duganella*; iii.) uncultivated β-Proteobacteria belonging to the family Rhodocyclaceae which was also found in trembling aspen rhizosphere; iv.) β-Proteobacteria distantly related to the genus *Rubrivivax (*Burkholderiales); v.) γ-Proteobacteria closely related to *Pseudomonas corrugata* SB4, an endophytic bacterium capable of growing on 4-chloroaniline; vi.) bacteria belonging to Fibrobacteres/Acidobacteria group.

Role of the Vetiver root-bacteria in essential oil biogenesis

Owing to the close association of bacteria with Vetiver root cells producing essential oil, the ability of the root-associated bacteria to grow using Vetiver oil as a sole carbon source was tested. Enterobacteriaceae strains VET-2, VET-7, VET-37 and VET-40 grew well in a mineral medium supplemented with Vetiver oil as a sole carbon source (SMR-oil medium) with generation times (μ) ranging from 0.22 to 0.23, *Arthrobacter* sp. strain VET-35 also demonstrated ability to grow in these media (μ = 0.19). On the other hand, Pseudomonadaceae strains exhibited poor growth (VET-5 [μ = 0.14], VET-8 [μ = 0.14] and VET-3 [μ = 0.09]), whereas VET-4 and *Aeromonas* sp. VET-1 were unable to grow [12].

The ability of several root-associated bacteria to grow using the essential oil as carbon source led the authors to investigate the modification in the oil molecular structure caused by the bacterial growth in vitro. To this purpose, VET-2, VET-3, VET-5, VET-7, VET-8, VET-35, VET-37 and VET-40 were individually cultivated in SRM-oil medium for 40 h. Then, the Vetiver oil constituents were extracted from the exhausted growth medium and subjected to quantitative analysis by Gas Chromatography-Mass Spectrometry (GC-MS). Interestingly, each given microorganism specifically metabolized the raw Vetiver oil by releasing into the medium a large number of compounds, some of which were absent (or present in very low amounts) in the raw oil, but typically found in other commercial Vetiver oils [12].

Bacteria were also incubated in the presence of individual compounds of the Vetiver oil, for instance (+)-cuparene, one of the main components of raw Vetiver oil. Interestingly, only VET-7 and VET-35 were able to metabolize (+)-cuparene, which was almost completely biotransformed by VET-7 yielding an array of compounds characteristics of Vetiver oil as well as new interesting compounds. Moreover, when bacteria were fed on β-caryophyllene, the main sesquiterpene produced by axenic Vetiver, they oxidized it to β-caryophyllene oxide, and some of them (in particular VET-7, VET-8 and VET-40) produced typical Vetiver oil constituents, which were not produced by axenic roots [12].

Root-associated bacteria were also able to induce host plant gene expression. Indeed, most of the root isolates induced transcription of sesquiterpene synthase-encoding gene *Vet733* in the host axenic Vetiver. This sesquiterpene synthase produces a mixture of at least 7 different sesquiterpene hydrocarbons that usually do not appear in the Vetiver essential oil [13]. Interestingly, the highest *Vet733* gene expression was found in axenic Vetiver plants colonized with VET-7, VET-8 and VET-35, which were also the most active in β-caryophyllene metabolism [12].

Perspectives

The results of these studies shed a new light on the ecological significance of the association between Vetiver and its root-associated bacterial community and open the intriguing and immediate possibility to maneuver the molecular structure of the Vetiver oil either *in vivo*, by acting on the bacterial colonization of the plant root, or *in vitro*, by means of strain-dependent bioconversion processes. The ability of the isolated bacterial strains to produce arrays of new interesting compounds is of relevance for biological struggle strategies and will find many industrial applications. Moreover, the knowledge gained on the Vetiver system can be translated to other plants of relevant interest in both biotechnological and agronomic field for their specific activities and products.

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PL-05

Biotechnology in plant essential oil production. Progress and perspective in metabolic engineering of the terpene pathway

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Plant essential oils and, more generally, plant-derived secondary metabolites are of great economic importance and are widely used for applications such as flavors, fragrances, cosmetics and pharmaceutics. Plant-derived materials are often subject to fluctuations in price, annual production volumes and qualities due to factors such as seasons, geographical area of productions, geopolitical issues, climatic disasters and plant diseases. In addition, the price of compounds obtained from plants can sometimes be high due to limited cultivation, low abundance of the molecules of interest, high labor requirement for the harvest, or the depletion of natural resources.

Organic chemistry can be used to overcome the problems encountered with plant-derived products by developing processes for the production of the active compounds, independently of the original plant. In fact, synthetic routes were developed for many natural compounds, but given their often complex structures, cost-effective synthetic processes are not possible for many molecules. In the last decades, considerable progress has been made in biotechnology and gene manipulation. Particularly, the development of molecular biology tools has been used to elucidate many biosynthetic pathways in plants. An outstanding volume of information has been accumulated concerning biosynthetic genes, enzyme functions and regulation of complex biosynthetic pathways in model plants such as arabisopsis, rice or maize. The recent development of rapid and cost-effective DNA sequencing techniques provided data for hundreds of plants and sometimes for those so far neglected by scientists but yet of economic interest. The combination of this knowledge with the tools available for genetic manipulation and metabolic engineering, now opens great opportunities for developing biochemical routes for the production of plant natural compounds.

The typical constituents of plant extracts or essential oils are mainly derived from fatty acid, phenylpropanoid or isoprenoid pathways. The latter represent certainly the largest class of plant secondary metabolites with the monoterpenes and sesquiterpenes being classical constituents of essential oils and which display an extremely wide diversity of structures and biological properties. Because of the implication of terpene compounds in many biological functions and their economical value, the isoprenoid pathway has been a focus for many scientists. In this review, the current state of knowledge of terpenoid biosynthesis is briefly summarized and the perspective of developing new biocatalytic routes to terpene compounds traditionally obtained from plants, is discussed.

The biosynthesis of terpenoids

In all organisms, terpenoids are derived from the condensation of the two 5-carbon units, isopentenyl-diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Two distinct pathways leading to these universal precursors have been identified. The mevalonate (or MVA) pathway and the recently discovered DXP (for 1-deoxyxylulose-D-5-phosphate), otherwise known as the MEP (for 2-C-methyl-D-erythritol-4-phosphate) pathway [1,2]. These two pathways are not ubiquitously distributed in all organisms. The MVA pathway is found in fungi, animals and in the cytosol of plants while the MEP pathway is used by bacteria and in the plant plastids. The condensation of IPP and DMAPP, is catalyzed by prenyltransferases which produce the linear prenyl diphosphate precursor for each class of terpenes: geranyl-diphosphate (GPP), farnesyl-diphosphate (FPP) and geranylgeranyl-diphosphate (GGPP) for the mono-, sesqui-, and diterpenes respectively. The next step involves the terpene synthases which are part of a very large enzyme family. Terpene synthases play a key role in terpene biosynthesis since they catalyze the cyclization of GPP, FPP and GGPP to form the carbon skeletons of the terpenes and are thus at the origin of the extremely wide diversity of final structures. Finally, enzymes such as cytochrome P450 monooxygenases (P450) and oxidoreductases are involved in further modification and decoration of the terpene backbone yielding to the thousands of terpene compounds found in nature (Fig. 1).

The MVA and MEP pathways have been extensively studied. In addition to the identification of the genes and the characterization of all enzymes in these two pathways, important regulatory mechanisms have also been elucidated. This knowledge provides valuable information for the metabolic engineering of terpene diphosphate precursor supply in any organism. Another key element for the development of biocatalytic routes is the availability of the key biosynthetic genes leading to the target compounds, particularly genes encoding for terpene synthases. Since the cloning and characterization of the first terpene synthases in the early 90s, thousands of sequences for plant and microbial terpene synthases have been deposited in public databases and enzymatic activities have been assigned to several hundreds [3,4]. The number of terpene synthase sequences has increased exponentially in recent years, essentially due to the growing number of genome sequencing projects. However, of the terpene synthases leading to economically-important compounds, only a limited number of genes are available. One example is the recently cloned patchoulol synthase from *P. cablin*, a plant used to produce the patchouli oil in annual volumes exceeding 1000 tons [5]. Given the high diversity of terpene structures in nature, many terpene biosynthetic genes (i.e. terpene synthases, P450,…) remain to be discovered. Alternatively, interesting terpene biosynthetic genes can also be accessed by engineering available enzymes. The structure of some terpene synthases has been elucidated and, as illustrated by several reports [6,7], this information can be used to modify the product profiles and to synthesize new molecules.

The knowledge accumulated in terpene biosynthesis opens now possibilities for metabolic engineering of all the steps in the entire pathway. As illustrated below, several examples of metabolic engineering of terpene biosynthesis in microorganisms and plants demonstrate the possibilities of developing cost effective biochemical routes for production of terpene compounds.

Engineering of the terpenoid biosynthesis in microorganisms.

The engineering of microbial cells for the biosynthesis of plant isoprenoids usually combines the regulated expression of one or more plant genes together with genetic alteration of the host metabolism. The production of complex terpene molecules in a microbial host (usually *E. coli* or *S. cerevisiae*) offers several advantages over traditional extraction methods in scalability, consumption of natural resources, and process sustainability. It also has the potential to provide these molecules at a lower cost through large-scale fermentation processes from inexpensive sugar-carbon sources. So far, this strategy has been mostly applied to the production of high-value therapeutic compounds such as the sesquiterpene lactone and anti-malaria drug, artemisinin [8,9]. However, recent progress in engineering the isoprenoid pathways of microbial cells combined with fermentation optimisation [10] suggest that this route should rapidly become economically viable in other areas such as the F&F industry.

In bacteria and yeast, the existence of two distinct biosynthetic pathways (i.e. the mevalonate and the MEP pathways in *S. cerevisiae* and *E. coli*, respectively) (Fig.1) has led to the consideration of complementary approaches to generate microbial platforms for isoprenoid biosynthesis. The first strategy has been to transfer a heterologous pathway into a foreign host system while the second one focused on the optimization of the endogenous pathway. The former has the main advantage of avoiding putative feedback regulations and/or branching pathways as the metabolites are foreign to the producing host cells.

Both approaches have been successfully used to increase isoprenoid production and are reviewed below.In a recent study, the seven bacterial genes of the MEP pathway were successfully introduced into *S. cerevisiae* and were found to be functionally expressed as the heterologous pathway was able to rescue the otherwise lethal inhibition of the yeast endogenous pathway [11]. Conversely, the transplantation of the yeast mevalonate pathway into *E. coli* was achieved using an elegant approach based on optimized synthetic operons[9]. The resulting strain was recently reported to produce commercially relevant titers (27g/L) of amorphadiene, the olefin precursor of artemisinin[10]. Transplantations of complete or partial mevalonate pathways of bacterial origin into *E. coli* were also shown to produce high levels of the targeted isoprenoids [12,13].

The complementary approach has also been thoroughly investigated. Initial attempts of engineering the MEP pathway in *E. coli* aimed at identifying the main rate-limiting steps. This led to the identification of four enzymatic steps that were subsequently targeted to improve the metabolic flux [14]. Although significant improvements in isoprenoid production were achieved, the results of these studies also highlighted that the flux through the native DXP pathway is tightly regulated by mechanisms that are still poorly understood. Applying the same strategy to *S. cerevisiae* turns out to be more complex because yeast cells have a higher endogenous use of isoprenoids in particular for membrane sterol biogenesis. Consequently, pathway optimization had also to deal with reducing the incorporation of the precursors into competing pathways. These observations led to the downregulation of the yeast squalene synthase that catalyzes the first committed step in sterol biosynthesis. Other genetic modifications include the overexpression of rate-limiting enzymes and of a mutant allele of a transcriptional activator of sterol biosynthesis. Various combinations of these genetic alterations provided yeast strains capable of producing up to 150mg/L of sesquiterpene [8,15,16]. Of interest, important F&F molecules such as patchoulol were successfully produced in such recombinant strains [16].

Currently, the transplantation of a heterologous mevalonate pathway into *E. coli* appears as the most promising route for the generation of a microbial terpene platform [9,10]. However, one of the remaining challenges relates to the engineering of the latest steps of the pathway and more specifically those related to the functionalization of the terpene olefin [17]. Indeed, most of the complex terpenes of industrial interest are selectively hydroxylated by enzymes from the cytochrome P450 family. From that perspective, *S. cerevisiae* is considered as a better host for the heterologous expression of plant P450 because yeast endogenously expresses membrane-associated P450 enzymes that are structurally similar to the plant enzymes. Alternative strategies to further exploit the highest productivity reached in *E. coli* have also been investigated; the most promising focuses on the engineering of bacterial P450 through mutagenesis to alter their catalytic properties [18,19]. Validity of this approach is further supported by the observations that bacterial P450 are more stable in prokaryotic systems and tend to have a higher turn-over rate than their eukaryotic counterparts.

Engineering of the terpenoid biosynthesis in plants.

Traditionally, improvement of plants to introduce desired traits is made by crossing, selection, or introduction of mutation by irradiation. More recently, the development of stable genetic transformations of plants allowed targeted modifications, by introducing new genes or altering the expression of existing genes, resulting for example in altered metabolic profiles.

The metabolic engineering of terpenoid biosynthesis in plants is more complex than in microorganisms, first because of the sub-cellular organization of the terpene biosynthesis with the MVA pathway leading to sesquiterpenes in the cytoplasm and the MEP pathway leading to monoterpenes and diterpenes in the plastids. Another important parameter is the tissue-specific distribution of terpene biosynthesis. Biosynthesis of terpenes can occur in any part of the plant; leaves, roots, fruits, seeds, flowers, wood and bark have all been reported to accumulate terpenes in various plant species.

Several examples of metabolic engineering of terpene biosynthesis in plants have been reported and illustrate the potential of this approach to selectively modify the terpene composition of plants. For example, the total oil content in mint could be increased by over-expressing enzymes controling carbon flux in the monoterpene pathway [20,21]. In another example, the monoterpene profile of mint was altered by modifying the expression of an enzyme diverting metabolites from the menthol biosynthetic pathway [22], thus producing plants with an essential oil of higher commercial-value.

Probably the most attractive application of metabolic engineering of terpene biosynthesis in plants is their use as platforms for the production of terpenes. Several reports showed the feasibility of engineering plants to produce new terpene compounds, not present in the wild type plants. This is generally achieved by expressing a heterologous terpene synthase. Thus, plants have been successfully engineered to produce novel monoterpenes [23,24,25], sesquiterpenes [26,27,28] and diterpenes [29]. Interestingly, transformation of tobacco plants with a patchoulol synthase gene isolated from patchouli resulted in plants accumulated a mixture of sesquiterpenes with a composition similar to patchouli oil [26]. Effort has been made to increase the levels of terpene produced by the plants. It has been demonstrated that the co-expression of the terpene synthase with enzymes controlling the carbon flux in the isoprenoid pathway can lead to increased accumulation of terpene by the transgenic plants [26]. The compartmentalization of the terpene biosynthesis in plants was also exploited and it was observed that changing the subcellular localization of the terpene synthases can result in increased production of terpenes [26,30].

In many of the examples of engineering the terpene metabolism in plants, the accumulation of the terpenes is associated with reduction of plant growth and chlorosis, indicating possible toxic effects and/or deviation of carbon flux from essential metabolites [25,26,29]. Furthermore, the plants evaluated so far, are plants that do not naturally accumulate large quantities of volatiles metabolites and consequently a significant proportion of the terpene synthesized is lost by emission into the atmosphere [25,26]. Therefore, further modifications are still necessary to increase the accumulation of terpenes in the engineered plants and achieve cost-effective terpene production. In general, the recovery of essential oils from leaves is less laborious and less costly than from other parts of the plants such as the wood or roots. For example, essential oils from mint or patchouli are amongst the least expensive essential oils. Thus, engineering of the leaves, preferably in high-biomass producing plants, seems the most advantageous approach. The accumulation of high levels of volatile and hydrophobic compounds in plant leaves is usually associated with the presence of specialized structures: glandular trichomes. The biochemistry of trichomes has been studied in several plants and extensive information is already available [31] providing opportunities for metabolic engineering of terpene biosynthesis in this specialized structures. Amongst the recent findings, the characterization of trichome-specific promoters [32,33,34] will be helpful for the targeted expression of proteins in the trichomes with limited toxic effects and increased accumulation of the terpenes.

Concluding remarks

Over the past decades, progress in biotechnology and gene manipulation techniques allowed the development of an in-depth understanding of terpenoid biosynthetic pathways in plants and microorganisms. This knowledge enabled the engineering of the terpene metabolism in plants and microorganisms, demonstrating the feasibility of developing new biotechnology routes for compounds traditionally extracted from plants.

Plants are attractive platforms for this purpose. The cost of producing molecules from plants can be very competitive as illustrated by the relative low price of some plant essential oils such as mint or patchouli oil. In addition methods for extraction of secondary metabolites from plants are well established industrially and would therefore require limited optimization and investment. One major drawback is the complexity of the terpene biochemistry in plants and the time necessary to generate stable genetic transformations.

On the other hand, microbial platforms also have their own advantages over engineered plant platforms. These include easier genetic manipulations (allowing many different modifications to be introduced, compared and combined in a limited amount of time), a smaller genome size and hence a lower degree of pathway complexity and branching, the independence from climatic and geo-political conditions, and a more flexible process, in particular, in terms of scalability.

Finally, engineering of terpene biosynthesis in microorganisms or plants could also provide an effective route for production of new compounds. For example, the demonstrated ‘plasticity’ of terpene synthases opens the possibility of generating enzyme variants with altered product profiles or even increased selectivity for new molecules [6,7]. Alternatively, co-expression of terpene synthases together with ‘non-native’ plant or microbial P450 enzymes would generate new oxygenated terpenes which may have novel olfactory properties.

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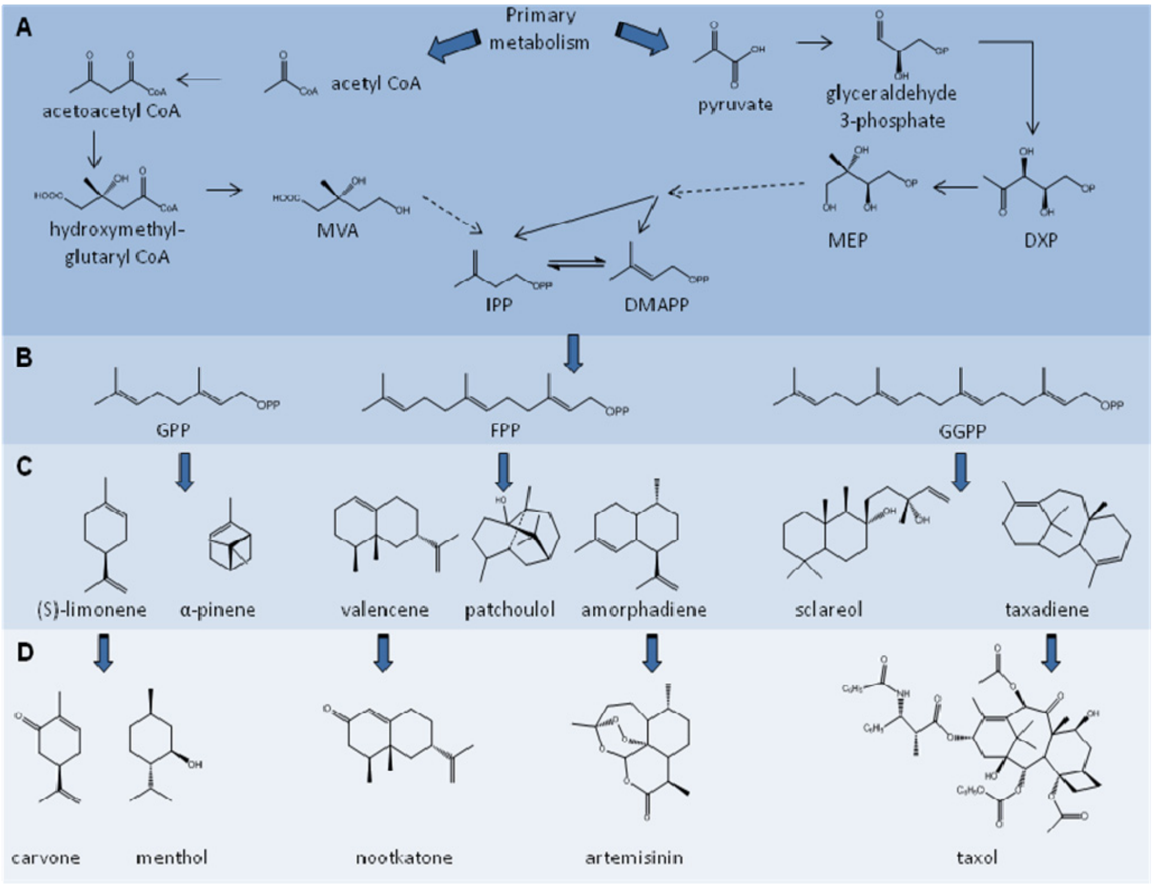
Figure 1. The successive steps in the biosynthesis of terpene compounds. (A) Biosynthesis of IPP and DMAPP via the MVA or the MEP/DXP pathway. (B) Condensation of IPP and DMAPP to GPP, FPP and GGPP, the linear prenyl diphosphate precursors of monoterpenes, sesquiterpenes and diterpenes. (C) Cyclization of GPP, FPP and GGPP by terpene synthases and formation of the carbon backbone of the terpene compounds. (D) Representative examples of terpene compounds generated by modification and decoration of the parental molecules. Dashed arrows represent multiple enzyme steps. MVA, mevalonate; MEP, 2-methylerythritol-4-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranlygeranyl diphosphate.



PL-06

Metabolomics: what is new?

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Metabolomics is the latest of the –omics. It is a major tool in functional genomics, quality control of botanicals, studies on the activity of medicinal plants, and systems biology type of studies of plants [1, 2]. Metabolomics has the ambitious objective to identify and quantify all metabolites in an organism. Numerous reviews have been pointing out the various advantages and limitations of the possible analytical methods. Where do we stand now? Do we have public databases for metabolomic data, similar as to gene and protein sequence databases? Can we expect these in the near future? What are the hurdles and can we overcome these? Many questions, few answers. Which is maybe not so surprising after all. The dream of all natural products people in the past 50 years was to develop reproducible analytical methods for compounds in plants. However, from literature we know that instead of a dream we have a nightmare. Some 25 years ago [3, 4], we reviewed the analysis of tropane alkaloids: 69 references on HPLC and 32 on GC analysis. But every year new studies are published on this separation, always with the same aim. Apparently standardization failed. The major reason is the improving quality of equipment, and chromatographic materials. But even a seemingly trivial aspect, the method of sample preparation, is not yet standardized. Harvesting, storage, grinding, extraction: steps that carry the risk of artifact formation or loss of compounds. For a total analysis of all metabolites we must conclude that we still have no suitable standard method. However, the basic idea of metabolomics, an unbiased analysis of the metabolome instead of a targeted analysis of certain compounds, has already shown to be a very powerful tool in life sciences, it is a novel gateway to new discoveries.

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Oral presentations

OR-01

Fragrance contact allergy: chemical reactivity of terpenic allylic hydroperoxides toward amino acids in iron systems.

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Allergic contact dermatitis associated with the use of scented products is an acknowledged health issue. Thus, it is important to better understand how certain fragrance ingredients may induce skin sensitization. Both terpenes *R*-(+)-limonene, the main component of citrus peel oil, and linalool, a major constituent of lavender essential oil, are frequently occurring fragrance chemicals in perfumed products. *R*-(+)-Limonene and linalool are not allergenic themselves. However, their oxidation on air exposure leads to the formation of several products, allylic hydroperoxides in particular, with a considerable sensitizing potency [1,2].

Terpene-derived allylic hydroperoxides are strongly believed to bind covalently to cutaneous proteins *via* a radical pathway. In order to better understand the molecular mechanism, the two hydroperoxides principally responsible for the allergenic activity of autoxidized *R*-(+)-limonene and linalool were synthesized. In presence of iron, the metal most involved in radical biological processes, the existence of radical intermediates issued from the prepared molecules was verified by ESR spin trapping. Afterwards, iron-catalyzed free radical reactions of *R*-(+)-limonene and linalool hydroperoxides with various amino acids were studied. Analysis of the reaction products were performed employing two-dimensional NMR spectroscopy in combination with LC-ESI-MS/MS methods. Investigations have shown the ability of these allergens to chemically modify amino acids in iron systems, either by formation of covalent adducts or through oxido-reduction phenomena.

This information further supports the hypothesis of a radical type mechanism for the hapten-protein binding of some allylic hydroperoxides derived from fragrance terpenes.

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OR-02

Tahitian liverworts – rich source of terpenoids and other volatile components

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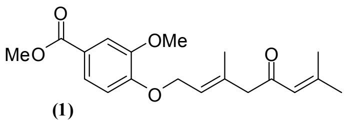
Liverworts are very small plants but they are known as rich sources of secondary metabolites, which contain mainly terpenoids with a variety of carbon skeletons [1]. The southern hemisphere is a region of extraordinarily high liverworts diversity with many endemic species [2]. In continuation of our investigation on the chemistry of southern hemispheric liverworts, we report in this communication the analysis of the volatile components of six liverwort species collected in Tahiti (French Polynesia).

All liverworts were extracted with diethyl ether and then each extract were analyzed by TLC and GC/MS. Remaining extracts were chromatographed on silica gel and Sephadex LH-20, and isolated compounds were purified by normal and reverse phase HPLC. Structures of the isolated compounds were determined by spectroscopic methods (^{1}H and ^{13}C NMR; HREIMS; FTICR-MS).

All investigated liverworts are chemically different, each species biosynthesizes own peculiar compounds and some of them are chemical markers of the liverworts species. *Trichocolea pluma* biosynthesized characteristic isoprenyl phenyl ethers (e.g. **1**), cembrane-type diterpenoids (e.g. **3**) are peculiar for *Chandonanthus hirtellus*, fusiccocanes (e.g. **4**) for *Plagiochila* sp. and herbertane-type sesquiterpenoids (e.g. **5**) for *Mastigophora diclados*. The main volatile component of *T. pluma* is vanillic acid methyl ester (**2**). This is the first report of the presence of compound **2** in the Marchantiophyta, and this compound could be the precursor of isoprenyl phenyl ethers occurring in this liverwort. *C. hirtellus* produces mainly diterpenoids. Besides cembranoids (e.g. **3**) this liverwort produces also fusiccocane- (e.g. **4**) and verticillane-type (e.g. **6**) diterpenoids. Compound **6** we also detected in *Jungermannia* species. Up to now verticillanes were found only in *Jackiella javanica* [3]. The Tahitian *C. hirtellus* also produces very volatile acetogenins (**7** and **8**) – pheromones occurring in marine brown algae and previously reported in *Fossombronia angulosa* [4]. The GC-MS analysis of ether extract of *Cyathodium foetidissimum* showed the presence of skatole (**9**), which is responsible for a very bad odor of this liverwort.



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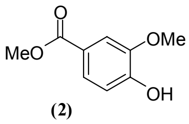

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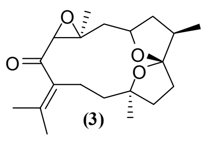

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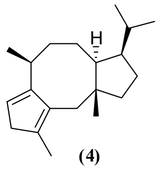

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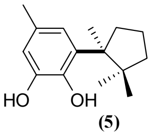

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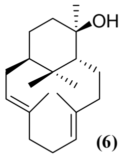

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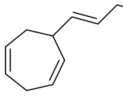
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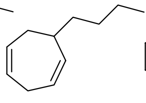
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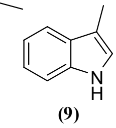

(9)

Acknowledgments

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OR-03

Structure-odor relationships of Santalane and Zizaane derivatives.

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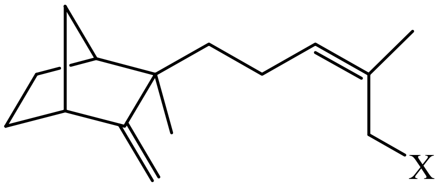
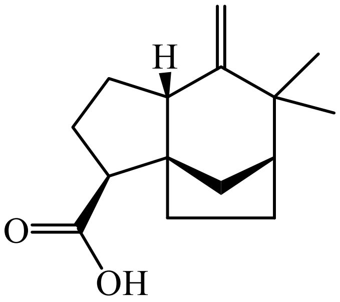
The main odorant constituents of Sandalwood essential oil are the sesquiterpenic alcohols α- and β-santalol, the latter (**1**) beeing responsible of the characteristic woody sandalwood note. In the course of our studies on structure-odor relationships (SOR), we isolated β-santalol from indian *Santalum album* essential oil, and used it as a starting material for the hemisynthesis of several santalane derivatives such as β-santalthiol (**2**), β-santalylmethyl ether (**3**) and other potent odorant compounds. We investigated also the SOR in the zizaane series, which includes many olfactively important components of vetiver essential oil. Then, several derivatives of zizaenoic acid (**4**) and khusimone (**5**) were prepared by hemisynthesis from pure **4** obtained from vetiver essential oil. All the odorant components described in this study were studied by GC-O in order to evaluate their olfactive character and potency.



X = OH : **1**
X = SH : **2**
X = OCH_3 : **3**



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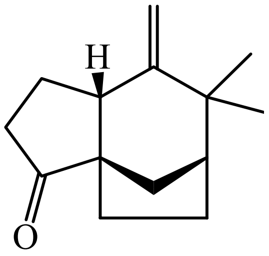

5

OR-04

Metabolomics and Genomics: combining tools for chemotyping and genotyping.

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The general approaches to herbal identification depend on morphological, anatomical, and chemical analyses, but these characteristics are often affected by environmental and/or developmental factors during plant growth. Molecular genetic methods have several advantages over classical morphological and chemical analyses. For instance, the genetic method requires genotype instead than phenotype, therefore DNA based experiments have become widely employed techniques for a rapid identification of herbal medicine. By using PCR approaches, nanograms quantities of DNA are required to amplify and yield sufficient amounts of template DNA for molecular genetic analysis. In this work, we demonstrate that molecular approaches using sequence analysis coupled to restriction mapping represent a powerful tool to distinguish different chemotypes barely distinguishable based on the sole morphology.

In higher eukaryotes, the 5S-rRNA gene occurs in tandemly repeated units consisting of an 120 bp coding region separated by a non-transcribed spacer of variable sizes. Although the coding region is highly conservative, the non-transcribed spacer varies from species to species in both sequence and length since it is apparently not under the same rigorous selection pressure as in the coding region. Thus, the diversity of the spacer region can be used as an identification basis.

Here we report on the successful use of the 5S-rRNA gene as a genomic tool for the unequivocal identifications of some important medicinal and aromatic plants such as *Acorus calamus* [1], *Salvia divinorum* [2,3], some chemotypes and species of *Artemisia* [4] and *Santolina*.

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OR-05

Evaluation of angiogenic activity of Lamiaceae essential oils by using the *in vivo* chorioallantoic membrane (CAM) assay

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The use of natural products, terpenes and essential oils for pain relieving, wound healing, anti-inflammatory properties and related features are well known since centuries and documented by numerous studies [1-3]. Angiogenic mechanisms and inflammation processes have correlations also to various tumor types. Angiogenesis is the physiological blood vessel formation and growth, where studies suggest that this can be a strategy with an important role in cancer therapy [3-5]. Within the frame of our ongoing project [6] on screening essential oils and their constituents as potential angiogenic and anti-angiogenic compounds the *in vivo* chicken chorio allantoic membrane (CAM) assay was used. The essential oils of some Lamiaceae plants such as *Ajuga chamaepitys* (L.) Schreber (18%, germacrene D), *Lavandula stoechas* L. ssp. *stoechas* (46%, camphor), *Origanum majorana* (L.) Karst. (85%, carvacrol), *Romarinus officinalis* L. (53%, 1,8-cineole), *Thymbra spicata* L. (65%, carvacrol) growing in Turkey were evaluated compared with standard substances suramine, thalidomide, cortisone for their angiogenic activities, which are traditionally used in folk medicine. The major compounds of the essential oils were also tested (conc. 0.5; 1 and 5 mg/ml) alone, in the same assay resulting in good and moderate inhibition of blood vessel formation in a scoring system. None of the oils, neither the pure compounds showed any membrane toxicity at the active concentrations. Membrane irritations (>50%) were observed in few cases. As a conclusion, the assay is suitable and reliable for all compounds polar or of lipophilic nature. The CAM assay is used as an animal alternative method for angiogenesis, membrane toxicity, systemic toxicity, and membrane irritation *etc* evaluations. On going *in vitro* cytotoxicity, cell migration and tube formation tests using specific cell lines will provide more information on the mechanism of the action of essential oils or their constituents.

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OR-06

Higher resolution comprehensive two-dimensional gas chromatography applied to the analysis of essential oils

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The present research is based on the full exploitation of the separation power of a 0.05 mm ID capillary, as comprehensive 2D GC (GC x GC) secondary column, with the objective of attaining very high-resolution second dimension separations. The aim was achieved by using a split-flow system developed in previous research, and a dual-oven GC x GC instrument. The column combination employed consisted of an apolar conventional column connected, by means of a T union, to a detector-linked high-resolution 0.05 mm ID polar analytical column and to a 0.05 mm ID retention gap; the latter was connected to a manually-operated split valve. The use of a split valve enables the regulation of gas flows through both analytical columns, generating the most appropriate gas linear velocities.

Comprehensive 2D GC experiments were carried out on essential oil samples with the split-valve closed (equal to what can be defined as conventional GC x GC) and with the split-valve opened at various degrees. The reasons why it is absolutely not convenient to use a 0.05 mm ID column as second dimension in a conventional GC x GC instrument will be discussed and demonstrated. On the contrary, the use of a 0.05 mm ID column as second dimension, under ideal conditions in a split-flow, twin-oven system, will also be illustrated and discussed.

OR-07

^{13}C NMR: a tool for essential oil analysis

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The analysis of natural mixtures of volatiles is commonly performed by GC (RI), GC-MS, GC-IRFT or a combination of these techniques. In a different approach, ^{13}C NMR spectroscopy could be used for the non separative identification of the individual components of complex mixtures such as essentials oils.
Following the pioneering work of Formácek and Kubeczka, a few research groups used ^{13}C NMR to confirm the presence, in the oil, of a given compound previously identified or suggested by GC or by MS. In parallel, we developed a computerized procedure, based on the analysis of the ^{13}C NMR spectrum of the essential oil, which allows the identification of the main components. The chemical shift value of each carbon in the experimental spectrum is compared with those of reference spectra compiled in a home-made spectral data library.
We will show the complementarity of NMR with conventional techniques, particularly for identification of compounds which possess close retention indices on polar and apolar columns and exhibit insufficiently differentiated mass spectra.
For instance, ^{13}C NMR allowed the identification of i) stereoisomers (and overall diastereoisomers) in various oils: cis- and trans-7-hydroxy-3,7-dimethyl-3,6-oxyoctanal isolyratols cedrene and funebrene α– and epi-α–bisabolol, bisepoxy-farnesatrienes and isopentenylindoles ii) thermolabiles compounds such as furanodiene .
Peucedanum paniculatum oil contained various components which were not identified by GC-MS. The essential oil was fractionated and the fractions were analysed by ^{13}C NMR. It appears that unassigned signals belonged to compounds bearing the β-cyclolavandulyl framework and various ester substructures. Therefore, eight esters were prepared from β-cyclolavandulol and β-iso-cyclolavandulol and then were identified.
Both subspecies of *Mentha suaveolens* grow wild in Corsica. Analysis of their essential oils followed by statistical analysis, led to the partitioning into three groups; all the samples of the *insularis* subspecies contained pulegone and cis-cis-p-menthenolide (reported for the first time as a natural product) as major components.

OR-08

Appling chemometric methods to identify volatile aroma active compounds of oregano

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Intentions of chemical analyses is amongst others to find a fast and efficient method for complex analytical questions, for example, in life sciences. Particularly, new analytical methods produce a flood of data, often very badly arranged. A powerful way to overcome this problem is the application of chemometric methods.

Within this investigations three oregano trade specimens were analyzed to identify the volatile aroma active compounds. Two techniques were applied – gas chromatograpy-olfactometry and human sensory evaluation.

With the aid of chemometric methods (principal component analysis, hierarchical cluster analysis, linear discriminate analysis, partial least-squares regression) aroma impact compounds of the main trademarks of oregano could be identify. Therefore it is possible the reduce the analysis of sensory and olfactometry to relevant attributes.

So a classification of new species is better and quite faster which is the premise for a fast identification of new potential genotypes for plant breeding of oregano.

OR-09

Microbial transformation of isopinocampheol and caryophyllene oxide as terpenoids with four carbon ring

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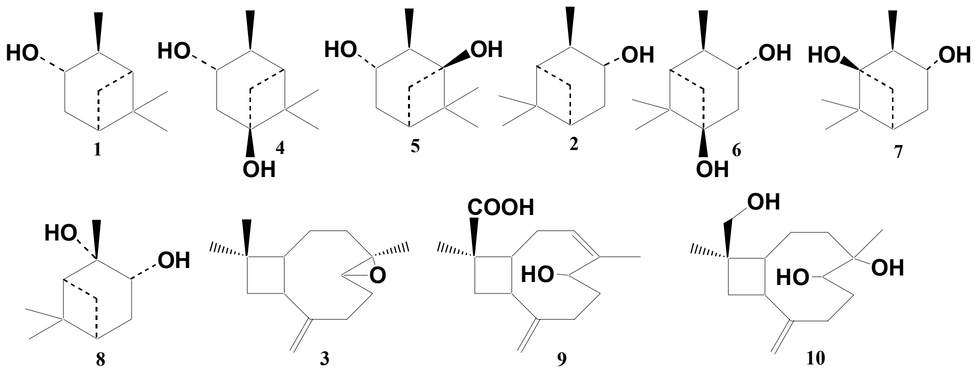
Authors are continuing the studies on microbial transformation of terpenoids in order to search the useful compounds [1-3]. This time authors investigated the transformation of (+)-(**1**) and (-)-isopinocampeol (**2**) in the relation of the metabolism of β-pinene and caryophyllene oxide (**3**) as terpenoids with four ring carbon ring by using *Aspergillus niger* TBUYN-2.

Microorganisms was cultivated rotatory (100 rpm) in the 200ml Czapek-pepton medium (500ml Erlenmyer flask) at 30°C for 3 days. After full growth of microorganisms each substrate (100mg) was added into the cultured broth and biotransformed under the same conditions described above for 4-7 days. 2 ml Aliquiots of cultured broth was applied to Extrelut NX column every day for the time course study and metabolites was extracted with ether. Ether extract was applied to TLC and GC-MS to check the manner of time course changes of the metabolites. The metabolites were isolated by silicagel CC and the stereostructures were established by a combination of high-resolution NMR spectrum and chemical reactions.

(+)- 5-Hydroxy- and 1-hydroxyisopinocampheol (**4** and **5**) from **1**, 5-hydroxy-, 1-hydroxy- and (+)-2-hydroxyisopinocampheol (**6** ~ **8**) from **2**, (-)-caryophyllene-12-oic acid (**9**) and (-)-caryophyllane-6, 7, 12-triol(**10**) from **3** were obtained. Comound **11** was obtained from **3** under the shaking for 4 days in the medium without microorganisms. This time we will discuss the the metabolism of (+)- (**1**) and (-)-isopinocampeol (**2**) and caryophyllene oxide (**3**) by *A. niger* TBUYN-2 as terpenoids with four ring carbon ring and the relation of the metabolism of β-pinene.



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OR-10

Essential oils and their major constituents as alternative antifungal agents: a South African perspective

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The demand for organically produced commodities is rapidly expanding throughout the world. Food producers face many challenges in providing products that are attractive, free of disease and toxic residues, and display a long shelf-life. Fungal pathogens are becoming increasingly resistant to fungicides, while legislation regarding residue levels and waste disposal is becoming more restrictive. Essential oils, generally recognized as safe, have the potential to address all these problems. In our investigation, several essential oils and their constituents were evaluated for their ability to control commercially important fungi of food crops. Essential oils from two indigenous South African *Lippia* species were included as potential mycobiocides. The oils were analysed by GC-FID and GC-MS to determine their chemical compositions. *In vitro* assays proved the efficacy of the oils against the pathogens. Commercial trials were conducted to access the efficacy of the essential oils for the postharvest management of avocado, mango and citrus fruit. Quality parameters measured indicated that the overall quality of the fruit was maintained. Fruit treated with essential oil-amended coatings displayed less moisture loss than their counterparts treated with conventional fungicides. In general, the oils from the two indigenous *Lippia* species, as well as spearmint and lemongrass oil, exhibited promising antifungal properties. Oils containing citral (a mixture of the E- and Z-isomers) as major constituents seem to be excellent candidates for the control of *Fusarium oxysporum* in soils and *Rhizoctonia solani,* a destructive pathogen of potato crops. Essential oils deserve more attention as mycobiocides, to use in conjunction with, or as alternatives to synthetic fungicides.

OR-11

Unattended odours influence facial attractiveness

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The effect of New Caledonian sandalwood oil on physiological, psychological, and endocrine parameters as well as the influence of the odor on attractiveness ratings was measured in 50 subjects. The study focused on the possible aphrodisiac effect of sandalwood oil. Geranium oil served as an odor control [1], odorless air as an additional control condition. Each participant was tested in three sessions on three different days. The order of the essential oils vaporized in the testing room and air was arbitrary, and in contrast to other studies [1, 2] the subjects were unaware of odors being presented. At the beginning and the end of each session, blood pressure was measured, and the participants had to fill in a subjective mood questionnaire (POMS). In addition, saliva samples were collected in order to determine salivary cortisol level. During the sessions the participants had to rate a number of faces presented on a computer screen for attractiveness while various physiological parameters were recorded. At the end of the final session the subjects were debriefed and had to rate the essential oils for pleasantness and familiarity. The data indicated no significant influence of sandalwood oil on physiological and psychological parameters compared to geranium or odorless air. Although the effect was not significant in terms ANOVA, salivary cortisol levels remained highest under the influence of sandalwood oil [3]. Even though the participants did not detect any odor during testing, they rated the presented faces significantly more attractive in the sandalwood condition in comparison to odorless air and geranium. Both essential oils were experienced as pleasant. Geranium oil was significantly more familiar than sandalwood oil.

In a supplemental study the effect of Haitian vetiver oil on the same parameters is now performed, and the results of this investigation will be presented.

Acknowledgements
All collaborators, post and present postgraduate students, who contributed to this study, are greatly acknowledged.

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OR-12

Fragrances and Flavours of Zululand

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There is a constant need for new flavours and aroma's in the world. Latest trends show an interest in blends of known and unknown, healthy and popular. There are more than 22 000 different species of seed plants indigenous to Southern Africa of which very few has been investigated for potential in the perfumery and flavouring industry. Zululand has a wide variety of flora, with well over 2000 plant species, many of which are endemic to the region of especially the Ongoye and Nkandla forests and coastal Zululand. It can be said that there is a wealth of species still to be studied and developed. The unspoilt rural environment of Zululand is particularly favourable for the production of novel essential oils and extracts. Zululand is a poverty stricken area and need opportunities for development e.g. natural products.

The aim was to identify the aromatic plants with potential of commercial production for the fragrance and flavour industry. The indigenous plants that have been investigated for potential production include: *Mondia whitei* (White's ginger*), Warburgia salutaris* (Pepper bark tree)*, Siphonochilus aethiophicus* (Wild ginger), *Osyris lanceolata* and *O compressa* (African sandalwood), *Cymbopogon validus* (Blue grass), *Spirostachys africana,*(Tamboti), *Tarchonanthus camphoratus* (Sagewood), *Helichrysum oderatissimum* (sweet everlasting*), Acacia nilotica* (Lekkerruikpeul, sweet smelling pod), *Croton gratissimus* (Bushman's perfume, lavender croton)*, Heteropyxis natalensis* (Lavender tree), *Lippia javanica* (Lemon fever bush), *Dovyalis caffra* (Kei apple) and *Sclerocarya birrea* (Marula). Chemical analysis has been done on most of the species and sensory evaluation is now done on some of the species.

Young Scientist Session

YS-01

Polymorphism of “katrafay”, a complex essential oil from Madagascar

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Cedrelopsis grevei (Rutaceae) is an aromatic and endemic tree to Madagascar, growing wild in the South and West parts of the island. This species is commonly referred to as ‘‘Katrafay’’ by the local people. The bitter bark is traditionally used as a febrifuge, to relieve muscular fatigue when it is soaked in bath water [1].

C. grevei essential oils are mainly produced from either the leaves or the barks. They are marketed locally and in the areas of the North (under the name of katrafay oils) for their invigorating, tonic, anti-inflammatory, febrifuge and antalgic properties. The few works carried out on these essential oils have shown great variability and complexity in their composition, dominated by sesquiterpenic structures [2,3].

We recently reported on the chemical composition of the bark oil of *C. grevei* stemming from six representative sites of the distribution area of the species [4]. 71 molecules were identified using a combination of chromatographic techniques [gas chromatography (GC)] and spectroscopic techniques [GC–mass spectrometry (MS)]. Four chemical patterns were distinguished (from 21 samples) showing a preferential geographic grouping of each of them: two in the North-West and two others in the South-West of Madagascar.

Otherwise, for a non destructive exploitation of the species, leaf oils have been analyzed showing the same main compounds as for the bark essential oils from a site. The corresponding results will be presented and discussed with a view for the sustainable exploitation of the resource.

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YS-02

Chemical characterisation and antimicrobial capacity of essential oils from *Eremophila longifolia* (F. Muell) (Myoporaceae): an indigenous Australian medicinal plant with high geographic chemovariabiliy

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Essential oils from the Australian Aboriginal medicinal plant *Eremophila longifolia* (emu bush) were characterised using GC/MS and NMR, and antimicrobial capacity investigated using disc diffusion and broth dilution. Leaves were collected from various locations within New South Wales (NSW, Australia) and hydro-distilled for volatile leaf oils. Overall yield and oil constitution differed markedly according to the geographical region from which the plants were collected. *E. longifolia* demonstrated a variety of chemotypes not yet recognised. Four further chemotypes are now recognised within NSW, in addition to the two previously characterised from other regions of Australia; the Northern Territory (NT) [1] and the Murchison district in Western Australia (WA) [2]. Characterisation of NSW chemotypes revealed that here *E. longifolia* does not produce the carcinogenic volatile compound, safrole, as previously described in the leaf oil from Murchison specimens (WA) [2]. Two separate chemotypes within NSW yielded oil as high as 7% w/w and 3.5% w/w consisting mostly of iso-menthone (70-90%) and karahanaenone (≈80%) respectively; marking these as the most abundant natural sources of these compounds so far described [3,4,5]. The two remaining chemotypes had a much lower yield, 0.2 and 0.7%, and were more similar to the chemotype found in the NT; leaf oils consisting of limonene (≈20%) and borneol (20-30%) respectively [6]. Antimicrobial assays of volatile oils from the four chemotypes revealed a moderate to high antimicrobial capacity, varying with species and chemotype. Traditional (location specific) indigenous applications of the oils are consistent with these results [6]. The essential oil from *E. longifolia* may thus be a likely candidate for further investigation into cosmeceutical use addressing a similar market niche to that already successfully occupied by the essential oil of *Melaleuca alternifolia* (tea tree oil) and more recently *Backhousia citriodora* (lemon myrtle oil). Further investigations (wound healing, anti-inflammatory and cultivar chemotype requirements) are in progress.

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YS-03

A thorough analysis of the chemical composition of treated bergamot oil (*Citrus bergamia*)

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Bergamot oil is one of the most valuable products used in cosmetics and fragrances, and, for its antimicrobial characteristics, in pharmaceuticals as well. An important fraction of the essential oil is constituted by oxygen heterocyclic compounds (psoralens and coumarins) which have phototoxic effects, therefore limiting its use in the final products [1]. Indeed, a series of treatments are applied to bergamot oils by producers in order to remove, partially or completely, these undesired substances. In this study, commercial samples of treated bergamot oils have been screened for their chemical variability depending upon the characteristics claimed onto the label (terpeneless, colourless, bergapten-free, furocoumarin-free) [2]. GC-FID, GC-MS, enantio-GC-FID and HPLC allowed for the achievement of the complete chemical characterization of these samples. Furocoumarin and bergapten-free oils showed a volatile fraction superimposable to that of untreated cold-pressed bergamot oil, which presented 58.3 g% of monoterpenes, 34.0 g% of oxygenated monoterpenes, 1.1 g% of sesquiterpenes, and the remaining part constituted by oxygenated sesquiterpenes, carbonylic compounds, esters and others. Terpeneless oils presented only 0.7 g% of monoterpenes which were higher in the terpeneless-colourless oils (9.8 g%); however, in both samples, oxygenated monoterpenes constituted the main fraction (94.9 and 86.6 g%, respectively). Quantification of compounds has been carried out by means of both peak area integration and internal normalization with the use of response factors. Chiral analysis allowed to establish the enantiomeric ratios for the most important constituents. Finally, by means of HPLC, it was possible to determine the ranges of ppm for herniarin, sinensetin, citropten, bergapten, bergamottin and 5-geranyloxy-7-methoxycoumarin. The analytical findings of this study have shown a specific correlation between the technological process and the composition of the essential oil, to the point that it becomes possible to "trace" the history of a sample [2]

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YS-04

New cyclodextrin derivatives to improve selectivity, specificity and speed of essential oil ES-GC analyses

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Since the end of the ’80, cyclodextrin (CD) derivatives have been shown to be highly effective chiral selectors in gas chromatography [1-3]. Many applications of enantioselective GC (ES-GC) with cyclodextrins as stationary phases are from the field of flavour and fragrance [4-6] where the enantiomeric excess (EE) and/or ratio (ER) determination is a very important task to define not only the biosynthetic pathway or the origin of a compound, but also to evaluate the authenticity of a sample or to detect frauds.

Recent studies [7] overcome the main limit of ES-GC analyses for routine application, i.e. the long analysis time, by short and narrow bore columns looking for the best compromise between analysis time gain and loss of resolution. The limiting condition to enable a correct EE and ER determination is a resolution of at least 1.5 for the investigated enantiomers.

Several successful 6-*tert*-butyldimethylsilyl cyclodextrin derivatives with the same substituent (symmetrically substituted CDs) in position 2 and 3 were synthesized as stationary phases for ES-GC analyses. However, the development of new CD derivatives is still necessary to improve their separation capacity and/or specificity and to speed-up ES-GC analyses for the routine Q.C.

This study is part of a project aiming to develop new CD derivatives with different apolar and polar substituents in position 2 and 3 (asymmetrically substituted CDs), i.e. 2-*O*-ethyl-3-*O*-methyl-6-*O*-*t*BDMS-β-CD (EM), 2-*O*-methyl-3-*O*-ethyl-6-*O*-*t*BDMS-β-CD (ME) and 2-*O*-methyl-3-*O*-acetyl-6-*O*-*t*BDMS-β-CD (MA) to increase both the number of separated chiral compounds and their resolution.

More than a hundred standard racemates of chiral molecules from the flavour and fragrance field and a set of real-world samples were analysed with the new derivatives and their performance was compared to that of the corresponding symmetrically substituted CDs (i.e. 2,3-di-*O*-ethyl-6-*O*-*t*BDMS-β-CD [8] and 2,3-di-*O*-methyl-6-*O*-*t*BDMS-β-CD [9], 2,3-di-*O*-acetyl-6-*O*-*t*BDMS-β-CD [9]).

The new derivatives showed good chromatographic properties and enantioselectivity providing the ME- and EM-CDs an higher separation capability while the MA-CD higher specificity for carbonyl compounds; they are therefore suitable to be explored to speeding-up ES-GC.

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YS-05

Antimicrobial activity of essential oils: the possibilities of TLC-bioautography

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Essential oils are well-known for their antimicrobial activity against different plant and human pathogenic microorganisms [1-3]. The results of the most commonly used antimicrobial assays cited in the literature are very different, sometimes their reliability is questionable. Direct bioautography is a laboratory technique to detect substances affecting the growth rates of test microorganisms in complex mixtures and matrices [4].

The aims of our research include the phytochemical characterization of the essential oil extracted from various medicinal plants that are important from therapeutic and economic aspect, the optimization of direct bioautography for selected strains and the microbiological investigation of the effect of essential oils against human and plant pathogenic microorganisms.

The antibacterial effect of the essential oils obtained from a few important medicinal plants (e.g. thyme, eucalyptus, spearmint, lavender, cinnamon) was investigated by TLC-bioautographic method. The chemical composition of the essential oils was analyzed by TLC and GC-MS. The solvents applied in thin layer chromatographic developing systems were also tested.

According to our results toluol, ethylacetate, ethanol and chloroform had no inhibiting effect on the test bacteria, thus they did not influence detection with direct bioautography [5]. Antibacterial activity of thyme, lavender and cinnamon oils, as well as their main components (thymol, carvacrol, linalool, eugenol) was observed against two plant pathogenic bacteria (*Xanthomonas campestris pv. vesicatoria*, *Pseudomonas syringae pv. phaseolicola*) and some human pathogens (*Staphylococcus epidermidis*, *S. saprophyticus*, *S. aureus*, methicillin-resistant *S. aureus*).

On the whole, the antibacterial activity of essential oils is supposed to be related with its most abundant components, but the effect of the minor components should also be taken into consideration. Direct bioautography is more cost-effective and faster compared to traditional microbiological laboratory methods (e.g. disc-diffusion, agar-plate technique). In future studies, complex antimicrobial effects will be modelled in vitro and in vivo.

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REACH Session

REACH-01

Perspective of the fragrance industry

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REACH, the EU regulation for the Registration, Evaluation and Authorisation of Chemicals, came 100 % into force on 1 June 2008. REACH requires EU manufacturers and/or importers of substances to register them with a central European Chemicals Agency (ECHA) unless the regulation indicates otherwise.

The chemicals currently on the EU market (phase-in substances) had to be pre-registered between 1 June and 1 December 2008 to benefit from extended registration deadlines depending on the tonnage band and the hazardous properties of the substance. The staggered deadlines are 30 November 2010, 31 May 2013 or 31 May 2018.

Companies that did not pre-register by 1 December 2008 can no longer be able to market their substances in the EU until they have submitted a full registration.

Pre-registration leads to the formation of a Substance Information Exchange Forum (SIEF) for each substance where all companies that have pre-registered the same substance come together to exchange studies and to organise the joint submission of certain part of the registration dossier.

Most NCSs are not exempt from REACH; they must be registered because of hazardous constituents. The producers and downstream users of NCSs promote the adoption of a special protocol for the registration of these substances that has been shared with ECHA.

ECHA has received more than 2,7 millions of pre-registrations covering more that 150,000 substances. SIEFs are much bigger than expected and industry faces serious problems to get organised in the SIEFs to be able to comply with the first registration deadline of November 2010.

Both EFEO and EFFA trade associations covering fragrances and NCSs currently cooperate for data exchange from the fragrance substances, which occur as constituents in the NCSs.

The presentation gives a state of play of REACH explaining the challenges being faced by producers and users of essential oils and aroma chemicals and a more specific description of the situation related to the registration of NCSs.

REACH-02

Toxicological Challenges for Essential Oils in REACH

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REACH requires that all substances produced or imported at >1 ton per year in the European Community are submitted for registration following a phased-in timetable according to tonnage until 2018. Depending upon the tonnage band a pre-determined set of toxicological and ecotoxicological information is required for submission along with a risk assessment (Chemical Safety Report) which considers consumer, occupational and environmental exposures. These requirements apply to Essential Oils, technically termed “Natural Complex Substances” (or NCS’s) in REACH, and presents a unique set of challenges, not only due to the cost and administrative burden for essential oils producers and importers, but also in the approach used for the toxicological evaluation of these oils. Specific technical problems are encountered for the NCS’s. The identification of representative specifications for the conduct of studies and natural variability of product composition is an important and sometimes complex area to resolve even before studies can begin. The relevance and adaptation of toxicological test methods to complex mixtures such as essential oils and the interpretation of results and use in risk assessment presents perhaps the greatest challenge. Alternative test and evaluation strategies for NCS’s, based on a components approach or toxic equivalency factors have been proposed and offer the potential for strategic testing and reduction of animal use in achieving REACH compliance. Finally, different approaches may also apply dependant on whether the NCS is a “simple” well-defined substance or one that it complex in nature with many components which is not fully characterised. Industry has already started to work on many of these aspects through REACH consortia and joint EFEO and EFFA activities [1], yet further refinement in the assessment approach and experience in dossier submission and acceptance with the European Chemicals Agency (ECHA) remains a real challenge for Essential Oils producers and importers.

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REACH-03

REACH-Driven developments in analysis and physico-chemistry

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The enforcement of the REACH regulation in the fragrance domain has created new challenges for the analytical and physical chemist. Many chemicals used as perfumery ingredient are hydrophobic because of their higher substantivity (*i.e.* persistence after application). As a result, the usual protocols may appear unsuitable and new methods must be developed. Biodegradation studies sometimes call for the quantification of traces of such hydrophobic analytes in complex media (waste water, aqueous surfactant solutions). Existing sample preparation techniques are either inefficient or time consuming. A new approach is proposed, based on single-use absorbants, which allows accurate quantification down to the 100 ppb range. This extremely simple technique allows high throughput analyses.

Determining the environmental profile of a compound requires the determination of some physical constants. Among these, solubility in water can be obtained from theoretical models, or experimentally, but the resulting values may greatly differ as a function of the model or the protocol. Several experimental approaches are critically discussed and compared to a reference technique. The air-to-water partition coefficients are determined using an improvement of the previously developed static-and-trapped headspace technique.

Poster Presentations
Session A

A-01

Composition of the essential oils of *Calamintha grandiflora* (L.) Moench

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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% [1-3]**.** *Calamintha* species are locally called as ''Güzel Nane, Dağ Nanesi, Miskotu,, Dağ Miskotu, Yabani Oğulotu'' and used as herbal tea [4-6].

In the present study, aerial parts of the *Calamintha grandiflora* (L.) Moench collected from the following regions of the Eskişehir; Efsunbaba (A) and Kütahya; Domaniç (B) province.

A : Eskişehir; Efsunbaba , 14.08.2002 (ESSE: 14413)
B : Kütahya; Domaniç, 18.07.2001 (ESSE: 14380)

The oils were extracted from aerial parts of *Calamintha grandiflora* using Eppendorf Microdistiller®. Oils were analyzed by GC and GC/MS.

Isopinocamphone (35.4%), pinocamphone (8.4 %) and linalool (7.6 %) were detected as main constituents in sample A; β-Pinene (19.4%), isopinocamphone (19.0%), pinocamphone (15.1%) and limonene (12.1%) were found as main constituents in sample B. The results are in accordance with the previous report [7].

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A-02

Flavour & Fragrance analysis: easy heart cut MDGC with mass spectrometric detection in 1st and 2nd Dimension

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In flavour and fragrance analysis coelutions are often observed in gas chromatography. In order to resolve those problematic regions they are transferred into a second column using heart cut multidimensional gas chromatography GC/GCMS [1]. A Carbowax column with 30 m, 0.25 mm i.D. and 0.25 μm film was coupled to a RTX-5 30m, 0.25 mm, 0.25 μm in the second dimension.
To have also identification of the peaks in the first dimension an FID/MS splitting was realised [2]. This was created by a capillary split connection to feed the effluent at the FID (1 dim) partly into MS simultaneously (1/25 relativ to FID). For this a desactivated fused silica tube (1m, 0.15 mm ID) was lead via the interface from the GC of the first dimension into the second dimensional GCMS. Both columns the split connection and the second dimensional column are mounted into the MS detector by using a special connector [3]. While the FID chromatogram can be used for an area normalisation report the MS full scan data can be used to identify coelution regions in the first dimension. Those can be then transferred to the second column in a subsequent run. In cut runs the FID/MS splitting transfer line has to be blocked to prevent coelutions of cutted peaks from the second dimension with first dimensional analytes. This is achieved by a pressure increase of an auxiliary pressure unit which reverses the flow in the splitting line. Three cuts were done on a perfume sample. Below major peaks of linalool, hexanal and diethyl phtalate in the first dimension small peaks of trans pinanol, isobornyl acetate and dimethyl jasmonate are resolved after transfer to the second dimension. The identification was done using the library FFNSC1.3 dedicated to Flavour and Fragrance compounds with linear retention indices.
As a conclusion this multidimensional GC/GCMS configuration offers easy and reliable analysis of flavour and fragrance samples.

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A-03

Volatile composition of predominant *Erica arborea* honeys from Corsica

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Honey is prepared by the honeybees from raw materials as nectars and/or honeydew; nectar is an aqueous sugar-containing and flavourful secretion, directly produce by plants. The workers bees have important effects as chemical changes and concentrations of the raw material. In general, aroma and flavour of honey are formed by volatile materials, which depend principally upon the nectar(s) whence it originates [1,2].

The Corsican honeys are certified by the National "A.O.C" and the European "A.O.P" denominations: "Miel de Corse-Mele di Corsica" divided into six ranges. In the current study, 14 honey samples of "Maquis de Printemps" range were selected according to their melissopalynological and sensorial characteristics [3]. These honeys have been prepared by bees from nectars issued of shrub vegetation dominated by *Erica arborea*.

For each sample, quantitative and qualitative melissopalynological data are established in order to produce the microscopic picture of the *Erica arborea* predominance variability and the importance of secondary nectariferous species representation. Moreover, the volatile compositions of *Erica arborea* flowers and honey samples were carried out using HS-SPME, GC and GC-MS. The aim of this work was to establish the relationships between volatile fractions of nectar from *Erica arborea* flowers and those of corresponding honey.

In the volatile fraction of *Erica arborea* flowers, 20 components were identified amounting to 85.5% of the total composition. The major components were oct-1-en-3-ol, (E)-β-ocimène and benzaldehyde. The volatile fraction of honeys was characterized by 18 components with octanal, benzaldehyde, phenylacetaldehyde, *para*-anisaldehyde, 4-propylanisol and *para*-menth-1-en-9-ol as main compounds. However, honey compositions showed qualitative and quantitative differences on their chromatographic profiles.

Finally, aromatic complexity of "Maquis de Printemps" honeys is linked to volatile compositions, sensorial properties and palynological associations. Furthermore, the variability of these characteristics appears to be dependant of the altitudinal repartition of *Erica arborea* and other melliferous sources.

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A-04

Volatile constituents of *Dictyopteris membranacea* native to Malta

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Marine algae are rich sources of structurally novel and biologically active primary and secondary metabolites which could be considered potential ingredients in the pharmaceutical, food and cosmetic products.

In fact, extracts of seaweeds from the Mediterranean Sea as well as the Atlantic, Pacific and Indian oceans, have shown antibacterial, antifungal and antiviral activities [1,2], as well as cytotoxicity and antimitotic properties [3]. Furthermore, some species of *Dictyopteris* genus are known for their characteristic odour and have been studied for their essential oils. The brown alga *D. menbranacea* showed the C_{11} hydrocarbons dictyopterene A and dictyopterene C, as the major constituents of its essential oil. These compounds are well known as sex pheromones of brown algae [4]. Studies of different species of the genus *Dictyopteris* have also demonstrated the importance of C_{11} hydrocarbon derivatives as deterrent feeding for several fish species [6]. In the present study, we reported the essential oil profiling obtained by steam distillation of *D. membrananacea* native to Malta. It was characterised by a high content of C_{11} hydrocarbons derivatives (87.5 %), while the sesquiterpenes were present in minor percentage (5.8%) and the monoterpenes only in traces. Nineteen components were identified (96.2% of the total volatile fraction). The main constituents were a series of C_{11} hydrocarbon derivatives such as 6-butylcyclo-1, 4-heptadiene (68.8 ± 1.03), dictyopterene A (12.06 ± 0.11) and dictyopterene C (2.8 ± 0.55). The analysed EO samples did not contain sulphur derivatives, which are known as other typical constituents in *D. membranacea* [1,4,5,7].

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A-05

Characterization of Sicilian mandarin (*Citrus deliciosa* Ten.) essential oil

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The essential oil obtained from mandarin is a valuable product employed in cosmetic and food industry due to its characteristic and appreciated organoleptic properties. Sicilian mandarin oil is industrially obtained by mechanical cold pressure (FMC, Sfumatrice and Torchi) of the fruit peel of *Citrus deliciosa* Ten., that belongs to the family of Rutaceae. The complexity of the mandarin group from the botanical point of view, the absence of the specification of the cultivar studied by different authors render the characterization of mandarin essential oil quite complex. Although many papers indicate *Citrus reticulata* Blanco, the Sicilian production is limited to *Citrus deliciosa* Tenore [1].

Recent literature lacks of a complete view on the volatile, non-volatile and chiral components and physico-chemical indices determined in a large set of samples of Sicilian industrial mandarin oil.

The samples were preliminarily investigated by the determination of specific gravity, refractive index, optical rotation and UV absorbance (CD value). The investigation was then completed by the qualitative and quantitative analysis of the volatiles by GC/MS-LRI and GC/FID, of the non-volatile oxygen heterocyclic fraction by reversed phase liquid chromatography RP-HPLC, the determination of the enantiomeric ratio (ER) of several monoterpene hydrocarbons and linalol by enantioselective gas chromatography (Es-GC/FID).

This study consists of the update of the information available in literature [2-5], on Sicilian mandarin (*C. deliciosa* Ten.) essential oil, and provides information on the composition and quality parameters for the evaluation of this product.

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A-06

Chemical composition of the wood oil and resin of *Cedrus atlantica* Manetti from Corsica

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Cedrus atlantica Manetti presents natural geographic distribution is limited to Morocco and Algeria (Atlas Mountains). It was introduced in Corsica forty years ago by the Office National des Forêts for reforestation.

C. atlantica wood oil (sawdust oil) from Morocco is reported to contain mostly β-himachalene (31-46%), α-himachalene (7-17%), γ-himachalene (7-11%) [1-4]. One sample was dominated by (E)-α-atlantone (29%) beside himachalene isomers [5]. Another study reported an oil sample (origin undetermined) containing α-cedrol (22 %) and α-cedrene (16 %) as major components [6].

In the course of our ongoing work on the chemical variability of essential oils of plants growing in Corsica, we investigated the composition of the wood oil of *C. atlantica*. Identification of the main components was achieved by ^{13}C NMR, without previous separation, using a computerised method developed in our laboratories and their quantitation was carried out by GC(FID). α-Pinene, β-pinene, α-himachalene, β-himachalene, γ-himachalene, himachalol and (E)-α-atlantone were found to be the main constituents of the samples. The results were submitted to Chemometric Analyses which allowed two groups of essential oils to be distinguished with respect to the content of himachalol and α-pinene / β-pinene.

The chemical composition of various resins from *C. atlantica* was also determined by ^{13}C NMR using the quantitative procedure developed in our laboratories [7]. The resins were mainly constituted by diterpenic acids (mainly abietic and isopimaric acids) and lignans (pinoresinol and lariciresinol).

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A-07

Combined analysis by GC, GC-MS and ^{13}C NMR and chemical varibility of the leaf oil of *Juniperus thurifera* var *thurifera* from Corsica (France)

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Juniperus consists of 67 species and 34 varieties. Two varieties of *J. thurifera* are recognized: var *thurifera* and var *africana. J. thurifera* var *thurifera* is a pyramidal tree up to 20m. It grows in Spain and France (mostly in Pyrenees and Alps) [1]. In Corsica it is found only in two mountainous locations. The composition of oil samples from France and Spain was investigated by Adams [2]. The aim of this work was to characterize Corsican *J. thurifera*, through the chemical composition of its leaf oil and to investigate if a chemical variability occurred in the well-delimited territory of the island.

One oil sample was fractionated over SiO_2 and all the fractions of chromatography were analyzed by GC(RI) and ^{13}C-NMR. The composition of the leaf oil of 16 individual trees was investigated by GC(RI). Some samples were also analyzed by GC-MS and by ^{13}C-NMR. In total, 42 constituents were identified and they accounted for 84.2-99.4% of the total amount of the oils.

Juniperus thurifera leaf oil was characterized by a high content of monoterpene hydrocarbons. Limonene, α-pinene, Δ-3-carene and Δ-2-carene were the major constituents. Although the amount of the major components varied drastically from sample to sample, only one group was distinguished by chemometric analysis (K-means clustering and PCA). However two samples differed substantially and appeared as atypic with respect to the main group. They contained unusual high quantities of elemol (up to 19.7%) and cedrol (up to 6.9%). Such a composition is reported for the first time in *J. thurifera* var *thurifera* oils.

The leaf oil of *J. thurifera* from Corsica was dominated by limonene as the oils from Pyrenees and Alps [2]. It differed by the higher content of Δ-3-carene and a lower content of linalyl acetate. It is drastically different from the sabinene-rich oils from Morocco (var *africana)*.

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A-08

Fast gas chromatography and time of flight mass spectrometry for essential oil characterization

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Natural flavouring materials, such as essential oils are complex hydrophobic mixtures usually steam distilled or solvent extracted out of plant materials. These oils are used in a wide variety of applications, such as perfumes, cosmetics, cleaning products, as also for food and beverage flavourings.

As widely acknowledged, due to the robustness and easy applicability, gas chromatography (GC) represents to be the most suitable characterisation technique. Though, the significant cost per analysis and the large number of samples to be analysed per-day, requiring faster response requirements, have highly increased the interest in minimising analysis time.

However, the obtainment of faster GC response has been hindered by both a lack of instrumentation presenting the proper features (high inlet pressure, fast heating rate, fast cooling rate, fast acquisition rate etc) and the high cost of the most powerful detection systems, such as the time of flight mass spectrometer (TOF-MS). Fortunately, today the technological implementations have allowed significant instrumental improvements.

In the present work the analytical performance of a dedicated fast GC system hyphenated to a new bench top time of flight mass spectrometer has been investigated on different essential oils. The analyses were properly supported by a dedicated and user friendly software which applies automatic deconvolution procedures and specific tools to speed up data processing.

A-09

2DChrom™ software for automatic GCxGC data comparison. A tool for essential oils quality assessment

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As industry for processed food has grown, the use of food additives has become a need for all type of foods to enhance natural flavors or create desired aroma for increased appeal. For this purpose, flavorants are formulated by manufacturers with the need to keep flavor consistency between different product batches or after recipe changes. The same consistency of organoleptic properties is required when natural essential oils are selected to be used as food aroma enhancers.

Currently, human nose and experience are still the best and reliable qualifying tool to discriminate acceptable oils which comply with the required organoleptic standard. However, a more objective and faster method for quality assessment would be desirable.

The analytical challenge is to highlight differences in chemical composition of such very similar mixtures and correlate them to the different organoleptic properties.

The number of compounds which are typically separated by using a mono-dimensional gas chromatographic approach, may result not enough to reveal critical compounds which could discriminate flavors very similar in composition but clearly different for taste and smell.

GCxGC is the best approach for increasing the separation capability and the probability to isolate minor discriminating compounds. Besides, the chromatogram visualization as a bi-dimensional plot facilitates the visual comparison of the data. An automatic comparative analysis would make this operation user-independent and would speed up data reprocessing.

The use of comparative tools available in the 2DChrom SW, a GCxGC data handling software developed by IFP (France) and recently available on the market, was demonstrated on different essential oils having very similar composition. In particular, samples of lemon and orange essential oils from different producers and different batches have been compared and classified for quality assessment.

Matching indices are generated for selected zones of the 2Dplot, highlighting where the differences occurs. Chemometric techniques are also available as principal component analysis (PCA), K-Means and Clustering analyses and were used for data processing.

A-10

Variation in essential oil yield and chemical composition of intact and powdered "atroveran" (*Ocimum selloi* Benth.) leaves under storage conditions

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Besides genetic and environmental characteristics, the cultivation and processing systems of medicinal plants are important factors in the product final quality [1]. Among such factors, storage has been scarcely studied regarding medicinal plant conservation. The aim of this work was to study the effect of storage time on essential oil yield and chemical composition in the dry matter of intact and powdered *Ocimum selloi* leaves. The experiment consisted of two fragmentation treatments (intact and powdered leaf) and five storage times (0, 3, 6, 9, and 12 months). Dry leaves were packed in plastic bags, which were sealed and stored in a dry and dark place. The essential oil was extracted by hydrodistillation in modified Clevenger-type device and the chemical composition was analyzed through gas chromatography using a flame ionization detector attached to a mass spectrometer (GC-MS-FID). There was an accentuated reduction in the essential oil yield of powdered and intact leaves stored over one year, but the essential oil yield of intact leaves was significantly higher than that of powdered ones. In general, the major compound methyl-chavicol was higher in intact leaves than in powdered ones and decreased over the storage time (Table 1). The fragmentation type and the storage time decisively interfere in *O. selloi* essential oil yield and composition.

Table 1. Relative concentration of peaks corresponding to the main chemical constituents of essential oil in dry biomass of intact and powdered leaves of *Ocimum selloi*, depending on storage time. (KI = Kovats index).

Constituinte	Time of storage (months)										
	Relative concentration (%)										
		Intact					Powder				
	IK	0	3	6	9	12	0	3	6	9	12
Methyl-chavicol	1198	93,9	92,5	76,6	64,3	7,2	97,9	81,4	61,5	28,8	1,6
Methyl-eugenol	1397	0,3	0,7	1,8	2,2	5,3	-	1,1	2,3	5,1	4,7
β-Caryophyllene	1418	-	1,8	4,0	5,9	11,0	-	3,4	5,5	10,6	6,5
Germacrene D	1480	-	1,6	4,9	7,1	18,5	-	3,4	5,8	11,3	8,9
Bicyclogermacrene	1494	-	1,9	6,4	9,7	28,4	-	3,8	6,4	13,5	11,8
β-Bisabolene	1506	0,1	0,3	0,9	1,3	3,9	-	0,8	1,7	4,2	4,1
Spathulenol	1575	0,9	0,8	1,9	2,9	10,6	-	2,2	5,7	14,8	32,9
caryophyllene oxide	1581	0,9	-	0,3	0,4	1,4	-	0,3	1,25	2,7	6,2
TOTAL		96,4	99,5	96,8	93,8	86,4	97,9	96,5	90,00	91,1	76,9

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A-11

New Advances in Ultrasound- and Microwave-assisted Extraction

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Conventional extraction processes for flavours and essential oils are often time-consuming, laborious, involve large amounts of solvents and, ultimately cause some thermal decomposition of the target molecules and partial loss of volatiles. Great improvements may be in the offing with non-conventional techniques [1] such as ultrasound-assisted extraction (UAE) [2] and microwave-assisted extraction (MAE) [3] and even their combination [4]; each matrix however will need careful optimization of operating conditions. The application of ultrasound (US) to enhance extraction yields began in the fifties at the laboratory level [5]. It has been recently shown to improve the extraction from plant materials of polysaccharides and volatile fractions, mainly through the phenomenon of cavitation [6].The mechanical effect of US accelerates the release of organic compounds contained within the plant body by disrupting cell walls [7], by enhancing mass transfer and facilitating solvent access to the cell content. This effect is much stronger at low frequencies (18-40 kHz). UAE has proved to be a versatile technique that can be scaled up to the great benefit of industrial production. Microwaves (MW) interact selectively with the polar molecules present in glands, trichomes or vascular tissues. Localised heating causes the expansion of cells and rupture of their walls, followed by the release to the solvent of volatiles [8]. Several recent studies showed the great advantages offered by MAE compared to conventional methods [9]. Both Authors' groups developed several devices and equipments for improved UAE and MAE.

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A-12

Two Novel sesquiterpenes from *Eryngium maritimum* essential oil

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Eryngium maritimum belong to *Eryngium* genus and A*piacees* family. *Eryngium* species have been reported to exhibit different therapeutic uses in folk medicine. *Eryngium maritimum* called in France "Panicaut maritime", is a species growing wild on the sand dunes of West and South of Europe [1]. Previous study results about *Eryngium maritimum* essential oil was exposed by Kubezka & al. at the 29th ISEO and reported (4S,7S)-muurol-9-en-15-al as new muurolane derivative [2].

In this work, two novel natural molecules were isolated from the aerial parts of *E. maritimum* essential oil from Corsica and Sardinia. After first analysis by GC and GC-MS(EI), both compounds were isolated by successive columns chromatography on a silica gel. Their structures have been established by joint informations from NMR (^{1}H, ^{13}C, DEPT, HMBC, HSQC, COSY and NOESY) and GC-MS(CI). We characterized unambiguously the structure of cadina-9-en-15-ol, a new primary alcohol with cadinane skeleton and its corresponding aldhehyde: cadina-9-en-15-al. In addition, a stereoisomer (4R,7S)-muurol-9-en-15-al was identified for the first time as natural product.

^{1}H-NMR, ^{13}C-NMR and MS (EI and CI) spectra suggested the functional groups and the molecular mass. The differenciation between cadinane and muurolane skeletons could be established from characteristics NMR chemical shift of both carbon bridges (C-1 and C-6) which differed by more than 5 ppm in the cadinane ring. 2D-HSQC and -HMBC sequences have confirmed the structures and 2D-NOESY allowed to establish the relative configuration of C-4 and C-7 of these three naturals products.

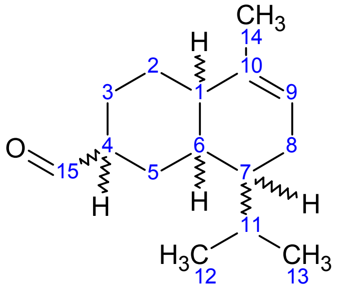

Figure: Sesquiterpenes aldhehyde skeleton

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A-13

Chemical variability of essential oils of *Artemisia herba-alba* Asso from Morocco

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Artemisia herba-alba (family: Asteraceae) is a dwarf shrub growing wild in semi-arid or arid areas all around the Mediterranean basin. In Morocco, this medicinal and aromatic plant is used in traditional medicine as anthelmintic agent especially against ascaris. Some studies have reported the essential oil composition of *A. herba-alba* from various countries (Spain, Egypt, Israel, Algeria and Morocco) and described the chemical variability of this species [1-4]. However, the chemical compositions of wild population from East Moroccan have been poorly explored.

In the present work, the chemical composition of sixteen *A. herba alba* oil samples harvested in ten East Moroccan localities were investigated by combination of Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS). The chemical variability of *A. herba alba* oils is also discussed according to a statically analysis.

Detailed analysis of the essential oils led to the identification of 51 components amounting to 80.5-98.6% of the total oil. The chemical composition exhibited high amount of monoterpene ketones (camphor, chrysanthenone and thujone) and showed important qualitative and quantitative differences.

Principal Component Analysis (PCA) and Cluster Analysis (Dendrogram) were applied on the matrix linking essential oil composition and sample locations in order to identify possible relationships between relative percentages of volatile compounds and geographical origins. The data analysis has established the existence of three main groups:

- The group **I** was characterized by high amounts of camphor (17.6-46.2%) and chrysanthenone (21.2-52.5%) and included seven samples from five localities.
- The group **II** was characterized by high amount of camphor (39.6-49.5%) followed by α- and β-thujone (10.7-30.8%) and included five samples from three localities.
- The group **III** (including four samples from two localities) exhibited higher amount of amount of β-thujone (44.2-73.8%) and borneol (6.4-9.6%) and lower amount of camphre (0.9-36.7%) than the others samples.

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A-14

Chemical composition of Corsican and Sardinian *Teucrium massiliense* L. essential oil

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The genus *Teucrium* (Labiateae) exhibit more than 300 species widespread around the world. Among them, *T. massiliense* grows in some areas of Mediterranean basin, particularly in Corsica [1] and Sardinia [2]. To our knowledge, only one study report the identification of the constituents of *T. massiliense* essential oil from Sardinia [3] in which 34 components were identified. The main components were 3,7-dimethyloctan-2-one (15.2%), butyl-2-methylbutyrate (12.1%), linalool (10.6%) and linalyl acetate (7.1%).

In the present work, the essential oils of Corsican and Sardinian *T. massiliense* L. were investigated using combination of capillary GC/Retention Indices, GC-MS and Carbon-13 NMR spectroscopy after fractionation on chromatography column (CC). After the preliminary analysis of each oil, the main component remained unidentified. Since its molecular ion was absent, the EI MS showed a base peak at m/z 43 that suggest the occurrence of an acetate. The identification of 6-methyl-3-heptyl acetate was unambiguously established by comparison of its ^{13}C-NMR spectral data in the CC fraction (98:2, n-C_2H_5:Et_2O) with those reference compounds. Finally, more than 138 compounds accounting for 93.7% and 87% of the total amount were identified from Corsica and Sardinia oils, respectively. The main components of both oils were 6-methyl-3-heptyl acetate (20,3% - 19,5%), 3-octyl acetate (7,1% - 7,1%), germacrene-D (7,6% - 1,4%), pulegone (6,7% - 1%), α-curcumene (0,5% - 6%) and linalool (4,8% - 5,8%), respectively.

The samples analyzed are significantly different from that of the literature.

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A-15

Determination of oxygen heterocyclic components in *citrus* products by HPLC with UV detection

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Furanocoumarins (psoralens) constitute a large family of natural components in plant extracts from Rutaceae, Umbelliferae, Solanaceae, used as ingredients in foods and fragrances. In particular, *citrus* oils present the biggest potential contribution to furanocoumarin content in fragrance products [1]. Furanocoumarins are contained in the non-volatile fraction of cold-pressed *citrus* oils together with other oxygen heterocyclic components (coumarins and polymethoxylated flavones) [2].

Due to their non volatile nature, liquid chromatography has been widely used for the analysis of oxygen heterocyclic components in *citrus* oils and in other natural matrices [2-5]. Because of structural diversity of oxygen heterocyclic components, RP-HPLC analysis is not easy, due to the possible coelutions between components. Recently, it has been demonstrated that the use of a ternary mixture of water, THF and acetonitrile under gradient conditions allowed to obtain good resolution of coumarins and furocoumarins [3,6] using C18 HPLC columns. On the basis of method optimization carried out by Frerot et al. [3], we used the same mobile phase mixtures to optimize a gradient program capable to baseline resolve all the oxygen heterocyclic components present in citrus oils (bergamot, lemon, lime, sweet orange, bitter orange, grapefruit, mandarin). An Ascentis Express C18 column was employed. This column is packed with partially porous particles of 2.7 um based on Fused-Core™ technology, that permits to obtain higher resolving power.

Due to the global shortage of acetonitrile, a new method that uses methanol instead of acetonitrile as solvent had to be developed. Gradient program remained unchanged and no relevant differences have been detected between the two methods.

The methods were validated in terms of detection limit, quantitation limit, linearity and precision in terms of repeatability and intra-laboratory reproducibility.

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Composition of the leaf and flower essential oils of *Stachys officinalis* (L.) Trevisan subsp. *officinalis* (Labiatae) growing in Italy and related secretory structures

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Stachys officinalis (L.) Trevisan (Labiatae/Lamiaceae), commonly known as wood betony, is a perennial herbaceous plant widely distributed in Europe, Western Asia and Northern Africa [1]. Wood betony was at one time commonly employed as a medicinal plant in the treatment of a wide range of disorders [2]; it is much less used nowadays [3]. Morphologically it is a well-defined species; in Italy two different subspecies are recognized [3]. The essential oil composition of this species was the subject of few previous studies [4-7].

In this work we investigate the composition of the leaf and flower essential oils and the related secretory structures from plants of the most diffused subspecies: *S. officinalis* subsp. *officinalis,* collected in Scarperia (Florence, Italy), on July 2007.

The epidermal surface presents simple or dendritic non-glandular hairs and abundant glandular trichomes of two different types (type A and type B).

Type A is the typical Labiatae peltate trichome; the histochemical and ultrastructural observations indicate a secretion of essential oil and polyphenols. Type B is a short capitate trichome secreting both hydrophilic (acid polysaccharides) and lipophilic (essential oil) substances, as it is evidenced by the histochemical and the ultrastructural investigations.

The essential oils were hydrodistilled in a Clevenger-type apparatus and analyzed by GC and GC-MS.

The major constituents of the leaf essential oil are (*E*)-caryophyllene (20.1%), (*E*)-nerolidol (14.3%), caryophyllene oxide (6.1%) and γ-cadinene (5.7%).

In the flower essential oil the major constituents are caryophyllene oxide (16.5%), (*E*)-nerolidol (15.4%), humulene epoxide II (9.2%) and α-pinene (7.0%).

The essential oil composition of Italian *S. officinalis* subsp. *officinalis* plants are here reported for the first time. Our study revealed significant differences in the essential oil profile of Italian *S. officinalis* samples with respect to the plants from Balkan regions [5-7], indicating the existence of a chemical polymorphism.

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A-17

Essential oil composition of three *Stachys* L. species from Turkey

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Stachys is one of the largest genera of Lamiaceae containing 270 species throughout the world, 72 being in Turkey. There are two main centres of diversity. One of them is South and East Anatolia, Caucasia, north western Iran and northern Iraq and other is Balkan Peninsula. Ten new species have recently been described in Turkey. [1-3].

The aerial parts of *Stachys euadenia* P.H. Davis (endemic), *S. pseudopinardii* Bhattacharjee et Hub.-Mor (endemic) and S. *iberica* Bieb. ssp. *iberica* were hydrodistilled for 3 h using a Clevenger-type apparatus and analysed by GC and GC-MS methods, simultaneously.

In *S. pseudopinardii*, sixty-four components were characterized representing 77.4% of the total oil. β-Eudesmol (29.5%), (*E*)–nerolidol (20.5%) were identifed as the major components.

Sixty-one components representing 83.6% of the total oil were characterized *in S. euadenia, with* β-eudesmol (46.6%) and caryophyllene oxide (4.6%) as the major constituents.

Thirty compounds comprising 95% of the oil with hexadecanoic acid (41.5%), phytol (8.2%) and germacrene D (9.7%) were characterized as major constituents in the oil of *S. iberica* ssp. *iberica.*

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A-18

Variability of essential oils in *Stachys officinalis* from Kosovo

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Aerial parts and roots of *Stachys officinalis* L. (Lamiaceae) were collected from three localities with different ecological condition to analyse essential oils. The leaves and inflorescences were collected in flowering time and the roots at the end of the vegetative period. Plant material was dried at room temperature. Essential oils were obtained by hydrodistilation and analyzed using gas chromatography (GC) and gas chromatography and mass spectrometry (GC-MS). 112 compounds were identified. The main components in samples from leaves and inflorescences were α-pinene, caryophyllene [2], 1-octen-3-ol, germacrene D [1, 3], whereas the main component in the roots was propanoic acid 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanester in all localities.

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A-19

Reevaluation of aroma profiles of sandalwood, frankincense, patchouli and vetiver

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Sandalwood, frankincense, patchouli and vetiver are typical scent materials in traditional Japanese scents. Although these materials possess valuable unique features such as providing the base notes for perfume, the key components of their characteristic odors have not yet been elucidated due to the following: (1) the fragrances of the scent materials are not formed by a mere superposition of individual aromatic components, and (2) the fragrances of the aromatic components of these materials are weak. In this study, we considered these problems and obtained the following results. By isolating the minimum odorant groups necessary to retain the characteristic fragrances of the scent materials, we found that the fragrances of these scent materials could be expressed through a merger of these groups The odors of the hexane extracts of these materials are similar to the odors of the materials themselves. We performed precise distillations to obtain the fractions that form the fragrance of the scented materials. Santalols have been regarded as the primary constituents of sandalwood odor; however, we found that the odor of sandalwood chips is formed by a combination of santalols and their aldehyde derivatives. In the same way, the odor of frankincense is formed by the association of aliphatic-chain acetates, diterpenes, and diterpene acetates. The fragrance of patchouli is formed by a combination of sesquiterpenes and sesquiterpene alcohols. The odor characteristics of vetiver are the same as those of patchouli. Thus, by using the reported method, the scent profiles of these materials were successfully characterized.

A-20

Comparison of aroma profiles of hexane extract and commercial essential oil of vetiver

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Vetiver is an important odor source for both perfume and traditional Japanese scents. The odor profile is difficult to elucidate because the characteristic fragrance is formed by many kinds of odor constituents.

The fragrance of commercial vetiver essential oil is clearly different from not only the scent of vetiver roots, but also the odor of the hexane extract. In this study, we investigated the source of these differences by clarifying the fragrance profile of the commercial essential oil.

Precise distillation of the essential oil yielded three fractions (groups A–C), each having different characteristic odors. We determined the main constituents of each group by purification and spectroscopic analysis. Group A contained hydrocarbons and aromatic compounds, group B contained aldehydes, and group C consisted of ketones and alcohols (which are thought to be the main odor constituents). An odor evaluation of groups A-C was performed to examine their similarity to the essential oil. We found that the contributions of Groups A and B to the vetiver fragrance of the essential oil are larger than that of Group C, even though Group C contains the alcohols usually regarded as the main aromatic components of vetiver. That is, the characteristic aroma of vetiver essential oil is due to the odorants in Groups A and B. On the other hand, the odorants belonging to Group B were hardly observed in the hexane extract of the vetiver roots. These sensory evaluations indicate that the scent of the hexane extract was formed by a combination of Group A and the alcohols of Group C. Thus, the aroma profiles of the hexane extract and the commercial vetiver essential oil were successfully determined.

A-21

Fragrance profiling of consumer products using a fully automated dynamic headspace system

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Accurate qualitative and quantitative analysis of perfumed or flavored products is essential to the flavor and fragrance industry. Especially when unknown samples need to be analyzed traditional methods of GC analysis often lead to only vague results and often require time consuming and cumbersome sample preparation techniques such as solvent extraction (liquid/liquid, Soxhlet, Likens-Nickerson).

The technique of dynamic headspace requires minimal sample preparation, and significantly reduces overall analysis time with improving data quality. It is applied to different types of consumer products, comparing the analyses of the neat perfume oil with those of consumer products containing this oil.

A-22

Essential oil composition of *Eremophila longifolia* (F. Muell) (Myoporaceae): evidence for new chemovarieties

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Eremophila longifolia is a woody shrub, endemic to arid and semi-arid regions of Australia, where it is employed in traditional indigenous medicine to treat a wide variety of conditions [1]. An early report examining *E. longifolia* leaf essential oil composition had indicated high levels of the hepatotoxic and carcinogenic phenylpropanoid safrole [2], and as a result, authors have urged caution in the use of traditional preparations derived from this species [3]. The present study was initiated after noting significant variations in morphology and odor profiles of wild *E. longifolia* specimens in the state of New South Wales, (NSW) Australia. Leaves from several specimens were collected across a range of biogeographic regions in NSW. Essential oils were obtained by hydrodistillation and analysed using GC-MS and NMR spectroscopy. Thirty-five compounds were identified with comparison of retention data and mass spectra with that of published values. Considerable variation was found among specimens in essential oil yield and composition, resulting in identification of three distinct types (here designated A, B and C). Type A specimens produced oils at relatively high yields (3.1% - 5.7 %) with major constituents isomenthone (61.1% - 86.7%), menthone (8.8% - 22.6%) and α-terpineol (8.4% - 11.0%). Type B specimens produced oils of relatively moderate yield (0.5% - 1.9% g/g) with major constituents karahanaenone (81.0% - 82.2%) and α -terpineol (4.1% - 11.7%). One specimen (designated type C) produced essential oil at relatively low yield (0.4% g/g fresh leaves) with major constituents identified as borneol (31.7%), fenchol (19.7%) and limonene (9.9%). No phenylpropanoids, including safrole, were detected in any of the specimens examined here. The relatively uncommon monoterpenoid karahanaenone is valued as a precursor in the fragrance industry and to the best of our knowledge the leaves of type B specimens described here represent the richest known natural source of this compound.

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A-23

Unravelling the complexity of fragrances in HPC* products using high throughput TruTOF GC-TOFMS

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The composition and analysis of perfume components in Home and Personal Care product fragrances is very complex and unquestionably time consuming.

Traditionally, significant GC-MS time may be consumed by running multiple separations using different phases. The resulting chromatograms are subject to labour intensive data interpretation in order to accurately resolve and quantify the composition of perfume raw materials, including essential oils in the product.

Now, with a new analytical approach, using a TruTOF GC-TOFMS, with automated true signal data deconvolution, it is possible to significantly increase the throughput. Additionally, in some cases where either there are a lot of interferences from the matrix or a complex mixture of essential oils, the ability to deconvolute full mass range spectra provides extra information. Moreover, this may also increase our ability to accurately characterise & interpret our data to unravel further the complexity of fragrance analysis in such products.

*HPC – Home & Personal Care

A-24

Changes in the composition of fennel and marjoram essential oils influenced by steam vacuum decontamination

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Reduction of micro-organisms in essential oil plants using steam is widely used in Germany because the application of short-wave radiation is often not very popular. A special process [1] is based on the combination of steam and very fast vacuum. Thereby bacterial spores can be removed.
Marjoram and fennel drugs were decontaminated by this process. The reduction of the total bacterial count was approx. 3 decades in both cases; the results vary slightly depending on the individual decontamination conditions.
The process had a major influence on the essential oil content of marjoram, while the oil content of fennel remained widely constant (Fig. 1). The decontamination conditions are insignificant in this context.

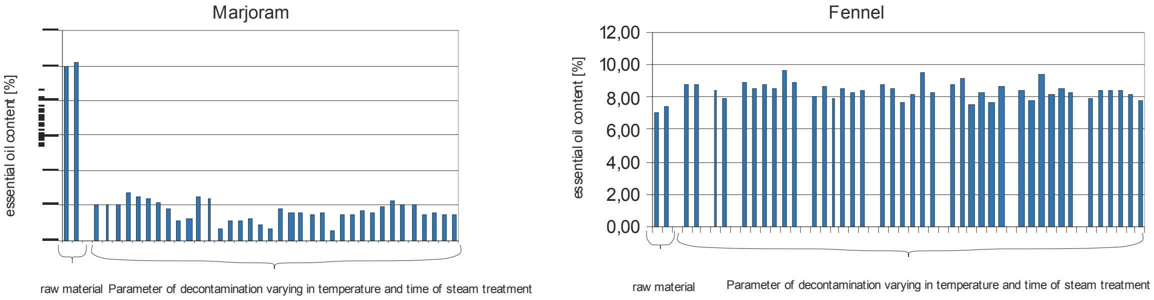

Fig. 1. Essential oil contents of marjoram and fennel before and after decontamination

The reasons for the diverse results obtained for fennel and marjoram are probably due to the differences in stability of the oil cells. Photomicrographs show the very different cell structures. The sensitive marjoram cells crack under vacuum influence and essential oil evaporates.

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A-25

Chemical composition of essential oil of *Ajuga laxmannii* (L.) Benth. (Lamiaceae) from Serbia

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The genus *Ajuga* L. (Lamiaceae), commonly known as bungle or bungleweed is comprised of more than 40 species widely distributed in temperate regions of both hemispheres.[1] Ten species of the genus *Ajuga* are distributed across Europe[2] and six are represented in the flora of Serbia[3].

This genus has been the focus of interest due to significant pharmacological and economical importance. *Ajuga* species are commonly used in folk medicine for the treatment of rheumatism, respiratory system illnesses, ulcers and diarrhea [4] and it is worth mentioning that the genus has been named after the condition for which the plants have been used most effectively- for revealing and curing gout (genus name *Ajuga* is derived from the word *aguja* meaning gout in Greek [5]). A vide spectrum of effectiveness against a number of conditions and the widespread usage of *Ajuga* plants in folk medicine resulted in numerous scientific investigations undertaken in order to reveal the extent of the possible application.

Ajuga laxmannii (L.) Benth.(syn. *Teucrium laxmannii)* was the subject of only one previous phytochemical study, reporting the isolation of six compounds: coumarin, two diasteroisomers of 14,15-dihydro-15-hydroxyajugahin, makisterone, melilotic acid methyl ester, 8-acethylharpagide and harpagide [6]. One literature source reporting the traditional usage of *A. laxmannii,* as the plant used in the treatment of respiratory infections, exists [7]. The composition of wild-growing *A. laxmannii* hydro-distilled essential oil was investigated by means of GC and a GC-MS analysis. The analysis resulted in the identification of seventy-seven components, accounting for 95.1% of the total oil. Coumarin (22.7%), l-octen-3-ol (15.5%), (*Z*)-3-hexenol (14.1%) and linalool (13.7%) were recognized as the main oil constituents, all together contributing more than two thirds (2/3) of the components of the oil. This is not only the first report on the composition of *A. laxmannii* volatiles but also a Lamiaceae essential oil with coumarin as the constituent present in considerable amount.

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A-26

Essential oil profiles of *Lippia scaberrima* Sond. from Highveld (South Africa) gold and uranium mines

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Lippia scaberrima Sond. is an aromatic South African shrub shrub which commonly colonizes soils contaminated by gold and uranium mine tailings. Mine rehabilitation strategies worldwide involve the use of indigenous plants that naturally inhabit the area of concern. Where such plants have commercially-valuable attributes, their use to stabilize contaminated soils and tailings also provides opportunities for local beneficiation. The production of essential oils from aromatic plants is considered a particularly safe option for mine waste, since these plants are generally unpalatable to grazers. Moreover, the steam distillation methods used for essential oil isolation yield products free from metal and radioactive contaminants.

In this study, the essential oil of bulk samples of *L. scaberrima* growing in a natural habitat was found to exhibit strong *in vitro* antifungal activity against commercially important postharvest spoilage pathogens of fruits. GC-FID analysis of the oil identified limonene (38%), 1,8-cineole (5%) and carvone (34%) as the main constituents of the oil. The antifungal activity of the oil was attributed to the presence of carvone, after antifungal assays of the individual terpenoids indicated that this terpenoid had the highest activity. These results were confirmed by commercial trials, using essential oil amended coatings on citrus, avocado and mango fruit. The potential application of *L. scaberrima* essential oil, rich in carvone, as a post-harvest fungicide of fruit, may necessitate the large-scale production of the oil. However, essential oil profiles of *Lippia* spp. in general have been shown to exhibit high intraspecific variability due to genetic differences. In this investigation, several *L. scaberrima* specimens from populations growing in mine-contaminated substrata were found to produce sufficient quantities of carvone for commercial production. However, no link between the metal contamination of the site and the carvone/limonene production could be found. Future studies will focus on propagation methods which will retain the genetic traits of these specimens.

A-27

Pattern recognition of volatiles from *Juniper communis* L. (mountain juniper). An investigation into the variability of the composition and the interpretation of analytical data

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The high intraspecific variability of the genus *Juniperus*, also strongly influenced by the climatic conditions, and above all, the complexity of the group of plants that may be related to the species *Juniperus communis* L. make the taxonomic classification of the species difficult. The morphological and geographical analysis are not able to reveal any substantial distinction [1-2]. An investigation of the achiral and chiral terpenes composition of *Juniperus communis* L. has been started. We hope to be able to distinguish between random and systematic variations of the volatile constituents of juniper species. Samples from Norway were collected at different altitude. HS-SPME-GC/MS coupled to statistical elaboration was applied to the analysis of the volatile fraction of needles and berries of Juniper [3-4]. The choice of the analytical method was made to facilitate future analysis of a larger number of samples and to minimize human errors in sample handling. 72 compounds were tentatively identified by comparing their mass spectra and retention indices with those reported in mass spectra libraries and literature, respectively. The results point out that the needles and berries show different volatile profiles and even the sampling at the same altitude does not always show a correspondence between berries and needles of the same tree bush [5]. Needle samples collected at the see level are distinct from the others by the percent contents of α-pinene and from higher ER(-)/(+) of β-pinene, sabinene, limonene and terpinen-4-ol. These results are very interesting in order to provide a more clear pattern recognition of juniper species and to determine the distribution in the Norway area. The results may also lead to possible correlations between the biochemical pathways and the analysis of the volatiles fraction due to automatic and faster analytical methods [6-7]. To achieve this goal further multidisciplinary investigation is needed [4].

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A-28

Composition of pine oil from Pinus *sylvestris* L. young shoots in different development stages

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Pinus sylvestris L.,Scots pine, is the most common pine species widespread in Europe, Northern Asia and Northern America. Pine oils are colorless or pale yellow mobile liquids with strong, dry-balsamic, turpentine-like aroma. They are very often used in medicine and aromatherapy, as well as in cosmetic and cleaning agents industry due to their flavour and numerous beneficial pharmaceutical properties, e.g.: antibacterial, antifungal, antiviral, antineuralgic, cholagogue, choleretic, diuretic, expectorant, hypertensive. Pine oils are obtained by hydrodistillation of needles or shoots. They are mixtures of volatile compounds especially mono- and sesquiterpene hydrocarbons [1].

Essential oil was isolated from young pine shoots collected in different development stages (from April to September 2008) of the same tree by hydrodistillation in Clevenger-type apparatus. Volatiles were analyzed by GC and GC-MS.

According to our investigations significant quantitative differences in the composition of the pine oil during development of young pine shoots were observed. The main difference was connected with the content of diterpenes that was very high at the beginning of shoot development and achieved 38.5% in May, and than gradually decreased to about 3% in June and trace amount in later stages. The content of monoterpene hydrocarbons increased from 49% in May to 73% in June and than decreased to 58% in September. In the same time the content of sesquiterpenes increased, for hydrocarbons from 4% to 20% and for oxygenated derivatives from 1% to 15%.

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A-29

Composition of the essential oils, supercritical CO_2 and hexane extracts of *Commiphora erythraea* (Ehnreb) Engl.

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The genus *Commiphora* (Burseraceae) comprises more than 150 species of small trees or large shrubs, native to some African countries. All the species produce essential oils that have a great importance in the perfumery industries. *Commiphora erythraea* is a less studied specie [1] that is often confused with *C. kataf*. [2]

The aim of our work was to define the composition of essential oils obtained from *Commiphora erythraea* with different techniques (hydro- and steam distillation, hexane and CO_2 extraction) and to show that *C. erythraea* and *C. kataf* [3] are two different species.

The oils were analysed by GC-MS and most of the components were identified by computer matching with commercial MS library (NIST98) and comparison of MS with those of our library of pure standard authentic compounds. The identification of the oil components was also possible by comparison of their linear RI [4] with those from the literature. The components of the furanosesquiterpenoidic fraction of the oils were isolated by column chromatography and identified by comparison of ^1H and ^{13}C NMR spectra with those from the literature. [5-7]

Gaschromatographic analysis of the essential oil of *C. erythraea* shows many differences with the analysis reported for *C. kataf*. [3] The main differences reside in the absence of a monoterpenoidic fraction in the hydrodistilled oil of *C. kataf* oil and in a very small percentage of 1(10),4-furanogermacradien-6-one and curzerenone (1,3-furanoelemadien-6-one) (together 1.0%) while in our case the two compounds amount to 10% ca in hydrodistilled oil, while in all the other oils the amount range is between 11 and 21%. The qualitative composition of the sesquiterpenoidic fraction is almost the same for all the oils, while the quantitative composition is highly dependent on the methodology used for the extraction.

Acknowledgements

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A-30

GC-MS analysis of volatile components of selected liverworts and Japanese medicinal plants

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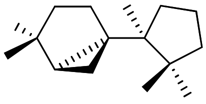
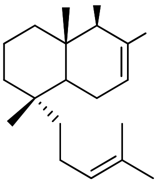
Liverworts belong to bryophytes which are taxonomically placed between algae and pteridophytes. Oil bodies, in which volatile components are stored, are characteristic for liverworts [1].

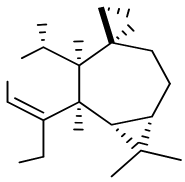
The ether extracts of some Austrian, Japanese, New Caledonian and Le Reunion Island liverworts have been used for the GC-MS analysis. Several parts of different medicinal higher plants and the essential oils from three of them have also been used.

Cyclopropanecuparenol (**1**) seemed to be the major component of all three samples of *Marchantia polymorpha*, collected in Austria and two different places of Japan. The Japanese and the Austrian *Conocephalum conicum* are chemically different. The diterpene dialdehyde, sacculatal (**2**), and its derivatives are predominant in the composition of volatiles of *Pellia endiviifolia* while *P. epiphylla* does not contain sacculatal. The Austrian *Radula* sp. showed the presence of a number of prenyl bibenzyls of which radulanin K is predominant. β-Patchoulene and drimenol have been identified from the New Caledonian *Bazzania* sp. *Plagiochila boryana* collected in the Le Reunion Island shows very pungent taste which is due to the presence of plagiochilline A (**3**) [1,2].

γ–Cadinene appeared in high percentage on the data obtained from the ether extracts of *Artemisia vulgaris* var. *indica* leaves and *Solidago altissima* stem and flowers. Thujopsene is the major component of the red-brown colored essential oil of *Thujopsis dolabrata*. The ether extracts red leaves of *Platanus occidentalis* showed a higher amount of monoterpenes and a smaller amount of chlorophyll decomposition products. The presence of mono- and sesquiterpenes in *A. vulgaris* var. *indica* was very high before the steam distillation. After the steam distillation a large amount of unidentified yellow-orange colored fungus grew on the residue. The obtained essential oil might have a fungicid or at least a fungistatic activity. The major component of the *Angelica keiskei,* γ–terpinene, was not detected in the excrement of the larvae of swallowtail butterfly that ate this plant.







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A-31

Aframomum dalzielii Hutchinson from Cameroon: a new potential source of (E)-nerolidol

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The Zingiberaceae is a tropical monocotyledons family, comprising about 1300 species, shared out among 53 genera [1]. Many of them play or have played an important role in folk medicine or as spices or flavouring agents, and produce essential oils mainly from their leaves or seeds.

In continuation of our investigations on volatiles from the *Aframomum* species belonging to this family and growing in Central Africa [2- 5], we have focused on a new species: *Aframomum dalzielii* Hutch., a perennial herb that can reach 1.5 to 3 m high [6]. The seeds or fruits of this species are traditionally used in indigenous medicine for many purposes.

The essential oils, obtained with yields of 0.3%, 0.06%, 0.6% and 1.2%, by hydrodistillation of fresh leaves, rhizomes, seeds and fruit pericarps collected in Fontem (West province of Cameroon), were analyzed by GC and GC/MS. The essential oils from the seeds and leaves were characterized by a high content of a single component: (E)-nerolidol (91.2%) or (E)-β-caryophyllene (81.4%) respectively. The other volatile extracts were more complex: the major constituents in rhizomes oil were β-pinene (37.9%), sabinene (22.9%) and α-pinene (7.8%), while pericarps extract was dominated by β-pinene (42.4%), sabinene (14.2%) and 1,8-cineole (13.3%). These results were compared to those of two other *Aframomum* species containing also a high amount of (E)-nerolidol in their seeds: *A. pruinosum* [3] and *A. letestuianum* [2].

To the best of our knowledge, this is the first report on the essential oil constituents of *A. dalzielii* which can be considered as a new potential source of (E)-nerolidol.

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A-32

Chemical investigations on essential oils from endemic Cupressaceae trees from New Caledonia

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Callitris is a genus of 19 species of which 17 are native to Australia[1] and only two are endemic in New Caledonia : *Callitris sulcata* and *Callitris neocaledonica*.[2] Comparative GC and GC/MS analyses of essential oils obtained by hydrodistillation of pieces of dead trunks of the two species have shown different chemical profiles: the first species was dominated by thujopsene and β-barbatene while *C. caledonica*, which was characterized by high levels of guaiol and eudesmols, was closely related to *Neocallitropsis pancheri* considering their essential oil composition, except the presence of minor content of β-barbatene in the *C. caledonica* essential oil.[3]
Chemical investigations, with a specific focus on this rare sesquiterpene, were performed on the leaf oils of the three species. The corresponding results will be presented and discussed in terms of clarification of the relationship of these botanical species within the Cupressaceae family.

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A-33

Highlighting of chemotypes of *Ravensara aromatica* and their repartition areas

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Ravensara aromatica (Lauraceae) is an endemic species of Madagascar. The leaf essential oils have a great chemical variability which was already detected in different papers published by various authors [1-4]. Chemical (GC, GC/SM) analyses were performed on essential oils obtained by hydrodistillation of leaves and bark of 316 trees from various areas in Madagascar [5-7]. Their statistical (PCA, Clustering, FDA) analyses made it possible to define five chemical patterns. Their stability in the year and from one generation to another allowed to determine that these five chemical patterns answer the definition of chemotype. These chemotypes are defined by their major compound, methyl chavicol, methyl eugenol, α–terpinene, sabinene or limonene [7]. The chemical variability of those essential oils of *Ravensara aromatica* induced the variability of their biological activities [7]. Bark essential oils of all chemotypes have a homogeneous chemical composition, mainly characterized by methyl chavicol [6-7]. These results highlight that for *Ravensara aromatica*, the major compounds can belong to two biosynthesis (shikimic or mevalonic acids) pathways. Furthermore, a variability related to the harvesting site could be displayed. In one hand, the distribution of the chemotypes is individual: two trees of different chemotypes can be met side by side in the same ecological context. On the other hand, for the seven studied sites, chemotype answers different patterns: only one biosynthesis pathway in certain sites while in other ones, the individuals are characterized by the coexistence of the two pathways.

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A-34

Chemical characterization, antiradical, antioxidant and anti-inflammatory potential of the essential oils of *Canarium schweinfurthii* and *Aucoumea klaineana* (Burseraceae) growing in Cameroon

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The essential oils obtained by hydrodistillation of the resin of *Canarium schweinfurthii* collected in Mbouda (West Cameroon) and Lolodorf (South Cameroon) as well as that of *Aucoumea klaineana* collected in Lolodorf (South Cameroon) with yields of 5.3%(w/w), 7.2%(w/w) and 3.3 %(w/w) respectively

The analysis of their chemical composition was analysed by GC and GC/MS [1, 2]. In both cases, the essential oils are dominated by monoterpenes: limonene (36.6-37.6%), p-cymene (25.3-10.5%) and α-terpineol (18-41.1%) were the major constituents of the volatile extracts of *C. schweinfurthii;* α-phellandrene (32.8%), α-pinene (23.6%), p-cymene (12.9%) and 1,8-cineole (12,3%) were the most abundant in the essential oil of the second species.

The antiradical properties of these oils were assayed by spectrophotometry using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [3, 4], giving SC_{50} of 5.4 g/l, 6.4 g/l and 4.6 g/l respectively for the volatile extract of *C. schweinfurthii* (West), *C. schweinfurthii* (South) and *A. klaineana* (South), while BHT, taken as reference, gave a SC_{50} of 6.6 mg/l.

Evaluation of their antioxidant properties by decoloration of β-carotene, gave an IC_{50} of 35mg/l and 0.25mg/l, respectively for the essential oil of *C. schweinfurthii* (South) and *A. klaineana* against 0.09 mg/l for BHT.

The evaluation, *in vitro*, of their inhibitory effect on soybean 5-lipoxygenase [5], comparatively to that of nordihydroguaretic acid (NDGA), indicated that only the essential oil of *C. schweinfurthii* from Lolodorf was active with an IC_{50} of 35 ppm, against 0.7 ppm for NDGA. This activity is of the same scale order as those evaluated by Baylac and Racine [6] or Alitonou et al. [5] for other essential oils presented as natural extracts with "significant potential anti-inflammatory activity".

In conclusion, the essential oil from *A. klaineana* could show the best antioxidant capacity, while that from *C. schweinfurthii* would present appreciable anti-inflammatory potential.

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A-35

Chemical constituents of the essential oil of *Ajuga austro-iranica* and *Biebersteinia multifida* from Iran

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Ajuga L. is an Old World genus of Lamiaceae family which comprises by about 50 species [1]. In Iran, this genus is represented by 5 species [2]. *Biebersteinia* Steph. is a small and distinctive genus of 5 species which distributed in central and western Asia and also in eastern Mediterranean regions [1]. In Iran, the only existing *Biebersteinia* is *B. multifida* which is widely distributed over the mountainous areas. Its rhizome is called "Chelledagh" in Persian is traditionally used for the treatment of rheumatoid disorders. The essential oil of *Ajuga austro-iranica* and *Biebersteinia multifida* growing wild in the Fars province of Iran was obtained by hydrodistillation and analyzed by GC and GC/MS technique. Thirty-nine components representing 91.5% of the total oil of *A. austro-iranica* and Forty-four compounds represent 96.9% of oil of *B. multifida* were identified. The main components of the oil of *A. austro-iranica* were trans-verbenol (7.0%), caryophyllene oxide (6.8%), 6,10,14,-trimethyl-2-pentadecanone (6.5%), myrtenol (6.3%), 1-octen-3-ol (6.2%) and β-pinene (6.1%). Pinene type monoterpenes were dominant by constituting 30.5% of the total oil. The major constituents of the oil of *B. multifida* were (E)-nerolidol (33.8%), phytol (17.7%), 6,10,14-trimethyl-2-pentadecanone (15.5%) and hexadecanoic acid (11.2%).

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A-36

Constituents of the essential oil of *Berula angustifolia* and *Johrenia paucijuga* from Iran

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Berula angustifolia (L.) Mertens & W. D. Koch (Apiaceae, Subfamily Apioideae) is a perennial, probably a monotypic species which distributed in damp places of Europe, Asia, E & S Africa, N America and many parts of Iran. [1, 2]. The genus *Johrenia* DC. (Apiaceae) comprises by 20 species which are mainly distributed in SW and Central Asia [3]. In Iran, this genus is represented by four species which all of them are endemic [2].

The essential oils of *Berula angustifolia* and *Johrenia paucijuga* were obtained by hydrodistallation of the aerial parts of the plant and analyzed by GC and GC/MS technique. Forty-four compounds representing 94% of the total oil was identified in the oil of *B. angustifolia* which the main components of it were piperitenone oxide (14.6%), limonene (13.9%), α-zingiberene (12.8%) and (E)-β-farnesene (9.6%).

Forty-seven Compounds representing 95.5% of the total oil of *J. paucijuna* were identified. Phenolics constituted more than 60% of oil composition which trans-anethole alone formed 58.1% of the total oil.

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A-37

Essential oils from Asteraceae (*Senecio gallicus* Chaix) and Apiaceae (*Prangos latiloba* and *Prangos ferulaceae* (L.) Lindl.) growing wild in Iran

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The genus *Senecio* involves 21 species in Iran, among which five are endemic [1] while the genus *Prangos* (Apiaceae) encompasses about 30 species [2]. Identification the components of the aerial parts oil of *Senecio gallicus* chaix and comparison of the stem volatile oils of *Prangos latiloba* and *Prangos ferulaceae* (L.) Lindl. is the main goal of this research. The plants were collected during the flowering stage in May 2008 from Shahmirzad in Semnan province of Iran, at an altitude of 1550 msl. A voucher specimen has been deposited at the herbarium of Research Institute of Forests and Rangelands, Tehran, Iran. The hydrodistilled oils were dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (4 ˚C) before analysis. The analyses of all oils were performed with a GC (Shimadzu GC-9A equipped with a SE30 fused silica column) and a GC/MS (Hewlett-Packard 6890/5973 fitted with a fused silica HP5MS capillary column) instrument. Fifteen components were identified constituting 98.1 % of the *Senecio gallicus* chaix oil and among them the main components were α-pinene (14.3 %), sabinene (5.1%), β-pinene (9.5 %), myrcene (2.2%), α-phellandrene (6.1%), β-phellandrene (7.8%), Cis-ocimene (4.1%), δ-3-carene (7.7%), α-terpinolene (3.3%), β-caryophyllene (6.8%), germacrene-*D* (27.1%), α-zingiberene (1.8%). On the other hand, The main components which were characterized in the stem essential oil of *Prangos latiloba* were α–pinene (25.5%), sabinene (12.6%), δ-3-carene (3.8%), limonene (5.4%), γ – cadinene (30.4%). Furthermore, the major compounds of *Prangos ferulacea* (L.) Lindl.*stem oil* were β-phellandrene (6.5%), α-terpinolene (5.5%), α–pinene (23.1%), δ-3-carene (4.6%), α-phellandrene (18.4%), trans-β-ocimene (21.3%) and γ-terpinene (12.7%).

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A-38

Chemical composition of the essential oils from stems of some Lamiaceae species of Iran: *Ajuga chamaecistus.Ging. subsp. scoparia*, *Salvia leriifolia* Bench. and *Salvia multicaulis* Vahl.

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Ajuga, commonly known as bugle or bugleweed, is one of the best-known genera within the Ajugoideae tribe of this family which is found in many parts of Iran and the world [1]. Furthermore, the genus *Salvia* comprises 700 herbs and shrubs, growing in the temperate and warmer zone of the world. Fifthy-eight species are found in Iran, among which 17 are endemic [2]. After drying the plants under the darkness, the essential oils of the stems of the plants grown in northern parts of Semnan were obtained by hydrodistillation and were analyzed by GC/MS for characterization their composition. The main constituent components of *Ajuga chamaecistus.Ging.subsp.scoparia* oil were found to be α- pinene (25.2%), myrcene (4.2%), ρ-cymene (29.1%), *β*-pinene (8.2%), Cis-ocimene (3.6%), α-phellandrene (12.7%), *β*-caryophyllene (7.3%) and Germacrene-*D* (3.1%). The stem oil of *Salvia leriifolia* Bench. was characterized by compounds constituting 92.4% of the total oil which were predominately α-pinene (8.9%), β--pinene (19%), 1,8-cineole (8.8%), (Z)- β- ocimene (6.3%), Germacrene-D (11%), Bicyclogermacrene (7%), γ-cadinene (10.5%), α-cadinol (5.7%) and benzyl benzoate (7.0%). Eight compounds representing 98.05% of stem oil of *Salvia multicaulis* were identified among them 1,8-cineol (24.83%), α-pinene (20.91%), camphor (24.20%) and camphene (13.03%) were the major ones. Accordingly, in all volatile oils monoterpenes predominated over sesquiterpenes.

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A-39

Chemical composition of the volatiles of *Artemisia* species from Kazakhstan

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Volatile metabolites obtained from the aerial parts of *Artemisia commutata Bess.*, *A. glauca* Pall. ex Willd., *A. santolinifolia* Turcz., *A. dracunculus* L., *A. laciniata* Willd. and *A. proceraeformis* Krasch. (Asteraceae) were analyzed by GC-FID and GC/MS methods. Plants were collected in June-August 2007 in the Northeastern and Eastern regions of Kazakhstan. The plant materials were subjected to microdistillation, using an Eppendorf MicroDistiller to obtain volatile metabolites.

p-Cymene (11.2%) in *A. commutata*, β-pinene (18.4%) and α-fenchene (14.6%) in *A. glauca*, 1,8-cineole (30.0%) and camphor (11.8%) in *A. santolinifolia*, sabinene (14.5%) and α-thujene (12.8%) in *A. dracunculus*, limonene (16.5%) and (*E*)-nerolidol (10.0%) in *A. laciniata*, α-thujone (55.2%) and selin-11-en-4α-ol (9.6%) in *A. proceraeformis* were found as the major volatile constituents.

A-40

Gas-chromatographic analysis of the essential oils of *Johrenia* species from Turkey

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Fruits and overground parts of *Johrenia alpina* (Fenzl) Fenzl, *J. dichotoma* DC., *J. polyscias* Bornm., *J. porteri* Post ex Boiss., *J. silenoides* Boiss. & Bal. and *J. tortuosa* (Fisch. & Mey.) Chamberlain (Apiaceae) were separately subjected to hydrodistillation by using a Clevenger type apparatus to obtain essential oils which were then simultaneously analysed by GC-FID and GC/MS [1, 2].

α-Pinene (39.2%) and dodecanoic acid (24.4%) in *J. alpina*, methyl farnesoate (16.9%) and bicyclogermacrene (13.2%) in *J. dichotoma*, α-pinene (23.0%), β-pinene (18.2%) in *J. polyscias*, bicyclogermacrene (25.7%) and hexadecanoic acid (17.4%) in *J. porteri*, α-pinene (33.8%), β-pinene (14.3%), spathulenol (12.8%) in *J. silenoides*, and methyl farnesoate (44.0%) in *J. tortuosa* were found to be the main constituents of the oils.

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A-41

Analysis by gas chromatography-mass spectrometry of the essential oils from the aerial parts of *Pimpinella dendrotragium* Webb & Berth., an endemic species to the Canary Islands, Spain.

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Pimpinella L. belongs to the plant family Apiaceae (Umbelliferae), subfamily Apioideae, and comprises about 150 species which occur largely in Europe and Asia extending to China [1]. The genus is present in the Macaronesian archipelago comprising four endemic species (*Pimpinella cumbrae* Link. *P. dendrotragium* Webb & Berth., *P. anagodendron* Bolle and *P. junoniae* Ceb. & Ort.) [2].

P. dendrotragium inhabits only in Tenerife and La Palma islands. With this work we complete our contribution to the knowledge of *Pimpinella* species endemic to the Canary Islands. This is the last part of the studies that Dr. Velasco-Negueruela started and published about the chemical composition of this genus [3-5].

The essential oils from the aerial parts of *Pimpinella dendrotragium* were studied by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MS). In essential oil from Tenerife population, the major components of the flowering tops were found to be, β-bisabolene (63.8%) and α-pinene (7.9%), while the leaves + stems only showed β-bisabolene (59.4%%). In samples from La Palma, the only fraction considered (leaves + stems + flowers + unripe fruits) had α-pinene (32.9%), β-pellandrene (5.7%), β-bisabolene (17.0%) and *epi*-biciclosesquiphellandrene (7.9%) as major compounds. Other characteristic constituents of Macaronesian species of the genus *Pimpinella* such as geijerene, pregeijerene, isokessane and β-dihydroagarofuran together with four pseudoisoeugenol esters (pseudoisoeugenyl 2-methylbutyrate, pseudoisoeugenyl tiglate, epoxy-pseudoisoeugenyl 2-methylbutyrate, and epoxy-pseudoisoeugenyl tiglate) were also identified.

Acknowledgements
In memory of the Professor Doctor Arturo Velasco-Negueruela. This work is his last contribution to the essential oils

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A-42

Essential oil composition of the different parts of *Eryngium aquifolium* Cav. from Spain

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The *Eryngium* L. genus belongs to the Apiaceae family and, with about 250 species, has a cosmopolitan distribution. In the Iberian Peninsula grow 14 of the 26 species described in Flora Europaea. *Eryngium aquifolium* Cav. is a perennial species that grows in dry places of the southern in The Iberian Peninsula (Fig. 1). The essential oil extracted from the different parts (stems + leaves, inflorescences and roots) of this species, gathered in Cadiz (Spain) has been extracted by steam distillation and analysed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MS).

The different parts of the plant yielded low amount of pale yellow oil. Qualitative and quantitative differences have been found between different fractions. The oil from the aerial parts showed sesquiterpenes as predominant compounds while the roots had diterpenes as principal ones. However, the different fractions contained the majority of the constituents although their percentage composition changed from one fraction to the other. The principal compounds of the inflorescences were found to be germacrene D (30.3%) and sesquicineol (26.7%). The stems and leaves shared one of the two main constituents, germacre D (46.0%), while the other one, myrcene (13.8%), appeared in low amounts in the inflorescences. Finally, the chemical composition of the roots was different, with phyllocladene isomer as unique major compound.

As far as we know this is the first report about the chemical composition of this species.

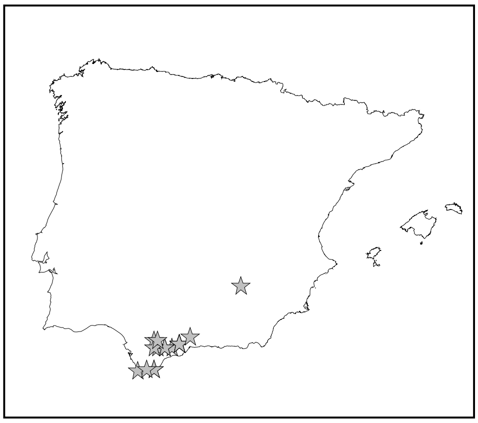

Figure 1. Distribution map of *E. aquifolium* in the Iberian Peninsula.

A-43

Essential Oil Composition of three species of *Darwinia* Rudge (Myrtaceae) an Australian endemic genus

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Darwina Rudge is an Australian endemic genus of shrubs that belongs to the family Myrtaceae. The genus consists of at least 45 named species and a number of undescribed taxa. *Darwinia* was named in 1816 after Dr Erasmus Darwin, an english physician and poet, and grandfather of the famous biologist Charles Darwin [1]. Three of the described species (*Darwinia collina* Gardn., *Darwinia procera* B.G.Briggs and *Dawina leiostyla* (Turcz.) Domin) have been gathered from different localities from Australia to analyse their chemical composition. A voucher or each species has been lodged at the Queensland Herbarium (BRI).

The essential oil extracted from the aerial parts of these Australian species has been extracted by steam distillation and analysed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MS). The chemical composition varied among species although all of them shared their principal compounds as α-pinene, limonene or globulol. The main compounds identified from the essential oils of *Darwinia collina* were found to be α-pinene (36.3%), β-pinene (20.4%), limonene (2.6%) and globulol (31.9%). The essential oil of *Dawina leiostyla* was quite different with bicyclogermacrene (23.6%) and α-pinene (12.9%) as the major compounds. Finally the oils of *Darwinia procera* were rich in myrteyl adetate (29.7%), α-pinene (23.7%) and bicyclogermacrene (8.1%).

All the species were gathered at flowering state, so the differences of the essential oils could be intraspecific or could be caused by environmental factors, because this genus has a wide distribution with different ecological conditions that could affect its chemistry.

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A-44

Composition of the essential oil of *Phyla dulcis* of Guatemala by GC-MS

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The composition of the essential oil of *Phyla dulcis* from Guatemala was determined. *P. dulcis* is a perennial plant, not taller than 40 cm with very aromatic leaves, which grows from Mexico to Colombia, from sea level to 1800m [1]. The plant is used in treatment of coughs, bronchitis, asthma, urinary retention among other diseases. Previous results on the essential oil of the plant from Mexico and Puerto Rico showed different compositions, consisting predominantly of monoterpenoids in Mexico and of sesquiterpenoids (79%) in Puerto Rico. The EO of *P. dulcis* from Puerto Rico showed the intensely sweet (+)-hernandulcin (36%) and (-)-epi-hernandulcin (22%) as main components [2]. In previous studies on the composition of the EO of Guatemalan *P. dulcis*, hernandulcin had not been identified [3]. In this work, the essential oil of leaves of *P. dulcis* was extracted by hydrodistillation for 3 h using a Clevenger type apparatus (yield: 0.82%) and analyzed by GC-FID and GC-MS using a HP5 (5% phenylmethylsilicone) column (25m x 0.2 mm, 0.25 μm film thickness). Temperature program was 60°-240°C at 3°C.min^{-1}, injector temperature at 260°C. The compounds were identified by their mass spectra, external standards as reference and retention indices. The first chromatographic analyses of the EO showed 6-methyl-5-hepten-2-one as main component (16.8%) followed by 3-methyl-2-cyclohexen-1-one (13.3%), *(E)*-caryophyllene (11.6%), α-bisabolol (7.8%), *(E,E)*-α-farnesene (6.7%) and α-copaene (5.4%) with no detection of hernandulcin. 6-methyl-5-hepten-2-one and 3-methyl-2-cyclohexen-1-one have been reported as the thermal degradation products of hernandulcin [2]. New analyses were performed with the injector temperature set at 220°C. A compound, apparently Hernandulcin was then found with its mass spectrum corresponding to a peak at high retention time in the chromatogram (10.9%). The spectrum showed no peak of molecular ion, but important peaks of hernandulcin showed high intensity at 218 m/z (elimination of water), 137 m/z (fragmentation of the previous ion) and other characteristic peaks (153, 110, 95, 82, 69 m/z).

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A-45

Quality assessment of some European caraway resources (*Carum carvi* L.)

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The quality of caraway fruits used in pharmaceutical and food industry is given by the essential oil content and carvone content respectively. The minimal limit of carvone content in the essential oils required by the Czech Pharmacopoeia V. [1] is 50 %. The specification in the application for designation of origin (PDO) for Czech caraway requires also minimum of 50 % of carvone [2]. Seven European sources of caraway fruits (Czech varieties Kepron, Prochan, Rekord, Czech annual caraway, Czech candivar (candidate of variety) OP-CC-02, Polish variety Konczewicki, raw material imported from Poland) were analyzed for essential oil content by steam distillation [1] and essential oil composition by GC/MS. The results were compared to an Italian caraway from literature data [3]. The requirements of the Czech Pharm. and of PDO for carvone content were met by three Czech varieties (52.40 – 56.87 %), the candivar (57.84 %) and Koncziewicki (59.81 %). Czech annual caraway and imported Polish material haven't reached the limit of 50 % (46.18 and 48.36 %). Italian non-specified material had 23.3 % of carvone [3]. Generally, D-limonene content negatively correlates with carvone, due to the fact that limonene is its precursor and decreases during the ripening [4, 5]. Another part of the work was to evaluate the spectrum of micro-components in the essential oils where fenchon, β-linalool, anethol exceeded 1.0 % and also showed the differences between various genetic materials.

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A-46

Essential oil composition in *Lippia citriodora* (Humb et Kuntze): the farmer point of view

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Lippia Citriodora Humb et Kuntze, Verbenaceae family, is used as natural product by different industries for cosmetics (aromatherapy), phytopharmaceuticals, in perfumery and personal care, liquor and it is often used as herb tea [1].

The aim work's was to analyze the variation of essential oil composition and yield in response to cultivation site and phenological stage of harvesting.

The experiment was conducted in plain site (Legnaro, PD) and in mountain one (Villiago, BL). Plants grown on plastic mulch at a density of 13.333 p/ha and were harvested at three stages: before flowering, at full bloom and after flowering stage. For simulating a variation in the final harvesting time in autumn, as it is mainly determined in open field by weather conditions, three harvests in a very short period were evaluated for essential oil composition (10, 15 e 17 october 2007). Essential oils were obtained by steam distillation immediately after cutting and analyzed with GC equipped with a capillary column.

The essential oil content in the above ground mass increased during plant development and it reached its maximum at full bloom stage [2]. Geranial, neral and limonene were the main compounds [3].

Some of the molecules present in the essential oils during the blooming period showed a great variation even if the distance from the first to the last cutting were very short (7 days).

In the last harvesting period in autumn, geranial and neral showed an increase while Limonene decreased. Essential oils derived from two sites were different in composition: oils from Villiago presented an increase in the number of molecules increasing thus the aromatic complexity. In particular in the mountain site there was an increase of the higher molecular weight components and the highest peak resulted geranial on the contrary in the plants of the plain site limonene was the major constituent.

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A-47

A novel approach for aroma components analysis using a monolithic hybrid adsorbent as a new generation medium. "Monotrap"

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A Conventional media for sample adsorption and stir bar sorptive extraction (SBSE) and solid-Phase micro extraction (SPME) are coated with an extracting polymerphase, for example, polydimethylsiloxane (PDMS).Disadvantages of conventional media are their small surface area and thin polymer phase coating resulting in low recovery and need for a long extraction time and long conditioning time. The newly introduced product, MonoTrap is a new generation media for adsorption and extraction developed by using silica monolith technology. The state-of-the-art silica monolith technology allows us to manufacture an innovative hybrid adsorbent of silica and activated carbon (graphite carbon) having a large surface area bonded with octadecyl silane (C18). This C18 bonded hybrid medium shows quite effective adsorption capability to a wide variety of compounds.
In this study, the aroma component of ground coffee (Tanzania Black) were analyzed by MonoTrap - TD(Thermal desorption) and HSSE$^{(*1)}$ methods.
Instrumentation. Analyses were performed GC2010 and GCMS, QP2010 (Shimadzu), Thermal desorption unit (T-Dex) and GC Capillary Column IC-PureWAX (30mX0.25mmi.d., df=0.25um),MonoTrap-TD$^{(*2)}$(graphite carbon) (GLSciences).

fig1,MonoTrap-TD(Including Graphite carbon)

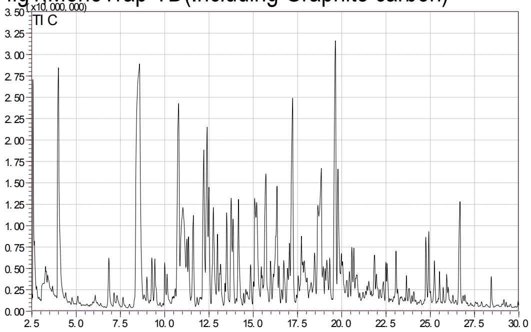

fig2,HSSE

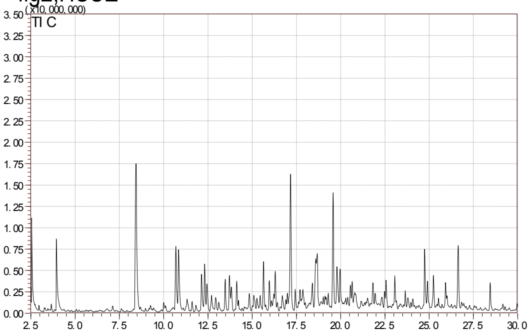

Table1,Area ratio

Compound	Ratio
1-Methylpiperidine	3.8
1-Methyl-1,2,5,6-tetrahydropyridine	4.8
(Dimethylamino)acetone	9.5
Dimethylpyrazine	5
Caffein	7.2

As a result, it was confirmed that MonoTrap has the capability of the Adsorption the Nitrogen compounds far better than HSSE$^{(*1)}$ method.

$^{(*1)}$; Head space sorptive Extraction.

$^{(*2)}$; At present, it is not a marketed production.

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A-48

Genuineness assessment of mandarin essential oils employing stable isotope ratio analysis (SIRA).

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Cold-pressed mandarin essential oils are products of particularly economic importance in many parts of the world and in Italy. They are used in perfumery as well as in food. So colled reconstituted mandarin oils are easy to find on the market. These oils are generally obtained by mixing monoterpenes and distilled oils of different origins, citrus oils other than mandarin with small amounts of natural mandarin oils, γ-terpinene and methyl N-methyl-anthranilate [1,2]. As well-known, useful information on the authenticity, quality, extraction technique, geographic origin, and biogenesis of the oils may be achieved through the classic non–chiral high resolution gas chromatography (HRGC) or employing different chiral stationary phases, to monitor specific pairs of enantiomers used as quality markers [1-5].

In recent decades, stable isotope ratio analysis (SIRA) has taken special interest for the detection of the adulteration of citrus oils. With respect the above mentioned, small differences in the isotopic composition of carbon can provide plenty of information: discrimination among products of different geographical origin, natural essential oils adulteration with synthetic or natural compounds, illicit application of farming practices or production methods etc [6]

The aim of this work is the assessment of genuineness of several mandarin essential oils, employing GC hyphenated to isotope ratio mass spectrometry (IRMS) together with classic GC-FID, enantioselective GC and HPLC analysis. Results obtained for commercial mandarin oils, and industrial “so declared” natural mandarin essential oils, belonging to different harvest period and geographic origin, were compared with those of genuine cold-pressed Italian mandarin oils obtained during the harvest season 2008-2009. The IRMS analyses were performed through δ^{13}C measurements via a combustion furnace (GC-C-IRMS), where the carbon atoms contained in the sample are converted into a simple gas (CO_2), and afterwards the attained carbon isotope ratio of the unknown sample is compared to that of a calibrated CO_2 reference.

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A-49

A Comparison Of Conventional And Multidimensional Gas Chromatography Methodologies In The Analysis Of Volatile Chiral Compounds In Mandarin Essential Oil

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The economical importance of Italian mandarin essential oils is widely recognized because of their quality thus many sophisticated adulteration techniques have been studied to maintain the qualitative or even the quantitative composition of the genuine oils [1]. Enantio GC is considered one of the most powerful techniques for the quality assessment of essential oils thanks to the characteristic enantiomeric excess of each chiral compound in different essential oils due to the specific biological pathway of the plant metabolism [2-4]. The advent of *enantio*- multidimensional capillary GC (enantio-MDGC), which was first described by Schomburg at al. [5], allowed unresolved target components to be heart-cut from the primary (achiral) column and delivered to the (chiral) analytical column [6].
The present research can be considered as a comparative study between conventional and multidimensional gas chromatography for the enantiomeric distributions of chiral compounds contained in mandarin essential oils in order to establish a genuineness range for quality control purposes. MDGC system, based on a Deans switch device, providing useful informations due to the possibility to accomplish a very high number of heart-cuts because of the lack of retention time shifts after each transfer thanks to the three restrictors configuration [7-8]. 113 natural Italian mandarin oils, belonging to the harvest period from september 2008 to february 2009, grown in Palermo and Reggio Calabria (Italy), were analyzed by enantio GC-FID and MDGC system.
The aim of the present work was to verify by means of the multidimensional system possible overestimations with respect to monodimensional enantio-GC thus to establish a genuineness range also for compounds partially coeluted and to obtain enantiomeric ratios of trace compounds commonly un-investigated for this reason.

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A-50

Chemical composition of the essential oil of *Achillea tenuifolia* Lam. (leaf), *Thymus caucasicus*, *Thymus kotschyanus* , *Thymus vulgaris* and *Pulsatilla albana* (Stev.) Bercht. & Presl., from Iran

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In Iran, the genus *Achillea* represented by nineteen species including 7 endemics. *Thymus* is one of the genuses in Labiatae family. It is represented in Iran by fourteen species including 4 endemics [1]. The leaf of *A. tenuifolia* was collected on 19 July 2008 in Khalkhal- Ardabil road northwest of Iran, at an altitude of 1700m. The plants materials of three species: *Thymus caucasicus*, *Thymus kotschyanus* and *Thymus vulgaris* were collected in North – west of Iran. Aerial parts of *Pulsatilla albana* were collected on 29 July 2007 in Khalkhal- Asalem road northwest of Iran. The air-dried of plants were subjected to hydrodistillation for 4 h, using a Clevenger type apparatus. The essential oil of plants was analyzed by GC and GC/MS methods. Twenty – one compounds in the leaf oil of *A. tenuifolia* (yield 0.5% V/W) were characterized, representing 92.2% of the total components detected and twenty constituents representing 94.8 % of the essential oil of *T. caucasicus*, thirty- one components 97.1% of the *T. kotschyanus* oil and twenty two compounds 98.2% of the *T. vulgaris* oil have been identified. The main components of the *A. tenuifolia* leaf oil were limonene (25.2%), α- pinene (14.4%), caryophyllene oxide (6.5%) and α- gurjunene (6.3%). The oil of *T. caucasicus* was characterized by higher amount of 1, 8-cineol (21.5%), thymol (12.6%), β- fenchyl alcohol (8.7%) and nerolidol (7.8%). In the oil of *T. kotschyanus*, carvacrol (24.4%), β- caryophyllene (14.5%), γ- terpinene (12.9%) and α- phellandrene (10.8%) were the predominant compounds. the main components of the oil of T. *vulgaris* were thymol (43.8%), ρ- cymene (15.2%) and germacrene-d (11.7%).Twenty- five compounds representing 97.5% of oil from *P. albana* were identified among them pulegone (39.1%), piperitenone (17.2%) and menthone (16.1%) were the major ones.

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A-51

Volatile composition of sage (*Salvia officinalis*) honey from the North West Adriatic region of Croatia by SPME-GC-MS analysis

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Sage (*Salvia officinalis*) honey is a traditional specialty of the North West Adriatic region of Croatia. [1] Since former times it is well known and also used in traditional medicine for the treatment of respiratory problems. Such importance notwithstanding, the honey is so far not well investigated.

The volatile profile is one of the typical features of honey. The volatile's composition is specific for each type of honey and closely related to the geographical region of production. [2] In order to characterize the volatile fraction of sage honey, SPME-GC-MS and SPME-GC-FID analysis were performed on 16 honey samples from different locations of the North West Adriatic region. The identification and quantification of the volatile compounds were done with the aim to find marker compounds useful for the quality and authenticity control of the sage honey of this specific geographical region. [3, 4]

The results show a high number of volatile compounds. In particular, most abundant is benzeneacetaldehyde with a mean value of 20.7% followed by benzaldehyde with a mean value of 10.1%. All the others compound's mean values range between 0.1-4.0 percent. Sage essential oil compounds could not be detected in the volatile fraction of sage honey.

The North West Adriatic region of Croatia is constituted by the islands of the Kvarner Bay and the part of coast under the North Velebit mountain range. Due the particular geographical characteristics and climate conditions of this region, a canonical discriminant function analysis (CDA) was applied to volatile compositions to distinguish honeys from the islands and the one from the coast.

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A-52

Chemo-taxonomical study of *Mentha suaveolens* ssp. *insularis*

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The genus *Mentha* L. (*Labiatae* family) includes 18 species and 11 hybrids among which several species are cultivated for essential oil production. In Europe and especially in France mints can be restricted only into six sexual species: *M. aquatica* L., *M. arvensis* L., *M. pulegium* L., *M. spicata* L., *M. longifolia* (L.) Hudson and *M. suaveolens* Ehrh [1]. The *M. suaveolens* Ehrh species is widespread in Corsica were it is represented by two subspecies (ssp.): *suaveolens* and *insularis* being an endemic species to Corsica and Sardinia.

Both subspecies, *insularis* and *suaveolens*, are botanically close. Leaves of *insularis* ssp are longer and greener than those of *suaveolens* ssp. They are not sessile and they have a short petiole (<0.5 cm) which differentiate both subspecies [2].

A detailed analysis of oil samples from individual plants which possessed all the botanical characters of the sub-species (*suaveolens* or *insularis*) was carried out by combination of GC(RI), GC-MS and ^{13}C NMR spectroscopy. *M. suaveolens* ssp. *suaveolens* essential oil exhibited two chemical compositions dominated by piperitenone oxide and piperitenone/isopiperitenone, respectively. The second composition is reported for the first time. Conversely, we demonstrated in a previous work, that *M. suaveolens* ssp. *insularis* oil presented an original composition, with pulegone and *cis-cis*-p-menthenolide as main components [3].

Fifty six individual plants were collected all around Corsica, and their compositions submitted to statistical analysis (K-means and PCA). Three groups could be clearly distinguished dominated by piperitenone/isopiperitenone (group 1, 5 samples, *suaveolens* ssp), piperitenone oxide (group 2, 15 samples, *suaveolens* ssp) and pulegone/*cis-cis*-p-menthenolide (group 3, 36 samples, *insularis* subspecies).Thus, the chemical composition of the oil has shown to be an additional tool to differentiate both subspecies.

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A-53

Sesquiterpene rich oils from leaves of five *Fortunella* species

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The so called *true citrus genera* are including three genuses: *Poncirus, Fortunella* and *Citrus*. Citrus essential oils have become an important by-product of the citrus industry. They are also used in the food and beverage industries to give citrus aroma and flavor to a variety of products. Kumquat (*Fortunella* sp), has been used in traditional Chinese herbal medicine, especially for colds and coughs. Literature reports the volatile constituents of essential from peel, flower and fruit from the *Fortunella* species, and only a few studies from leaves [1, 2]. The aim of the present work was to characterize the *Fortunella* species through the chemical composition of their leaf oil.

Five species, growing in the experimental orchard located at the Station de Recherches Agronomiques of INRA-CIRAD, San Ghjuliano, France, were studied: *F. hindsii* (Champ.) Swing., *F. obovata* Hort. ex Tan), *F. japonica* (Thunb.) Swing., *F. margarita* (Lour.) Swing. and *Fortunella crassifolia* Swing. [*F. japonica x F. margarita*]. A detailed analysis of essential oil from leaves was carried out by combination of GC(RI), GC-MS and ^{13}C NMR spectroscopy. The 70 identified components accounted for 92.5-97.0% of the total amount of oil: 41 hydrocarbons (8 monoterpenes, 3.2-12.8% and 33 sesquiterpenes 30.6-55.7%) and 29 oxygenated compounds (1 monoterpene tr-0.6% and 28 sesquiterpenes 23.4-52.2%).The five oils from *Fortunella* species exhibited a chemical composition dominated by germacrene D (14.9-28.7%) and β-elemol (7.5-26.8%). No qualitative differences were observed between the five chemical compositions, however quantitative variations were observed, for instance, δ-elemene (0.1-10.6%), limonene (2.8-7.4%), eremophil-9-en-11-ol (0.7-8.0%) and guai-6-en-10β-ol (0.4-5.8%). ^{13}C-NMR appeared as a powerful tool for identification of sesquiterpenes which possess various structures such as amorphane, cadinane, elemane, eremophilane, eudesmane, guaiane and stereoisomers such as: guai-6-en-10α and β-ol, amorph-4-en-10α and β-ol.

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A-54

Comparison between essential oil and volatile constituents of *Otanthus maritimus* (L.) Hoffmanns & Link isolated by HS-SPME/GC/GC-MS

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The genus *Otanthus,* found mainly in the Mediterranean region, belongs to *Asteraceae* and is represented by a single species [1], *O. maritimus* (L.) Hoffmans & Links, which is a strongly aromatic perennial herb, 15–30 cm tall, that has been reported in the past to exhibit a significant array of biological and pharmacological activities [2]. Only few studies have described the chemical composition of *O. maritimus* essential oil, obtained from the aerial parts [3, 4] and no studies are present about *O. maritimus* growing in Sardinia.

The fresh aerial parts (mixture of whole flowers and leafy stems) were cut and subject to hydrodistillation (4 h), to obtain the essential oil (EO), and to headspace-solid phase microextraction (HS-SPME). The SPME device (Supelco) coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 30 lm) was used for extraction of the plant volatiles. The optimization of HS-SPME sampling parameters was carried out using fresh aerial parts. The maximum sum of total peak area was acquired for a temperature of 70°C, an equilibrium time of 60 min and an extraction time of 30 min [5].

We made a comparison of hydrodistillation and HS-SPME extraction of volatile components in term of isolation time, plant-consuming and chemical composition. HS-SPME technique was fast in contrast to hydrodistillation. HS extraction was performed with a much smaller amount of plant than hydrodistillation. HS-fractions and oils showed quantitative differences in relative amount of components.

Both techniques evidenced that with the exception of camphor (20.8% in EO, 10.3% in HS-SPME), the main constituents were irregular monoterpenes: yomogi alcohol (22.8% in EO, 1.2% in HS-SPME), chrysanthenone (3.4% in EO, 2.8% in HS-SPME), artemisia alcohol (17.4% in EO, 12.0% in HS-SPME) and artemisyl acetate (5.4% in EO, 52.3% in HS-SPME). The quantitative analyses of hydrodistillated essential oil and HS-SPME showed large differences in relative amount of constituents.

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A-55

Comparison between compositions of peel and petitgrain essential oils from 17 *Citrus medica* L. cultivars

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The revival of the market for Corsican citron liqueur, and new information about the medicinal properties of citron essential oil [1] have led us to study the chemical variability of citron cultivars cultivated in Corsica. To our knowledge, little attention has been paid to assay the variability of citron essential oils [2, 3].

The aim of the present work was on the one hand, to get a knowledge of chemical composition of essential oils from citron cultivars in order to consider a potential utilization of these oils, and on the other hand, to purpose a chemotaxonomic method using data analysis for the differentiation of Citron cultivars.

The chemical compositions of peel and petitgrain essential oils, obtained from seventeen citron cultivars were established by GC and GC/MS. Combined analysis of essential oils led to the identification of 42 components amounting to 97.0-99.9% of the total oil. The peel and petitgrain oils were qualitatively close. However, the chemical composition of these oils showed important quantitative differences. For each cultivar, the peel oil exhibited higher amount of hydrocarbon monoterpenes and lower amount of oxygenated monoterpenes than the petitgrain oil.

To synthesize the chemical composition data, statistical analysis was applied to examine the relative distribution of cultivars according to their production of volatile compounds. For peel essential oils, the data analysis suggested the existence of three chemotypes: - the first was characterized by limonene and γ-terpinene; - the second characterized by limonene, geranial and neral; - the third characterized by limonene.

For petitgrain essential oils, the statistical analysis suggested the existence of four chemotypes: - the first characterized by limonene, geranial and neral; - the second characterized by limonene, geraniol and nerol; - the third characterized limonene, nerol, neral, geraniol and geranial and the last chemotype was characterized by limonene and β-pinene.

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A-56

Chemical characterization of four germplasm mint accessions (*Mentha* spp.)

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Essential oils of *Mentha* species are valued commercially as additives for food products, cosmetics and pharmaceuticals. Mint species are widely cultivated with production mainly in the United States, China, Eastern Europe, India and Mediterranean countries. Germplasm of mint species and cultivars have been recently introduced in Brazil by Embrapa Genetic Resources and Biotechnology, Brasília, DF. The objective of this work was to evaluate agronomic and chemically four genotypes of Mint: *M. aquatica* (CM01), *M. spicata* (CM07 and CM54), and *M. citrata* (CM47). The experiment was carried out at greenhouse conditions at the Department of Agronomy, University of Brasília, Brazil in a completely randomized design with four treatments (genotypes), each with four replications (5 plants per replication). The essential oil was extracted using a modified Clevenger apparatus and essential oil constituents were analyzed by gas chromatography and GC/MS. ANOVA and Tukey test were performed for statistical analysis. Considering morphological characters, both genotypes of *M. spicata* (CM07 and CM54) have presented differences related to leaf hair covering and rugosity. Accession *M. spicata* (CM07) showed leaves with the more rugose surface and a petiole size smaller than the other accessions. Leaf area did not show any significant difference among accessions. The average yield of essential oil from all accessions varied from 1.5 (CM1) to 2.52% (CM47). The highest values were presented by CM54 (2.25%) and CM47 (2.52%), which were significantly different from the other two accessions. The major constituents found in *M. aquatica* were limonene (73%) and 1,8-cineole (10%), respectively. Both accessions of *M spicata* (CM07 and CM54) and accession CM47 of *M. citrate* presented linalool as a major constituent, followed by linalyl acetate, representing around 70-80% of total essential oil content. The best results for fresh and dry matter, and essential oil yield were found with *M. citrata* (CM47) e *M. spicata* (CM54).

A-57

Chemical composition of the essential oil and hydrolat from leaf buds of *Populus maximowiczii* Henry × *berolinensis* (K. Koch) Dippel.

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Populus is a genus of between 25-35 species of dicotyledonous flowering trees in the family Salicaceae, native to most of the Northern Hemisphere. English various names applied to different species include poplar, aspen, and cottonwood. In Poland three *Populus* species and a hybrid grow naturally and many other species in this plenty of hybrids are cultivated [1]. *Populus maximowiczii* Henry (Japanese poplar) and *Populus* × *berolinensis* (K. Koch) Dippel (Berlin poplar) belongs to *Tacamachaca* division. In Poland a hybrid of that two species, *Populus maximowiczii* Henry × *berolinensis* (K. Koch) Dippel., as well as many others were brought by Arboretum in Kornik and now they are valuable species. Characteristic features of the hybrid under investigation are fast growth, regular corolla, light bark and dark green leaves [2].

Poplar leaf buds were collected in April 2006 from the trees growing in the Northern Poland. Essential oil and hydrolat were obtained in the same step by industrial steamdistillation (30 min.) from 134kg of fresh buds in the amount of 270ml and 10l, respectively. Essential oil was colourless liquid with characteristic, pleasant fragrance. Hydrolat was colourless and has got similar odour to the essential oil. Volatiles from hydrolat were recovered during laboratory hydrodistillation in Clevenger-type apparatus from 0.5l of hydrolat with the yield 0.067%. The essential oil and volatiles from hydrolat were analyzed by GC and GC-MS. *ar*-Curcumene and γ-amorphene composed together over 31% of the oil. Other sesquiterpenes were α-humulene (7.5%), (*E*)-β-caryophyllene (6.7%) and *trans*-α-bergamotene (6.3%). Oxygenated sesquiterpenes constitute over 22% with guaiol (7.0%) and bulnesol (4.6%) as the main ones. Major components of the hydrolat were also *ar*-curcumene and γ-amorphene (12.9%), guaiol (6.0%) and bulnesol (4.8%) but hydrolat contained more oxygenated monoterpenes than the oil, e.g. linalool (12.4%), borneol (2.1%) and α-terpineol (1.6%). Essential oil and hydrolat from that hybrid were not studied before and are interesting mainly for the content of sesqiterpene alcohols. Both products have got very pleasant fragrance so could be used in perfumery.

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A-58

Essential oil from fruits of Japanese cork tree

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Japanese cork tree (*Phellodendron japonicum* Maxim., *Rutaceae*) is a small (5 m high), deciduous tree with ornamental, exotic appearance. It is native to east and northeast Asia. In Europe and North America it is planted in parks as a decorative tree. The plant has pea-sized, black, aromatic fruits.

Essential oils from fruits of Japanese cork tree were investigated. The plant material was collected in Rogow arboretum from a single tree, unripe fruits in July, ripe fruits in October. Fresh unripe fruits, fresh ripe fruits and air-dried ripe fruits were crushed and hydrodistilled separately. The volatile oils were obtained in yields 1.1% (v/w) from fresh unripe fruits, 1.3% (v/w) from fresh ripe fruits and 3.4% (v/w) from air-dried fruits. The oils had intensive, terpenic aroma with fruity note.

Chemical composition of the oils was analyzed by chromatographic and spectral methods. Components were separated by vacuum distillation and repeated flash chromatography (FC). They were identified by gas chromatography (GC), gas chromatography coupled with mass spectroscopy (GC-MS) and some of them by ^{1}H-NMR spectra.

More than 60 components were identified in the oils. Monoterpene hydrocarbon myrcene was the dominant constituent (79.5-86.1%) in the samples. The other significant constituents were germacrene D (2.5-11.8%) and β-caryophyllene (1.1-4.0%). The other components occurred in amount of less than 1%. The composition of the oils obtained form ripe and unripe fruits varied slightly. The oil from unripe fruits contains more monoterpenes and less sesquiterpenes than oil from ripe fruits. Drying of fruits had no influence on the oil composition.

Poster presentations
Session B

B-01

Antimicrobial activity and chemical composition of the bark essential oil from *Anthonotha isopetala* var. *macrantha*, an endemic species from Gabon

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The genus *Anthonotha* (syn. *Macrolobium*) which belongs to the family of Leguminosae-Cesalpinoideae includes 28 species found in the tropical african forest [1-3]. In the Gabonese flora, this genus is represented by 11 species. Our investigations concern an endemic species growing in Gabon: *Anthonotha isopetala* (Harms) J. Leonard var. *macrantha* (J. Leonard) J. Leonard (syn. *Macrolobium conchyliophorum* Pellegr.; *Macrolobium isopetalum* Harms var. *macranthum* J. Leonard). This species is commonly used by healers in traditional medicine to treat spleen disorders; nevertheless, to the best of our knowledge there are no previous reports on the chemical investigations and biological activity of its volatile (or non volatile) extracts.

The essential oils, obtained by hydrodistillation of two samples of bark from *A. isopetala* var. *macrantha*, were analyzed using GC and GC/MS. The essential oils yield was 0.18% (v/w) with sabinene (5.7-21.8%), limonene (41.2-49.9%) and terpinen-4-ol (11.2-15.1%) as major constituents.

The antimicrobial activity of the essential oils was investigated using 3 strains of bacteria (*Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus)* and 2 fungi (*Aspergillus niger* and *Fusarium moniliforme).* A first evaluation using the agar disc diffusion method [4] indicated that the more resistant strain was *P. aeruginosa*, the two fungi and *S. aureus* being the most sensitive.

These results were confirmed by a second evaluation using a broth microdilution method [5] where the MIC was found higher (2%) for the Gram-negative bacteria and equal to 1%, 1% and 0.5%, respectively, for *S. aureus, A. niger* and *F. moniliforme.*

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B-02

Secondary metabolites as protection against mango gall fly

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Volatile organic compounds (VOC) and non-volatile leaf compounds such as tannins, phenols and terpene lactones are known to act as insect attractants, repellants or inhibitors of insect development [1]. The aim of this study was to determine the role of secondary metabolites on the resistance of mango cultivars to gall fly attack. Headspace profiles of VOC's of three mango cultivars were obtained by thermal desorption-GC-FID and TD-GC-MS, over two seasons. Cultivars susceptible to gall fly were found to present similar profiles, while profiles of susceptible and resistant cultivars differed substantially. Most of the terpene concentrations, in ratio to Δ^3-carene, stayed constant throughout the season. The concentrations of some terpenes increased relative to that of Δ^3-carene as the season progressed, reaching optimal levels in February, which corresponds to flush and the timing of gall fly hatch. Possible mango gall fly attractants were identified from this data. The second aspect of this study was to determine whether non-volatile organic compounds play a role in the resistance/susceptibility of the cultivars to gall fly. Extraction and HPLC determination of these organic compounds in leaf material showed concentration differences between the extracts from susceptible cultivars 'Heidi' and 'Keitt'. The phenolic profiles of the resistant cultivar, 'Sensation', were found to differ significantly from those of the susceptible cultivars. This study proves that there is a link between the secondary metabolite production of mango cultivars and mango gall fly attack and development.

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B-03

The aroma profile of the volatiles from edible marine green algae *Ulva lactuca*

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The green algae *Ulva lactuca* L. (Ulvacea), known as sea lettuce, are popular food in many of the places where they grow, including Scandinavia, Great Britain, Ireland, China, and Japan (where they are known as Aosa and used in the popular Sushi). They can be eaten raw in salads, or cooked in soups, and are high in protein, soluble dietary fibers, and a variety of vitamins and minerals including especially iron [1, 2], in addtion to their medicinal values as antibacterial (MRSA) [3] and immunostimulating activities [4]. The aroma profile of characteristic volatile components isolated by hydro-distillation from fresh *Ulva lactuca* obtained from Red Sea were investigated for the first time by GC and GC/MS using CP-Sil-5-CB fused silica capillary column. Sixty-six compounds (representing 84 %), including monoterpenes 5.38 %, sesquiterpenes 8.08 %, diterpenes 0.97 %, fatty acids 11.06 % with palmitic acid as major component 9.8 % were identified. The total oxygenated compounds 66.67 % with major long chain aldehydes; tetradecanal 2.63 %, hexadecanal 17.57 %, octadecanal 0.2 % , eicosanal 0.83 %, docosanal 0.12 % were confirmed to be important compounds responsible for the sensory characteristic aroma of the *U. lactuca..*

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B-04

Antioxidant activity of Lemon essential oils

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In order to evaluate antioxidant activity, twelve samples of lemon essential oil (LEO) produced from November to July were considered. Cold pressed essential oils were obtained from the fruits of *Citrus Limon* L. Burm. f. processed by an FMC In-Line system. The volatile fraction was measured by GC/MS and residue composition by HPLC. The total antioxidant activity (TAA) was determined with the β-carotene-linoleic acid bleaching method. The free and stable radical DPPH° (2.2'-Diphenyl-1-picrylhazyl) was used to measure the radical scavenging activity (RSA) of essential oil samples. Superoxide anion scavenging activity (SSA) was measured using the ipoxanthine-xanthine oxidase method with NBT as an indicator. Preliminarily it was verified that LEOs do not inhibit the xanthine oxidase.

The results show that the composition of the volatile fraction and residue is variable and the highest percentage of distinctive aldehydes, neral and geranial, are in winter samples. Traces of 5-MOP and 8-MOP in the residue were not measurable for all samples. Volatile and non-volatile components of LEO samples were used for principal component analysis. More than 70 % of variance was explicated from the first two components. Sample positions in the score plot are influenced by the production period and the typical trend is obtained according to previous research (data not published). Correlations between TAA%, RSA%, SSA% and the composition of essential oils were carried out. The loading plot shows two groups of essential oil components (positive and negative correlations) that are well correlated with TAA% and two other groups correlated with SSA%. No significant correlations were found between DPPH radical scavenging activity and LEO composition.

The results of this research show that cold pressed lemon essential oil, thanks to its antioxidant capacity, could be used for improving the shelf life of food that is subject to oxidative alterations.

B-05

Molecular mechanistic evidences of the anti-arthritic activity of α-pinene

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Nuclear Factor-κB is a key transcription factor activated by pro-inflammatory signals, like interleukin-1β, being required for the expression of many inflammatory and catabolic mediators, namely nitric oxide (NO) that plays an important role in arthritic diseases.

Aiming an explanation for the alleged anti-arthritic proprieties of some essential oils and the detection of natural inhibitors of IL-induced NF-κB activation and NO production, several oils were screened by measuring their ability to inhibit IL-1-induced NO production in human articular chondrocytes isolated from the femoral condyles and NF-κB activation in the human chondrocytic cell line C-28/I2.

Both cell cultures were incubated with the oils, their fractions or pure components in different dilutions, followed by treatment with IL-1β to evaluate the NO production (Griess reaction) or the cytoplasmic levels of IκB-α (western blot).

From this screening we report now on the activity of an α-pinene enriched fraction (F3) isolated from the oil of leaves of *J. oxycedrus,* as well as, on the activity of racemic α-pinene, (-)-α-pinene and (+)-α-pinene.

Effects on IL-1-induced NO production					
IL-1β, 30 ng/ml	Fraction F3 (α-Pinene >90%)	α-Pinene (racemic)	(+)-α-Pinene	(-)-α-Pinene	Test dilution
100.0 ± 6.0 %	51.4 ± 7.3 %	58.5 ± 7.5 %	56.2 ± 10.8 %	63.4 ± 7.8 %	1:7500
	11.3 ± 0.4 %	40.6 ± 3.4 %	30.1 ± 2.9 %	57.3 ± 6.0 %	1:5000

At non cytotoxic (MTT reduction assay) dilutions, fraction F3 achieved the greatest inhibition of both NF-κB activation and NO production, followed by (+)-α-pinene.

At the test dilution 1:2500, α-pinene reduced IκB-α degradation by 44%, whereas at a concentration two fold lower (dil. 1:5000) NO production was inhibited by nearly 90%. This indicates that α-pinene acts both at the transcriptional level and at iNOS enzyme activity. This dual mode of action may represent an advantage over other agents that inhibit NF-κB activation or iNOS activity, making α-pinene a promising anti-arthritic lead compound.

Results also contribute to explain the anti-arthritic effects assigned to some α-pinene containing essential oils. Since fraction F3 is more active than racemic α-pinene or anyone of its enantiomers, becomes plausible that other minor components of such fraction can also influence the observed activity.

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B-06

Effects of phenolic essential oils and their major compounds on the morphology and adherence of *Giardia lamblia* trophozoites

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Flagellated protozoan *Giardia lamblia* is a cause of human and animal disease. It is responsible for intestinal infection that may lead to significant morbidity and mortality, among children, elderly and immune compromised. Current chemotherapy is unsatisfactory due to high incidence of undesirable side effects and failure in clearing parasites from gastrointestinal tract. *Giardia* attachment to enterocytes is essential for colonization of the intestine and a prerequisite for clinical disease. Earlier we showed that phenolic rich essential oils are particularly active inhibiting the growth of *G. lamblia* in vitro [1, 2]. Now we report on the changes induced by essential oils and pure phenolic components on the morphology and functional aspects of the parasite cell. Trophozoites (ATCC 30957) in culture (5x10^4) were incubated at 37 ºC with essential oils -*Thymus zygis subsp. sylvestris* chemotype thymol, *Thymbra capitata*, *Origanum virens, Lippia graveolens* and *Syzygium aromaticum* - or of their major phenolic components - thymol (DBH), carvacrol and eugenol (Fluka) - at IC50 concentrations. *Syzygium aromaticum* oil was acquired at commerce (Segredo da Planta), all other oils were prepared at laboratory by hydrodistillation from fresh plant material. Compositions were determined by GC and GC-MS analysis. Cell morphology was studied by optical and transmission electronic microscopy; adherence capacity was evaluated by cell counting and expressed as percentage of attached trophozoites. Major morphological alterations were observed on cell shape together with precipitates and cytoplasmatic protrusions, intracellular clearing and membrane disruption. Impairment on cytoskeleton, disorganization and fragmentation of the adhesive disc were also observed. These morphological damages justify effects on viability, likewise on functional aspects as adherence capacity.

Effects on *G. lamblia* attachment and total cell number (at 7 hours of incubation)

	Thymus zygis	*Thymbra capitata*	*Origanum virens*	*Lippia graveolens*	*Syzygium aromaticum*	Thymol	Carvacrol	Eugenol
IC$_{50}$ (μg.ml^{-1})	185	71	85	257	134	47	51	101
Adherence ability	10%***	10%***	10%***	10%***	20%***	20%***	15%***	5%***
Viable cells	50%***	50%***	50%***	50%***	50%***	50%***	70%**	100% ^{ns}

***p<0.001 **p<0.01 ^{ns} not significant

These mechanisms support the potential of phenolic essential oils and phenolic components as therapeutic agents for giardiasis once they influence both, *Giardia* viability and attachment to the host tissues, limiting infection and improving the intestinal clearance of the parasite.

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B-07

Composition and cytotoxic activity of essential oils of *Croton matourensis* and *Croton micans* from Venezuela

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Continuing a research project in which the chemical and pharmacology studies of *Croton* species from Venezuela are investigated [1-2], the analysis of volatile constituents from the species *Croton matourensis* and *Croton micans*, has been undertaken. In our pharmacological screening, the aqueous and dicloromethane extracts from flowers and leaves of these plants, showed strong inhibition on three different leukemia cells. *C. matourensis* is locally known as *tabaquillo* and the isolation of a diterpene known as maravuic acid had been reported from this specie [3]. *C. micans*, is endemic in our country and was misidentified as *C. caracasana* in our previous publications in which we reported, the isolation of two 3,4-*seco*-entkaurenes [4]. The chemical composition of the oils were examined by means of GC-FID and GC-MS. The identification of volatile components was assigned by comparison of mass spectra with the Wyley and Nist libraries, and by the Kovats indices. Forty six different compounds accounting 95.5% of the total oil were identified in the oil from the leaves of *C. matourensis* and among the major components, we found: fenchyl acetate (19.5%), methyleugenol (14.2%), isoelemicin (11.3%), elemicin (7.6%), spathulenol (6.9%) and valencene (5.8%). The main components identified from the flowers oil of *C. micans* were: fenchyl acetate (41.0%), α-caryophyllene (12.6%), α-cubebene (5.3%), β-cubebene (5.5%), β-elemene (4.7%), β-caryophyllene (5.5%) and valencene (4.6%). The oil from the leaves from *C. micans* gave: fenchyl acetate (25.3%), caryophyllene (20.7%), α-selinene (12.8%), β-bourbene (9.3%) as major constituents. The essential oils from flowers and leaves showed moderate activity against the cancer cell lines and the normal cell line. Some of the components found in these oils such as: terpinen-4-ol, D-limonene, spathulenol, cadinol, β-elemene have been reported with activity against breast, colon, gastric, lung, ovarian and laryngeal cancer cell lines [5].

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B-08

Volatile constituents and antimicrobial activity of *Leptospermum scoparium* J.R. et G. Forst from New Zealand, *Ravensara aromatica* Sonn. and *Cinnamomum camphora* (L.) J. Presl. from Madagascar

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Ravensara aromatica Sonn. is an evergreen tree belonging to the family of *Lauraceae*, endemic to Madagascar [1]. In the same environment of *R. aromatica*, although not endemic to Madagascar, *Cinnamomum camphora* (L.) J. Presl. finds optimal conditions for spontaneous growth. Much confusion has arisen from the name *Ravintsara* which is given by Madagascan people to *C. camphora*. Furthermore, *C. camphora* from Madagascar has been scarcely investigated up to now, despite of the Asian species [2]. A completely different ecosystem can be found in New Zealand, where *Leptospermum scoparium* J.R. et G. Forst has its own habitat [3]. This shrub is sometimes confused with *Melaleuca alternifolia*, commonly known as tea tree, because of the similar use made in the past of these two species.

Scope of the work was the chemical investigation through GC-FID and GC-MS of the plant extracts, in order to bring clarity to the uncertainty of data present in literature; also, the antimicrobial activity of some constituents has been studied. Area normalization with response factors has allowed to determine the detailed chemical composition of the three oils. Major compounds were limonene (28.8 g%), methyl chavicol (9.9 g%), linalool (8.9 g%) and methyl eugenol (3.7 g%) in *R. aromatica*; 1,8-cineole (47.8 g%), limonene (35.0 g%) and p-cymene (7.4 g%) in *C. camphora*; α-copaene (35.5 g%), (E)-caryophyllene (13.0 g%), trans-calamenene (5.7 g%), isoleptospermone (1.6 g%) and leptospermone (6.3 g%), in *L. scoparium*. An nteresting and quite diverse sesquiterpene fraction has been found in *R. aromatica* (10.5 g%). The screening for antimicrobial and antifungal activity was carried out through the determination of Minimal Inhibitory Concentration and Growth Inhibition Factor (Kirby-Bauer method). *L. scoparium* was highly effective against *Bacillus subtilis* and *Candida albicans*, whereas the same level of activity was shown by *C. camphora* and *R. aromatica* against *Staphylococcus aureus*.

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B-09

Compositions of the Essential Oils of Six *Salvia* Species from Turkey

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The genus *Salvia* (Lamiaceae) is represented in Turkey by 95 species, of which 48 are endemic. The rate of endemism in the genus *Salvia* is *ca.* 50%, in Turkey [1]. The leaves of *Salvia* species are also used in Turkish folk medicine. They are generally known as 'adaçayı' in the regions where they grow and consumed as a hot drink [2]. In our continuing research on *Salvia* species we have investigated *Salvia divaricata* Montbret & Aucher ex Bentham (Endemic), *S. huberi* Hedge (Endemic), *S. hydrangea* DC. ex Benth., *S. kronenburgii* Rech. fil. (Endemic), *S. macrochylamys* Boiss. & Kotschy, and *S. nydeggeri* Hub.-Mor. (Endemic) essential oils. The *Salvia* essential oils were obtained from the aerial parts by hydrodistillation and were subsequently analyzed both by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems, simultaneously. The major components characterized in the oils were as follows: 1,8-cineole (30.9%), α-pinene (17.1%), camphor (10.1%) and camphene (7.7%) in *S. divaricata*; naphthalene (22.1%), β-pinene (13.3%) and α-pinene (9.9%) in *S. huberi*; camphor (46.9%), camphene (9.4%) and 1,8-cineole (7.4%) in *S. hydrangea*; geranyl acetate (16.0%), 1,8-cineole (12.5%), carvone (12.0%) and limonene (6.2%) in *S. kronenburgii*; β-caryophyllene (26.4%) and caryophyllene oxide (22.2%) in *S. macrochylamys*; α-pinene (15.8%), β-pinene (9.2%), cubebol (6.2%) and caryophyllene oxide (5.6%) in *S. nydeggeri.* Blank distillation and analysis was performed to exclude pollution from the laboratory.

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B-10

Chemical constituents and antibacterial activity of *Choisya ternata* Kunth essential oil

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Choisya ternata Kunth is member of the Rutaceae family. It is an evergreen shrub native to North America particularly the South Western states and Mexico. In a continuous search for antibacterial, antifungal and antivirus compounds from essential oils of plants growing in Ireland, the leaves of *Choisya ternata* were collected on the 20th October 2008 from the Trinity College Botanical Gardens, Dartry, Dublin. A portion of the plant material was extracted for essential oils immediately. The other portion was allowed to air dry in a dark unheated room with no heat source. After one week the sample was then extracted. This provided two oils from this plant, oil 1 and oil 2 (after drying). The chemical composition of the oil was determined by GC and GC/MS. Oil constituents were identified by comparison of their linear retention indices (relative to C_7-C_{31} alkanes [1] on the HP-5MS column) with literature values [2] and their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3, and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils, and wherever possible, by co-injection with an authentic sample. GC (FID) analysis was carried out under the same experimental conditions using the same column as described for the GC/MS. The percentage composition of the oil was computed from the GC peak areas without any corrections. The main difference between the two samples was the fact that sample 2 showed a slightly enhanced concentration of sesquiterpenoids. Samples 1 and 2 had a total of 157 compounds identifiable being 0.1/0.2% of hemiterpenoids, 86.4/83.8% of monoterpenoids, 11.8/13.6% of sesquiterpenoids, 0.2/0.2% of diterpenoids and 0.7/0.9% of other molecules, respectively. Sample 2 was assayed for antibacterial activity using MRSA *Staphylococcus aureus*, showing an inhibition halo of 2.5 cm at a concentration of 1mg/ml. This oil is being evaluated at this moment for antifungal and antivirus activities.

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B-11

ACF-DHS/GC vs SPME/comprehensive GC: two approaches for a rapid evaluation of the volatile fraction of aromatic plants.

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Essential oil plants are aromatic crops used in perfumery, cosmetic and food industry which extracts were obtained by hydrodistillation or steam distillation. During extraction process, essential oil storage vesicles were explosed due to temperature and mechanical factors and consequentatively aromatic compounds could be more easily extracted by water vapors. Flavorists and farmers duplicated this fact when they crush between their fingers a couple of leaves or flowers followed by sniffing of the generated odor in order to set up a rapid evaluation of the aromatic potential of such plants. In the framework of the AROMATIC Program, we develop/adapt instruments to be used for a rapid evaluation of aromatic potential of essential oil plants based on headspace analysis. Two strategic approaches were studied; either the use of a classical sampling technique coupled to a specific/ultra sensitive separation/detection system nor a specific/high effective sampling system coupled to a classical separation/detection ones. If the first methodology was based on the coupling of Static HeadSpace device to Comprehensive GC (GC x GC / MS-TOF), the second ones was based on a self designed apparatus, allowing to copy the crushing of leaves between fingers. The so-called Artificial Crushing Finger device (ACF) was based on a modified artificial mouth and has the objective to perform a rapid evaluation of aromatic potential of essential oil plants with only a couple of leaves through a controlled Dynamic HeadSpace (DHS) concentration of volatiles emitted by crushed fresh leaves followed by GC-MS analysis [1,2] In the present poster, we reported the comparison of volatiles chromatographic profiles of essential oil of our model plant , balsamite (*Tanacetum Balsamita*), obtained by hydrodistillation with the ones obtained with entire fresh leaves volatiles concentrated by SPME (PDMS-CAR) and analyzed by Comprehensive GC/MS-TOF and with ACF treated fresh leaves volatiles concentrated on Tenax trap and analyzed by GC-MS [3]

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B-12

Calamintha Grandiflora native oil vs extracted essential oil: chemical composition and biological activities

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As Midi-Pyrénéees region in France and Lithuania have a rich and unvalorized wild vegetal heritage, the BALTMIPAROM Trek was conducted in order to collect and analyse various regional medieval aromatic plants. Among these, greater calamint (*Calamintha grandiflora)*, belonging to the Labiatae family, is a perennial herb having a strong and penetrating mint odor which is presently used in fresh state as a condiment in french “nouvelle cuisine”. So in order to study native” oil (equivalent to leaving plant odor for flowers), a special designed apparatus, i.e. Artificial Crushing Finger (ACF) device, allowing to copy the crushing of leaves between fingers practiced by farmers and flavorists for evaluation of aromatic potential of herbs was self-developped [1]. By explosing the storage vesicles located on leaves, ACF, coupled with either a Tenax trap or SPME system, allowed to perform a rapid evaluation of native oil content with only a couple of leaves. Major components and odor active compounds of *Calamintha grandiflora* native oil were analysed by ACF-SPME and ACF-DHS coupled to GC-FID and GC-MS and compared to extracted (hydrodistillated) essential oil ones. The optimization of sampling conditions (duration, fibers type) were set up and DVB/CAR/PDMS fiber appeared as giving the best representation of emitted volatiles. On the otherhand, a bioreffinery approach, i.e. valorization of the entire plant, was applied to greater calamint in order to better valorize extraction by-products. The residues obtained after hydrodistilation were separated into liquid and solid fractions. The solid fraction was dried and then extracted with acetone, methanol and ethanol, while the liquid fraction (aromatic waters) was freeze-dried. Antioxydant and antibacterial activities of concentrated extracts obtained were evaluated and comparated to ones of both methanol extract of entire plant and essential oil [2].

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B-13

Composition - antimicrobial activity relationship of essential oils from some apiaceae species cultivated in Egypt using different methods of extraction

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The fruits and leaves of many species belonging to Family Apiaceae have been widely used for medicinal and culinary purposes worldwide. This is mainly attributed to their rich essential oil content of diverse constituents and hence, various medicinal use [1]. In this study, the essential oils of the fruits of six Apiaceae species, commonly used and cultivated in Egypt, including *Pimpinella anisum*, *Carum carvi*, *Apium graveolens*, *Anethum graveolens*, *Foeniculum vulgare* and *Petroselinum crispum* were investigated to determine and correlate the optimum oil composition for antimicrobial activity. Essential oils fractions were collected using hydrodistillation at constant time intervals as well as by SFE methods and the composition of each fraction was analysed using GC and GC-MS techniques. The collected fractions were screened for their antimicrobial activity and showed significant inhibition of Gram-positive bacteria and to less extent, Gram-negative bacteria, yeast and filamentous fungi. The MIC of each fraction was determined along with the major component for each oil. The data obtained was compiled and comparative interpretations regarding the oil composition and the effect it had on the antimicrobial activity was substantially different for each oil. In some cases, the first fraction collected after 30 min. showed the most potent antimicrobial activity as in the case of *Pimpinella anisum* which also constituted the highest percentage of its main component *trans*-anethole (96.31%). The study concluded that even though the antimicrobial activity was largely dependent on the major oil component in most of the oil fractions, yet in others, the synergistic effect of certain components contributed to the highest antimicrobial activity.

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B-14

Propolis volatile composition from beehives maintained in Algarve (Portugal)

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Propolis is a resinous substance collected and processed by bees from the local flora, and includes, among others, phenolics, mono-, sesqui-, di- and triterpenes. Propolis is reported to have diverse biological activities [1], but its chemical composition and properties vary considerably, according to the bees' botanical sources. *Varroa destructor* is an ectoparasitic mite that attacks *Apis mellifera* and may lead to the death of a honey bee colony. Thymol has been used in the integrated *Varroa* control. Nevertheless, long-term use of thymol can lead not only to bees' death but also to the presence of thymol residues in beehives products such as propolis. The aim of this study was to determine the composition of propolis volatiles and to ascertain if the use of thymol leads to higher amounts of this compound in propolis.

Twenty-three samples of propolis of *Apis mellifera* were collected, in October 2007, from beehives maintained in three different areas of Algarve. Three of the beehives had no treatment, two were treated with thymol and the remaining ones were submitted to flumethrin (pyrethroid ester acaricide). The most abundant species in the surrounding natural flora were *Cistus* spp., *Rosmarinus officinalis*, *Quercus* spp. and *Arbutus unedo*. Volatiles were isolated by hydrodistillation and analysed by GC and GC-MS [2].

The volatiles composition from the majority of the samples was dominated by viridiflorol (9-20%), *n*-nonadecane (4-9%) and *n*-tricosane (5-8%). A separate group of two samples was characterized by high amounts of thymol (17-29%) and viridiflorol (10-11%). Data showed that variations in propolis volatiles were mainly quantitative and not qualitative, which may indicate that bees collected propolis from a similar botanical source. The presence of higher thymol levels in some propolis samples may reflect the long use of an acaricide with thymol as main active ingredient.

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B-15

Volatiles from *Plicanthus hirtellus and Radula boryana* grown in São Tomé and Príncipe

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São Tomé and Príncipe is an Archipelago located in the Gulf of Guinea, comprising two islands, São Tomé the largest one where the highest mountain is also located (2024 m) and Príncipe. The climate is tropical, hot and very humid, with small thermal amplitude. In 1988, the forest of São Tomé and Príncipe was classified by the International Scientific Association as one of the most important in Africa, due to the high plant diversity, including many endemic species, namely bryophytes.

Plicanthus hirtellus (F.Weber) R.M.Schuster is a robust liverwort belonging to the Jungermanniaceae Rchb that grows on rocks or on the tree trunks and branches in full light habitats [1].

Radula boryana (F.Weber) Nees is a Radulaceae (Dumort.) Müll.Frib. liverwort that grows on boles and large branches of forest trees in humid habitats [2].

The volatiles of the *P. hirtellus* and *R. boryana*, collected on São Tomé, were isolated by distillation-extraction from samples of the two species and analysed by GC and GC-MS, as previously reported [3].

P. hirtellus volatiles were dominated by spathulenol (12%), bicyclogermacrene (9%) and one unidentified sesquiterpene hydrocarbon (14%). Carvone (4%) and 1,8-cineole (2%) were also detected in this species volatiles.

Interestingly, *R. boryana* volatiles were characterized by high amounts of *p*-cymene (17%), along with other monoterpene hydrocarbons and by the unusual presence of the oxygen-containing monoterpenes, thymol and carvacrol. Sesquiterpenes and fatty acids were also present in *R. boryana* volatiles. This composition is markedly different from that usually found in *Radula* species organic-solvent extracts [4].

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B-16

Combined volatile and molecular analysis of *Juniperus brevifolia*, an endemic Azorean species

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Juniperus brevifolia (Seub.) Antoine (Cupressaceae) is an Azorean endemic species that occurs on all islands, except on S. Maria and Graciosa [1]. The present study reports a combined analysis using *J. brevifolia* volatiles composition, and RAPDs and ISSRs as molecular markers, evaluating the relationship between both data sets.

A total of forty-two individual twigs samples were collected on seven Azorean islands (S. Miguel, Terceira, S. Jorge, Pico, Faial, Flores and Corvo). Volatiles from all samples were isolated by distillation-extraction and analyzed by GC and GC-MS, as previously reported [1]. DNA fingerprinting was performed using fifty-one RAPD primers (Operon Technologies) and twenty-five ISSR primers (UBC set Nº. 9), according to [2, 3]. A sample of *Juniperus chinensis* was included as an out-group on molecular analysis. NTSYS software [in 1] was used for volatile oils and DNA data cluster analysis.

Eighty one components, representing 97-100% of the total oils, were identified. The oils consisted mainly of monoterpene hydrocarbons (84-96%), limonene (33-87%), α-pinene (1-48%) and β-mircene (1-5%) being the main components, in accordance with previous studies [1]. The diterpene fraction (1-9%) was dominated by 13-*epi*-manool (1-6%) and *trans*-totarol (0.1-3%), while 1-octen-3-ol (trace-4%) was the major constituent of the non-terpenic fraction (trace-4%). Cluster analysis of chemical data showed a high correlation among all samples (S_{corr}=0.84).

The selected RAPDs and ISSRs primers generated a total of 882 bands representing 94% of polymorphism between individual samples. In contrast to chemical data, cluster analysis of molecular data showed a low correlation between accessions (S_{corr}=0.32). Plants were grouped according to their geographical location.

According to the present results, *J. brevifolia* samples from seven Azorean islands showed a homogeneous volatile composition. The molecular approaches tested were useful in assessing genetic diversity although no straight correlation could be found between both data sets.

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B-17

Antioxidant, anti-inflammatory and antiacetylcholinesterase activities of essential oils and remaining water-distillation extracts of some aromatic plants

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Essential oils and/or other plant extracts have been shown to act as antioxidants and acetylcholinesterase inhibitors, among other biological activities [1, 2].

In the present work, the antioxidant (scavenging of free radicals and superoxide anion), anti-inflammatory (inhibition of lipoxygenase capacity) and antiacetylcholinesterase (acetylcholinesterase inhibitor) activities of essential oils and remaining water-distillation extracts of eight aromatic plants (*Thymus camphoratus*, *Th. carnosus*, *Th. mastichina*, *Thymbra capitata*, *Origanum vulgare*, *Salvia officinalis*, *Foeniculum vulgare* and *Dittrichia viscosa*) were studied. Essentials oils were isolated by hydrodistillation and analysed by GC and GC-MS, as previously reported [3]. Biological activities of essential oils and remaining water-distillation extracts were assessed as reported in [4].

The essential oils were dominated by 1,8-cineole in *S. officinalis* (59%), *Th. mastichina* (49%) and *Th. camphoratus* (21%); borneol (20%) in *Th. carnosus*; carvacrol in *T. capitata* (68%); γ-terpinene (49%) in *O. vulgare*; α-pinene (26%) in *F. vulgare*; and *trans*-nerolidol (8%)+β-oplepone (7%) *in D. viscosa*. The remaining water-distillation extracts of *O. vulgare* had the highest amount of phenols (45±3mg GAE/ml) while *F. vulgare* only had 5±0mg GAE/ml.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of samples revealed that the remaining water-distillation extracts were more effective than the essential oils. *O. vulgare* remaining water-distillation extract had the best activity (IC_{50}=3±0μg/ml), while the most effective essential oils were those of *T. capitata* (IC_{50}=61±2μg/ml) and *O. vulgare* (IC_{50}=156±5μg/ml). *T. capitata* (IC_{50}=6±0μg/ml) remaining water-distillation extract showed the best superoxide anion scavenging activity. The essential oils interfered with this method.

F. vulgare remaining water-distillation extract and essential oil showed the best capacity for inhibiting lipoxygenase activity (IC_{50}=27±1μg/ml and (IC_{50}=68±2μg/ml, respectively).

Due to the negative interference, the remaining water-distillation extracts could not be assessed for antiacetylcholinesterase activity. The most effective antiacetylcholinesterase activity was found with the essential oils of *Th. mastichina* (IC_{50}=46±4μg/ml), *S. officinalis* (IC_{50}=51±4μg/ml), *T. capitata* (IC_{50}=52±1μg/ml) and *Th. camphoratus* (IC_{50}=137±2μg/ml).

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B-18

Antioxidant activity from five Portuguese thyme species essential oils

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The capacity for preventing lipid peroxidation of some Portuguese thyme species (*Thymus mastichina*, *Th. albicans*, *Th. camphoratus*, *Th. carnosus* and *Th. serpyllum*) collected in different regions of Portugal has been evaluated [1]. The results showed that some of these species are effective antioxidants. In the present work the capacity of the essentials of *Th. carnosus*, *Th. camphoratus*, *Th. capitellatus*, *Th. caespititius*, *Th. zygis* subsp. *zygis* and *Th. zygis* subsp. *sylvestris* collected in different regions of Portugal, including Azores islands, for preventing lipid peroxidation and scavenging free radicals, hydroxyl and superoxide anion were evaluated. The essential oils were isolated by hydrodistillation and analysed by GC and GC-MS [2]. Antioxidant activities were evaluated according to previously reported methods [3].

Th. *zygis* subsp. *zygis* oil possessed the best capacity for preventing lipid peroxidation (IC_{50}=29.9±2.8 μg/ml), immediately followed by the oils isolated from *Th. zygis* subsp. *sylvestris* collected in different regions of Portugal. IC_{50} values ranged from 65.6±2.8 to 86.7±1.5 μg/ml in the oils isolated from samples from Alcanena and Duas Igrejas, respectively. The oils isolated from *Th. zygis* subsp. *sylvestris*, independent on the harvesting region, revealed to be the most effective for scavenging free radicals (365-452 μg/ml). Due to oil shortage, the activity of *Th. zygis* subsp. *zygis* essential oil of was not evaluated. The best capacity for scavenging hydroxyl radicals was found in the oils of *Th. camphoratus* of Espartal and with those of *Th. caespititius* of Lordelo, with IC_{50}<500μg/ml) The low oil amount did not allow the IC_{50} values for the superoxide anion scavenging determination, therefore and considering an unique oil concentration (5 mg/ml), the highest inhibition percentages (>50%) were registered with the essential oils of *Th. zygis* subs. *sylvestris* from Duas Igrejas and Covão do Coelho and with those of *Th. capitellatus* from Sines.

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B-19

Investigation of antimicrobial activity of selected aroma samples

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Results on anti-tumor and antiviral activities of selected aroma compounds, like cinnamic acid, β-carophyllene, caryophyllene oxide, longifolene, nerolidol (synthetic and natural samples) have been reported in a previous study [1]. This investigation has shown a remarkable inhibition of viral activity by synthetic nerolidol and natural nerolidol. But data about antimicrobial properties of the tested compounds are rare.

Therefore, a further investigation to examine antimicrobial activitiy of these substances was carried out. Antimicrobial testing was performed against Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis)* and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Salmonella sp.*) and the yeast *Candida albicans,* using a modified agar dilution and agar diffusion method. Antimicrobial effects were compared with those of the phenolic compound eugenol and some synthetic antibiotics. In addition, the purity of the investigated samples was determined by GC and GC-MS analyses and olfactory characteristics were further described by professional perfumers.

Compared to the synthetic nerolidol, the natural compound showed higher antimicrobial activity against the gram-(+) bacteria *Staphylococcus aureus*. Against all other investigated microorganisms similar antimicrobial activities for natural and the synthetic nerolidol were observed. Although, highest inhibiting activity upon the growth of Gram-positive, Gram-negative bacteria as well as the yeast, was found for cinnamic acid.

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B-20

Composition of the essential oil of *Dionysia diapensifolia* Boiss. and micropropagated *Haplophyllum Stapfianum*

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The genus *Dionysia* Fenzl (Primulaceae) is represented by about 50 species that confine to the mountainous regions of western and central Asia [1]. Among them, more than 30 species are found in Iran and except four species, all the Iranian *Dionysia* are endemic [2]. They are suffrutescent herbs, forming loose tufts or dense cushions in crevices of rocks and cliffs [1]. *Haplophyllum stapfianum* is a beautiful perennial herb endemic to Iran and is worth to be used as an ornamental plant. It is a critically endangered species that confines to a very restricted area in the NE of Shiraz city in the SW of Iran and a few dramatically scattered populations of a couple of individuals.)[3] In a previous bio-assay study, this species showed outstanding cytotoxic activity against some tumor cell lines [4]. Volatile oil constituents of *Dionysia diapensifolia* were investigated by GC and GC/MS. 38 Compounds representing 93.3% of the oil of *D. diapensifolia* were identified. Phenolic compounds were dominant in this oil and (E)-Chalcone constituted 85.6% of the total oil. The identity of this compound was confirmed by ^{13}C-NMR. *Halophyllum stapfianum*, a critically endangered species endemic to Iran was micropropagated in order to find its optimized in-vitro cultural condition and its essential oil composition was investigated by GC and GC/MS and compared with wild plant oil composition. Hexadecanoic acid (31.0%) and phytol (21.9%) were the major compounds of wild plant oil, in contrast with the oil of in-vitro sample which γ-palmitolactone comprised more than half of the total oil (53.5%).

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B-21

Microbial biotransformation of some monoterpene hydrocarbons

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Aromas and fragrances are used everywhere in the modern world, they find a wide range of applications in the food, cosmetic, chemical and pharmaceutical sciences. Globally, the market demand for natural flavorings continues to increase. Most available flavor compounds are now produced via chemical synthesis or extraction. Drawbacks of such chemical processes are the formation of undesirable racemic mixtures and the growing aversion of the consumer towards chemicals added to his food, cosmetics and other household products [1]. The biotechnological approaches suggest advantages over such conventional methods [2]. Monoterpene hydrocarbons such as α-pinene, β-pinene and p-cymene are inexpensively available in large quantities. Oxygenated monoterpenes are valuable compounds and used in flavor and fragrance industries. The biotransformation of monoterpene hydrocarbons to oxygenated ones is of interest because it allows the production of stereoselectively pure compounds under mild conditions and also the products may be considered as natural products [3]. High commercial value compounds can be obtained through the microbial biotransformation of monoterpenes. Some of these monoterpenic substances are not expensive and produced in a variety of plant species. Biotransformation of some monoterpenes hydrocarbons such as α-pinene, β-pinene, myrcene and p-cymene by 7 strain bacteria and 2 strain fungi was investigated. It was observed that some of microorganisms transformed monoterpenes to oxygenated monoterpenes in a good yield which among them *Staphylococcus epidermidis* showed higher yields.

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B-22

Nepetalactones as chemotaxonomic markers in the essential oils of *Nepeta* L. (Lamiaceae) species

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The genus *Nepeta* L. comprises 75 species in Iran and is the largest genus of the Lamiaceae family found in the area. Furthermore, the area is one of two throughout the entire world for which the genus is endemic [1]. From a taxonomic point of view, it is a complicated genus and the range of morphological variation within *Nepeta* can lead to difficulties of specific classification and identification [2]. In Flora Iranica [3] the *Nepeta* species has been divided in 13 sections based on morphological characters. In a recent ITS sequence analysis, two clades were informally recognized for 34 studied Iranian species, where one clade was further divided to four new clades [1]. We analyzed the essential oils of twelve *Nepeta* samples from eight species, representing three sections based on morphological characters. Samples were evaluated by GC and GC/MS; the chemical data were classified using the counter propagation neural network (CPNN), cluster analysis and principal component analysis (PCA). The chemotaxonomic importance of the results is discussed; from this perspective, the value of nepetalactone isomers as characteristic constituents of volatile oil obtained from the *Nepeta* species is assessed.

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B-23

Geographical variation of *Ajuga laxmannii* (L.) Bentham essential oil

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The genus *Ajuga* L. consist of about 90 species, mostly distributed in the north temperate zone of the old world. The genus also occurs in South Africa and Australia [1]. In flora of Turkey *Ajuga* represented by 14 species and 27 taxa [2]. Some *Ajuga* species have been widely used for their diuretic, antipyretic, tonic, diaphoretic, astringent properties in the Turkish folk medicine [3]. Aerial parts of *Ajuga laxmannii* collected from five different localities in Turkey were hydrodistilled for 3 h using a Clevenger-type apparatus to produce a small amount of essential oil which was trapped in *n*-hexane. Oils were analysed by gas chromatography (GC) and mass spectrometry (GC/MS). Computer matching against commercial libraries (Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library) [4-5], and in-house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data [4-7], was used for the characterization of the constituents.

Table 1. Main components of *A. laxmannii* oils from different localities

Collection place	Main components (%)
1	Nonacosane (17.9), heptacosane (12.0), hexahydrofarnesylacetone (11.2), phytol (7.3)
2	Hexadecanoic acid (21.2), dodecanoic acid (12.3), tetradecanoic acid (8.9), nonacosane (8.3)
3	Phytol (12.5), hexadecanoic acid (10.0), hexahydrofarnesylacetone (8.6), nonacosane (7.4)
4	Hexahydrofarnesylacetone (9.1), hexadecanoic acid (8.9), nonacosane (7.6)
5	Hexadecanoic acid (13.6), phytol (13.3), hexahydrofarnesylacetone (8.3), nonacosane (7.7)

% calculated from Flame Ionization Detector (FID) data

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B-24

Biotransformation of monoterpene bicyclic diketone

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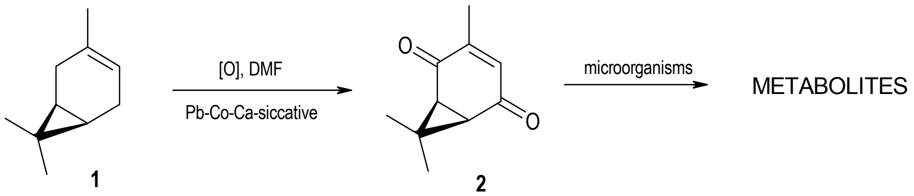
Biotransformation is an effective tool for the structural modification of bioactive natural and synthetic compounds. Due to its versatility and simplicity this method is useful in bioorganic chemistry.

In our previous paper we presented bioconversion of bicyclo[3.1.0]hexane ketone [1], obtained in two step synthesis from monoterpene bicyclic hydrocarbon, (+)-3-carene **1**, one of the major constituent of resinous extract from Scotch Pine (*Pinus sylvestris* L.).

Continuing our studies on the biotransformation of carane derivatives, car-3-ene-2,5-dione **2** was carried out by different kinds of microorganisms (*Aspergillus niger, Saccharomces cerevisiae, Pseudomonas putida, Penicillium sp).* Bicyclic diketone **2** has been isolated from the roots of asiasari radix [2]. This extract prepared from *Asiasarum sp.* is one of the most important crude drug in Chinese medicine and it has been the subject for extensive chemical investigations for many years. That research results in isolation of a number of essential oils, lignans and amides [3].

The key-compound, bicyclic diketone **2** was synthesized in one step reaction. Autooxidation of **1** in DMF with Pb-Co-Ca-siccative as a catalyst afforded desired car-3-ene-2,5-dione **2**, which was distilled under reduced preasuer and then purified by crystallization in mixture of hexane:ethyl ether:methanol (2:2:1).



Microorganisms were cultivated rotatory (200 rpm) at 28°C for 24-48 hours in the 250 ml erlenmyer flask. After the full growth of microorganisms car-3-ene-2,5-dione **2** was added (50 mg) and incubated for 12 days under the same conditions. The progress of biotransformation was monitored by TLC. After filtration of the curltured broth the aqueous layer was extracted with ethyl acetate. The concentrated ethyl acetate extract was evaluated by GC/MS and then applied to silica gel chromatography column for isolation of the metabolites. The structure of isolated metabolites were established by ^{1}H, ^{13}C NMR and IR spectroscopy.

Synthetic and biotransformation details of the applied procedures will be presented.

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B-25

Larvicidal activity of essential oils from *Cyperus* species against *Culex quinquefascitus*

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The larvicidal activity of essential oils from three *Cyperus* species (*Cyperus distans, C. papyrus* and *C. rotundus)* obtained by hydrodistillation and analyzed using GC and GC/MS was tested against fourth-instar larvae of *Culex quinquefascitus* [1, 2]. Different concentrations of the essential oils were studied and compared with the controls [3]. Results showed that the essential oil of *C. papyrus* had an excellent inhibitory effect (LC_{50} 24.04; LC_{90} 33.33 µg/mL) followed by *Cyperus distans* (LC_{50} 42.49; LC_{90} 119.07 µg/mL) and *C. rotundus* (LC_{50} 42.77; LC_{90} 145.06 µg/mL).

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B-26

Chemical composition and antimycobacterial activity of the essential oil from *Anemia tomentosa var. anthriscifolia* (Pteridophyta).

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The family Anemiaceae comprises only one genus with more than 100 terrestrial species, distributed mainly in the New World [1] with many species endemic to Brazil.[2] *Anemia tomentosa*, is popularly known in Brazil as "espiga-de-ferrugem", being its very aromatic leaves used to treat bronchitis.[3] The essential oil from plants of this species were previously studied in Argentina and Brazil with α-bisabolol and isoafricanol as the main constituents [4,5]. In this study, *Anemia tomentosa var. anthriscifolia* was collected at two different rocky hillsides in Rio de Janeiro and Espírito Santo States, Brazil. The essential oils were obtained from fresh aerial parts by hydrodistillation in a Clevenger-type apparatus for 2 hours, yielding 0.6 and 0.3% of essential oil, respectively. Antimycobacterial activity was assayed by REMA method using *Mycobacterium tuberculosis* ($H_{37}Rv$) [6]. A final concentration of oils/substances was 200 µg/mL. GC-MS/EI analysis of both essential oils showed basically the same chemical composition, with the marked presence of two triquinane type sesquiterpenes - silphiperfol-6-ene (13.4% and 14.7%, respectively) and presilphiperfolan-8-ol (12.7% and 8.3%, respectively). The main constituent of these oils (31.2% and 30.6%, respectively) was a sesquiterpene alcohol, which structure could not be identified by comparison with literature mass spectral data nor by comparison with reference mass spectra databases. Silica-gel column chromatography fractionation led to the isolation of (-)-*epi*-presilphiperfolan-1-ol, a new triquinane sesquiterpene. Its structure elucidation was accomplished by extensive 1D and 2D-NMR analyses, chiral bidimentional GC, dehydration reactions, and a comparative (GIAO/DFT) theoretical study of the ^{13}C-NMR chemical shifts of this sesquierpene and its known isomers (presilphiperfolan-1-ol, presilphiperfolan-8-ol, and presilphiperfolan-9-ol). The essential oils from *A. tomentosa var. anthriscifolia* showed MIC values (Minimal Inhibitory Concentration) of 100 µg/mL, while for an oil fraction containing α-guayene (17.25%) and silphiperfol-6-ene (39.64%) the MIC value was 25 µg/mL. Assayed MIC for (-)-*epi*-presilphiperfolan-1-ol was 120 µg/mL.

Aknowledgements
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B-27

Comparative analysis of the essential oil of *in vitro* and e*x vitro* Plants of *Lippia lacunosa* (Verbenaceae)

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Lippia lacunosa (Verbenaceae) is a strongly aromatic shrub that reaches up to 2.5m height. Previous chemical investigation of the essential oil from cultivated plants, originary from Diamantina, Brazil, showed that monoterpenes were the major components of this oil (above 80%), being myrcene (11.9%), myrcenone (64.2%), *(Z)*-ocimenone (5.2%) and *(E)*-ocimenone (4.1%) its main constituents [1]. The oil from this plant has revealed commercial value since it exhibits a strong and pleasant mango aroma, which was related to the presence of myrcene and myrcenone.[1] However, the commercial use of plants involves standardization in order to overcome variations in the genetic expression of their phytochemical potential which can drastically affect the production of metabolites. Tissue culture methods are important biotechnological tools to provide multiplication of specific genotypes and circumvent the influence of environmental factors in the production of secondary metabolites. In the present work tissue culture protocol for *L. lacunosa* was established for comparison of the phytochemical profiles between *in vitro* and *ex vitro* plants. *In vitro* propagation was achieved through the culture of nodal segments on MS medium. The volatile oil from fresh leaves of *in vitro* plants was obtained by Simultaneous Distillation and Extraction (SDE), for four hours, and analyzed by GC-FID and GC-MS. Twenty five compounds were detected. Comparative analysis of the essential oils from *ex vitro*[1] and *in vitro* plants showed that the phytochemical profile was maintained, with exception to the relative percentages of its main components, according to Table 1. Apart from these differences, its characteristic mango aroma was kept.

Table 1. Comparison between the chemical composition (%) of the essential oils from *ex vitro* an *in vitro Lippia lacunosa* leaves.

Constituents	Lippia lacunosa	
	Ex vitro	*In vitro*
myrcene	11.9	8.8
limonene	0.8	6.1
myrcenone	64.2	7.7
(Z)-ocimenone	5.2	6.2
(E)-ocimenone	4.1	31.8
(E)-caryophyllene	0.7	11.0
α – humulene	0.1	2.8

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B-28

The efficacy of plant essential oils on postharvest control of rot caused by fungi on apples and actinidia *in vivo*

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The ultimate aim of the recent research in postharvest control of fungal decay on fruit and vegetables is the development and evaluation of alternative strategies to reduce dependency on synthetic fungicides [1]. The essential oils have shown, generally *in vitro* studies, a significant antifungal activity [2-4]; their advantage is the bioactivity in vapour phase, that characteristic makes them attractive as possible fumigants for stored product protection [1]. The efficacy of the essential oils was evaluated on apples cv. Golden delicious, Granny smith, Red chief , Royal gala and on actinidia cv. Hayward. The essential oils from basil (*Ocimum basilicum*), fennel (*Foeniculum sativum*), lavender (*Lavandula officinalis*), marjoram (*Origanum majorana*), oregano (*Origanum vulgare*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), savory (*Satureja montana*), thyme (*Thymus vulgaris*) and wild mint (*Mentha arvensis*) were tested against *Botrytis cinerea* and *Penicillium expansum* on apples and against *B. cinerea* on actinidia. The fruits were artificially wounded and inoculated with 10 µl of pathogen suspension at 1 X 10^{-5} conidia ml^{-1} into each wound. After 12 hours, 10 µl of an emulsion at 1% and 10% of each essential oil were dropped into each inoculated wound. A tebuconazole control (for apples), a boscalid control (for actinidia) and an inoculated control were also carried out. Immediately all the treated fruit were conservated at 4,0±1°C. After 30 days, the diameter of the rot around each wound was measured. The efficacies of the essential oils from oregano, peppermint, sage, savory and thyme at 1% were statistically similar to the chemical control against *B. cinerea* and *P. expansum* on apples, meanwhile the efficacies the essential oils from marjoram and thyme at 1% were statistically similar to the chemical control against *B. cinerea* on actinidia. The treatments with essential oil emulsions at 10% were sometimes phytotoxic for the fruit.

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B-29

Effect of essential oils on *Leishmania infantum* promastigotes

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Parasitic protozoa of the genus *Leishmania* are able to cause disease affecting millions of people around the world. Diseases like leishmaniasis were classified by the World Health Organization as "neglected", once they have been ignored by governments and pharmaceutical companies. Currently employed chemotherapy for these diseases has serious limitations due to lack of efficacy, toxic side effects, drug-resistances and high costs. The development of new effective, safe and accessible agents is urgently needed.

Motivated by previous results on the efficiency of some essential oils and their components as inhibitors of the pathogenic protozoa *Giardia lamblia* [1-4] we undertook a screening of selected essential oils as inhibitors of *Leishmania infantum* promastigotes growth. Essential oils from *Crithmum maritimum, Cymbopogom citratus, Distchoselium tenuifolium, Eryngium maritimum, Lavandula luisieri, L. viridis, Juniperus oxycedrus* leaves, *J. oxycedrus* berries, *Mentha cervina, M. piperita, Lippia graveolens, Origanum virens, Rosmarinus officinalis, Seseli tortuosum, Syzygium aromaticum, Thymbra capitata, Thymus capitelatus, T. mastichina, T. zygis subsp. sylvestris* chemotype thymol and *T. zygis subsp. sylvestris* chemotype geraniol, as well as, some common volatile components, α-pinene, 1,8-cineole, linalool, menthol, geraniol, borneol, iso-borneol, citral, thymol, carvacrol or eugenol, were evaluated.

Antileishmanial screening tests were performed on culture promastigotes of *L. infantum* (10^6 cells.ml^{-1}) incubated with different oil or compound concentrations. Viability of promastigotes was assessed by the tetrazolium-dye colorimetric method. Experiments were performed in triplicate and in at least six independent assays. Results were expressed as the concentration that inhibits parasite growth by 50% (IC50).

Cymbopogom citratus revealed the most active oil with IC_{50} value of 25µg/ml. Other oils, as those from *Lavandula luisieri* and *L. stoechas* are also effective inhibitors with IC_{50} values under 100 µg/ml. Citral (mixture of *Z*- and *E*-citral), is the most active (IC_{50} = 72µg/ml) among the tested components and the plausible responsible of the activity of *Cymbopogom citratus*.

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B-30

Essential oils from aerial parts of *Zhumeria majdae* and *Salvia sahendica* (Two rare endemic species of the Iranian flora)

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The monotypic Iranian *Zhumeria majdae* Rech. f. & Wendelbo (Lamiaceae), was recently described as the first member of a new genus (*Zhumeria*). It has a limited geographical rang in southern of Iran at Hormozgan province [1].The leaves have been used for many years as a curative for stomachaches, an antiseptic and for treatment of painful menstruation. Antiniciceptive, anti-inflammatory and acute toxicity of the extracts of *Z. majdae* were reported [2]. In this research, the chemical compositions of the essential oils of *Zhumeria majdae* aerial parts collected from Geno mountain (southern Iran) were isolated by hydrodistillation clevenger type apparatus and analyzed via GC and GC-MS [3]. The oil yield of the dried aerial parts by hydrodistillation were 7.5%, (v/w). 22 compounds, were identified. The main components in essential oil were linalool (35.6%), camphor (42.1%), α-pinene (1.6%), camphene (4.1%), limonene (3.4%), α-terpineol (2.8%), caryophyllene oxide (0.3%) and myrcene (1.7%).
Salvia, the largest genus of Lamiaceae, is represented in Iranian flora by 58 species, which 17 specie of them are endemic [1]. Some of these species have been used in folk medicine all around the world for their antibacterial and antitumor activities, as well as flavoring agent in perfumery and cosmetics [4]. The aerial parts of *Salvia Sahendica* Boiss. & Buhse were collected at vegetative and flowering stages from Sahend mountains near to Sardroud city (Azarbayjan province, Iran). The essential oils were isolated by hydrodistillation clevenger type apparatus and analyzed by GC and GC-MS [3]. In vegetative stage, Thirty-two components were identified, representing 98.5 % of the total oil. α-pinene (28.5%), β-pinene (26.1%), sabinene (10.3%), germacrene-D (9.6%) and 1,8-cineole (4.9%) were the main components in vegetative stage. In flowering stage, Thirty-five components were identified, representing 99.8% of the total oil. α-pinene (18.9%), β-pinene (18.5%), 1,8-cineole (13.9%), linalyl acetate (8.4%), bicyclogermacrene (8.2%), sabinene (7.6%) and linalool (5.3%) were the main components at flowering stage.

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B-31

Biological activity of essential oils and their main volatile compounds on seed germination

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The phytotoxicity of the essential oils from twelve Mediterranean species has been studied *in vitro*. Hyssop, lavander, marjoram, lemon balm, basil, oregano, sage and thyme (Lamiaceae), anise, fennel, caraway (Apiaceae), and vervain (Verbenaceae) were grown in the Campus of Salerno State University. Five-hundred g of freshly picked aerial parts of each lamiaceous and verbenaceous species and five-hundred g of fruits of fennel, caraway and anise were submitted to hydrodistillation for 3 h, according to the standard procedure reported in *European Pharmacopoeia* [1]. Their chemical composition was achieved by GC and GC/MS methods. Essential oils, at doses ranging from 0.06 and 2.5 µg/ml, were tested for their effects on the germination and the radical elongation of garden cress, lettuce and radish [2]. The oils showed generally an inhibitory activity, in a dose-dependent way, against the three test species. At the highest dose used, radish germination was inhibited by 100% by caraway, lemon balm, hyssop, lavender, oregano, marjoram, verben and thyme essential oils. Lettuce resulted sensitive to caraway, lemon balm, hyssop, marjoram, oregano, thyme and vervain oils; garden cress germination was inhibited by caraway, lemon balm, hyssop, oregano, thyme and vervain oils. A positive correlation beetwen the total oxygenated monoterpene content in the essential oils and the inhibitory activity has been found. For this reason, twenty-eight monoterpenes, main constituents of the above mentioned oils, were assayed at concentrations ranging between 10^{-3} and 10^{-6} M. The compounds showed different potency in affecting both germination and radical elongation, in a dose-dependent way. Alcohols and ketones resulted the more effective inhibitory monoterpenes. Geraniol was the most active both against radish and garden cress germination. At 10^{-5} M aldehydes showed inhibitory activity on germination of radish. Carvone inhibited by 93,6% the root growth of radish; cress radical elongation was inhibited by alcohols, ketones and phenols.

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B-32

Biological activity and chemistry of *Piper claussenianum's* essential oil

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Brazil, a country of megadiversity encompasses the richest flora in the world. A great number of the Brazilian plant species are used in folk medicine to treat many types of conditions. The family Piperaceae is formed by five genera: *Piper, Peperomia, Ottonia, Sarcorhachis* and *Pothomorphe*; including 2500 species widely distributed in the tropical and subtropical regions [1]. Species of *Piper,* the largest genus of the family, are the most studied on chemical and pharmacological point of view. The essential oils from Piper species are chemically diversified and show several biological activities [2]. Fresh leaves and inflorescences of *Piper claussenianum* were separatedly extracted by hydrodistillation during 2h in a Clevenger-type apparatus. The essential oils were recovered in about 1% yield. Analysis of the plant volatile fractions were carried out on a GC-MS QP5000 Shimadzu with a ZB-5MS fused silica capillary column (30m x 0.25mm, 0.25μm film thickness). The operating temperatures were: injector 260°C; detector 290°C and column oven 60°C up to 290°C (3°C/min). Identification of the detected analytes was accomplished by matching their mass spectra with the available databases. The presence of *trans*-nerolidol was remarkably in both organs being above 80.0% in the leaves and 22.0% in the flowers in which the main constituent was the linalool (50.0%). The essential oils extracted from leaves and from flowers were asseyed against *Leishmania amazonensis* as promastigote forms during the experiment. The MIC and IC_{50} calculated values of leaf essential oil were respectively 57.6μg/mL and 30.4μg/mL (± 3.2 for both). The inflorescence essential oil tested showed a MIC and IC_{50} 1.05 μg/mL (±0.24) and 0.597 (±0.16). The results corroborate the nerolidol's activity on *Leishmania*'s strains and stimulate the scientific interest about the potential value of this plant species on neglected diseases.

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B-33

Antimicrobial and antioxidant properties of the essential oils of *Pimpinella barbata* Boiss. and *Pimpinella tragioides* (Boiss.) Benth. & Hook.

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Members of *Pimpinella* are well known since ancient times for their carminative, antispasmodic, antiseptic, expectorant, diuretic and tonic effects [1, 2]. The aerial parts of two species of *Pimpinella*, *P. barbata* and *P. tragioides* grown wildly in Iran were collected and the essential oils were obtained by a Clevenger-type apparatus. They were subjected to essential oil study by GC and GC-MS. Thirty-four and 29 compounds representing 96.1% and 92.7% of the total oil of *P. barbata* and *P. tragioides* respectively were identified. Major constituents of *P. barbata* oil were methyl eugenol (36.9%), geijerene (17.1%) and limonene (14.0%). On the other hand, germacrone (26.9%), trans-β-elemenone (18.8%), γ-elemene (14.2%) and isospathulenol (10.3%) were the main compounds in the oil of *P. tragioides.* Antimicrobial activities of two oil samples were tested against seven bacteria and two yeasts by disc diffusion method. The antimicrobial effects of the oil samples were tested by disc diffusion method. The following microorganisms were used in this study: *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Candida albicans*, *Aspergillus niger*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The oil samples were also assessed for antioxidant activity by FRAP method. *Pimpinella barbata* and *P. tragioides* were most active against gram negative bacteria. The antioxidant activity of the oil samples which were measured by the FRAP assay were equal to 15.8 ± 2.1 and 14.6 ± 1.9 (μM quercetin/g essential oil) respectively. *P. barbata* oil was more active and this trend can be contributed to the amount of phenylpropanoid constituents in this oil. In conclusion, remarkable antimicrobial and antioxidant activities of these oils of *Pimpinella* as medicinal plants give promise that these oil samples could be used as potent preservatives in food, drug and other related industries but for a definite statement more studies should be carried out.

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B-34

Chemical composition and antimicrobial activity of essential oils of Stems from *Sanguisorba minor* Scop. grown in Iran

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Sanguisorba L. genus is one of the important plants in Iran, some of them are endemic. In this study stems of *Sanguisorba minor* Scop**.** were collected from Neyshabur in Khorasan-e-Razavi Province in Iran, chemical constituent of essential oils of stems of *Sanguisorba minor* Scop determined. Air-dried stems (185gr) were subjected to hydrodistillation in a Clevenger – type apparatus until there was no significant increase in the volume of the oil collected (3h). The yield of the yellow oil was 0.70% (w/w). The essential oil was analyzed by GC and GC/MS.

Identification of the components was based on GC retention indices computer matching with Wiley GC-MS library, and by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [1]. 34 components were identified constituting more than 92% of the oil.

Camphene(2,3%), 2,2,3-trimethyl-3,6-heptadiene-2-ol (6.4%), *p*-Cymene (1%), 1,8-cineole (13.1%), Artemisia alcohol (4.2%), β-Thujone (1.2%), Camphor (9.3%), Santolina alcohol (4.1%), Borneol (23.7%), Piperitone (3.1%), Bornyl acetate (13.7%),and Germacrene D (3.1%) were major components in stems of *Sanguisorba minor* Scop [2,3].

The oil was evaluated against 4 strains of bacteria (Gram-Positive and Gram-Negative) and yeasts**.** The Oil showed mild to moderate antimicrobial activity associated mainly with Gram-Positive and Gram Negative bacteria and yeasts.

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B-35

QSPR study of essential oils of Metasequoia glyptostroboides by genetic algorithms (GA) and partial least squares (PLS) method

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Plant essential oils and their extracts have been greatly employed in folk medicine, food flavoring, fragrance and pharmaceutical industries [1]. Metasequoia glyptostroboides is a deciduous conifer of the redwood family of Cupressaceae [2, 3]. The plant is used in various folk medicines such as anti-fungal activity and anti-inflammatory [4].In the present report, the quantitative structure-retention relationship (QSRR) of the essential oil components against the retention time (R_t) has been studied, using the variable selections of the genetic algorithm (GA)- PLS. At first, a data set was selected, consisting of the retention times for 58 essential oil molecules. Then, the suitable set of the molecular descriptors was calculated and the important descriptors were selected with the aid of the genetic algorithm method. The predictive quality of the QSRR model was tested for an external prediction set of 12 compounds, randomly chosen out of 58 compounds. The GA-PLS model with nine selected descriptors were obtained. The GA-PLS model, demonstrating high statistical qualities ($R^2_{train} = 0.940$; $F = 69.654$, $R^2_{pred} =0.8904$; SEP(%) =3.07), could predict the retention time of the molecules with a low prediction error.

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B-36

A quantitative structure retention relationship for prediction of retention indices of essential oil of *Ammoides atlantica* using GA-based MLR

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Essential oils are mixtures of more than 200 compounds that can be grouped basically into two fractions, a volatile fraction, that constitutes 90-95% of the whole oil and contains monoterpenes and sesquiterpene hydrocarbons and their oxygenated derivatives, along with aliphatic aldehydes, alcohols and esters, and a nonvolatile residue, that constitutes from 5-10% of the whole oil and contains hydrocarbons, fatty acids, sterols, carotenoids, waxes, coumarins, psoralens and flavonoids [1]. Ammoides atlantica is of Apiaceae family. The plant is used in infusions to treat headache, fever and diarrhea. It is also used in compresses, alone or soaked in alcohol or vinegarand mixed with henna, to treat children affected by mental debility as reported by Laouer et al.[2]. GC and GC–MS are the main methods for the identification of these plant oils. A simple, strong, descriptive and interpretable model, based on a quantitative structure-retention relationship (QSRR), is developed using genetic algorithm multiple linear regression (GA-MLR) approach for prediction of the retention index (RI) of essential oil components. By molecular modeling and calculation of descriptors, three significant descriptors related to the retention index values of the essential oils, were identified. A data set was selected, consisting of the retention index for 32 essential oil molecules with a range of more than 931 units. Then, the suitable set of the molecular descriptors was calculated and the important descriptors were selected with the aid of the genetic algorithm and multiple regression method. A model with low prediction error and good correlation coefficient was obtained. This model was used for the prediction of the RI values of some essential oil components which were not used in the modeling procedure.

Key words: Chemometrics, QSRR, Genetic algorithms, Multiple linear regression, Retention index, Essential oils

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B-37

Linear and nonlinear quantitative structure–property relationship for prediction of retention indexes of the essential oils constituent components of *Tanacetum* Podl. *Polycephalum* Schultz Bip. Subsp. *farsicum* Podl.

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Plant essential oils and their extracts have been greatly employed in folk medicine, food flavoring, fragrance and pharmaceutical industries [1]. GC and GC–MS are the main methods for the identification of these plant oils. Seeking quantitative relationships between the molecular structure and the gas chromatographic retention indices has been a basic task in chemistry. Correlations between the GC retention indices and the molecular structures can provide more profound insights into the interactions between the eluents and the stationary phases from a theoretical approach. In addition, they can provide valuable information about the effect of the chemical structures on the retention behavior and can lead to accurate and proper mechanisms of absorption and elution. In the present report, linear and nonlinear quantitative structure–property relationship (QSPR) models have been developed for the prediction of kovatz indexes of the essential oils ingredients by using multiple linear regression (MLR) analysis and feed-forward artificial neural networks (ANN). At first, a data set was selected encompassing the retention indices of the volatile oil constituents comprising the amounts more than 927. Then, the suitable set of the molecular descriptors was calculated and the important descriptors were selected with the aid of the stepwise multiple linear regression (MLR) methods. Descriptors which were chosen by stepwise MLR technique were utilized as inputs for artificial neural network (ANN) evaluation. The statistical parameters by MLR model indicated considerable potential for prediction, while the predictive ability of ANN model is somewhat superior.

Key words: ANN, QSRR, MLR, *Tanacetum* Podl. *Polycephalum* Schultz Bip. Subsp. *farsicum* Podl., Retention index, Essential oils

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B-38

Quantification of (–)-linalool in human blood by GC-MS. A pilot study

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Although several studies have suggested pharmacological effects of linalool in human beings have been carried out [1, 2], information on the bioavailability of this volatile compound is still rare.

In order to substantiate the observed effects, it is necessary to determine the amount of the monoterpene that actually migrates into the blood of a subject. Thus, the aim of this work was to develop a convenient method, to determine the levels of a commercial (–)-linalool in human blood after inhalative or transdermal application.

All analyses were carried out on a GC-MS system using a TR1-MS capillary column. Three different sampling methods, i.e., liquid injection, head space-injection (HS) and head space-solid phase microextraction (HS-SPME), were compared. A temperature program from 60° C to 160° C and a heating rate of 20° C per minute were employed. Sample preparation for liquid injection was carried out by transferring the samples on Extrelut ®NT3 cartridges and eluting (–)-linalool with n-hexane as a solvent. In case of HS and HS-SPME no sample preparation was necessary. For the HS-SPME investigations a 100µm polydimethylsiloxan fibre was used.

(–)-linalool was quantified in selected ion monitoring (SIM) mode, using α-terpineol as an internal standard. Calibration curves were established with either water or blood as the medium.

Our investigations showed that the HS method was most suitable, because of the short preparation and analysis time. Both other methods also complied with the requirements, but had the drawbacks of an expensive and long sample preparation for liquid injection and a time-extend analysis of about 60 minutes for HS-SPME.

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B-39

Chemical composition and antimicrobial Studies of *Croton pseudopulchellus* and *C. gratissimus* from South Africa

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Isolation of the essential oil from the leaves of *C. pseudopulchellus* and *C. gratissimus* were performed using an all glass Clevenger apparatus according to British Pharmacopeia method. Golden yellow oil was obtained with percentage yield of 1.03 -1.25 respectively (w/w). Analysis of the oils was performed using GC and GC/MS. The leaf oil of *C. pseudopulchellus* had germacrene (24.2%), β-phellandrene (17.4%), myrcene (13.4%) and β-caryophyllene (11.4%) as the prominent compounds identified in the oil. The chemical composition of the leaf oil of *C. gratissimus* was characterized by sabinene (14.6%), α-phellandrene (12.3%), β-phellandrene (10.7%), α-pinene (6.0%) and germacrene D (5.9%). Chemical profile of the essential oils of *Croton* species reported in literatures are specific to their geographical location [1, 2]. This is the first time that the South African species are been reported.

The minimum inhibitory concentrations [3, 4] of the oils was assessed against the seven different strains of bacteria: *Bacillus pimitus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Serratia marcescens* and *Entarobacter cloacae* using micro dilution technique on a 96 well microtitre plate. The essential oil of *C. gratissimus* had a broader inhibitory effect of the bacteria when compared to the oil of *C. pseudopulchellus*.

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B-40

The effect of GA3 and NAA on the essential oil composition in tarragon (*Artemisia dracunculus* L.)

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The genus *Artemisia* belongs to the *Asteraceae* family and is present in Iran with 34 perennial and annual species of which two are endemic [1]. Previous investigations have been carried out on chemical composition [2,4,8,9], biological activity of essential oil [2,6,8], anticonvulsant activity [3], antioxidant activity [4], loss of essential oil due to drying [5] and effect of NAA on the accumulation of volatile oil components in cell-cultures of tarragon (*Artemisia dracunculus*) [7].

Aerial parts of tarragon plants treated under field condition were sprayed with NAA (100µM) and GA3 (100µM) separately three times in a week.

After a week, aerial parts of plants were harvested and dried in the dark with air stream .The essential oil of dried aerial parts were obtained by hydrodistillation. The main compounds of essential oil of control plants.were methyl chavicol (82.3%), cis-β-ocimene (5.15%), trans-β-ocimene (4.01%). It was seen that NAA decreased the precentage of methylchavicol in the hydrodistilled essential oil and increased other main compounds. On the opposite side, GA3 increased the amount of methylchavicol in the obtained volatile oil.

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B-41

Phytochemistry and pharmacology of volatile components of *Callitris glaucophylla* Wood

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During the last few years the importance of plants as source of medicine has increased, mostly depending on the idea that herbal remedies are safer and less damaging to the body than synthetic drugs. Australia's flora has always been used as medicine: by the Aborigines as well as by European settlers (or in some rare cases by other immigrant groups). There are a lot of plants, whose essential oils are used for healing purposes [1]. One of them is *Callitris glaucophylla*, a small to medium-sized tree which usually grows to about 18 m tall and 0.45 m in diameter, but occasionally can reach a height of 30m.

The aim of this study was to explore whether there are differences in the *Callitris glaucophylla* wood oil from different accessions (PIF 31973, PIF 32352 and PIF 32209) or not. The oils obtained from three different accessions have been profiled by GC/MS and LC/MS to obtain MS and UV data and they showed similarities but also differences in their composition. Guaiol, a sesquiterpene, was the principal component in the two oils (PIF 31973 and PIF 32209), as well as bulnesol and α-eudesmol. β-Eudesmol, 10-epi-γ-eudesmol and the two sesquiterpene lactones columellarin and dihydrocolumellarin were found in all three wood oils. The oil from *Callitris glaucophylla* PIF 31973 showed dihydrocolumellarin as principal component, then a variety of other compounds such as limonene, 1,8-cineole, methyl myrtenate, citronellic acid, α- and β-selinene, γ- and β costol and sandaracopimarinal.

Pharmacological assays were performed on the crude oils and on fractions as well. Antioxidant activity in the plant material was measured using the ORAC assay. ORAC values were situated between 300 and 1400 µmolTE/g of crude oils, some fractions of different compositions showed even more antioxidant activity than their oil of origin. The cytotoxicity assay was based on adenosine triphosphate (ATP), however the results should be seen as preliminary. Anti-inflammatory properties were tested with a competitive ELISA assay, but none of the tested fractions showed any anti-inflammatory activity.

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B-42

Chemical composition, olfactory evaluation and antioxidant effects of essential oils from *Mentha canadensis* L. and *Mentha* x *piperita* L.

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It has been reported that free radicals and reactive oxygen species (ROS) cause cellular injuries and initiate peroxidation of polyunsaturated fatty acids in biological membranes [1, 2]. To prevent and retard lipid oxidation, often synthetic antioxidants are added to lipid-containing foods. However, potential health hazards, including possible carcinogens formation, have been reported so far [3, 4]. By this reason, special interest is directed towards naturally occurring antioxidants, including volatile compounds in herbs and spices. [5-7]. Therefore, our study stresses the characterization of the chemical composition, the olfactory profile and the antioxidant effects of the essential oil from *Mentha canadensis* L. (cornmint, *Lamiaceae*) and *Mentha* x *piperita* L. (peppermint, *Lamiaceae*) with their unique combination of main and minor compounds. [8] The chemical composition of the essential oils from cornmint/peppermint were analyzed by GC/FID and GC-MS. Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH), evaluation of antioxidant activity in linoleic acid model system, determination of conjugated dienes formation, detection of hydroxyl radicals in the deoxyribose assay, and the modified thiobarbituric acid reactive substances (TBARS) method were used to measure the antioxidant activity of the oil. GC analyses identified in cornmint/peppermint oil menthol (41.2/40.7%) and menthone (20.4/23.4%) as the main constituents of both oils. Main distiction between the two oils is the lack of menthofuran in cornmint oil. It was further established that both oils showed antiradical activity with respect to the DPPH and hydroxyl (OH•) radicals. The concentrations necessary for 50% neutralization of the respective radicals (IC_{50}) in cornmint/peppermint oil were 365.0/860.0µg/ml (DPPH) and 0.326/0.256µg/ml (OH•). Cornmint/peppermint oil also demonstrated antioxidant activity in a linoleic acid emulsion model system, where at 0.1% concentration it inhibited the formation of conjugated dienes by 57.14/52.38% and the generation of secondary oxidized products of linoleic acid by 76.12/76.86%. The antioxidant properties of cornmint oil as well as peppermint oil established in this study expand the scope of its implementation as natural preservatives in food and pharmaceutical products exercising stronger antioxidant impact on OH• radical.

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B-43

Chemical composition and antibacterial activity of the essential oil from three chemotypes of *Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart growing wild in Campania (Southern Italy)

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Among the plants colloquially named *oregano* or *origanum*, four main groups commonly used for culinary purposes can be distinguished: Greek oregano (*Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart), Spanish oregano [*Coridothymus capitatus* (L.) Hoffmanns & Link], Turkish oregano (*Origanum onites* L.) and Mexican oregano (*Lippia graveolens* HBK) [1-3]. The most commonly found species belong to the genus *Origanum*. Within this genus, Ietswaart, based on morphological criteria, recognised 3 groups, 10 sections, 38 species, 6 subspecies, 17 hybrids [4]. *Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart is a typical East Mediterranean taxon. Though very variable in morphological aspects, it can be distinguished from other *O. vulgare* ssp. by its hairy stems, compact inflorescences, densely glandular leaves and calyces, green bracts, which are usually as long as calyces, and white flowers [5]. As a continuation of our research on the oils from Lamiaceae growing wild in Southern Italy [6-8], in this study we have examined the essential oils from three *O. vulgare* L. ssp. *hirtum* populations growing wild in Campania and their antibacterial activity on ten micro-organisms. The oils have been obtained from plants growing wild in different areas of Salerno province (Southern Italy) and collected in the June 2008: Furore, 600 m s.l. (F), Sansa, 500 m s.l. (S) and San Giovanni a Piro, 450 m s.l. (SG). The essential oil composition of the three populations of plants appeared quite different. The F and S oils were characterized by great amounts of phenols (45.3% F and 40.5% S); the SG oil was characterized by linalyl acetate and linalool (15.9% and 12.5%). The oils showed action mainly against the Gram-positive pathogens, among which *S. epidermidis* was the most affected. Among Gram-negative bacteria, only *E. coli* was affected by the oil F. The oil F and S resulted more active than oil SG.

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B-44

Growth inhibition of intestinal pathogenic bacteria of livestock using essential oils and their main components

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Since the antibacterial properties of certain essential oils and their main components are commonly known, their use as feed additives in animal nutrition as an alternative to previously used antibiotics is emerging. The growth inhibition of intestinal pathogenic bacteria in the animal avoids diseases as postweaning diarrhea in piglets and improves the intestine's as well as the animal's health. Relating to this, ten essential oils were tested *in vitro* against representatives of intestinal pathogenic bacteria, comprising three *Escherichia coli*, one *Salmonella typhimurium* and one *Salmonella enteritidis* strain. Two different methods, an agar diffusion assay and a broth microdilution assay, were carried out. The agar diffusion assay acted as a qualitative pre-screening whereas the microdilution assay provided quantitative results by determination of the optical density. Concentrations of test substances ranged from 0.5 % to 0.0078 % and the minimal inhibitory concentration that inhibited at least 50 % of the bacterial growth (MIC_{50}) was considered as effective inhibition of the strain, compared to the control. In accordance with literature data, carvacrol, oregano essential oil, clove essential oil and cinnamaldehyde inhibited bacterial growth of all five strains at low concentrations. Also eugenol highly affected the strains except *E. coli* O55:K59(B5):H which was less inhibited. *E. coli* O55:K59(B5):H showed also less susceptibility to tea tree oil and estragon oil compared to the other strains. Lemongrass had only a slight antibacterial effect on *S. typhimurium* and *E. coli* O128:H2 whereas basil essential oil and coriander essential oil did not affect the growth of *E. coli*, *S. typhimurium* and *S. enteritidis* at all. Comparing the five strains, *E. coli* O157:H7 and *S. typhimurium* were the most susceptible regarding all test substances. In conclusion, these tests support the usefulness of certain essential oils as feed additives for the control of pathogenic bacteria in the animal's intenstine.

B-45

Examination of plant extracts regarding their potential to modify rumen fermentation using an *in vitro* rumen fermentation model

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Plant extracts are considered as promising alternatives to previously used antibiotics in ruminant feeding. For examination of eight plant extracts an *in vitro* model was established covering a 24 hours batch fermentation. Using rumen fluid, synthetic saliva, ground corn and hackled hay, the model simulated a discontinuous form of rumen fermentation. Plant extracts were added at final concentration of 1 ml/l and an incubation period under standardized conditions followed. The effects of the treatments were examined at 0 and 24 hours of incubation including quantification of ammonium using a photometric test kit and HPLC analysis for the determination of acetic acid, propionic acid and butyric acid. The ionophore antibiotic monensin served as positive control, whereas the negative control represented the fermentation without any additives. Incubation with carvacrol, eugenol and cinnamaldehyde resulted in a marked decrease of ammonium and butyric acid concentrations. Additionally, the treatment with palmarosa oil also resulted in lower acetic acid but higher butyric acid and ammonium concentrations. Sweet orange oil decreased the acetic acid to propionic acid ratio as requested but increased ammonium concentration. Regarding tea tree oil, citronellal and 1,4-cineol, the ratio of fermentation end products was not affected at all. In conclusion, a slim majority of the examined plant extracts had the potential to modify the ratio of rumen fermentation end products which consequently may lead to their use as natural feed additives for ruminant farm animals.

B-46

Following up the influencing factors of drug quality of *Melissa officinalis* L. during primary processing

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Within the frameworks of project cooperation samples were taken during primary processing of *Melissa officinalis* at two producers in Hungary. The aim was to follow the practical steps of post-harvest processing and to unfold their influencing effects on quality.

In Dunaföldvár the samples were dried in plate-chamber drier with the drying air of 40C^0, while in Kondoros a drier with forced ventilation was used with the air-flow of ~30C^0 through the ducts covered with plant material. As controls, samples with natural drying were used, and the comparison of hand and machine cutting were made as well. For every sample the essential oil and cinnamic acid content (PhHg. VIII.) were measured.

The essential oil content was generally the highest in case of fresh materials (Dunaföldvár: 0.068 ml/100g; Kondoros: 0.104 ml/100g) and decreased during the post-harvest process due to damage, effect of heat and storage, especially in case of plate-chamber drier (0.015 ml/100g). The careful hand cutting and natural drying gave the highest essential oil content among the samples collected in Dunaföldvár (0.101 ml/100g). According to the results of post-harvest process in Kondoros the samples from the drier with forced ventilation were not substantially different from the results of natural drying (0.052 and 0.056 ml/100g, respectively). This method working with high air-flow and extensive ventilation surface ensured careful drying.

For both post-harvest cases it was proved, that the cinnamic acid content of dried *Melissa* is the largest (Dunaföldvár fresh: 1.30%, dried: 5.35%; Kondoros fresh: 2.23%, dried: 6.00%). Comparing the two genotypes of the two production sites it can be stated, that samples from Kondoros primarily have more favourable cinnamic acid content. However the cinnamic acid contents of the drugs from both producers were appropriate exceeded a good deal the allotted min. value of 4 % of the VIII. Hungarian Pharmacopoeia.

B-47

Bioactive compounds from *Scleroderma vulgare*

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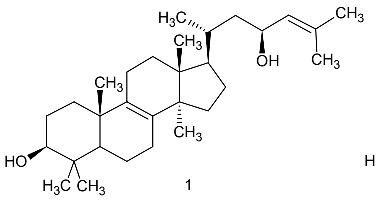
Scleroderma vulgare, is the most common species of earthball in the Central Europe and occurs widely in woods, heathland and in short grass from autumn to winter. Some of isolated compounds possesses cytostatic activity, antiviral activity against Herpes simplex type 1 and antibacterial activity towards the pathogen *Mycobacterium tuberculosis* [1].
We are interested in chemical composition and biological activities of *Scleroderma vulgare.* The column chromatography of hexane extracts allowed to obtain, in the high yield, the ergosterole, (S)-23-hydroxylanosterole (1), (23*E*)-lanosta-8,23-dien-3β,25-diol (2) and it's linolenic ester, ergosterol endoperoxide (3) and 9,11-dehydroergosterol endoperoxide (4). Their structures were elucidated on the basis of one- and two-dimensional NMR. The steam distillation on the Deryng apparatus of Scleroderma shows the oct-1-en-3-ol and octan-3-ol as a main components of essential oil obtained.



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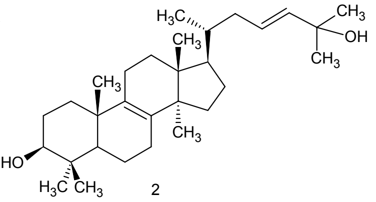

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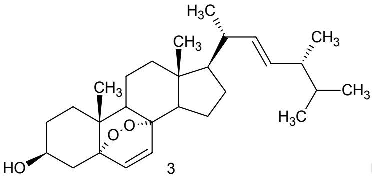

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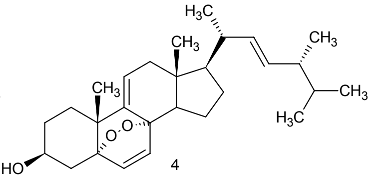

4

Selected compounds were examined for their antiproliferative activity *in vitro* against the cells of human cancer cell lines (HL-60 leukemia, HT-29 colon and MCF-7 breast cancer) using cytotoxic MTT or SRB assays. The IC_{50} values were calculated and compared with those of referential ergosterol. The compounds tested revealed the highest antiproliferative activity against the cells of HL-60 cell line. It appeared that all extracts revealed stronger or comparable with that achieved with ergosterol antiproliferative effect. Moreover, when the effect against individual cell lines is considered, two compounds 1 and 4, revealed a marked effect against HL-60 and HT-29 cells. The activity of these two extracts against this two cell lines was higher as compared to ergosterol.

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B-48

Chemical composition and cytotoxic activity of the essential oil from *Seseli andronakii* Woron.

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The genus *Seseli* L., well known in Europe and Asia, is represented in Turkey by 12 taxa (11 species and 1 subspecies), 4 of which are native to the region [1-3]. The phytochemistry of the genus *Seseli* has been investigated especially concerning the coumarins, which show interesting medicinal properties. In addition to that, several *Seseli* species are reported in ancient literature for various healing effects.

Here, we report on the analysis by GC and GC/MS of the essential oils of *S. andronakii* growing wild in Turkey. The main constituents are carotol (51.4%) and α-pinene (6%) in the fruit oil of *S. andronakii.* The cytotoxic activity of the oil was measured by MTT assay and cell death kinetic effect was observed by modified AlamarBlue assay and SYBR Green staining. The crude oil from *S. andronakii* exhibited moderate cytotoxic effect (IC_{50} = 74 ug/ml in 24h and 48.3 ug/ml in 72h) to cancer cell lines with pronounced exposition time-dependency suggesting pharmacologically sought active (specific) cell death.

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B-49

Screening of insecticidal activity of essential oils of *Mentha* species

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The essential oils from 17 *Mentha* species and cultivars (*M. aquatica, M. longifolia* in 2 types, *M.longifolia* ´Budleia´, *M. spicata, M. suaveolens* ´Variegata´, *M.* x *piperita* in 2 types, *M.* x *piperita* ´Persephone´ and ´Krasnodarskaja´, *M.* x *piperita* var. *citrate* ´Lemon´, *M.* x *piperita* var. *crispa, M.* x *piperita* var. *piperita* ´Agnes´ and ´Eau Cologne´, *M. pulegium* ´Repens´, *Pulegium vulgare, Pulegium vulgare* ´Nanum´) were obtained by hydrodistillation of the dry herb and were used for evaluation of the insecticidal activity. The composition of essential oils was not evaluated yet. The test animals used were the house fly (*Musca domestica* L.) and larvae of the tobacco cutworm (*Spodoptera littoralis*). Acute toxicity of the essential oils was determined by topical application to adult females of *Musca domestica* and to early fourth-instars of the tobacco cutworm. Acetone was used as a carrier such that each adult or larvae received 1 µl of oil solution per treatment, with acetone alone as the control. A range of five doses were used to approximate the active dose range to establish the lethal doses. Doses were applied to the thorax of *Musca domestica* and to the dorsum of *Spodoptera littoralis.* Four replicates of 20 adults or larvae were tested per dose. Nine oils were lethal for adults of *Musca domestica* in doses ranging from 180 to 250 µg/fly. The lethal doses of another 4 oils were higher than 250 µg/fly. The lowest lethal dose were found in essential oils distilled from *Pulegium vulgare* (120 µg/fly), *M. suaveolens* ´Variegata´ and *Pulegium vulgare* similar 136 µg/fly. In the case of larvae of *Spodoptera littoralis* the lowest lethal doses ascertained were from *M. suaveolens* ´Variegata´ (31 µg/larvae), *Pulegium vulgare* (34 µg/larvae) and *Pulegium vulgare* ´Nanum´ (38 µg/larvae). For another 14 oils the lethal dose ranged from 44 to 102 µg/larvae.

Acknowledgements
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B-50

Chemical composition, olfactory analysis and antibacterial activity of selected essential oils (mainly used in perfumery products)

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The chemical composition of essential oils of cabreuva (*Myrocarpus fastigiatus* Allemao, Fabaceae) from Brazil, cedarwood (*Juniperus asheii*, Cupressaceae) from Texas, ginger (*Zingiber officinale* Roscoe Zingiberaceae) from Ecuador, lemon balm (*Melissa officinalis* L. Lamiaceae) as well as essential oil from Juniper berries (*Juniperus communis* L., Cupressaceae) and myrrh essential oil (*Commiphora myrrha* (Nees) Engl., Burseraceae) were analyzed using GC/FID and GC/MS. Chemical analysis led to the identification of the following major compounds in cabreuva oil from Brazil *trans*-nerolidol 77.0%, α-bisabolol 16% and *trans,trans*-farnesol 2.1%; in cedarwood oil from Texas thujopsene 38.4%, cedrol 27.2% and α-cedrene 8.2%; in ginger oil α-zingiberene 14.3%, geranial 13.4%, camphene 9%, β-sesquiphellandrene 5.5%, β-bisabolene 5.1% and α-farnesene 4.2%; in the essential oil of lemon balm β-caryophyllene 23.5%, geranial 19.1%, neral 13.0% and germacrene D 10.1%; in the essential oil of juniper berries: α-pinene 38.1%, myrcene 15.4%, sabinene 7.9% and limonene 7.2% and in myrrh oil furanoeudesm-1,3-diene 37.3% and curzerene 24.7% were identified as main compounds. The samples were olfactorily evaluated by two professional perfumers and one aroma chemist. The antimicrobial activity of all essential oils mentioned was tested against eleven different genera of gram-positive and gram-negative bacteria by using agar diffusion [1] and agar serial dilution [2] methods. Of them, animal and plant pathogens, food poisoning and spoilage bacteria were selected. The volatile oils exhibited considerable inhibitory effect against all the tested organisms except against *Pseudomonas* by using disc diffusion methods as well as broth dilution methods. Antimicrobial tests of essential oils showed better overall reduction of gram-positive strains in comparison to gram-negative bacteria. Following this trend, cabreuva oil of Brazil showed resembling activities, but in comparison to further evaluated oils its activities were only accomplished by higher concentrations of oil.

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B-51

Biotransformation of racemic piperitone by fungi

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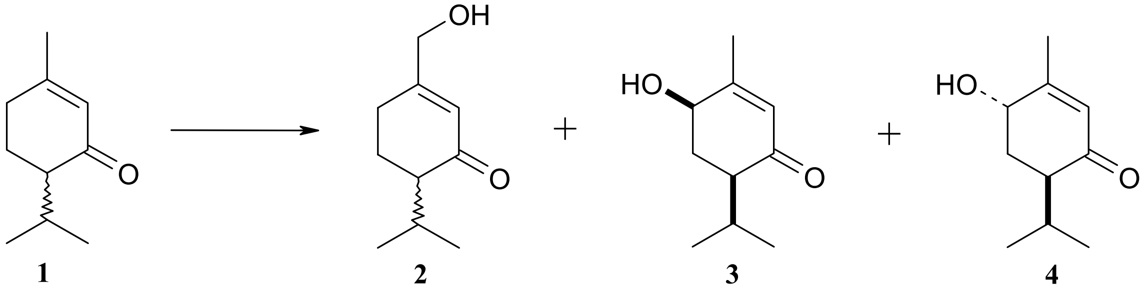
Monoterpene ketones like piperitone, menthone, carvone are very important to flavour and fragrance industry. Piperitone is a major constituent of essential oil from some *Eucalyptus*, *Mentha* and *Cymbopogon* species [1]. The literature studies afforded many informations that microbial hydroxylation of piperitone was the main transformation of this substrate. However the enantioselectivity of this processes were not determined [2-4].



Here we present the results of microbial transformations of piperitone (**1**) by cell suspension culture of *Absidia cylindrospora, Absidia coerulea* and other fungi strains. The results obtained indicate that many strains catalyzed regio- and stereoselective hydroxylation of substrate in allyl position (**2**, **3**, **4**). Biotransformations of natural, enantiomerically enriched, (-)-piperitone were also carried out in the same conditions. The results of chiral GC analysis allowed us to ascribe absolute configuration to corresponding hydroxypiperitones formed.

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B-52

Allylic alcohols as precursors in the synthesis of terpenoid lactones with potential antifeedant activity

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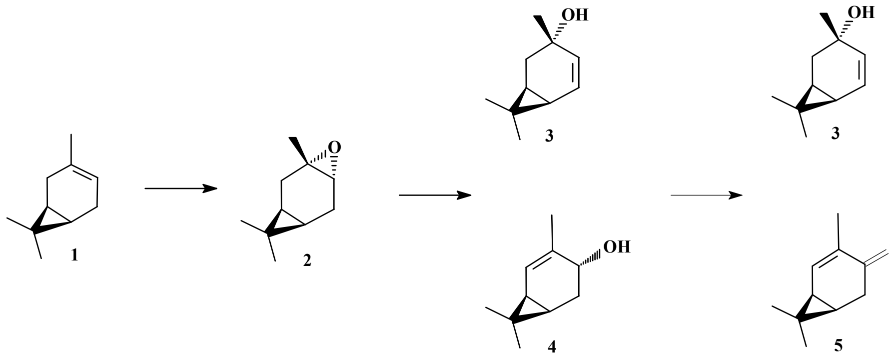
Allylic alcohols constitute key-compounds in the synthesis of derivatives with lactone moiety, possessing various biological activities, which can be insecticidal or odoriferous. We are interested in investigation of the structure-activity relationship for terpenoid lactones with antifeedant activity.
Antifeedants (feeding deterrents) exemplify natural class of pesticides, which can become alternative for increasing contamination of natural environment [1].



Herein, we present the synthesis of allylic alcohols with preserved carane system. The starting material was bicyclic monoterpene hydrocarbon, (+)-3-carene **1**, natural component of turpentine, obtained from some species of pine (in Poland from *Pinus sylvestris* L.) The mixture of allylic alcohols **3, 4** were obtained from **1** through the epoxidation by mean of *m*-CPBA and next conversion with potassium *tert*-butoxide in pyridine [2]. The next step of our investigation was the resolution of obtained secondary and tertiary alcohols using chromatographic and chemical methods. In this case column chromatography turned out to be useless. The mixture of alcohols was also subjected to the oxidation with Brown-Garg and Jones reagents. Secondary alcohol **4** underwent oxidation giving ketone **5,** whereas tertiary alcohol **3** did not react. Obtained products were separated by column chromatography and next ketone was submitted to reduction reaction.
Synthetic details will be presented with special emphasis on stereochemical aspects.

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B-53

Nematicidal activity of *Lavandula viridis* essential oils against the root-knot nematode *Meloidogne javanica*

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Root-knot nematodes (*Meloidogyne* spp.) are one of the most widespread pests limiting world agricultural productivity due not only to their pathogenic effects but also to their wide host ranges. Nematode control relies mainly on the use of synthetic nematicides that are likely to be reduced due to environmental concerns and some are able to act as hormone disruptors causing loss of fertility, carcinogenesis and mutagenesis. Therefore, there is a need to search for naturally occurring alternatives with less toxicity and more friendly to the environment. Aromatic plants are a valuable source of bioactive compounds that can be used as nematicides or serve as model compounds for the development of chemically-synthesized derivates [1, 2]. The composition and the nematicidal activity of the essential oils of *L. viridis*, collected in the south of Portugal, were investigated against hatching of the root-knot nematode *M. javanica.*

Essential oils, isolated by hydrodistillation and analysed by GC and GC/MS, were characterized by high contents of 1,8-cineole (34.5%), camphor (13.4%), α-pinene (9.0%) and linalool (7.9%). For nematicidal bioassays, different concentrations of the oils were prepared by serial dilution with distilled water containing Triton X-100 (5000ppm). Each treatment consisted of 15 eggs embryonated with second-stage juveniles (J2), in 1mL of each oil concentration. All treatments and controls were replicated five times and hatched J2 were recorded every 24h, up to 360h. Data on hatching were converted to percentage cumulative hatching inhibition, corrected by Abbott's formula [3], and subjected to probit analysis [4]. The essential oils inhibited egg hatch (50% inhibition by 8.01μL/mL).

Our results showed that *L. viridis essential* oils have potential to be used as nematicides against *M. javanica*. Further research is being conducted in order to assess the effect of the major compounds of the essential oils on hatching and mortality of *M. javanica*.

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