ISEO 2008
Quedlinburg, Germany
September 7 – 10, 2008

39th International Symposium on Essential Oils

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- **FINAL PROGRAMME**
- **BOOK OF ABSTRACTS**

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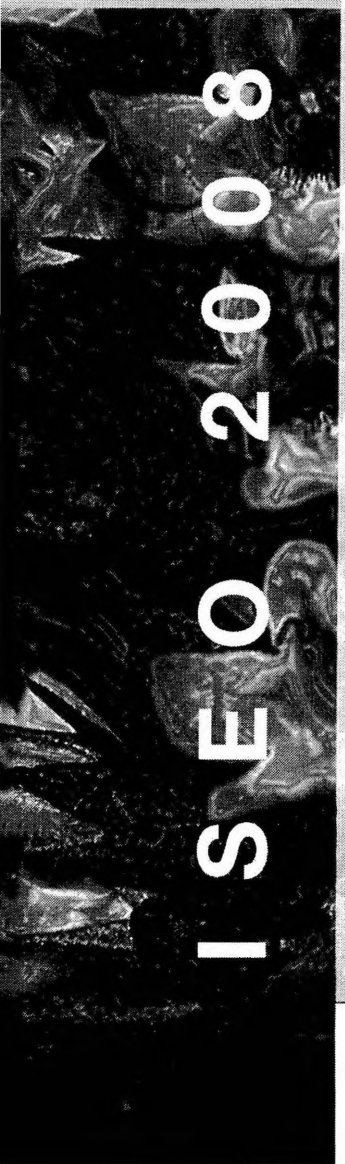

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TABLE OF CONTENTS

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LECTURE PROGRAMME

Sunday, September 7, 2008

Foyer Congress Centre, Historical Palais Salfeldt — Page

14.00-18.00 **Registration and mounting of posters**

Lecture Hall, Palais Salfeldt

18.00-18.30 **Opening ceremony**

Georg Friedrich Backhaus
President of the Julius-Kühn Institute, Quedlinburg/D

Eberhard Brecht
Mayor of the City of Quedlinburg/D

Hartwig Schulz
Head of the Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Quedlinburg/D

Günter Schumann
Head of the Institute for Breeding Research on Horticultural and Fruit Crops, Quedlinburg/D

Lecture Hall, Palais Salfeldt

18.30-19.20 **OPENING LECTURE** — 33
PL 01 **The scent of vanishing flora: new and uncommon volatile compounds in most diverse endangered plant species**
Kaiser, R., Dübendorf/CH

Cathedral Quedlinburg

19.20 **Walk to the romanesque cathedral**

19.45 **Organ recital**

Castle Museum

ca. 20.15 **Get-together**
(Entrance in front of the cathedral)

PL = Plenary Lecture KL = Keynote Lecture L= Lecture

LECTURE PROGRAMME

Monday, September 8, 2008

Lecture Hall, Palais Salfeldt — Page

BIODIVERSITY OF ESSENTIAL OIL PLANTS

Chairs: K. Hüsnü Can Baser and Friedrich Pank

12.20-14.00 LUNCH BREAK

PL = Plenary Lecture KL = Keynote Lecture L= Lecture

LECTURE PROGRAMME

Monday, September 8, 2008

Lecture Hall, Palais Salfeldt — Page

BREEDING AND CULTIVATION STRATEGIES

Chairs: Eva Nemeth-Zambori and Ana Cristina Figueiredo

16.10-16.40 COFFEE BREAK

Lecture Hall, Palais Salfeldt

YOUNG SCIENTIST WORKSHOP

Chairs: Eva Nicolas Baldovini

PL = Plenary Lecture KL = Keynote Lecture L= Lecture

LECTURE PROGRAMME

Tuesday, September 9, 2008

Lecture Hall, Palais Salfeldt — Page

ANALYTICAL METHODS AND ACTIVE PRINCIPLES

Chairs: Carlo Bicchi and Patrizia Rubiolo

10.30-11.00 COFFEE BREAK

Chairs: Luigi Mondello and Stanislaw Lochynski

12.50-14.10 LUNCH BREAK

PL = Plenary Lecture KL = Keynote Lecture L= Lecture

LECTURE PROGRAMME

Tuesday, September 9, 2008

Lecture Hall, Palais Salfeldt — Page

BIOGENESIS AND IDENTIFICATION OF SELECTED SUBSTANCES

Chairs: Chlodwig Franz and Elisabeth Stahl-Biskup

14.10-15.00 PL 04 **Metabolic regulation of essential oil formation in the Lamiaceae** 71
Gershenzon, J., Jena/D, Asbach, J., Jena/D, Crocoll, C., Jena/D, Degenhardt, J., Jena/D

15.00-15.20 L 22 **Distribution of volatile bibenzyls and tocopherols in liverworts and pungent medicinal plants** 72
Asakawa, Y., Tokushima/J, Nishiki, M., Tokushima/J, Ludwiczuk, A., Tokushima/J, Toyota, M., Tokushima/J

15.20-15.40 L 23 **Labelling with $^{13}CO_2$ reveals differences in the stored and recently synthesised fractions of terpenoids within *Eucalyptus globulus* leaves** 73
Winters, A. J., Sydney/AUS, Hocart, C., Canberra/AUS, Schnitzler, J.-P., Garmisch-Partenkirchen/D, Zimmer, I., Garmisch-Partenkirchen/D, Kreuzwieser, J., Freiburg/D, Rennenberg, H., Freiburg/D, Adams, M. A., Sydney/AUS

Historical Palais, Palais Salfeldt

15.40-17.00 **POSTER SESSION**

Railway Station Quedlinburg

17.30 **Departure for symposium dinner** (historical train)

Restaurant Habichtstein

19.00 **Symposium dinner**

PL = Plenary Lecture KL = Keynote Lecture L= Lecture

LECTURE PROGRAMME

Wednesday, September 10, 2008

Lecture Hall, Palais Salfeldt — Page

COMMERCIAL UTILIZATION

Chairs: Gerhard Buchbauer and Karl-Heinz Kubeczka

08.30-09.20 PL 05 **Encapsulation and other programmed release techniques for essential oils and volatile terpenes** — 77
Karlsen, J., Oslo/N

09.20-09.40 L 24 **Plant extracts to modify insect behaviour in stored product protection** — 78
Adler, C., Berlin/D

09.40-10.00 L 25 **GC/MS determination of IFRA-restricted substances in fine fragrances, cosmetics and household products** — 79
Brachet, A., Petit-Lancy/CH, Weidenauer, M., Petit-Lancy/CH, Chaintreau, A., Brussels/B, Vey, M., Brussels/B

10.00–10.30 COFFEE BREAK

Chairs: Erich Schmidt and Alain Chaintreau

10.30-11.00 KL 05 **Authentication of essential oils and aroma extracts** — 80
Hammerschmidt, F. J., Holzminden/D, Krammer, G. E., Holzminden/D, Meier, L., Holzminden/D, Brennecke, S., Holzminden/D, Lückhoff, A., Holzminden/D, Schäfer, U., Holzminden/D, Schmidt, C. O., Holzminden/D, Bertram, H.-J., Holzminden/D, Weber, B., Holzminden/D

11.00-11.20 L 26 **Intensification of the extraction phenomena** — 81
Besombes, C., La Rochelle/F, Allaf, K., La Rochelle/F

11.20-11.40 L 27 **Acid hydrolysis/catalysis (AHC) in essential oil production by hydro/steam distillation: improvements in yield and chemical composition analyses and change in biological activities** — 82
Kücük, M., Trabzon/TR, Akyüz, E., Rize/TR, Burnaz, N. A., Trabzon/TR, Baltas, N., Rize/TR, Ekinci, A. P., Rize/TR, Sarikaya, A., Trabzon/TR, Yasar, A., Trabzon/TR, Karahalil, F. Y., Trabzon/TR, Ertürk, Ö., Ordu/TR, Coskuncelebi, K., Trabzon/TR, Kolayli, S., Trabzon/TR, Yayli, N., Trabzon/TR

11.40-12.20 **Summary of poster highlights**
Lawrence, B., Winston-Salem/USA

12.20-12.40 **CLOSING CEREMONY**
Schulz, H., Quedlinburg/D

12.40 **Lunch**

14.00 **Excursion**
Meeting Point: Congress Centre Palais Salfeldt

PL = Plenary Lecture KL = Keynote Lecture L= Lecture

LIST OF POSTERS

LIST OF POSTERS

LIST OF POSTERS

LIST OF POSTERS

LIST OF POSTERS

LIST OF POSTERS

LIST OF POSTERS

LIST OF POSTERS

ANALYTICAL METHODS AND ACTIVE PRINCIPLES

LIST OF POSTERS

LIST OF POSTERS

LIST OF POSTERS

LIST OF POSTERS

LIST OF POSTERS

LIST OF POSTERS

COMMERCIAL UTILIZATION

LIST OF POSTERS

LIST OF POSTERS

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LECTURES

Biodiversity of essential oil plants

PL 01

The Scent of the Vanishing Flora: New and uncommon volatile compounds in most diverse endangered plant species.

Kaiser R., Givaudan Schweiz AG, Fragrance Research, Ueberlandstr. 138, CH-8600 Dübendorf

As a part of our broad and ongoing search for new scent molecules and scent concepts in nature during the past 25 years, we have encountered an astounding number of interestingly scented species which, today, have to be considered as endangered.

In our appreciation of these wondrous plants and in the hope to sensitize people for the many reasons making conservation activities so important, we decided in 2001 to focus even more on highly endangered scented species worldwide and to compile their scent compositions as well as complimentary information in an upcoming book entitled "The Scent of the Vanishing Flora". The purpose of this lecture is to give with a series of interesting examples from whole over the world an introduction to the concept of this future book.

Discussed among others are new derivatives of (E)-4,8-dimethylnona-1,3,7-triene found in the flower scent of *Abeliophyllum distichum*, an Oleaceae native to Korea, the scent of the famous *Rothmannia annae*, a Rubiaceae endemic to the Seychelles, new compounds of the scent of South African and South American orchid species, as the *trans*-(Z)-3-methyl-7-dodecen-4-olide dominating the floral scents of many *Kefersteinia* species or the *trans*-(Z)-3-methyl-6-dodecen-4-olide occurring in related genera as well as the scent concepts of highly endangered Hawaiian species as those of *Brighamia insignis* and *Hibiscus waimeae ssp. waimeae.* Finally, we will end this fragrant journey around the world in one of the most arid regions, in the Death Valley, and discover also here new or uncommon plant volatiles.

KL 01

South Africa's aromatic flora – an unexplored Eden of opportunity

Viljoen AM
Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria, 0001

The fragrance and flavor industry remains challenged and under immense consumer pressure to produce innovative products for this lucrative industry. Like the pharmaceutical industry, the flavor and fragrance industry turns to nature for guidance, inspiration and as a source of novel compounds for commercial development.

South Africa represents a global epicenter of aromatic plants. Despite this rich botanical diversity (matched by chemical diversity) it is surprising that many of the indigenous assets remain latent and are not systematically studied which is an obvious pre-requisite for these unique botanical assets to be transformed into consumer products. South Africa has offered the world two indigenous aromatic plants which have both been developed into a commercial success. Geranium oil (obtained from various cultivars of *Pelargonium graveolens*) and Buchu oil (from *Agathosma betulina*) are only two species out of the near 700 aromatic plants indigenous to South Africa.

An extensive exploratory phase and excellent researching infrastructure underpins any commercial development. In the quest to explore this extra-special flora of South Africa a project devoted to indigenous flavor and fragrances at Tshwane University aims at creating a strong scientific base and expertise in the study of the indigenous aromatic plants which hitherto remains poorly investigated. The paper is a brief reflection of past and present research and will unequivocally confirm the value (and privilege) in exploring one of the most biodiverse areas in the world.

Evaluation of a Parsley (*Petroselinum crispum*) World Collection – Essential Oil Components in Relation to Genetic Distances

Marthe F[1], Krüger H[2], Budahn H[1], Struckmeyer T[1], Ulrich D[2], Lohwasser U[3]

[1] Institute for Breeding Research on Horticultural and Fruit Crops (ZGO) and [2]Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection (ÖPV), Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, Erwin-Baur-Str.27, D-06484 Quedlinburg, Germany, [3]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, D-06466 Gatersleben, Germany

Parsley (*Petroselinum crispum* (Mill.) Nyman) belongs to the Apiaceae family and was used already in the ancient world mostly as a medicinal plant. Today parsley is the most important spice plant in Germany, cultivated on more than 1700 ha.

The complete assortment of parsley genotypes from the German Gene Bank at IPK, Gatersleben (200 accessions) and 19 genotypes from the ZGO, Quedlinburg were cultivated and characterized morphologically as well as for resistance traits, aromatic components and amount and composition of essential oil of leaves and seeds.

The investigation of hexane extracts of parsley seeds showed that methoxymethylbenzene derivates constitute more than 50% in all essential seed oils. The variability ranges from pure myristicin / elemicin types to pure apiole / allyltetramethoxybenzene types. The essential leaf oils of the first cut of all accessions were isolated by hydrodistillation and the contents of the principle components α-pinene, β-pinene, myrcene, limonene, β-phellandrene, α-terpinolene, 1,3,8-p-menthatriene, α,p-dimethylstyrene, β-elemene, myristicin and apiole were analysed by GC. The main component was 1,3,8-p-menthatriene in 96, apiole in 55, myristicin in 50, β-phellandrene in 12, α,p-dimethylstyrene in 3 and myrcene also in 3 accessions. Most of the leaf and seed oils correspond to the well-known chemotypes [1].

In parallel genomic DNA of the 219 genotypes were isolated and characterized by AFLP-, SRAP-, RAPD- and dp RAPD analysis [2]. Two hundred and six polymorphic markers were scored as 1/0 matrix. Using the program NTSYS pc 2.2, genetic distances were calculated. After cluster analysis by UPGMA method, the tree matrix was converted into a dendrogram. Molecular analysis shows that the 219 tested accessions split clearly into two groups (132 and 87 genotypes) with a distance of approx. 0.6. Root parsley genotypes clustered into the smaller group and all genotypes with curled leaves, except one, belong to the other group. Genotypes with smooth leaves can be found in both groups. In some cases clustering of genotypes from one region (or country) of origin is obvious, for example all 11 accessions from northern Africa (Tunisia and Libya) cluster in the smaller group.

The correlation of grouping based on molecular markers, and compounds of the essential leaf oil showed correlation in the case of apiole, myrcene, β-phellandrene and β-elemene. For the major compound apiole, the smaller group (87 accessions) has a mean value of 0.96% apiole in the essential oil, whereas the bigger group (132 accessions) has a mean value of 15.61%. The smaller group contains only 3 accessions with a high apiole level and the bigger group contains 48 accessions with a low apiole level. For the minor compound myrcene, the smaller group has a high mean value of 15.59% and the bigger group has a low mean value of 6.53%. The smaller group contains only 6 accessions with a low myrcene level and the bigger group contains 24 accessions with a higher myrcene level.

The distance analysis based on molecular markers is the first for parsley. It distinguished clearly between morphological groups of curled leaves and root parsley. The two main groups are clearly found also for compounds of the essential oil as secondary metabolites.

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On the study of essential oils obtained from four plant species growing in Nigeria

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As part of an extensive research into the biodiversity of Nigerian flora, we report on the compounds isolated from the essential oil plants *Vernonia amygdalina* Delile., *Eclipta prostrata* L., *Acacia nilotica* L., and *Acacia albida* Delile., growing in Nigeria. The oils were obtained by hydrodistillation in an all glass Clevenger-type apparatus and characterized comprehensively for their constituents by means of GC-FID and GC-MS. The present study on the oil composition of the stem bark *V. amygdalina* showed it to contain α-muurolol (45.7%) as the most singly abundant constituents. The results even become more significant considering the fact that this compound was either present in trace amount or absent from previous studies on *Vernonia* oil samples [1-3].
Monoterpenes (4.7-11.1%), sesquiterpenes (47.4-89.3%) and straight-chain aliphatic hydrocarbons (3.8-25.6) were the classes of compounds more represented in the oils. The quantitatively significant oil constituents present in stem and leaves are β-caryophyllene (15.9-47.7%) and α-humulene (12.9-31.8%), respectively. *(E)*-β-farnesene (10.0%) was also identified in significant amount in the stem oil. The oil of *E. indica* earlier reported consisted mainly of 2-tridecanone [4], which was conspicuously absent from the present study.
Menthol (34.9%) and limonene (15.3%) were the compounds occurring in higher proportions in *A. nilotica*. However, α-pinene (18.6%) dominated the essential oil of *A. albida*. The two oil samples contained mainly monoterpenoids. We had earlier reported that a sample of *A. tortilis* was rich in sesquiterpenoids consisting of α-humulene, α-cadinol, nerolidol and γ-cadinene [5].
Furthermore, this paper will discuss the chemotaxonomy of the analysed Nigerian plant species.

References

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Phytochemical characteristics of *Achillea* species and biological activities of their essential oils

Németh, É., Bernáth J.
[1] Corvinus University of Budapest, Dept. of Medicinal and Aromatic Plants, Budapest, Villányi Str. 29. H-1518, Hungary

Yarrow (*Achillea*) species had been utilized in ethno-pharmacology for thousands of years all around the world. As evidence is accumulating and scientific information is increasing, in several aspects their therapeutical value seems to be justified.

Yarrow species are rich in biologically active compounds. The content of essential oil in flowering shoots varies 0,1-1,0% d.w. depending on species, genotype and several abiotic factors. In the last decades, 50-60 compounds as a mean had been identified in different samples. Accumulation of azulenogenic compounds seems to be a characteristics of the members of *Millefolium* group, especially of *A. asplenifolia* Vent., *A. roseo-alba* Ehrend. and *A. collina* Becker. 1,8-cineole is hardly missing from any of the oils and it is often the main component. Camphor, borneol, futhermore α- and β-pinenes are universal components of the genus, too. Beside chamazulene, the most frequently identified sesquiterpenes are caryophyllene and its oxides, α-bisabolol, cubebene and germacrenes [1]. Beside the essential oil, sesquiterpene lactones (guaianolides, eudesmanolides, germacranolides, longipinenes) and flavonoids (mainly glycosides of apigenin, luteolin and quercetin. furthermore free flavonoid-aglyca) are considered as active constituents, which have also chemotaxonomic significance. Other compounds, like amino-acid derivatives, alkamides and polyacetylenes, polysaccharides, saponines, sterols, sugars, fatty acids, coumarins, tannins, polyines had been identified in several species, too.

Scientific references on biological activities had been accumulated especially in the last 10-20 years. However, human clinical trials are still rare. *Achillea* species seem to represent valuable therapeutic tools. They had been found active at many therapeutic areas: antioxidant, anti-inflammatory, antinociceptive, spasmolytic, antidiabetic, antiulcer, choleretic, hepatoprotective, antispermatogenic, phytoestrogenic, cytotoxic, wound healing, antihyperlipidemic, antihypertensive effects were proved several times. Essential oil and its compounds may contribute to these activities. Essential oil proved to be highly effective in antioxidant reactions, but the effect of individual components (1,8-cineole, camphor, β-pinene, borneol, terpinen-4-ol, α-pinene) is contradictious [2]. Sesquiterpene lactones, especially proazulenes contribute to the anti-inflammatory activity. [3] However, guaianolides are known to cause allergenic reactions (dermatitis) [4]. Essential oil and some components (1,8- cineole, α-bisabolol) showed in vivo antinociceptive effect in mice. [5]. As a new therapeutic area, sesquiterpenes compounds are supposed to be active as antitumor agents [6]. Essential oil may have the greatest practical importance as antimicrobial principle. Several oil samples had been shown to be active against different human pathogens, bacteria (mainly Gram-negative ones), fungi, and recently also against protozoa and viruses [2]. The results show, that in most cases not the main components themselves but the complexity of the oil is necessary for providing the appropriate activity.

The lecture presents an overview on pharmacological activities of yarrow, evaluates new scientific information - with special respect on those connected with the essential oil;

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L 04

Jatropha curcas L., a Stress Resistant Biodiesel plant

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Environmental stress severely restricts the distribution and productivity of the plants; in particular salinity, drought and heavy metal are the major abiotic factors that limit gross production worldwide. Adaptations to the condition may involve passive tolerance or active homeostatic mechanisms for maintaining water balance. Plants have stress specific adaptive responses which protect the plants for more than one environmental stress. Given the importance of stress tolerant plants in soil reclamation programmes, it would be thus profitable to adopt tissue culture approach. Cell and tissue culture technique can be fruitfully applied to induce tolerance and also it is an efficient means to study salinity and drought, and heavy metal stress effects in cell cultures. One way of increasing productivity in stressful environment is to breed crops that are more tolerant in stress. The tolerant level of the different abiotic stress under *in vitro* condition is under consideration in context to the morphological, biochemical and molecular aspects. During stress accumulation of certain organic metabolites solutes takes place. Increased levels of some compatible solutes such as proline appears to be a promising approach in efforts to increase the ability of plants to tolerate environmental stress.*Jatropha curcas,* the biodiesel plant has been used as model plants to study abiotic stress.

Aim of the study : *Jatropha curcas* L. (Euphorbiaceae) or physic nut is an all-purpose, zero-waste perennial plant , surviving with minimum inputs in many parts of the country. *Jatropha*, the wonder plant, produces seed with an oil content of 37%. *J. curcas* is gaining importance commercially as a green fuel source and is being advocated for development of wasteland and dry land. The present study was conducted on *Jatropha curcas* to understand the morphological, physiological and biochemical changes on abiotic stress under controlled *in vitro* conditions .

Conclusion :The plant exhibited distinct response on subjecting the plants to abiotic stress viz. salinity, drought and heavy metal under *in vitro* conditions . The increase in the osmolyte proline concentration and the subsequent decrease in the protein concentration were evident in all the cultures under stress. There were decreased growth at lower stress with no growth and necrosis of in *vitro* cultures at higher stress. $HgCl_2$ was found to be most toxic for the growth of *J.curcas* . Thus it was clear that heavy metal cause toxicity *and* impair the normal metabolism of the cell and creates an imbalance in the plant homeostasis. These results can be correlated with the other responses.

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KL 02

Essential oil bearing trees of Turkey

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Flora of Turkey is rich and diverse with over 12.000 taxa recorded. The ratio of endemism is also high with 30%. Forest trees as well as those introduced as garden or avenue trees constitute a large number. Many of these trees are providers of non-wood forest products and their sustainable utilization is crucial for rural development projects and also for food security. Many of such trees are aromatic and therefore have potential for the production of essential oils. Our group has been tapping this resource and has so far investigated essential oil yield and composition of a wide range of aromatic trees of Turkey.

Essential oils of genera like *Pinus, Abies, Betula, Eucalyptus, Juniperus, Liquidambar, Laurus, Schinus, Citrus, Viburnum, Cedrus, Pistacia* will be covered in the scope of this presentation.

Ethnobotany, Medicinal and Antioxidant potential of Essential oil plants in parts of Eastern India

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Aromatherapy was popular in ancient India in *ayurveda, siddha, unani* systems of medicine and among village and tribal people. Present practice on aromatherapy observed and recorded among local *bengalees, santhals, assamese, manipuris* and inhabitants of some hilly tracts in eastern India.
The light essences of many flowers and some other plant parts are sensitive to nasopharyngeal epithelium, and affect central nervous system. The plant materials recorded as antiseptic, anti inflammatory, sedative, muscle relaxant, analgesic, anticonvulsant, and memory enhancing.
A list of 130 plants including flowers and essential oil plants in plains and hilly tracts of eastern India presented. *Madhuca* flowers, *Hedychium coronarium* Koenig., many member of Lamiaceae, neem, and fragrant rice are considered as mild sedative whereas *Ocimum* spp., clove, *Eupatorium odoratum* L., *Ichnocarpus frutescens* R.Br., *Jasminum* spp. *Pandanus* inflorescence, *Plumeria acuminata* Ait., *Mangifera indica* L., *Murraya paniculata* (L.) Jack, *Tagetes* spp., *Wedelia calendulacea* Less. are considered as stimulant. *Aganosoma dichotoma* (Roth.) Schum, *Artemisia vulgaris* L., *Citrus* spp., *Holarrhena pubescens* Wall., *Mesua ferrea* L., *Mimusops elengii* L., *Nelumbo* sp. *Nyctanthes arbor-tristis* L., *Pterospermum acerifoiium* Willd. and rose essence considered as neuroprotective. Practice and uses of plants are declining in recent years due to the loss of habitat and discontinuity of the chain of acquired knowledge.
Plant materials used in different doses and combinations; oils extracted by various means that include simmering, steaming, and condensation, diluted in a carrier oil or water before use.
In vitro assay: Aromatic plant materials with local uses screened for total antioxidant activity, based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH [1]; and 2, 2-diphenyl-1-picrylhydrazyl radical scavenging capacity [2]. Phenol determined by Folin-Ciocalteau reagent in alkaline medium.
Out of 35 different aromatic plant sources aqueous extracts of 12 plants showed good antioxidant capacity. Among these materials, highest antioxidant capacity was in *Mangifera indica* blossom equivalent to ascorbic acid. It also exhibited neuroprotective action. A good correlation (r=0.9) between radical scavenging capacities of the extracts with total phenol content was observed. DPPH-TLC screening shows antioxidant activity compared with standards. It appears that pure compounds (α-pinene, quercetin, gallic acid) have less activity than the same amount when present along with other compounds in the plant extract. A synergistic/ additive effect of the compounds is responsible for high activity of the extracts. Synergistic and antagonistic interactions of the essential oils from different plant combinations recorded. The traditional formulations exhibited synergistic effects.

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Exploration of wild populations of *Salvia lavandulifolia* Vahl. from Castilla-La Mancha province (Spain): compilation, chemical composition and storage of the seeds.

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An exploration of 16 natural populations of *Salvia lavandulifolia* has been carried out in Castilla-La Mancha province (Spain). Our principal goal has been the chemical characterization of these natural populations. The seeds from these populations were stored in our Germplant Bank in the Agronomical Research Centre of Albaladejito (Cuenca, Spain).
The essential oil from different aerial parts (inflorescences, stems and leaves) of this species gathered in Castilla-La Mancha (Spain) has been extracted by steam destillation and analysed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MC).
Differences between natural populations have been found. Despite, all of them showing α-pinene, 1-octen-3-ol, limonene and cineol/eucalyptol as principal constituents amounting around 50% of the total essential oil.
Salvia lavandulifolia crops are an excellent alternative to conventional herbaceous crops in Castilla-La Mancha province. The aromatic and medicinal plants crop need to find effective chemotypes adapted to the market demand to be used in breeding and selection programmes.

L 07

Genetic diversity and classification of spring safflower *(Carthamus tinctorius)* cultivars using morphological characters

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Assessment of genetic diversity is primarily useful to utilize the genetic materials through breeding programs. In this survey, genetic diversity of 30 spring genotypes of safflower (*Carthamus Tinctorius*) was assessed by morphological traits. Studying of morphological traits was conducted in two years (2005 and 2006) based on a randomized Complete Black Design with three replications. The results of analyses of variance for each year and combined analysis of data for two years demonstrated that the differences among genotypes were significant for more traits. Cluster analyses by ward method classified the genotypes based on morphological traits in two groups. The first group included 11genotypes. The second group was divided into two subgroups with 11 and 8 genotypes. According to the results, land races genotypes, lines and improved genotypes were separately grouped as groups or subgroups. The result of principal component analyses introduced three principal components with eigenvalue more than one which in contributed 72.92 present of the total variability.

Key words: *Carthamus tinctorius*, Cluster analyses, Genetic diversity, Morphological traits , Principal component analyses.

ISEO 2008

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LECTURES

Breeding and cultivation strategies

Selection and breeding of essential oil plants: different approaches for annuals and perennials

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Essential oil can be obtained from plants belonging to different families and different species. In the past, these oils were collected from wild populations. The increased demand by the industry for uniform and quality raw material, besides the fact that less and less people continue to collect plants from the wild, and some of the wild species plants became protected, has necessitated – the cultivation of various species. This shortage of raw material has encouraged seed companies, researchers and farmers to select cultivars that could substitute for the raw material that came from the wild. Selection of new cultivars is somewhat different if the plant is annual or perennial and it depends on the propagation methods. With annual species propagation normally is done by seeds but with perennial species it could be done not only by seeds but also by cuttings. Same of the most important annual aromatic species come from the *Umbelliferae* family (caraway, coriander, dill, etc.) but also from other families such as the - *Labiatae* (basil), *Fabaceae* (fenugreek), *Brassicaceae* (mustard) and *Compositae* (chamomile). The first step in breeding a new cultivar is to select single plants with high performance (yield and quality) from as many as possible sources that are tested in the same soil and climate conditions that later on will be the place of commercial production. The second step is to produce "families" (by self-pollination) from each one of the selected plants, and again to choose only the best ones during 4 to 7 generations until a uniform line is obtained. Thus a new cultivar is "born". The same process could be done with perennial species if there is a commercially viable possibility of propagating the species by seeds. This process will take a longer time compared to annual species, as the performance tests take a longer time, at least two years. If there is a possibility of using vegetative propagation, the selection time could be shorter. The selected plants can be tested in plots after they are propagated by cuttings, as is necessary - before commercialization. This method has been used for oregano, thyme, onion, *Artemisia* and many others species. Hybridization can be conducted through artificial crossing or through genetic engineering when the selection process alone does not produce a variety that has all the characteristics that are needed for commercial production. In this lecture we give examples of each one of the methods and emphasis the advantage and the disadvantage of each.

Designing levels of volatile substances in essential oil plants by breeding

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One of the most important breeding aims is to improve the essential oil content of medicinal plants and to design the oil composition in compliance with the requirements of quality standards. In the following, results achieved by application of classical breeding methods are presented for caraway, fennel, savory and thyme. In Central Europe, biennial caraway is conventionally cultivated. An annual form (*Carum carvi* L. var. *annuum* hort) is endemic in the Mediterranean. It provides economic advantages due to the shorter vegetation period, but the level of its essential oil content is unsatisfying compared to biennial caraway. In a period of twenty years, the essential oil content of an annual caraway population could be raised from originally 3.37% to 7.42% by recurrent selection keeping the required level of its carvone content with 53.3% [1].

Selection towards maximum essential oil content of bitter fennel fruits (*Foeniculum vulgare* MILL. ssp. *vulgare* var. *vulgare*) with a minimum portion of estragole has been accomplished in a crossing progeny with improved variability. The essential oil content could be raised to more than 12%. Estragole is claimed to be a potential health risk. Due to an obviously genetically fixed positive correlation between the oil compounds anethole and estragole [2], the estragole content could not be lowered to more than 2% whilst keeping an anethole content of at least 60% as required by Ph.Eur.

Carvacrol containing essential oils are an alternative to antibiotic performance-improving additives to animal feed which have been prohibited in the EU since 2006. The short developmental vegetation period of summer savory (*Satureja hortensis* L.) facilitates its flexible arrangement in the crop rotation and provides good prerequisites for raw material production with low costs. The conventionally cultivated varieties suffer from low essential oil content with an insufficient portion of carvacrol. Evaluation of different accessions and subsequent recurrent selection with the best populations resulted in the generation of genetic material with an essential oil content of > 4.5% in the dry leaves and a carvacrol content in the essential oil of > 60%.

After selection of male sterile and hermaphroditic elite plants among high performance thyme accessions (*Thymus vulgaris* L.) [3], F_1-hybrid populations were generated to exploit their hybrid vigour. The essential oil content of the best F_1-hybrid populations exceeded the oil content of the standard variety by 50% with 3.5% in dry leaves. The breeding procedures did not result in a decline of volatile phenols in the essential oil. Their level could be kept above 70%.

The examples show that the essential oil content of medicinal and aromatic plants can be successfully improved by the exploitation of the available natural variability and additional variability increased by crossing. Also the composition of the essential oil can be maintained on the required level. However, the breeder has to face also genetically caused limitations. One prerequisite of a good selection response – as in the presented examples – is the inclusion of a high number of individual plants in the selection procedures. This could be achieved by rationalising the analytic methods using near infrared spectroscopy [4], simplified distillation procedures [5] and effective sample preparation.

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Influence of gibberellic acid on the essential oil formation in *Salvia officinalis* L.

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Gibberellic acid is a plant hormone which regulates a number of processes in plant development. In this study we present the effect of gibberellic acid on the essential oil formation (content as well as compositional changes) in garden sage (*Salvia officinalis* L.). The main proportion of the essential oil of *Salvia officinalis* is composed of monoterpenes derived from the general monoterpene precursor GPP via three distinct monoterpene synthases, namely 1,8-cineol synthase, (+)-sabinene synthase en route to α- and β-thujone and (+)-bornyldiphosphate synthase en route to camphor (Wise et al., 1998).
The availability of DNA-sequence information of all three monoterpene synthase genes enabled to quantitatively study their mRNA-expression by qPCR and to directly correlate the mRNA-expression levels to the quantitative levels of these essential oil compounds and the overall essential oil content.

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Ecological agriculture: essay of weed control management on *Salvia lavandulifolia* Vahl. and *Lavandula latifolia* Medikus. Culture from Castilla-La Mancha (Spain).

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An essay of the weed control on ecological agriculture has been carried out during the last three years in the Agronomical Research Centre of Albaiadejito (Spain). Three different cover managements have been tested: fresh plants of *Vicia sativa*, distil plant residues from aromatic plants and barley straw, on the tillage of two aromatic and medicinal plants from Castilla-La Mancha Province: *Lavandula latifolia* Medikus and *Salvia lavandulifolia* Vahl. Weed control with cover management is considered an alternative technique to conventional soil working. All the cover managements were found to reduce the weed in all the tested tillage, although the barley straw cover showed to be the most efficient one. Besides, cover management involves several benefits as an erosion control, maintenance of the soil moisture or increasing the organic part of the soil. The weed control with this type of covers could be an effective alternative to the use of herbicides and the most suitable one in ecological agriculture. As far as we know this is the first report about the weed control in the tillage of these two aromatic and medicinal plants.

Geranium oil from Pelargonium var Rose in the South Eastern part of South Africa

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There is a desperate need in South Africa for information on suitable small farmer crops with low risk, alternative choices for commercial farmers, and for drought tolerant crops. Meanwhile, South Africa is importing € 5.5 million worth of essential oils per year. It is used in the culinary, perfumery, industrial and in the cosmetic and pharmaceutical industries. The international trend of natural products is growing at 15% in demand world wide. South Africa's share of the world exports in essential oils is estimated at 1.03%. There is an increasing world demand for the high quality rose geranium essential oil of *Pelargonium* species which contains geraniol, linalool and citronellol. It is mainly used as, substitutes for the expensive attar of roses in the perfume trade and to treat cellulite. *Pelargonium* species are indigenous to South Africa. Geranium oil production was not sustain ably done in the past in African countries. Most rural areas of South Africa is densely populated, and poverty stricken. These communities rely on subsistence farming as livelihood. Limited information on Geranium cultivation methods, plant density, yields, harvesting methods and co-operative farming and markets has led to the rationale of this trial in this subtropical, South Eastern area of South Africa.

The aim was to determine maximum yield and farming potential for this area. This study focuses on planting density, harvesting techniques, and comparing yields and quality with the objective to transfer this technology and information to emerging farmers. The results were obtained from trials in Mpumlanga, at the Lowveld College of Agriculture. Higher plant densities than the prescribed South African norm of 15 000 plants per ha, and harvesting methods were investigated. Chemical analysis of different harvests, and different distillation units were compared. It is put into a collective data base and can be seen as a sign of the growing industry of essential oils in South Africa by commercial and emerging farmers. Comparisons were made with producers from other countries.

In conclusion: Improved agricultural practices and the known chemical analysis could make a difference in the profitability of geranium as a crop in South Africa. The success and experience of producers who have access to chemical analysis and relating data proved successful and formed part of their marketing strategy. The Essential Oil and Medicinal Plants Industry in South Africa need to get chemical information on order to have a good idea of expected yields, quality, market requirements and income per ha per year. In order to get the products up to a world standard and sustainable quantity, the chemical database of geranium oils in South Africa has to be coordinated and all information made available to new entrants.

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LECTURES

Young scientist workshop

Novel insights into the structures and formation pathways of bitter-tasting terpenoid degradation products generated during storage of beer

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Although the typical taste of beer is widely appreciated by consumers all over the world, the typical bitterness of fresh beer is well-known to slightly decrease in intensity and to change in quality with increasing age of the beer. During the wortboiling process the isomerization of α-acids from hops (*humulus lupulus*) leads to the formation of *trans*- and *cis*-iso-α-acids which are mainly responsible for the bitter taste of beer. Multiple studies demonstrated that during aging of beer the amount of *trans*-iso-α-acids decreases, whereas the *cis*-iso-α-acids seem to be rather stable. Based on this observation, the *trans*/*cis*-ratio was proposed as an aging indicator for beer. Although the decrease of the amounts of *trans*-iso-α-acids upon storage of beer, for example in cans, takes place under quasi oxygen-free conditions, the degradation of these bitter compounds is considered to be an oxidative process. As scientific data on these degradation are lacking in literature, the objective of the present study was to elucidate the molecular mechanisms involved in storage-induced *trans*-iso-α-acid degradation and formation of harsh and unpleasant bitter compounds. Aqueous model solutions of purified *trans*-iso-α-acids, isolated from a commercially available iso-α-acids product, were incubated under forced storage conditions, and the reaction products formed were isolated by means of preparative HPLC. For example, storage of the *trans*-*isocohumulone* solution resulted in the formation of five major reaction products, the structure of which were determined by means of sophisticated 1D/2D-NMR as well as LC-MS/MS experiments. Based on the chemical structures of these previously not reported degradation products and guided by stable isotope labeling experiments, a novel conclusive mechanism was proposed explaining why and how the trans-α-acids are degraded in a non-oxidative way whereas the *cis*-*iso*-α-acids stay rather stable during beer storage. By means of HPLC-MS/MS operated in the multiple reaction monitoring mode, all these previously not reported compounds were identified and quantified in various beer samples and were demonstrated as the major beer aging indicators explaining almost the complete loss of *trans*-iso-α-acids in aged beer samples. Furthermore, human sensory studies performed with the purified compounds revealed a more harsh bitter quality of some of these degradation products as well as somewhat higher bitter recognition thresholds when compared to the value the trans-iso-α-acids, thus being well in line with the slight bitterness decrease as well as the change in bitter quality during long-term storage of beer.

Are Odorants Psychopharmacological Agents? – Aspects of Fragrance Effects on Human Attention

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Odorants are ubiquitous in our environment, our food and also in many cosmetic products we use. In addition, many medicinal plants contain volatile molecules that are the active principle of their therapeutic properties. Nevertheless, our knowledge of these substances and their influence on the human organism is still very limited. In particular, scientific research on the effects of odorants on human attention, physiological processes, and ratings of mood and arousal has yielded rather heterogeneous results. In addition, findings on the modulation of physiological and behavioural arousal levels after inhalation and transdermal absorption of substances are very often contradictory For instance, the monoterpenes 1,8-cineole (the major component of Eucalyptus essential oil) and linalool (a main compound of Lavender essential oil) showed no effects on the performance of healthy human subjects in a standard sustained attention (vigilance) task or on physiological parameters after inhalation, whereas both fragrances changed evaluation of subjective well-being. On the other hand, after percutaneous absorption without olfactory stimulation effects on both vigilance and autonomic nervous system parameters were detected, but no influence on self-evaluation was determined [1, 2].
The problem of understanding the nature of attention seemed intractable only a few years ago, but developments in neuroimaging and cognitive psychology now allow us to provide specific anatomical and cognitive details about the attention systems of the human brain. When neurons are active, they change their own local blood supply and this process can be monitored by means of magnetic resonance imaging. This method, called functional magnetic resonance imaging (fMRI), depends on the BOLD (Blood oxygen level dependent) signal and is a non invasive method which makes it possible to track areas of the brain involved in cognitive processing by detecting changes in regional cerebral blood flow (rCBF).
In our study, we investigated the effects of 1,8-cineole and (–)-linalool after transdermal (20% solution in peanut oil) and inhalative (10% solution in propylene glycol) application by using fMRI. The two fragrances and corresponding placebo formulations were tested in 69 young healthy volunteers which were randomly divided into four groups. To prevent any olfactory stimulation in the transdermal groups odourless air was provided via breathing masks. In the inhalation groups, the odorants were administered by means of these breathing masks. In addition, both physiological parameters and the influence on self-evaluation were determined.
Preliminary results indicate a significant effect of 1,8-cineole on the central nervous system. Furthermore different activation patterns as a function of both administration mode and sex were observed.

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New analogues of sandalwood odorants – synthesis and olfactory characterization

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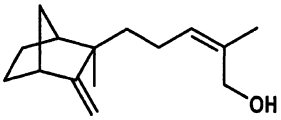
Sandalwood essential oil is an inescapable precious ingredient in perfume formulations. α- and especially β-santalol (1), the main odoriferous constituents, are responsible of its characteristic warm, milky, spicy and woody note. Nonetheless, many structurally different synthetic molecules possess the famous "sandalwood like" smell [1], among which a lot of compounds like Polysantol® are prepared from (*R*)- and (*S*)-campholenal (2). In the course of our studies in the field of structure-odour relationships based on previous works on sandalwood olfactophores [2,3,4], we synthesised a Polysantol® analogue (3) showing an interesting sandalwood note from trans-α-necrodyl acetate, the main component of *Lavandula luisieri* essential oil. We also prepared some new santalane analogues by hemisynthesis starting from pure natural (-)-β-santalol isolated from Indian sandalwood essential oil.



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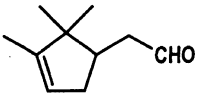

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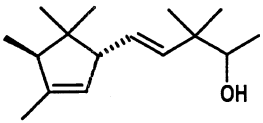

3

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Beyond Odour Activity Values: Dynamic Aspects of Aroma Release and Perception

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Historically, the odour activity value concept and the idea of aroma extract dilution analysis (AEDA) present a milestone in aroma analysis by introducing a measure of relevance rather than just concentration of aroma compounds in different foodstuffs [1-3]. Carrying the idea of relevance forward, aroma reconstitution experiments have always shown that additionally to correct concentrations of aroma compounds, an adequate simulation of the food matrix is crucial for acchieving an aroma impression that is most similar to its template. For example Kirchhoff et al. compared models based on citric acid, corn starch and desodorized rye bread crumb for simulation of sourdough rye bread aroma [4], whereas Jagella et al. applied water, starch and paper strips as model matrices for aroma reconstitution of pepper [5]. Furthermore, Buettner et al. have shown that the addition of 0.1% fat brings an orange juice recombinate closer to the original by reducing its terpene-like aroma impression while its fruity aroma is enhanced [6]. Consistent with these findings, current studies show that the detection threshold of the terpenoid compound limonene increase by a factor of 70 when reverting from aqueous to solutions in sunflower oil, while 2-methylbutanoic acid and diacetyl show lower detection thresholds in oily solutions. Numerous investigations on flavour-matrix interactions confirmed complex effects on aroma release and perception. For example, it was shown that higher fat contents lead to retention of lipophilic compounds in the food matrix [7-9], that proteins can form specific protein-ligand complexes with individual aroma compounds [10,11] and polysaccharides have an impact on aroma retention due to gelation as well as - in the case of starch - specific helical inclusion complexes [11-13].
Apart from the composition of the food matrix itself, dynamic flavour release and perception under oral conditions are investigated by different approaches, one of which is the simulation of the chewing and swallowing process by artificial mouth systems with increasing sophistication [14-18]. Other approaches quantify aroma compounds in the exhaled breath of panelists (EXOM) [19] or determine the persistence of aroma compounds in the oral cavity by a modified stir-bar sorptive extraction system (BOSS) [20].
Methods of choice for real-time analysis of flavour release are Atmospheric-Pressure Chemical Ionisation - Mass Spectrometry (API-MS) and Proton Transfer Reaction - Mass Spectrometry (PTR-MS) [21, 22]. Application of these analytical tools has shown the importance of inter- as well as intrapersonal variation for retronasal aroma release and perception [23, 24]. Through combination with sensory analysis, further insights were gained on dynamics of retronasal aroma release and perceptual phenomena [25-27].

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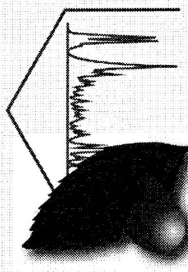

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LECTURES

Analytical methods and active principles

Coupling different retention mechanisms in chromatography: from simple to complex configurations

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For the separation of mixtures of extremely high complexity e.g. natural product research, optimal selectivity and peak capacity are mandatory and, in this respect, comprehensive techniques received much interest in the last years and the chromatographic community seems excited about these developments.
In this presentation, recent developments from simple to complex configurations in GC, LC and SFC will be reviewed with emphasis on hardware design, software approaches and column configurations.
The applicability of the different combinations and configurations will be illustrated and guidelines on "what configuration is preferred for a given sample" will be presented. Fluid-based separations will be highlighted in this contribution and configurations that will be discussed in detail are: simple selectivity optimization by column coupling (HILIC-RPLC and RPLC-HILIC), heart-cutting techniques and the comprehensive techniques NPLC×RPLC, SFC×RPLC, RPLC×RPLC, NPLC×2RPLC and RPLC×2RPLC.
Although the theoretical limits of comprehensive techniques are known, some fundamental questions still remain unanswered. How does the performance of currently available comprehensive techniques compare to that of one-dimensional separations taking into account that nowadays peak capacities as high as 1,000 can be reached in 1-D LC and SFC and what is required in instrumentation and approach for comprehensive techniques to reach their ultimate potential? To help start answering these questions, high resolution 1-D separations under optimal conditions will be compared to comprehensive separations under typically employed conditions. Applications from natural product research will be shown.

Acknowledgements (italic): Institution 1, Institution 2, Person 1. (Arial Narrow/10 pt/normal/single line spacing).
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Near infrared spectroscopy analysis of essential oils produced from indigenous South African aromatic plants

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Essential oils are distilled from various South African aromatic plants, renowned for their application to treat a wide range of ailments and their potential as flavour enhancers. Buchu (*Agothosma betulina*) which grows in the Western Cape region is mainly used in the flavour industry to enhance fruit flavours such as black currant [1]. Two other aromatic plants of increasing popularity are Cape chamomile (*Eriocephalus punctulatus*) found in the north-east slopes of the Drakensberg in the Freestate province [2] and sage (*Salvia stenophylla*) growing in the high altitude areas of the central and eastern part of South Africa [3].

As oils from these plants are increasingly being processed into commercial herbal products, quality control for optimisation of compounds and confirmation of authenticity of the correct species being used is becoming essential. Within a processing environment one of the requirements is rapid analysis of raw materials and the final product. A technique being used with great success in the food and agricultural industries is near infrared (NIR) spectroscopy. This technique correlates spectral data (being collected within seconds) with chemical data, or other known information of the sample e.g. species type, where after the developed calibrations can be used to predict content of a specific compound or species type. It has been shown that NIR spectroscopy can quantify diosphenol, pseudo-diosphenol, limonene, menthone, iso-menthone, pulegone and iso-pulegone in buchu oil accurately [4].

In this study NIR spectra of the hydrodistilled oils of 40 Cape chamomile (*E. punctulatus* and *E. tenuifolius*) and 32 *Salvia* species (*S. stenophylla, S. repens and S runcinata*) were recorded on a Büchi NIRFlex N-500 Fourier transform near infrared (FT-NIR) spectrophotometer (Büchi Labortechnik AG, Flawil, Switzerland) with the NIRWare Operator software programme (v1.0). Transmittance spectra were recorded from 10 000-4000 cm^{-1} at a resolution of 16 cm^{-1}. Spectra were measured in a quartz Suprasil cuvette (Hellma, Müllheim, Germany) with a path-length of 0.2 mm. A background spectrum of air was taken before each sample and the quartz cuvette was cleaned with acetone and air-dried between analysing samples. In addition spectra were collected from dried plant material samples of *Salvia* in glass chromacol vials in reflectance mode. The α-bisabolol content in the *Salvia* oils was quantified using GC-MS. Data analysis was performed using The Unscrambler® (v9.2) software.

Using principal component analysis (PCA) oil distilled from Cape chamomile species (*E. punctulatus* and *E. teniofolius*) could be distinctly classified. In addition, it was observed that commercial Cape chamomile, until now being believed to be *E. punctulatus*, was classified as belonging to *E. tenuifolius*. Partial least square (PLS) regression models were developed from the *Salvia* NIR spectral and GC-MS reference data. In spite of the small data set it was possible to predict the α-bisabolol content in *Salvia* oil accurately with a standard error of prediction (SEP) of 6.97% and R^2 of 0.96. After deleting spectral outliers it was possible to predict the α-bisabolol content from the dried plant material with a reduced accuracy (SEP = 13.87%; R^2 = 0.85), although with great potential.

Although NIR spectroscopy is not used in the herbal industry as yet, together with other spectroscopic techniques it has the potential to make a huge contribution to efficient in-process quality control.

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Volatile Organic Nitrogen-Containing Constituents in Ambrette Seeds *Abelmoschus moschatus* Medik (Malvaceae)

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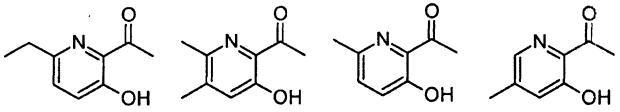
Volatile nitrogen compounds occur in low concentration in natural isolates, but some contribute significantly to the sensory properties of natural extracts [1-2]. The essential oil obtained from seed of *Abelmoschus moschatus* Medik, commonly known as ambrette, is a very expensive raw material, noted for its rich, sweet floral-musky, distinctly wine-like or brandy like odour which finds application both in flavour and fragrance formulations [3]. Despite a long history use of ambrette seeds oil in perfumery, there are few published studies about the constituents in the oil and nothing about volatile nitrogen compounds [4-6]. A detailed investigation of basic fraction of a CO_2 extract of ambrette seeds revealed a total of 58 nitrogen-containing compounds. The identification of these compounds was carried out on the basis of their mass spectral (MS) characteristics, GC/MS retention indices (RI) or NMR. All the identified nitrogen-containing compounds are reported here for the first time in ambrette seeds. Among these are 27 pyrazine derivatives and 12 pyridines, including four new natural compounds, 1-(6-ethyl-3-hydroxypyridine-2-yl) ethanone (**1**), 1-(3-hydroxy-5,6-dimethylpyridine-2-yl) ethanone (**2**), 1-(3-hydroxy-6-methylpyridine-2-yl) ethanone (**3**), 1-(3-hydroxy-5-methylpyridine-2-yl) ethanone (**4**). The odour of the basic fraction was assumed to be due to these pyrazines and pyridines and also the presence of seven thiazoles. The odours described suggest that these nitrogen-containing compounds contribute to what is described in perfumery terms as the 'natural and rounded' character of the ambrette extract.



1 **2** **3** **4**

New compounds **1-4** in ambrette seeds extract

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Further investigations of anticancer and antiviral properties of selected aroma samples

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Anti-tumor and antiviral activities of various aroma samples, e.g. synthetic and natural nerolidol, *trans,trans*-farnesol, farnesol (isomer mixture) and a farnesol-rich ylang-fraction, were investigated on HeLa and Jurkat cell lines in previous studies [1].
Based on these results, further oxygenated sesquiterpenes, *cis*- and *trans*-nerolidol, α-bisabolol and patchoulol, were tested for their anti-tumor effects using CytoTox-96R-Assay on HeLa and Jurkat cell lines. It was observed that an effective compound concentration required for a reduction of the number of the tumor cells by 50% (CC50) was 2.2-5μM for *trans*-nerolidol and 5-10μM for *cis*-nerolidol. Patchoulol and α-bisabolol possessed the same activity in concentrations of 10-20μM and 5-10μM, respectively.
The antiviral studies were performed to investigate inhibitory effects of these four samples on the mouse polyomavirus propagation in 3T6 cells. Our preliminary results show that both *cis*- and *trans*-nerolidol are active against mouse polyomavirus propagation in 3T6 cells in concentrations below the cytotoxic limit.

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Application of ATR-IR and Raman methods for analytical characterisation of aromatic plants

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In comparison to near-infrared reflectance spectroscopy, which has been used extensively for analytical characterisation of various components in aromatic plants, the use of ATR-IR and FT-Raman spectroscopy for the same purposes has been more limited. Until recently, both spectroscopic techniques were mainly used in academic research as a qualitative technique for identification and verification of unknown pure substances. Usually vibrational spectra obtained from plant samples are very complex because each functional group in a molecule contributes more or less to the spectral output. However several plant components can be successfully detected due to their predominant key bands.

Spectroscopy combined with microscopy can provide molecular information with a high spatial resolution at the cellular level. Raman spectra can be obtained in a single point or by mapping either along a line (1D) or in a specified area (2D). The results can be presented as a spectroscopic map and directly compared to the corresponding visual image of the investigated sample. Raman maps provide additionally detailed information regarding the distribution of the analyzed molecules occurring in a surface layer of the plant sample.

ATR-IR and FT-Raman spectroscopes were applied to a vast analyses of various aromatic plants. These methods were particularly informative in detection of essential oils. Other components such as carotenoids, fatty acids, polyacetylenes, polysaccharides etc. can be simultaneously observed and compared.

Vibrational spectroscopy appears to be a fast, safe and non-destructive method. Both dried and fresh samples can be analyzed at ambient temperature without any need for pre-processing.

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Characterisation of volatile metabolites in a gene bank collection of parsley by a non-targeted analysis approach

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In special cases of plant metabolite analysis like biodiversity studies, metabolite profiling or plant breeding it is of special advantage to use a so-called non-biased or non-targeted analysis approach because during the mentioned processes unexpected changes of the plant volatile patterns may occur. Using traditional strategies of targeted analyses which include the steps of separation, compound identification and creation of calibration tables the possible metabolic differences may then be overlooked. Non-targeted analysis strategies were developed to overcome these limitations and make the metabolic data analysis unbiased [1, 2]. A rapid method of plant volatile analysis was developed and applied as tool to study the metabolite diversity in a parsley collection as well as in strawberry, apple and carrot [3, 4].
A collection of 220 accessions mostly provided by the German Gene Bank of the Institute of Plant Genetics and Crop Plant Research in Gatersleben (IPK), Germany was cultivated in field. The patterns of volatile metabolites were determined effectively by a rapid, non-targeted analysis approach in leaf homogenates. The used method is a combination of an effective sample preparation and a non-targeted data processing. It consists of automated headspace solid phase microextraction (HS-SPME), gas chromatography (with FID and MS detector) and data processing by pattern recognition. This technique fulfills the requirements for rapid analysis of hundreds of samples including also those of small sample size. By this way in principle the area of all peaks (above a certain threshold) of a chromatogramme set are detectable. Overlooking of unexspected substances caused by a high diversity of the volatile metabolites is prevented using this non-targeted method.
First results show that the investigated accessions belong to two different basic chemotypes. The two types differ mainly in metabolites deriving from the lipoxigenase pathway, apiol and minor compounds like β-myrcene, p-cymene, allocimene and γ-terpinene. The metabolomic data are in total accordance with molecular marker data.

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Influence of PAR and UV-B radiation on quality and quantity of monoterpenoid essential oil from peppermint

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Plant secondary metabolism is characterized by an enormous variety of products and a high plasticity concerning regulation of pathways. A well-known example of differential regulation is the isoprenoid family: According to the requirement for a certain substance, it is synthesized constitutively in specific organs or at specific stages of development or its synthesis is induced by exogenous elicitors, e.g. herbivores or pathogenes. Monoterpenes synthesized in the glandular trichomes of peppermint, for example, are commonly regarded as constitutive barrier against herbivore or pathogene attack but also respond to changing climatic conditions like global radiation and temperature [1]. Our work focuses on the role of photosynthetically active radiation (PAR, 400 - 700 nm) and ultraviolet-B radiation (UV-B, 280 – 315 nm) for quality and quantity of monoterpenoid essential oil from peppermint (*Mentha* x *piperita* L.).

Plants of the variety 'BLBP 02' were grown in sun simulators (Helmholtz Zentrum München) providing exposure values very close to natural sun light. In order to study the influence of global radiation, twelve different regimes were simulated differing in PAR (approx. 250, 500, 750 and 1000 $\mu mol*m^{-2}*s^{-1}$) as well as in UV-B radiation (approx. 0, 0.3, 0.6 and 1.2 $W*m^{-2}$). Plants were sampled in four different stages of development: during vegetative phase, bud formation, emerging flower and flowering. Essential oil was extracted from fresh leaves by steam distillation and analyzed by gas chromatography. Additionally enzyme activity was determined using a specific terpene synthase assay.

Data obtained from the current study are supposed to give precise information on the relationship between monoterpene content and PAR and UV-B radiation, respectively ("action spectrum") and provide an insight into the role of PAR/UV-B ratio. Monoterpene content is expected to correlate with the activity of the corresponding enzymes.

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Controlled release study of encapsulated fragrance materials in detergent formulation

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Fragrance is not a single material of clearly defined properties, but rather a mixture of individual chemicals, each behaving according to its own unique attributes, characterizing these chemicals seperately & then combining their effects allows the behaviour of the complete fragrance composition in diverse media to be understood[1].

Detergents act like soap but, unlike soaps, they are derived from organic acids rather than fatty acids[2]. Microencapsulation is a process where droplets of liquids, solids, or gases (core) are coated by thin films (coatings), which protect the core until it is needed. Microencapsulation and controlled release of flavors and fragrances have revolutionized the food and fragrance industries, constantly interested in improving the flavour, aroma, stability, nutritive value, and appearance of their products[3].

The detergent powder was prepared and comparison study of encapsulated fragrance materials and as such fragrance materials in detergent powder formulation were carried out. For this study fragrance materials of different classes such as rosy, musky, woody, jasmine, orange, muguet, fruity and sandal were studied for properties like hedonic scale rating, substantivity scale rating and intensity. As the temperature varies from one area to another one, the temperature effect on the chemical reactivity also must be considered. And therefore the samples were kept at different temperatures (at 48°C in oven and at ambient temperatures) for the lab studies. The substantivity of fragrance material incorporated in detergent formulation was also studied on cotton fabrics. During study it was found that most of the fragrant materials that were tested at ambient temperature as well as 48°C show good stability, whereas few of chemicals were found to be unstable and gave off odor during storage under ambient conditions and 48°C temperature. The results indicate the advantages of encapsulated fragrance materials in detergent powder formulations.

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Innovative approaches for the analysis of essential oils (Fast-GC, MDGC, GCxGC, LCxLC)

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Essential oils are mainly used in food and perfume industries, while several isolated components are also employed for their pharmacological and anti-microbial properties and they can be classified as moderately to highly complex samples. It is well known that analytical time costs may be very high in standard GC applications. In the last few years, fast GC methods have been shown to be suitable for routine laboratory work, in this respect, the employment of narrow bore columns is of particular interest. These analytical tools enable a considerable reduction of analyses times while peak resolution is essentially maintained. Nevertheless, a single capillary GC column often proves to be insufficient for complete separation of all the compounds of interest, and therefore there has always been a strong interest in the research of more powerful separation methods. To overcome this problem, many advanced analytical solutions have been studied concerning multidimensional (MDGC), comprehensive GC x GC and LC x LC approaches. These chromatographic techniques can be considered innovative methods, only quite recently developed. Since their introduction to the chromatographic community, these techniques have been used in several fields and have gained an excellent reputation as valuable and powerful analytical tools.
The development of a series of methods has allowed not only the characterization of several essential oils but also, as a consequence, accurate judgements on genuineness, geographic origin, possible contamination and adulteration. These aspects regard not only essential oil analysis but also a vast amount of matrices in different fields.
The present contribution is focussed on the most advanced monodimensional (micro-bore column GC) and multidimensional chromatographic techniques (comprehensive GC, comprehensive LC, heart-cutting multidimensional GC) today employed in essential oil analysis. A series of applications on different essential oil samples will be described in order to demonstrate the effectiveness of these approaches.

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Biogenesis and identification of selected substances

Metabolic regulation of essential oil formation in the Lamiaceae

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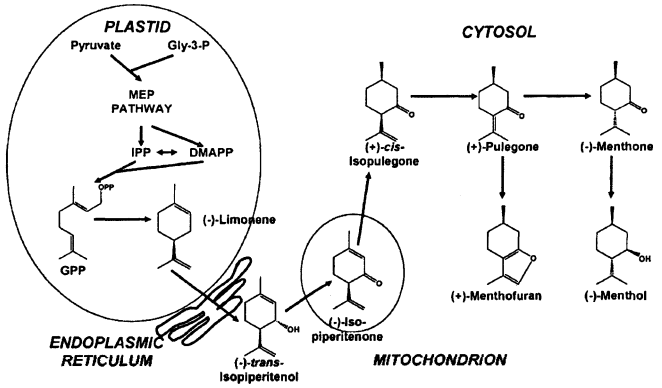
A few of the commercially-important essential oil-producing species of the Lamiaceae have been studied intensively in recent years to learn more about the formation of essential oil constituents. As a result, we now know a great deal about the pathways of monoterpene biosynthesis and the identities of most of the enzymes and genes involved. However, to manipulate the yield and composition of essential oil we need to learn more about when and where monoterpenes are formed and which steps of the pathway exert most regulatory control over biosynthesis.

I will begin with an overview of the cellular and subcellular compartmentation of monoterpene biosynthesis in the Lamiaceae and what is known about its timing with respect to tissue development. Next, I will describe our current understanding of monoterpene pathway regulation at the level of specific enzymes and report on the success of efforts to genetically transform Lamiaceae species with genes encoding monoterpene biosynthetic enzymes. Finally, I will consider possible future directions to alter essential oil biosynthesis. Whether or not genetically-altered plants are employed, increased knowledge of the molecular regulation of essential oil formation can help improve all breeding efforts directed at improving the yield and composition of Lamiaceae essential oil.

SUBCELLULAR LOCALIZATION OF MONOTERPENE BIOSYNTHESIS IN PEPPERMINT SECRETORY CELLS



Distribution of volatile bibenzyls and tocopherols in liverworts and pungent medicinal plants

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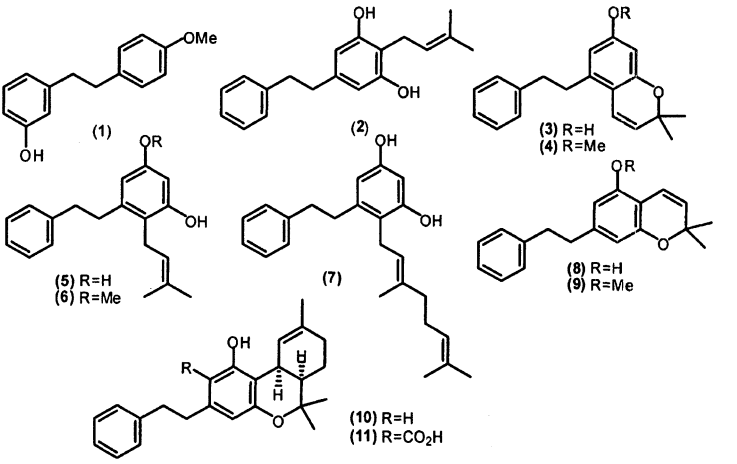
Liverworts contain cellular oil bodies which are composed of lipophilic terpenoids, phenolics and acetogenins. The *Radula* is one of the isolated liverwort groups chemically since this genus produces mainly not only simple bibenzyls, such as 3-methoxybibenzyl and 3-hydroxy-4'-methoxybibenzyl (1), but also various prenyl bibenzyls [1-4]. Here we wish to report the distribution of bibenzyls in *Radula* species, and tocopherols in the selected liverworts and pungent medicinal plants.

1)R. buccinifera (New Zealand), *R. bryana* (Germany), *B. brunnea* (Ehime, Japan), *R. campanigera* (Kagoshima, JPN), *R. chinensis* (Okayama, JPN), *R. grandis* (NZ), *R. javanica* (Kagoshima , JPN), *R. tokiensis* (Tokushima, JPN), *R. kojana* (Wakayama and Mie, JPN), *R. lindenbergina* (Saudi Arabia), *R. obtusiloba* (Gunma and Ehime, JPN), *R. okamurana* (Mie, JPN), *R. oyamensis* (Mie, JPN), *R. emarginata* (NZ), *R. uvifera* (Peru), and two unidentified Peruvian *Radula* species. The most popular bibenzyls in *Radula* species is 2,2-dimethylallyl-3,5-dihydroxybibenzyl (**5**) and 2-geranylbibienyl (**7**). Bibenzyl cannabinoids (**10,11**) are very characteristic compounds in a few Japanese and New Zealand *Radula* species.

2) Among 726 liverwort species collected in the world, 266 species (36.3%) produce α-tocopherol. Particularly, 65% of *Pollera* and *Pellia* species containing the pungent sesqui- or diterpene dialdehyde elaborate α-tocopherol. On the other hand, pungent medicinal plants, *Polygonum* hydropiper, (Polygonaceae) and *Cinnamosma fragrans and C. macrocarpa* (Cannelaceae) produce α-tocopherol and tocotrienol, respectively. These tocopherols may play important role as antioxidants against dialdehydes, the other highly saturated terpenoids and the other lipids.



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Labelling with $^{13}CO_2$ reveals differences in the stored and recently synthesised fractions of terpenoids within *Eucalyptus globulus* leaves.

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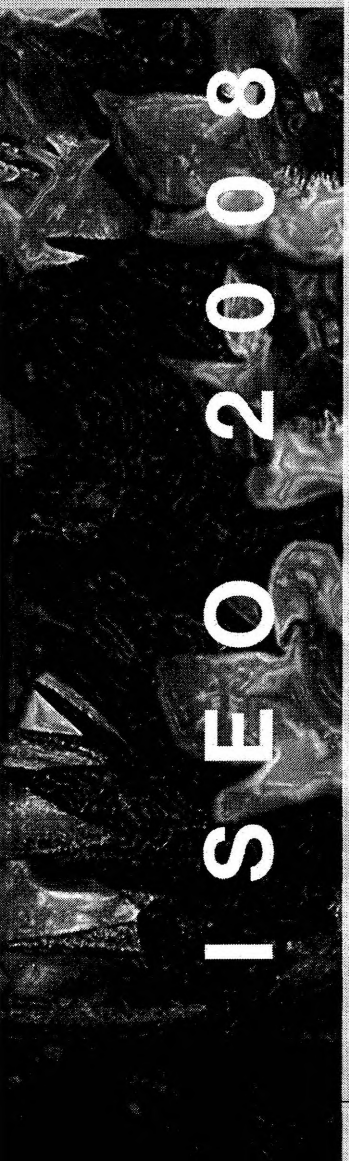
Leaves of most species of *Eucalyptus* possess terpenoid-filled subdermal oil glands, which are believed to constitute the sole source of terpenoid (C10 and greater) emissions from these leaves. However, we have observed differences in the emission rate of some compounds between light and dark at constant temperature; observations that have been obscured by the large pools of oils within leaves. We sought to identify the source(s) of volatile organic compound emissions from one year old leaves of *Eucalyptus globulus* subsp. *globulus* (Tasmanian blue gum) by fumigating leaves with air containing 99% labelled $^{13}CO_2$. Incorporation of the heavy carbon isotope was determined by monitoring volatilised compounds with proton transfer reaction-mass spectrometry (PTR-MS) and sorbent cartridges analysed by GC-MS. In addition, terpenoids stored within individual oil glands as well those dispersed throughout the remaining leaf tissue were also tested for incorporation of recently assimilated carbon. Of the many mono- and sequiterpenoids contained within, and emitted by, eucalyptus leaves, only the two isomers of ocimene (C10) and trans-caryophyllene (C15) incorporated recently assimilated carbon. This uptake of ^{13}C was only detected in compounds emitted from the leaf. Analysis of monoterpene synthases from these trees showed activity only in the enzyme responsible for ocimene production. The rate and extent of ^{13}C incorporation was greatest in isoprene, decreasing further in ocimene and lowest in trans-caryophyllene. These results indicate a shared, but not single, source of carbon for these compounds. While sharing recently fixed carbon, they do not exclude the possibility of transfer of this carbon source from the plastid to the cytosol. The results also suggest that biosynthesis of the majority of terpenoids is inactive for part of the leaf lifespan in this species, although these putative periods of activity remain to be determined.



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LECTURES

Commercial utilization

Encapsulation and other programmed release techniques for essential oils and volatile terpenes

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In drug delivery research microparticles,nanoparticles,cyclodextrin complexation,micelles,covalent bonding to polymeric matrices have been used to produce medical products with preset properties of an active ingredient. In the applications of essential oils, terpenes and other volatiles these techniques may give rise to a wide variety of products. Even if there have been reports on products using these methods to change the volatility of terpenes, relatively few research papers are seen covering this area. The volatility of our essential oils and terpenes make these compounds very useful for many products if combined with excipients and matrices carefully chosen.

The industrial applications of flavours, essential oils and volatiles requires, among other parameters, control of the volatility or release of the volatile components. This can be done by microencapsulation, nanoencapsulation, complexation etc, etc,..to facilitate the introduction into toothpaste, chewing gum, plastics, films, on skin, on textiles to mention a few applications. The encapsulation can also be a solution to stability problems of the volatile compounds.

However, just like to introduction of nanoparticles into human medicine, the use of nanoparticles and volatiles will give us particles which can cross biological barriers and give rise to toxicity reactions. Encapsulated compounds into a nanoparticle may give the volatile other physiological activities than the pure compound itself.

In ISEO we have been discussing mainly separation techniques, structure analysis of terpenes, separation of compounds in essential oils, biochemistry of terpenes – all of these areas of great interest to us. It is my opinion that we should turn our attention towards the applications of the volatiles. In this way we can make use of our long-standing experience in the terpene field to new active research areas.

In this lecture I will use examples from my own field of formulation research to illustrate how the combination of different research fields can lead to very interesting results and new industrial applications. The use of various types of micro/nano particles (1-1000 nm) will be the main topic.

Plant extracts to modify insect behaviour in stored product protection

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Traditional subsistence farmers in a number of African and Asian countries utilize leaves and other plant parts of aromatic plants to protect their harvest against stored product pests (1,2). Laboratory studies showed that many of these plants had repellent and/or toxic effects on stored product insects. In tests with pure compounds a marked toxic action of cineol, eugenol (3), anisole, and cinnamaldehyde (4) against certain species could be shown. However, only pyrethrins are authorized for stored product protection in Germany at present. This may be in part due to the fact that repellent or toxic compounds are difficult to purify or synthesize, often have a rather short shelf-life, a bitter or unpleasant taste if applied onto food, and are potentially hazardous to man, as well.
On the other hand, one of the key characteristics of stored product insects, crucial for their reproductive success and ecological function, is their capability of detecting and orientating towards gradients of food odours. In order to avoid an infestation, it would be important to know the chemical compounds most attractive to important stored product pest species. First trials with food grade aromas showed a high attractiveness of apple aroma to the Indianmeal moth *Plodia interpunctella* (5). It would be important to identify the attractive volatiles in goods such as wheat bran, broken almonds, and dried fruits. Such compounds may be optimised and utilised to lure pest insects into traps. The attractiveness to female moths, responsible for proliferation, would be a significant improvement to the sex pheromone traps used at present that are attractive to male moths only. Food lure traps on the basis of liquid volatile dispensers may attract all pest insect species of a given product without allowing reproduction. Furthermore, filter systems could be developed to remove attractive odours from the air around food and feed producers or chemical agents may be used to transform or cover attractive odours.

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GC/MS determination of IFRA-Restricted Substances in Fine Fragrances, Cosmetics and Household Products

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A GC/MS method has been developed for the determination of 70 substances that are restricted in fragrances by the self-regulatory policy of IFRA. The procedure was validated for most of them in six different kinds of consumer products: fine fragrances, washing powders, shampoos, deodorants, air fresheners and cosmetic creams.

For the validation step, each blank matrix was spiked at two different concentration levels (5 and 50 ppm) with a mixture of IFRA-restricted substances. After extraction of the fragrance constituents from the matrix, each sample was injected into a non-polar column using GC/MS under SIM conditions (three ions recorded for each substance: one quantification ion and two qualification ions). When co-elution occurred, all ions were taken into account for the quantification and a second column with a polar phase was used. The linearity, specificity, accuracy and precision of data were determined in the different matrices.

Two campaigns have been run to monitor the possible occurrence of these IFRA restricted substances in cosmetics and household products from ten different countries. Commercial samples representative of fine fragrances, washing powders, shampoos, eaux de toilette, fabric conditioners, liquid detergents and cosmetic creams were selected randomly from a list of top market products. This list was based on information received from selected countries covered by IFRA member associations.
Down to the limit of 100 ppm recommended by IFRA, none of the restricted substances were detected.

Authentication of Essential Oils and Aroma Extracts

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Essential oils and aroma extracts are commonly defined as complex mixtures of flavor and fragrance substances originating from plants.
The authenticity of natural compounds is an important topic for the flavor and fragrance industry because of regulatory and commercial aspects. The analytical authentication is a permanent challenge due to the complexity of the matrices.
The analysis of enantiomeric purity by enantioselective multidimensional gas chromatography is well-known as an efficient tool for the authenticity control of chiral compounds.
Over the last 15 to 20 years stable isotope ratio analysis became the most important tool for authenticity testing of non-chiral compounds. This analysis can be performed by isotope ratio mass spectrometry (IRMS) or by site-specific natural isotope fractionation nuclear magnetic resonance spectroscopy (SNIF-NMR®). The latter method is based on the fact that the deuterium content at specific positions of the molecule under scrutiny is different.
Mass spectrometers which allow the determination of isotope ratios of $^{2}H/^{1}H$, $^{13}C/^{12}C$ and $^{18}O/^{16}O$ and are coupled to gas chromatographs are sometimes the only tool available to assess the authenticity of non-chiral ingredients of essential oils or aroma extracts.

It is of great interest to check whether it is possible to detect the blending with synthetic substances or with isolates from origins other than from the named plant.
Examples are presented for the use of the different methods for authentication [1] of oregano oil (carvacrol) [2], laurel leaf oil (α- terpinyl acetate), sandalwood oil (cis-α-santalol, cis-lanceol), garlic oil, benzaldehyde, and vanillin.
The presentation demonstrates the merits of enantioselective capillary GC, of isotope ratio mass spectrometry (IRMS) coupled on-line with capillary GC, and of SNIF-NMR for the authentication process but it also illustrates that conventional GC/MS analysis still has its importance.

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Intensification of the extraction phenomena

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Traditional process for extracting essential oils, more especially steam distillation of plants, is usually very long time operation, which implies decreasing of quality, increasing energy consumption... A fundamental analysis of transfer phenomena intervening during such processes allows researchers to identify external and internal, heat and mass transfers. Heat transfer assuring the energy for vaporizing essential oils intervenes by convection (from outside towards surface), and by conduction (from the surface towards the granule heart). Essential oils must diffuse inside of the granule and be removed by external diffusion and/or convection.
The slowest phenomenon, which must control and define the whole kinetics, generally is the mass diffusion of essential oil vapour from inside towards the surface of the granule. By swelling the natural plant structure, increasing both porosity and specific surface area, it would be possible to highly intensify the extraction process. Our research team has already realized experiments confirming this observation in the cases of various plants.
However, the main process which highly lows down the operation is correlated to the "AL-HADDAD paradox" (1): the presence of a temperature gradient inside of the granule from the surface toward the heart must induce a similar essential oil partially pressure gradient; such a situation implies transferring vapour from the surface towards the heart of the granule reducing the whole kinetics of extraction. Thus, for intensifying the extraction of concerned compounds, one has to create an opposite partially pressure gradient. Two solutions were proposed:

- heating by microwaves, establishing temperature gradient going from inside towards surface of granule,
- extraction by successive DIC processes.

This second solution allows the mass transfer to be realized by permeation which is controlled by the gradient of total pressure. Experiments we already carried out allowed us to get a complete extraction process in some minutes.

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Acid hydrolysis/catalysis (AHC) in essential oil production by hydro/steam distillation: improvements in yield and chemical composition analyses and change in biological activities

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Plant essential oils are obtained by various methods, including steam distillation, and used for many purposes especially in aromatherapy and cosmetics. Steam distillation, generally followed by organic solvent extraction, is widely used in both scientific investigations and industrial processes.
We here report an improvement in steam distillation method by acidifying the aqueous medium. Under the proposed acidic conditions, oligomerik and glycosidic structures are hydrolized to their smaller building blocks. In addition, some of the components are transformed into new compounds by acid catalytic mechanisms.

In order to show the advantages and disadvantages of Acid Hydrolytic/Catalytic (AHC) procedure, the method was applied for the production of essential oils from the fresh samples of *Lamium purpureum*, *Primula vulgaris*, and *Actinidia deliciosa* (kiwi fruit).

The plant samples were frozen in liquid nitrogen, homogenized with a blender, and steam distilled for 4 hours in a Clevenger type apparatus. The experiments were repeated under acidified conditions. Hexane extracts of the essential oils were analyzed with GC-MS method. The yields of the essential oils were also calculated. In addition, the essential oil samples were tested for radical scavenging and antimicrobial activities.

The yields of AHC method were 1.8 to 5.4 times more as compared to the classical method. In addition to the increased yield, the chemical composition was also changed in the way that would improve biological activity. The quantities of many of the compounds observed in the classical method were found to increase. Some of the compounds were shown to be transformed into different compounds probably by acid catalysis. A good example for this is the acid catalytic conversion of carvone to carvacrol. Normally non-volatile oligomeric or glycosidic compounds were hydrolized and became volatile through acid hydrolysis and catalysis.

The biological activity tests revealed that the antioxidant and antimicrobial activities of the essential oils produced by AHC method were generally better than the samples from classical method.

The so-called Acid Hydrolytic/Catalytic (AHC) method has many advantages including higher yield, formation of better biologically active products, reduction in costs, and being an alternative method for chemical compositional analyses especially for biomarker and chemotaxonomic investigations.

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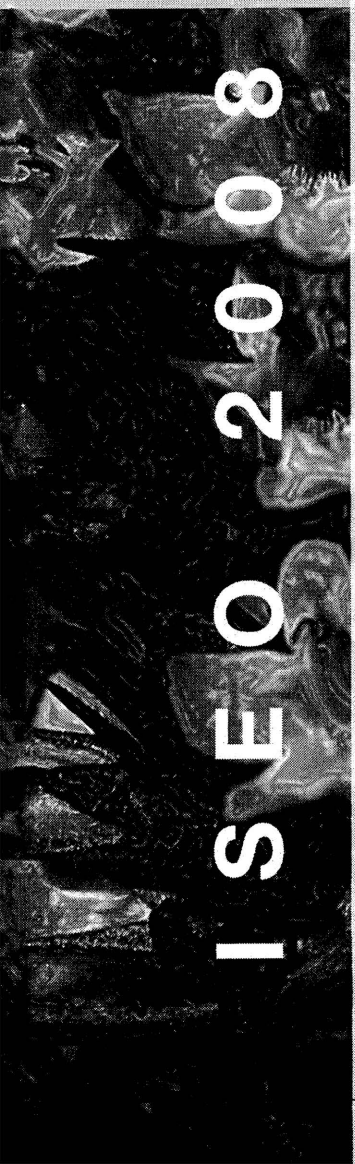

POSTERS

Biodiversity of essential oil plants

A 001

Blood Pressure Lowering Action of Active Principle from *Ocimum basilicum*

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Ocimum basilicum belongs to the family labiatae and commonly has known as Basil (Tulsi). It is a widespread plant cultivated in the world. In Indo-China, the ashes of the roots are suggested as a remedy for skin disease. The plant is used as aromatic, anti-microbial, astringent in dysentery, while the leaves are antipyretic. The seeds are laxative, particularly in case of habitual constipation. The juice of the leaves and flowers are a treatment of cough. A decoction may be given after parturition as emmenagogue and febrifuge. The leaves are carminative, antispasmodic and sedative. Preparations of basil are used fir supportive therapy for feeling of fullness and flatulence, for the stimulation of appetite and digestion, and as diuretic.

In anaesthetized rats, methanolic extract, fractions, and pure compound Eugenol (0.3-3.0 mg/Kg) produced dose-dependent fall in blood pressure and heart rate. These effects were not blocked by atropine (1 mg/Kg) and Eugenol did not modify presser response of norepinephrine which rules out the possibility of cholinergic stimulation or α-adrenergic blockade. In spontaneously beating atria, Eugenol caused decrease in force and rate of contractions. These effects remain unaltered in presence of atropine. In rabbit aorta, Eugenol caused relaxation of norepinephrine and potassium induced contractions in a concentration-dependent manner. These results suggest that the direct relaxant action of Eugenol on myocardium and on blood vessels may be responsible for its hypotensive and bradycardiac effects observed in the *in vivo* studies.

Determination of MIC values and Antimicrobial Activity and Volatile Components from leaves *Mentha longifolia* L. Growing in Iran

Motavalizadeh kakhky Alireza[1], Akhlaghi Hashem[2], Larijani Kambiz[3], Shafaghat Ali[4], Sharifi moghaddam Shohreh[5], Masoudi Shiva[6], Rustaiyan Abdolhossein[3]

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The genus *Mentha* is widespread in Iran and so has been commonly used for along time in folk medicine by the local population. The genus *Mentha*, which belongs to the lamiaceae family, is represented in the flora of Iran by 5 species [1,2]. Here the chemical composition of the essential oils from air-dried leaves of *Mentha longifolia* L. growing wildly in Neyshabur was analyzed by GC and GC/MS. The oils (92.55%) were obtained by hydrodistillation of samples. 14 constituents were identified in this plant. Among them cis-Piperitone Oxide (7.08%), Piperitenone (27.16%) and Piperitenone Oxide (52.48%) were the major components of the oil. The yield of essential oils (w/w %) in dry leaves was 0.85.
Another aim of this research is to evaluate the in vitro antimicrobial properties of the essential oil obtained from leaves *Mentha longifolia* L. .

In vitro antimicrobial activities were determined by using MIC values. Then antimicrobial of this sample was investigated on two strains of microbes: staphylococcus. Epidermidis and Escherichia. Coli. .The antimicrobial test results showed that the essential oil of leaves *Mentha longifolia* L. strongly inhibited the growth of them. The MIC values reported.

***Acknowledgements** : Address for correspondence: Amotavalizadeh@yahoo.com*
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Chemical Composition and Antimicrobial Activity of Essential Oils from leaves of *perovskia abrotanoides* karel

Motavalizadeh kakhky Alireza[1] , *Shafaghat Ali* [2], *Akhlaghi Hashem* [3], *Larijani Kambiz* [4], *Dolatabadi Samaneh*[1], *Masoudi Shiva*[5], *Rustaiyan Abdolhossein*[4]

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Perovskia abrotanoides Karel belongs to labiataes family that has been grown wildly in some regions of Iran. *Pervouskia abrotanoides* is a plant which is also used in folk medicine and vegetation [1,2]. This plant was collected from Neyshabur Mountains of khorasan-e-Razavi province in Iran. The air-dried leaves of *perovskia abrotanoides* were extracted by hydrodistillation, and their chemical composition was determined by GC-MS (in a yield 0.66 % w/w) and 21 components were identified.
The major component is α-pinene (15.97%), camphene (5.04%), δ-3-carene (6.16%), 1, 8-cineole (18.01%) and camphor (32.44%).

The antimicrobial activity was determined by using micro broth dilution and passage agar methods on Geram positive and Gram Negative bacteria. The MIC values reported for essential oil of leaves *perovskia abrotanoides*.

Acknowledgements *: Address for correspondence: Amotavalizadeh@yahoo.com*
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A 004

Volatile Constituents of the Essential oils of flowers from *Hypericum perforatum* L. Grown in Neyshabur area in Iran

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Hypericum genus one of the most important medicinal plants that contain 17 species in Iran, three of them are endemic [1,2,3]. In this study flower of *Hypericum perforatum* L. was collected from Neyshabur in Iran, chemical constituent of essential oils of flowers *Hypericum perforatum* determined. Air-dried flowers (220gr) were subject 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 0.9% (w/w). The essential oil was analyzed by GC and GC/MS.

Indentification of the component was based of GC retention indices computer matching with wiley GC-MS library, and by comparison of the fragmentation patterns of the mass spectra those reported in the literature. 20 components were identified constituting more than 92.43% of the oil. α-Pinene (34.03%), β-Pinene (14.55%), β-Caryophyllene (3.99%) , (E)-β-Farnesene (4.85%) , β-Selinen(10.07%) and α-Selinen (9.93%) were major components that in flower *Hypericum perforatum* identified.

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A 005

Comparative Study on the Essential Oils Composition of *Achillea Wilhelmsii* C.Kokh. in flowers and leaves

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Achillea Wilhelmsii C.Kokh is a rare species of *Achillea* that is native (Endemic) to Iran and grows exclusively in East of Iran [1,2]. In this study, in order to compare between oil composition of flowers and leaves of *Achillea Wilhelmsii* C.Kokh that collected from "Neyshabur" central area in Khorasan-e-Razavi province in 2005. After drying, essential oils of the samples were extracted by Clevenger- type apparatus by hydrodistillation. Essential oil constituents determined by GC and GC/MS. The results showed that essential oils yield in flowers and leaves 0.85% and 0.80% w/w respectively. In flowers of *Achillea Wilhelmsii* 14 compounds (89.74%) identified that major component were: α-Pinene (7.76%), α-Terpinene (5.34%), p-Cymene (6.63%), 1,8-Cineole (11.14%) and Camphor (43.11%).

Twenty components (93.20%) determined in leaves of *Achillea Wilhelmsii* and the main components were: α-Pinene (4.99%), p-Cymene (9.29%),1,8- Cineole (15.16%) , Camphor (23.49%) and Piperitenone oxide (15.74%).

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Chemical Composition and Antimicrobial Activity of Essential Oils from flowers of *perovskia abrotanoides* karel.

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Perovskia abrotanoides Karel belongs to labiataes family that has been grown wildly in some regions of Iran. *Pervouskia abrotanoides* is a plant which is also used in folk medicine and vegetation [1,2]. This plant was collected from Neyshabur Mountains of khorasan-e-Razavi province in Iran. The air-dried flowers of *perovskia abrotanoides* were extracted by hydrodistillation, and their chemical composition was determined by GC-MS (in a yield 0.65 % w/w) and 24 components were identified.
The major component is α-pinene (15.97%), camphene (5.04%), δ-3-carene (6.16%), 1, 8-cineole (18.01%) and camphor (26.25%).

The antimicrobial activity was determined by using micro broth dilution and passage agar methods on Geram positive and Gram Negative bacteria. The MIC values reported for essential oils of flowers *perovskia abrotanoides.*

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Leaf trichomes, yield and composition of the essential oil of *Ocimum selloi* Benth. cultivated under coloured netting

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Shading with coloured plastic netting modifies the quality of natural radiation by selective filtration and may be employed as a means of manipulating physiological responses in plants. The objective of the present work was to determine the effects of coloured shading on the density of glandular and tectorial trichomes, and on the yield, productivity and composition of the essential oil of *Ocimum selloi* Benth. Plants were cultivated for 90 days under full sunlight or under ChromatiNet® 50% red or blue netting. Leaf trichomes were analysed by SEM. The essential oil was obtained by dry leaf biomass hydrodistillation and the chemical composition was performed by GC/FID/MS analysis. Individual components were identified by comparison of mass spectra with those cited in the literature (1). The highest density of glandular trichomes was observed in plants that had received full sunlight. None of the light treatments altered the yield of essential oil, although productivity was higher in plants grown under full sunlight by virtue of the greater leaf biomass that accumulated under such conditions. The compositions of the essential oils varied according to the quality of light. Whilst the qualitative profiles of the oils of plants grown under full sunlight or red shading were similar, that obtained from plants grown under blue shading presented a larger number of constituents. The highest level of methyl chavicol, the major component of the essential oil (2), was observed in plants grown under full sunlight. The density of leaf trichomes, together with the yield and chemical composition of the essential oil of *O. selloi*, were influenced by the quality and intensity of light applied to the plants, confirming the importance of the environment on the production of secondary metabolites.

Compounds	Retention index	Full sunlight	Red shading	Blue shading
		Relative area (%)		
methyl chavicol	1198	93.2	87.6	86.1
α-copaene	1375	-	-	0.2
methyl eugenol	1397	0.6	1.1	1.1
β-caryophyllene	1418	2.2	2.4	2.0
trans-α-bergamotene	1432	-	-	0.3
α-humulene	1454	-	-	0.2
germacrene-d	1480	1.3	3.5	2.9
bicyclogermacrene	1494	1.2	4.4	3.3
β-bisabolene	1506	-	-	0.2
γ-cadinene	1517	-	-	0.3
germacrene b	1558	-	-	0.1
p-n-methoxy-cinnamaldehyde	1567	-	-	0.1
spathulenol	1575	1.3	-	1.8

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Chemical Composition of the Essential Oil of *Myrcianthes fragans* McVaught from Venezuelan Andes

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Myrcianthes genus (Myrtaceae family) is known to have few species in American continent. Several of its species have been reported to have ethnomedicinal properties [1]. The essential oil of several *Myrcianthes* species has been reported to have *1,8 cineole*, α-pinene, α-terpineol and limonene [1-5]. The composition of the essential oil of *Myrcianthes fragans* from Jamaica is dominated by limonene (60 %) and α-terpineol (11 %) [6] whereas *Myrcianthes fragans* from Cuba contains α-pinene (42%) and limonene (30%) [7] . The present work reports the chemical composition of *Myrcianthes fragans*'s essential oil collected in Venezuela. The essential oil of fresh leaves of *Myrcianthes fragans* was obtained by hydrodistillation using a Clevenger-type apparatus [8]. The plant was collected in February 2008 at Pueblo Hondo, Táchira State, Venezuela, yielding 0.08 % of the oil. Chemical constituents were identified by GC-MS analysis [9]. Forty three components (91.8 % of the sample) were identified, of which the seven major ones were β-caryophyllene (11.5 %), myrcene (8.9 %), β-phellandrene+limonene (8.7 %), α-humulene (6.7%), α-copaene-8-ol (6.7 %), and α-selinene (5.3 %).

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Essential oil composition of two new species of *Phebalium* (*Rutaceae*) from north-eastern NSW, Australia

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The Rutaceae is an important family of plants with a wide distribution throughout the world, with 150 genera and 1800 species. It is commonly known as the Rue or Citrus family and includes species with flowers divided into four or five parts, usually with strong scents. In Asutralia, this family occurs as trees, shrubs or more rarely herbs, all of them belonging to any of the 40 and 320 described genera species respectively. A few native genera have been cultivated as ornamentals, particularly species of *Boronia*, *Crowea*, *Eriostemon*, *Phebalium* and *Correa*.

The genus *Phebalium* includes shrubs, more or less covered, at least when young, with silvery to rust-coloured, circular to stellate scales, unarmed; branchlets often glandular-warty. Although seven species have been traditionally described in New South Wales, recent investigations have allowed the description of two new unpublished species for this genus: *Phebalium stellatum* I. Telford ms is particularly rare and only known from a small area in the Dorrigo district, northern NSW. It is a medium sized shrub which grows in moist areas such as rainforest margins and in wet sclerophyll forest. *Phebalium sylvaticum* I. Telford ms is less restricted with a distribution ranging from the Gibraltar Range in northern NSW to the McPherson Range in south-eastern Qld. Its also a shrub 3-4 m tall that prefers wet forests.

The essential oil extracted from the aerial parts of both 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 of both species differed although both of them contained sesquiterpene and monoterpene compounds.

Chemical Composition and Antimicrobial Activity of Essential oils of Stems from *Boissiera squarrosa* Grown in Neyshabur area in Iran

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Abstract:

Boissiera squarrosa genus is one of the most important plants in Iran, some of them are endemic. In this study root of *Boissiera squarrosa* was collected from Neyshabur in Khorasan-e-Razavi in Iran, chemical constituent of essential oils of stems of *Boissiera squarrosa* determined. Air-dried stems (180g) were subject 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 0.75% (w/w). The essential oil was analyzed by GC and GC/MS.

Indentification of the component was based of GC retention indices computer matching with wiley GC-MS library, and by comparison of the fragmentation patterns of the mass spectra those reported in the literature. 15 components were identified constituting more than 96.07% of the oil. δ-Cadinene (7.70%), Camphor (26.29%), β-Caryophyllene (20.19%),Borneole(5.33%) and α-Humulene (10.71%) were major components that in stems of *Boissiera squarrosa* identified.

The antimicrobial activity was determined by using micro broth dilution and passage agar methods on Geram positive and Gram Negative bacteria. The MIC values reported for essential oil of roots of *Boissiera squarrosa* .

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A 011

Chemical Composition and Antimicrobial Activity of Essential oils of Stems from *Boissiera squarrosa* Grown in Neyshabur area in Iran

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The antimicrobial activity was determined by using micro broth dilution and passage agar methods on Geram positive and Gram Negative bacteria. The MIC values reported for essential oil of roots of *Boissiera squarrosa* .

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Volatile constituents of *Salvia sclareopsis* Bornm. ex Hedge. and *Salvia brachysiphon* Stapf. growing wild in Iran

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Salvia, the largest genus of the family Lamiaceae, includes about 700 species widespread all over the world. Fifty-eight species of the genus are found in Iran, seventeen of which are endemic. There are a number of literature reports on phytochemical analysis of this genus. The most well-known of this genus is *Salvia* officinalis, which has been credited with a long list of medicinal uses: spasmolytic, antiseptic and astringent.
Previous chemical investigations on different species of *Salvia*, have shown the presence of flavonoids, diterpenoids and even the rare sesquiterpenes. This is the first report on the oil compositions of *S. sclareopsis* Bornm. ex Hedge. and *S. brachysiphon* stapf, which are endemic to Iran.
We have previously studied the essential oil compositions of *S. hypoleuca* [1], *S. lereifolia* [2], *S. eremophila* [3], *S. rhytidea*, *S. limbata* and *S. palaestina* [4].
The aerial parts of two *Salvia* species were collected during the flowering stage at the following places: *S. sclareopsis* was collected in May 2005 in Ghali kooh of Lorestan Province and *S. brachysiphon* was collected from Ghahiz protected area in Isfahan Province, Iran, in June 2005.
The dried aerial parts of the plants were subjected to hydrodistillation for 3h using a Clevenger type apparatus. The oils were analyzed by GC and GC/MS.
Sixteen constituents, representing 96.4% of the total components in the oil of *S. sclareopsis* were characterized by β-caryophyllene (22.5%), germacrene D (16.5%) and benzyl benzoate (13.7%) as the main compounds.
β-Caryophyllene (28.1%), α-pinene (20.6%), limonene (11.5%) and β-pinene (10.6%) were the main constituents among the eleven characterized, comprising 88.2% of the total components detected in the oil of *S. brachysiphon*.
Sesquiterpenes comprised (74.3%) and monoterpene consisted of (2.6%) of the oil of *S. sclareopsis*, while in the oil of *S. brachysiphon* monoterpenes (54.3%) predominated over sesquiterpenes (33.9%).

Acknowledgment: We are grateful to D.V.Mozaffarian for collecting and identifying plant material.

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Chemical composition of essential oils of the aerial parts of *Nepeta prostrata* Benth. and the flowers of *Nepeta* straussii Hausskn. Bornm. from Iran

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The genus *Nepeta*, which belongs to the Lamiaceae family, consists of about 280 species. In Iran 67 species are present, among which 39 are endemics.
Some of Iranian *Nepeta* species has been of great interest to Iranian folk and traditional medicines and used in the treatment of various disorders, such as some nervous, respiratory and gastrointerest diseases.
Previous chemical investigation on the constituents of *Nepeta* species indicated the presence of monoterpenoids, diterpenoids and triterpenoids.
We have previously studied the essential oil compositions of *N. ispahanica*, *N. binaludensis* [1], *N. racemosa* [2], *N. denudata*, *N. cephalotes* [3] and *N. makuensis* [4].
The aerial parts of *N. prostrata* and flowers of *N. straussii* were collected from Isfahan, Province of Isfahan, Iran, in May 2005, during the flowering stage.
The dried aerial parts of *N. prostrata* and flowers of *N. straussii* were subjected to hydrodistillation for 3h using a Clevenger type apparatus. The oils were analyzed by GC and GC/MS.
Twenty-three components representing 98.1% of the oil of *N. prostrata* were identified of which 1,8-cineole (26.1%), β-pinene (13.6%), myrtenol (11.8%) and *trans*- pinocarveol (10.7%) were found to be the major constituents.
Twenty-one components were identified in the flower oil of *N. straussii* making up 91.3% of total composition. 1,8-cineole (22.1%), germacrene D (18.5%) and β-pinene (12.1%) were the major components in this oil.
As can be seen from the above information the essential oils of two *Nepeta* species; *N. prostrata* and *N. straussii* monoterpenes (94.2% and 51.1%) predominated over sesquiterpenes (3.9% and 40.2%), respectively.

Acknowledgment: We are grateful to Dr. V. Mozaffarian for collecting and identifying plant material.

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Phytochemical analysis of essential oil of *Teucrium pruinosum* Boiss. growing wild in Lebanon

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Teucrium (Lamiaceae) is a cosmopolitan genus, with more than 300 species, that differs from other related genera because its corolla is formed of one lip. Plants of this genus have been used medicinally since ancient Greek times, when they were used for coughs and asthma; most of these are bitter, astringent, anti-rheumatic herbs that were used to reduce inflammation, stimulate the digestion, lower fever, as diuretic, antiseptic, antipyretic and antihelmintic agents. [1]. *Teucrium pruinosum* Boiss. is a rare plant growing wild in Lebanon. No report on the essential oil of this species has been found in the literature so far. Therefore, as a continuation of our research on plants from Lebanon [2,4], we report on the chemical composition of the essential oil of aerial parts of *T. pruinosum* collected on June 2006 at Hermel, a town in Beqaa Governorate, Lebanon. The oil from air-dried and ground aerial parts of plants was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus according to the method recommended in the *European Pharmacopoeia* [5]. The analysis of volatile constituents by GC and GC-MS led to the identification of 89 compounds, with hexadecanoic acid (10%) and naphtalene (8.3%) being the most abundant ones. On the whole, the oil is rich of sesquiterpenes (42.7%), particularly sesquiterpenes hydrocarbons (31.1%). In this fraction α-cubebene (5.6%), δ-cadinene (5.2%) and β-cubebene (4.2%) prevail. Among the oxygen containing sesquiterpenes (11.6%), spathulenol (3.2%) was the most abundant.

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Use of a university field for growing medicinal plants and the qualitative and quantitative analyses of their essential oils

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The university field with an area of 0.75 hectares at Prešov University in Prešov, belonging to the Department of Ecology, in Slovakia was used mostly for growing vegetables and fruit. The agro-technology development included medicinal and aromatic plant introduction. The objective of this study was the qualitative and quantitative analysis of the essential oil composition in selected medicinal plants (*Matricaria recutita* L., *Mentha x piperita* L. and *Acorus calamus* L.) grown on the university field. We studied the essential oil composition of chamomile over four consecutive years. The essential oil composition of peppermint from plants grown on the university field was compared with the plant material originally cultivated in Egypt. We analysed roots of sweet flag cultivated in Great Britain and the Czech Republic, then planted them on the university field and compared the essential oils. Soil chemical and climatic conditions of the university field were studied. After the four year study of cultivated chamomile variety "*Bona*", a diploid genotype, the essential oil composition reported high content of two active substances, which are *l*-*l*-α- bisabolol and chamazulene belonging to a bisabolol chemotype [1]. The essential oil from peppermint herba grown on the university field consisted 70 – 74 % of menthol and only trace amounts of pulegon and karvon which makes it an interesting chemotype. Roots analyses of sweet flag brought from Great Britain and the Czech Republic showed significant differences between the essential oil components and small amount of azarones were recorded. The introduction of further medicinal plants – *Stevia rebaudiana Bertoni, Tribulus terrestris L., Rhaponticum carthamoides Ilja.* - to the university field is in progress after it has been proved that it has the potential for research, cultivating and educational activities.

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Comparative study of the chemical profiles of the essential oils of ripe and rotten fruits of *Citrus aurantifolia* Swingle

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Most often during the processing of lime fruits for essential oil extraction, some rotten fruits are used along with the ripe fruits. In this study, we examine the volatile constituents of the essential oils from both ripe and rotten lime fruits *(Citrus aurantifolia* Swingle) from Nigeria. The oils were isolated by hydrodistillation and analysed using GC-MS. The ripe and rotten lime oils contain 55 and 50 components respectively. Both oils were rich in limonene (21.02%, ripe lime; 21.28% rotten lime) and β-terpinene (8.29%, ripe; 8.88% rotten lime) and E, E-α-farnesene (6.28% ripe lime; 4.83% rotten lime). The other major components α-pinene (11.12%), α–terpineol (11.70%) and linalool (5.52%) were identified in ripe lime oil only. Whereas, α– terpineolene (8.48%) and linalyl propionate (14.07%) were present in rotten lime oil only. Limonene and citral which are believed to be the two major citrus odour contributors [1,2] were present in both ripe and rotten lime oils. Aldehydes like decanal and the farnesenes which are also important in citrus flavour [2,3] were represented in the two lime oils. But some notable components of lime ripe fruit oil like trans-β-ocimene, linalool, myrcenol, dodecanal, trans -β –Bergamotene and trans-γ– bisabolene are absent in the rotten fruit oil

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Chemical composition of the essential oil from flowers and leaves of *Achillea nobilis* L., subsp. *neilreichii* from North of Iran

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The genus *Achillea* (Compositae) is comprised of about 90 species in widespread over the world. In the Flora Iranica this genus is represented by ninetheen species [1]. The genus *Achillea* are known for medicinal value, antibacterial, antioxidant, anti-inflammatory and antinociceptive properties [2].
Achillea nobilis L., subsp. *neilreichii* were collected during the flowering stage from Damash village (Height 1700 m), East of Roudbar, Gilan Province, located North of Iran in July 2007.
Flowers and leaves of *A. nobilis* L., subsp. *neilreichii* were separately hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 1.8% (w/w) and 1.0% (w/w) of yellowish oil, respectively.
The components of the essential oil were identified by comparision of their mass spectra with those of a computer library or with authentic samples and confirmed by comparision of their retention indices, either with data published in the literature [3].
forthy five compounds were identified in the oil of flowers, representing 94.2% of the total oil, of which 1,8-cineol (10.3%), geranyl isovalerate (8.4%), δ-cadinol (7.5%), camphor (6.4%) and 2,2,4-trimethyl-4,5-dihydro-1,3,8-H-azulene-6,7-dicarboxilic anhydride (5.8%) were find to be the main constituents.
In contrast, forty compounds were characterized in the oil of leaves, representing 92.9% of the total oil, of which 1,8-cineol (17.0%), *trans*-verbenol (14.2%), cadin-4-en-10-ol (7.8%), *cis*-chrystantenyl acetate (4.0%) and α-terpineol (4.0%) were the most abundant.

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Portuguese bryophyte *Radula* species: chemosystematic evaluation of volatiles composition

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Eight *Radula* species are known to occur in Portugal, including the Macaronesian archipelagos of Madeira and Azores. From these, four species are referred for the mainland (*R. aquilegia*, *R. complanata*, *R. holtii* and *R. lindenbergiana*, seven for Madeira (*R. aquilegia*, *R. carringtonii*, *R. holtii*, *R. lindenbergiana*, *R. nudicaulis* and two Macaronesia endemics, *R. jonesii* and *R. wichurae*) [1] and, six for the Azores (*R. aquilegia*, *R. carringtonii*, *R. holtii*, *R. lindenbergiana*, *R. nudicaulis* including one Macaronesia endemic, *R. wichurae*) [2].

The solvent extracts of about twenty *Radula* species have been evaluated [3 and references therein]. Based on these studies it was found that *Radula* species produce flavonoids, several linear and cyclic prenyl bibenzyls, cyclopropanochroman derivatives, sesquiterpenes and clerodane diterpenoids. It is considered that *Radula* is a genus chemically characterized by the presence of prenylated bibenzyls and very low levels or almost total lack of terpenoids [3].

To the best of our knowledge only two studies have addressed the volatile fraction, obtained either by hydrodistillation or distillation-extraction, of *Radula* species. Suire [4] extracted the essential oil from *R. complanata* [2% (w/f.w.)] and identified α-pinene, β-pinene, camphene, six sesquiterpenes of which β-caryophyllene was identified, and 3-methoxybibenzyl. Tesso *et al.* [5] isolated the essential oil of *Radula perrottetii* and identified several mono-, sesqui-, diterpenes and aromatic compounds.

As part of a chemosystematic survey on Portuguese *Radula* genus we report in this study on the comparison of the volatiles isolated from eight *Radula* species: *R. aquilegia* (Hook. f. & Taylor) Gottsche *et al.*, *R. carringtonii* J. B. Jack, *R. complanata* (L.) Dumort., *R. holtii* Spruce, *R. jonesii* Bouman *et al.*, *R. lindenbergiana* Gottsche ex C. Hartm., *R. nudicaulis* Steph. and *R. wichurae* Steph. Several populations of *Radula* were collected, in different years, on mainland Portugal and on Madeira and Azores archipelagos.

The volatiles were isolated by distillation-extraction and analyzed by GC and GC-MS. The percentage composition of the volatiles was used to determine the relationship between the different samples by cluster analysis using the NTSYS-pc software and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition as previously reported [6].

Although the presence of bibenzyl compounds was frequent in the volatiles isolated from the different *Radula* species, such as in *R. complanata* and *R. lindenbergiana*, other species showed high relative amounts of sesquiterpenes, such as *R. aquilegia*, *R. carringtonii*, *R. nudicaulis* and *R. wichurae*.

The volatile analyses of *Radula* species provide different chemical data from that obtained by solvent extracts evaluation but constitute a helpful tool for *Radula* species identification and characterization, in addition to molecular and taxonomic research in progress.

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Combined RAPD and volatile analysis of *Laurus azorica* from the Azores archipelago

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Laurus azorica (Seub.) J. Franco is an endemic Lauraceae tree of the Macaronesian forest, whose essential oils [1, 2] and genetic biodiversity [3, 4] have been previously studied. To the best of our knowledge, however, no integrative study using both genetic and chemical analyses has been performed. Here we report a combined study using Random Amplified Polymorphic DNA markers (RAPDs) and the volatile component analysis.

Forty-eight *Laurus azorica* individual leaf samples were collected on seven Azorean islands (Faial, Flores, Graciosa, Pico, S. Jorge, S. Miguel and Terceira, from 2004 to 2006). Following DNA extraction, 51 RAPD primers were tested and 23 were selected for PCR amplification, based on band intensity and polymorphism. *L. nobilis* was used as the outgroup for the molecular analysis. Volatiles from all samples were extracted by distillation-extraction and analysed by GC and GC-MS, as previously reported [5]. For both molecular and chemical data, cluster analysis was performed using NTSYS software [6], and their dendrograms compared.

On the molecular analysis, a total of 364 well resolved bands, with sizes between 300bp and 2.8Kb were generated, representing a total of 98% polymorphism. Cluster analysis grouped the plants mainly according to their geographical location, and interestingly the outgroup *L. nobilis* clustered together with some *L. azorica* accessions, maybe due to its genetic similarity, as mentioned before [3]. In fact, some assessments from S. Miguel were genetically more dissimilar among themselves than to the outgroup.

On the volatile analysis, forty-nine components, representing 71–97% of the total oils, were identified. All oil samples were dominated by their monoterpene fraction (22–87%), α- pinene (4–48%), 1,8-cineole (3-36%) and β-pinene (3-23%), being the main components, in accordance with previous studies on populations [1]. The sesquiterpene fraction (8-33%) was dominated by β-elemene (0.2–19%) and β-caryophyllene (traces–14%). Some phenylpropanoids were also present (0.1–16%), *trans*-cinnamyl acetate (0.1–15%) being the main component of this fraction. Cluster analysis of the volatile oils components showed a high correlation among all samples (S_{corr} = 0.86), with exception of four individuals: two from S. Miguel island (S_{corr} = 0.56) and the only two individuals from Graciosa island (S_{corr} = 0.4). The very low correlation of the latter samples can be explained by the higher relative amounts of 1,8-cineole (24-36%). β-Caryophyllene (3-9%), linalool (6-8%) and α-terpenyl acetate (3-6%) were also among the main oil components, suggesting a volatile composition similar to that of *L. nobilis* [7, 8].

No straight correlation could be found between the molecular and volatiles clusterings. However, it would be interesting to gain further knowledge on the molecular and chemical profiling of this species and to understand why *L. azorica* accessions collected in S. Miguel showed higher molecular resemblance to *L. nobilis* used as the outgroup than with the other *L. azorica* samples and why Graciosa samples showed such marked chemical differences from the other *L. azorica* samples.

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Essential oil of *Cacalia briquetii* by GC/MS (EI & CI) and ^{13}C-NMR

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The essential oil of *Cacalia briquetii* (family Asteraceae), an endemic species from Corsica, has been studied by GC and GC/MS. After fractionation of essential oil on chromatographic column, 156 components were identified amounting to 94.5% of the total composition. This oil was characterized by sesquiterpene hydrocarbons (52.8%) and oxygenated sesquiterpenes (25.9%). The major components were germacrene D (18.5%), zingiberene (12.9%) and β-oplopenone (10.8%).

GC/MS in chemical ionization mode (CI) has been carried out for the determination of molecular mass and the differentiation of compounds with similar EI-mass spectra; i.e. monoterpene ester or non-terpene esters. Moreover, structures of three components were ensured using ^{13}C-NMR as being albene, β-oplopenone and cacalol. The identification was based on the comparison of chemical shifts in the mixture with those reported in the literature.

The β-oplopenone, a major component of essential oil, was reduced by $LiAlH_4$. Thus, we have obtained the two corresponding epimers alcohols. After purification, the structural analysis was carried out by 1D and 2D NMR (^{13}C, ^{1}H, DEPT, HSQC, HMBC and COSY). To our knowledge, these two molecules were never been described.

Keywords:

Cacalia briquetii, Essential oil, GC/MS(EI, CI), Cacalol, β-Oplopenone, β-Oplopenol

Chemical Composition and Antibacterial Activity of Essential Oil of *Kelussia odoratissima* Mozaff. From Different Locations of Iran

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Kelussia odoratissima Mozaff., locally called "Karafs-koohi" in Iran, is commonly used in some parts of Iran as a popular garnish. It is also used as a folk medicine to treat hypertension, inflammation, ulcer, and cardiovascular diseases(2). The seeds of *K. odoratissima* were collected from Isfahan and Ize (Khuzestan province of Iran). The essential oil was isolated by hydrodistillation and analyzed by combination of capillary GC and GC-MS(1). In total 39 and 37 components were identified and quantified for Ize and Isfahan Sample, representing 99.8 and 99.1% of the oil, respectively. 3N buthyl phthalide (26.2%), γ-muurolene (18.7%), β-phellandrene (8.1%) and γ-Elemene (5.1 %) were the main components of Ize sample and 3N buthyl phthalide (33.2%), γ-muurolene (11.5%), β-phellandrene (13.8%) and γ-Elemene (4.9 %) were the main components of Isfahan sample. The *in vitro* antimicrobial activities of the essential oils of *K. odoratissima* from two different locations were studied against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*)(3). The results of the antimicrobial activities of the essential oils according to the disc diffusion method and minimum inhibition concentration (MIC) values indicated that the oils exhibited moderate to high antimicrobial activity.

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Comparison of Chemical Composition from *Hyssopus officinalis* L. Cultivated in Different Locations of Iran

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Hyssop (Hyssopus officinalis),belonging to the family Lamiacea, is an aromatic perennial herb native to southern Europe and some temperate regions of Asia. It is usually found on dry banks and among rocks and ruins [1].
It is one of the most important pharmaceutical herbs, is extensively cultivated in central and south European countries such as Russia, Spain, France and Italy). Despite having a bitter taste, hyssop is used as a food flavor and also in sauce formulations). Hyssop oil possesses anti-fungal and anti-bacterial properties, which are essential in such industries as canning, beverages and cosmetics.
The aerial parts of *Hyssopus officinalis* cultivated in two culture sites in Iran, Shiraz (sample A) and Karaj (sample B) were hydro distilled for 3 hours using a Clevenger-type apparatus.
After decanting and drying of the oils over anhydrous sodium sulfate, the corresponding oils were isolated in yields of 0.6 % (w/w) for the sample A and 0.5 % (w/w) for the sample B of yellowish oils. The oils were analyzed by GC and GC-MS. The components of the essential oils were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [3].
two components were characterized in the essential oil of sample A and isopinocamphone (53.12%), α-terpineol (7.4%) and pinocamphone (4.7%) were found as the major constituents. In contrast, Forty components were identified in the oil of sample B of which isopinocamphone (24.87%), pinocamphone (14.41%), elemol (8.55%) and β-pinene (7.81%) were reported as the main constituents.

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Chemical composition and antioxidant activity of essential oils from *Salvia virgata* Jacq. and *S. verticillata* L. from Iran

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Previous investigations revealed that the essential oils and organic extracts of Salvia species exhibited antioxidant, antimalarial, antibacterial, anti-inflammatory, anticancer and anticholinesterase activities both in vitro and in vivo (6-10=1). Therefore the aims of this study were first to determine the chemical composition of essential oils of *Salvia virgata* and *S.verticillata* (lamiaceae) collected from Mazandran Province, Chalus (Gachsar), second to investigate the antioxidant properties of the Salvia oils using the DPPH free radical method (2).

Hydrodistilled essential oil from the leave and flowers of *S. virgata* and *S. verticillata* were analyzed by GC and GC-MS (3). The oil yield for leave and flower of *S. virgata* were 0.15% and 0.19% (v/w), 19 and 30 compounds of the essential oils were identified respectively. The main constituents of the leave oil were phytol (29.1%), β-caryophyllene (19.15%), caryophyllene oxide (16.96%), hexadecanoic acid (8.21%), hexahydrofarnesyl acetone (4.52%), and germacrene-D (3.05%). The Major components of flowers oil were β-caryophyllene (21.05%), germacrene-D (13.6%), bicyclogermacrene (6.99%), α-humulene (6.71%), β-pinene (6.70%), β-phellandrene (4.81%), and α-pinene (4.76%).

The oils yield for leave and flowers of *S. verticillata* were 0.1% and 0.12% (v/w); which 54 and 36 compounds were identified respectively. β- caryophyllene (13.64%), β-phellandrene (10.2%), germacrene-D (11.47%), β-pinene (7.53%), α - humulene (5.58%), α-pinene (4.8%), sabinene (4.49%) and spatulenol (3.27%) were dominant substances of leave oil. Spathulenol (23.56%), β-caryophyllene (17.15%), caryophyllene oxide (16.38%), sabinene (8.35%), caryophyllenol-II (5.22%), terpinen-4-ol (4.53%), α-thujene (4.1%), γ-terpinene (3.03%) were the main compounds of flowers essential oil.

Antioxidant capacity of the oils was measured by the modified radical scavenging assay (6), using DPPH (2, 2-diphenyl 1-picrylhydrazyl) as oxidizable substrate. Results showed that the antioxidant properties of 0.05 ml of Salvia virgata flower oil have equal activity with BHA (200 µg) ($P>0.05$), but flower oil of *S. verticillata* and leave oils of *S. virgata* and *S. verticillata* is not as potent as BHA ($P<0.003$).

In conclusion antioxidant activity of S. *virgata* essential oil may be correlate with different therapeutic uses of salvia in traditional medicine.

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Chemical composition and antioxidant activity of *Otostegia persica* essential oil from Iran

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Otostegia is a genus of labiatae that comprises about 31 species in the world and 4 species of them grow in south of Iran (1, 2). *Otostegia persica* (Burm.) Boiss. is an endemic plant of Iran and Pakistan (2). Aerial parts of this plant are used in traditional medicine as anti-rheumatic, analgesic in toothache and treating oral infections (3, 4). Previous studies pointed that antioxidant activity of *O. persica* was comparable to vitamin E, BHA, methanol extract of Green tea and *Ginkgo biloba* using beta carotene bleaching and ferric ammonium thiocyanate methods. Methanol extract of the plant exhibited the highest antioxidant activity. Five compounds were separated and purified from the methanol extract by column and paper chromatography respectively. Three isolated flavonols (morin, kaempferol, and quercetin) showed significant antioxidant activity comparable to BHA and vitamin E. The C-glucoflavone (isovitexin) also exhibited interesting antioxidant activity, but weaker than the flavonols. Cinnamic acid showed no activity with this method (4, 5, 6).

The essential oil of *O. persica* flowers and leaves from Sistan & Baluchestan (OSB) and top flowered aerial parts of Kerman (OK) Provinces were prepared by hydrodistillation, using a Clevenger type apparatus, yelding (0.08, 0.1, 0.12 % v/w respectively). The essential oils were analyzed by GC and GC/MS (7). The antioxidant activities of essential oils were measured using free radical scavenging method with 2-2-diphenyl 1-picrylhydrazyl (DPPH) (6).

17 out of 19 compounds were recognized from the essential oil of OK sample (92.06 %), the major compounds being cycloisolongifolene (5.93 %), hexadecanoic acid methylester (4.76 %), hexadecanoic acid (31.73 %) and pentacosane (29.51 %). From the flowers and leaves oils of OSB 33 and 26 compounds representing 90.29 %, 83.27 % of the oils were characterized respectively. α-pinene (13.62 %), linalool (6.76 %), verbenol (9.22 %), trans-carveol (4.00 %), pentadecane (4.56 %), caryophyllene oxide (4.84 %) and hexadecane (5.52 %) were the abundant compounds of the flower and α-pinene (4.48 %), verbenol (10.16 %), trans-anethole (4.47 %), geranyl acetone (6.47 %), pentadecane (5.94 %), hexadecane (5.86 %) and hexahydrofarnesyl acetone (14.34 %) were the most important components of leaves. The antioxidant potential of 20 µl of essential oil was 94.96±2.07 % that in comparison to 50 µg vitamin E (95.32±2.2 %) and 200 µg BHT (96.66±2.5 %) showed equal antioxidant activity (P>0.05), in a 30 min period.

In conclusion the antioxidant power of essential oil showed that the anti-rheumatic activity of this plant could be due to this property.

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Compositional characteristics of essential oils of wild populations of *Hypericum perforatum* (St. John's Wort) from Lithuania and France

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Hypericum (family *Guttiferae*) is a genus of about 450 species that occurs in all temperate parts of the world. Five species of the genus *Hypericum* grow wild in Lithuania (*H. perforatum*, *H. maculatum*, *H. montanum*, *H. hirsutum* and *H. humifusum*) [1] whereas twenty-eight species of the same genus occur in France [2]. *H. perforatum* is the most abundant and frequently used as a medical plant and it is very significant in pharmacology. Many studies confirmed its antidepressant, antimicrobial, antiviral, anticancer and other activities [3-5].

Numerous classes of bioactive chemical constituents, such as hypericins, flavonoids and biflavonoids, xanthones, tannins, proanthocyanidins as well as phenolic acids have been investigated in St. John's wort. Low amount of essential oils in the species of *Hypericum* could explain why there is a limited number of the studies on volatile chemistry of this genus.

The aim of this study was to evaluate characteristics of volatile oils in various wild populations of *H. perforatum* from Lithuania and France.

Essential oil qualitative and quantitative analyses were performed on aerial parts of *H. perforatum* plants collected at flowering in different places from Lithuania (2002-2007) and France (2000-2003). The volatile oils isolated by hydrodistillation were analysed by gas chromatography (equipped with FID and capillary columns HP-FFAP and CP Sil) and gas chromatography-mass spectrometry GC-MS (with nonpolar capillary column DB-5). Qualitative analysis was based on a comparison of retention times, indices and mass spectra with the corresponding data in the literature [6] and computer mass spectra libraries.

The yield of St. John's wort essential oils was found ~0.01-0.2%. Analyses allowed identifying more than 100 compounds in the samples from both countries. A great variability was pointed out between the compositions of the essential oils from the various localities of collection. Even if trans-caryophyllene and its oxide, germacrene D, spathulenol and several aliphatic compounds were the major compounds in all the oils, those from Lithuanian plants were rich in α-pinene, while this compound was almost not present in oils from France.

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Identification of chrysanthenyl esters by GC-RI, GC/MS (EI and CI) and NMR from *Anthemis maritima* essential oils.

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The combination of Column Chromatography, capillary GC (Retention Indices, RI), GC-MS (EI and CI) and ^{13}C-NMR analysis applied to 11 *A. maritima* oils from Sardinia allowed the identification of 122 components which accounted for 85.2% to 91.9% of the total amount. The major components were trans-chrysanthenyl acetate (68.5%-34.9%), trans-chrysanthenol (14.1 %-4.8 %), cis-chrysanthenyl 2-methylbutyrate (15.4 %-11.2 %) and 6-methylhept-5-en-2-one (8.6 %-4.3 %). Oxygenated monoterpenes were accounted for 43,2%- 81,9% of the global composition.

15 compounds were remained unidentified after computer research against MS libraries; their IE-mass spectra exhibited a common mass pattern similar with those of trans-chrysanthenyl acetate. These observations suggested the occurence of several chrysanthenyl esters. The determination of the molecular mass and the acid part were carried out using PCI and NCI-MS modes with ammonia. The PCI-methane mass spectra allowed separating two series of compounds according to their stereochemistry. Finally, comparison of their MS and NMR spectral data with synthetic material allowed the identification of 6 trans- and 6 cis-chrysanthenyl esters. To our knowledge ^{13}C-NMR data of 6 chrysanthenyl esters and ^{1}H-NMR data of 7 chrysanthenyl esters were described for the first time.

Moreover, principal components analysis suggested 3 clusters of essential oils. One cluster differed of the two others by hightest hydrocarbon compounds 26.1%-28.3% and low abundance of trans-isomers of chrysanthenyl esters which accounted for only 2.1%-4.4%, while it was always more than 16.9 % in the samples of other clusters. The two other clusters differed by trans-chrysanthenyl acetate percentages.

Key words:

Anthemis maritima, chrysanthenyl ester, stereochemical differentiation, GC/MS-CI.

ANALYSIS AND DETERMINATION OF DIFFERENT INGREDIENTS OF THE ESSENCE OF IRANIAN RICE [HASHEMI VARIETY]

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THE AIM OF THIS RESEARCH WAS ANALYSIS AND DETERMINATION OF DIFFERENT INGREDIENTS OF THE ESSENCE OF IRANIAN RICE [HASHEMI VAREITY].
WE ALSO COMPARED THESE INGREDIENTS IN NEW CROPPED RICE AND ONE WHICH HAS BEEN KEPT FOR ONE YEAR.THE ANALYSIS WAS DONE WITH GC.MS SYSTEM AND ASSESED AND CORRECTED WITH REGARD TO THE RESOURCES.THE PRECEDURE OF ESSENCE EXTRACTION WAS DONE WITH WATER STEAM DISTILLATION METHOD AND PURIFICATION WAS DONE WITH THE USE OF VOLATILE ORGANIC SOLVENT PETROLIUM ETHER.
THE ANALYSIS OF GC.MS SYSTEM SHOWED THAT THE VOLATILE INGREDIENTS IS COMPLEX AND NO SOLITARY MATERIAL CAN INDICATE RICE ESSENCE.
THE ULTIMATE RESULT SHOWED THAT ESSENCE INGREDIENTS OF THIS VARIETY CHANGE SIGNIFICANTLY TO ITS PRIMARY INGREDIENTS WITH PASSING OF TIME.
THE MOST IMPORTANT INGREDIENTS IN THESE 2 SAMPLES ARE:
NEW RICE: HEXADECANOIC ACID , OCTADECANOIC ACID
OLD RICE: NAPHTALENE DECAHYDRO DIMETHYL , DECANE

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Essential oil composition of some *Salvia officinalis* varieties

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Salvia officinalis L. is one of the most important medicinal plants of *Lamiaceae* family. Modern evidence supports its effects as an antibiotic, antifungal, antispasmodic, estrogenic and tonic. The strongest active constituents of sage are within its essential oil, which contains cineole, borneol and thujone. Internal uses: liver complaints, excessive lactation, depression, menopausal problems. External uses: insect bites, throat, mouth, gum infection [1]. The present study focuses on the isolation and identification of essential oil composition of different sage (*Salvia*) taxa.
Materials and methods: The aerial parts of different *Salvia* taxa (*Salvia officinalis*, *S. officinalis var. tricolor*, *S. officinalis var. purpurascens*, *S. officinalis var. Kew Gold*) were collected at full flowering stage from the Botanical Garden of University of Pécs. The essential oils were isolated by hydrodistillation and analyzed by combination of capillary GC-FID and GC-MS. GC-MS parameters: Agilent 6890N GC instrument, 30m x 0.25mm I.D. capillary column, gradient programme, helium carrier gas, HP-5MS stationary phase, 280 °C injector temperature, 1 µl injection volume, 5973N mass selective detector. Identification of compounds: MS of standards, NIST spectrum library.
Results: Variations in the essential oil composition of *Salvia officinalis* L. varieties were determined. The essential oil yield was in the range of 0.2-1.3 mL/100 g. The main components of the essential oil are α-thujone, α-thujone and camphor, and α-humulene in *Salvia officinalis*, *S. officinalis var. tricolor* and *S. officinalis var. Kew Gold*, and *S. officinalis var. purpurascens*, respectively. Our results could promote the application of new taxa from the *Salvia* genus in phytotherapy, according to their medicinal value, and also provide valuable information for plant breeders.

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Biodiversity of vanilla: aroma and fatty acid composition of cured beans from different origins

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Vanilla tahitensis is known for its original aromatic flavour which is strongly appreciated and different from *Vanilla planifolia* due to an original anise note. Moreover, the beans of *Vanilla tahitensis* are very attractive thanks to their oily texture and appearance. Two main cultivars are produced in French Polynesia plantations. The morphological traits of their leaves, flowers and beans are different from those of *V. planifolia* cultivars. In order to assess the chemical biodiversity of commercial *V. planifolia* and *V. tahitensis* beans, different cured vanilla samples were collected in various producing countries (Central America, Indian and Pacific Ocean).
The study analysed the aroma and fatty acid content. Aroma extraction was performed in a Soxhlet apparatus with ethanol, and lipids were cold-extracted with chloroform. The aroma and fatty acid content were then assessed by HPLC analysis with external calibration.
Fourteen aroma molecules were quantified showing a uniquely high content of anise molecules for *V. tahitensis* samples and substantial composition differences depending on species, and sample origin. Besides common fatty acids, four very long-chain monounsaturated fatty acids, rarely found in plants, were identified: nervonic acid (24:1), ximenic acid (26:1), octacosen-19-oic (28:1) and lumequeic acid (30:1). Statistical analysis was performed on vanilla samples, aroma and fatty acids variables. Differentiation of *V. tahitensis* and *V. planifolia* samples is well achieved and can be explained either by genetic variations, agronomic factors or curing method.
Results of both aroma and fatty acid compositions could be used as a characteristic for the authentication of *V. tahitensis* enabling its protection as a unique species.

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Antioxidant Activity of *Mentha pulegium* liquid Extract in Humans: A cross- sectional Before/ After clinical trial

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Many plants contain natural antioxidants that act in metabolic response to the endogenous production of free radicals and other oxidant species(1). The present cross- sectional before/ after clinical trial was carried out to investigate the antioxidant properties of the decoction of aerial part of Mentha pulegium in human. A group of 20 healthy subjects was invited to use the M. pulegium (60mgkg^{-1}) twice a day for 14 days. Blood samples before and after entering the study were measured for lipid peroxidation level (LPO)(2), total antioxidant capacity (TAC)(3) and total thiol (SH)(4) molecules. A reduction of blood LPO (9.7 ± 5.7 versus 7.3 ± 5.7, $p= 0.2$) was observed after 14 days of M. pulegium consumption. Blood TAC (1.9 ± 0.3 versus 3.3 ± 0.5, $p= 0.0001$) and total thiol molecules (0.2 ± 0.12 versus 0.27 ± 0.16 $p = 0.168$) increased after 14 days of M. pulegium consumption. In conclusion, the phenolic content of M. pulegium is argued to be the possible scavenger of reactive oxygen radicals and reduction of oxidative stress. In recent years theimportance of oxidative stress in the pathophsiology of many human disorders has been confirmed, thus use of this plants as a dietary supplement is highly recommended.
Key words: antioxidant- oxidative stress- decoction- Mentha pulegium.

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Volatile constituents of the ether extracts of three Balkan *Micromeria* species

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The composition of the ether extracts of aerial parts of *Micromeria kosaninii* Šilić, *Micromeria parviflora* (Vis.) Reincheb. and *Micromeria juliana* (L.) Benth. ex Reich was analyzed by GC and GC/MS. Twenty-two compounds, representing 81.8% of the *M. juliana* extract, were identified. The major components were oplopanone (13.2%), caryophyllene oxide (9.5%) and α-pinene (6.4%). Eighteen compounds, representing 79.4% of the *M. kosaninii* extract, were identified. The major components were heptacosane (19.5%), caryophyllene oxide (12.1%) and β-caryophyllene (6.1%). Ten compounds, representing 80.0% of the *M. parviflora* extract, were identified. The major components were spatuhlenol (16.7%), eicosanal (16.4%) and heneicosanal (10.8%).

The terpene fraction of all three extracts was a significant one, reaching 71.0% in the *M. juliana* extract. The other two extracts contained comparable relative amounts of terpenes (52.9% and 32.6%, respectively). The n-alkanes were the second most abundant compound class in *M. juliana* and *M. kosaninii* extracts (8.7% and 19.5%, respectively), while in the *M. parviflora* extract the alkanes were the third (7.3%) class, however still being equal to the percentage of the *M. juliana* alkanes. Strangely, the aldehydes were the main fraction of *M. parviflora* extract (40.1%), but were completely absent from the *M. juliana* extract.

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Essential oil composition of *Micromeria kosaninii* Šilić, *Micromeria parviflora* (Vis.) Reincheb. and *Micromeria juliana* (L.) Benth. ex Reich

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The composition of the essential oil of aerial parts of *Micromeria kosaninii* Šilić, *Micromeria parviflora* (Vis.) Reincheb. and *Micromeria juliana* (L.) Benth. ex Reich was analyzed by GC and GC/MS. One hundred and twenty four compounds were identified in *M. kosaninii* oil, one hundred and forty three in *M. parviflora* oil and one hundred and eleven in *M. juliana* oil, accounting for more than 96.0 %, 97.5 % and 97.9% of the total oils, respectively. Sixty three compounds were common for all three oils. The major constituents of both *M. kosaninii* and *M. juliana* oils were borneol (8.2%, 9.3%), isomeric verbenols (11.7%, 8.7%) and furanoid linalool oxides (9.8%, 6.5%). The oil of *M. parviflora* was marked by a high content of the main constituent spathulenol (29.9 %). The main terpenoid fraction of *M. parviflora* oil consisted of oxygenated sesquiterpenes, and this contrasts to the oxygenated monoterpenes identified as the dominant class of terpenoids in *M. juliana* and *M. kosaninii* oils.

In conclusion, approximately equal small yield (0.1%) of oils characterized all three plant species. *M. juliana* and *M. kosaninii* oils are similar one to each other (a large number of compounds with low content), while *M. parviflora* oil contains a single dominant component – spathulenol (29.9%).

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Chemical characterization of essential oil constituents from four populations of *Piper aduncum* from Distrito Federal, Brazil

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Piper aduncum is considered a priority species for conservation of genetic resources in Cerrado due the commercial interest in the essential oil (Vieira & Silva, 2002). It is commonly known as aperta-rução and it is used as digestive and against flu, stomachaches, chronic ulcers and as insect repellent (Burke & Nair, 1986; Vieira, 1992).

Leaf samples of *Piper aduncum* were collected in December, 2004, in four different places at the Distrito Federal, Brazil: (1) Brazlândia, (2) córrego Bananal, (3) Parque do Guará, and (4) Água Limpa farm. The essential oil was extracted by hydrodistillation in a modified Clevenger apparatus. The oil composition was analyzed in an Agilent 6890N gas chromatograph fitted with a HP-5 (25m X 0.32mm X 0.25μm) capillary column. The injector was kept at 250°C and 0.05μL of pure oil was injected in split mode (1:100). The oven temperature was programmed from 60°C to 240°C at 3°C/min. Detector (FID) was kept at 280°C. Mass spectra were obtained in an Agilent 5973N system operating in electron impact mode (EIMS) at 70 eV, using the same injection procedure and oven temperature program as above.

The yield of essential oil observed in the four populations of *Piper aduncum* ranged from 0.7% to 1.3%. Individual populations collected at Brazlândia and Parque do Guará presented the same chemical profile, being similar even quantitatively, despite the geographic distance between both locations. The major constituents in both populations were: 4-terpineol, varying between 13.5 and 19.9%, respectively, and piperitone (18.6% and 26.6%). Other constituents were α-pinene, α-terpinene, p-cimene, β-phelandrene, γ-terpinene, sarisan and miristicine.

The essential oil from Córrego Bananal population showed an unique chemical composition, and also differences among individuals within the population, clustering into two groups, based on chemical constituents from the essential oil. The first cluster is formed by major constituents, such as *trans*-β-ocimene, bicyclogermacrene, safrol, and sarisan. The second group presented high content of β-phelandrene, *trans*-β-ocimene, piperitone, γ-terpinene, and 4-terpineol. Água Limpa farm population showed a unique essential oil composition being the only one with high content of dillapiole, ranging from 36.5% to 79.0%. Piperitone (5.9% to 20.0%) and 4-terpineol (2.7% to 9.0%) were also present in this population.

Essential oil composition and antibacterial activity of *Zataria multiflora* growing wild from Iran

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Zataria multiflora Bioss. is an endemic species grown in Iran [1]. Variation in the quantity and quality of the essential oils of wild population of *Z. multiflora* from different locations was reported. The oils of air dried samples were obtained by hydrodistillation. The oils were analyzed by GC and GC-MS [2]. The ranges of major constituents were as follow: linalool (27.4-52.9%), carvacrol (27.2-51.5%), p-cymene (2.1-3.9%), thymol (1.9-3.2%), beta-caryophyllene (1.4-2.8%), and caryophyllene oxid (1.1-1.5 %). Antibacterial activity of the oils and their main compounds were tested against seven Gram-positive and Gram-negative bacteria. Their minimum inhibitory concentration (MIC) values were determined. The maximal inhibition zones and MIC values for bacterial strains, which were sensitive to the essential oil of *Z. multiflora* , were in the range of 25-46 mm and 0.2-7.0 mg/ml. The oils showed high activity against all tested bacteria. Thus, they represent an inexpensive source of natural antibacterial substances that exhibited potential for use in pathogenic systems.

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GC-MS characterisation of kakuti oil obtained from *Ziziphora clinopodioides* Lam.

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Different species of three genera, *thymus, ziziphora* and *zataria* are very popular in Iranian families as flavouring agents and also well-known as medicinal herbs.
Ziziphora clinopodioides Lam. belongs to the *Labiatae* family; this species is endemic to Iran and grows in different parts of the country. Everybody knows the herb and it is called "kakuti" in Persian. Kakuti is used as stomachic, antipyretic, sedative, and anti-inflammatory drug. Besides the application as dried powdered herb it is also used as a common flavouring for drinking yogurt and other dairies. In this research study the essential oils of different kakuti samples were isolated from the plant material by applying the hydro-distillation method according to Clevenger and the main components of the oil were identified by GC and GC-MS. The main components were pulegone (51-55%), p-mentha-1-en-1-ol (20-22.4%), 1,8-cineole (10-12.3%), p-mentha-3,8-diene (3-4.7%), *cis*-isopulegone (1.2-1.7%), bornyl acetate (1-1.5%) and beta-pinene (1.2-1.7%).

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Exploration of the wild populations of *Rosmarinus officinalis* L. and *Lavandula latifolia* Medikus. from Castilla-La Mancha province (Spain): compilation, chemical composition and storage of their seeds.

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An exploration of natural populations of *Rosmarinus officinalis* and *Lavandula latifolia* has been carried out on Castilla-La Mancha province (Spain). A total of 74 populations have been located in this region. The principal aim of this exploration has been to know the state of these species and to analyse the variations of their chemical compositions. Besides, seeds proceeding from these populations have been included in our Germplant Bank in the Agronomical Research Centre of Albaladejito (Cuenca, Spain).
The essential oils from different aerial parts (inflorescences, stems and leaves) of both species have been extracted by steam distillation and analysed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MC). Quantitative and qualitative differences between the analysed populations have been found although the main compounds were the same. *Lavandula latifolia* showed linalool, cineole and camphor as principal constituents amounting around 80% of the total essential oil, while the populations of *Rosmarinus officinalis* contained 1,8-cineole, camphor, α-pinene and camphene as main compounds, constituting around 65% of the oil.
The aromatic and medicinal plants crop needs to find effectives chemotypes adapted to the market demands to be used in breeding and selection programs.

Essential oil composition of the different parts of *Eryngium dilatatum* Lam. from Spain

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The *Eryngium* L. genus belongs to the *Apiaceae* family and, with about 250 species, has a cosmopolitan distribution. In the Iberian Peninsula grow 14 of the 26 species described in Flora Europaea. *Eryngium dilatatum* Lam. is a perennial species that grows in dry places of the mid south in The Iberian Peninsula. 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. However, all of them showed the same principal compound, germacrene D (9.1-46.5%). In the same way, all the fractions shared most of their representative constituents although their percentage compositions were different from one sample to the other: α-cadinol (3.8%), bicyclogermacrene (3.5%), octanal (3.1%) and spathulenol were found in the inflorescences; octanal (8.1%), α-cadinol (3.7%), δ-cadinene (3.6%), (*E*)-caryophyllene (2.6%), bicyclogermacrene (2.5%) and spathulenol (2.4%) in the stems and leaves and, spathulenol (4.6%), α-cadinol (4.4%), khusinol (3.2%), α-muurolol (3.1%) and δ-cadinene (2.6%) in the roots.

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

Essential oil composition of *Eryngium galioides* Lam. from Spain

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The *Eryngium* L. genus belongs to the *Apiaceae* family and, with about 250 species, has a cosmopolitan distribution. In the Iberian Peninsula grow 14 of the 26 species described in Flora Europaea. *Eryngium galioides* Lam. is an small annual plant (15-30 cm) that grows in open dry places of the mid west in The Iberian Peninsula. The essential oil extracted from all the plant (aerial parts and roots) of this species, gathered in Guadalajara (Spain) has been extracted by steam distillation and analysed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MS).

It is worth noting that this species showed a relative high yield (0.48) in comparison with other species of this genus. The essential oil of this species consisted in a complex mixture of more than 70 compounds. The main constituents of the oil were identified as valencene (49.7%) and phyllocladene isomer (23.7%) representing more than the 70% of the total oil. Other representative compounds of the oil were found to be β-chamigrene (6.0%), γ-muurolene (3.4%), (*E*)-caryophyllene (3.0%) and β-elemene (1.6%).

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

Organ- and season-dependent variation of Spanish sage (*Salvia lavandulifolia* Vahl.) essential oil composition

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Spanish sage (*S. lavandulifolia* Vahl.) grows wild in Spain and Southwest France and is used in perfumes and food flavouring [1]. Several reports indicate that extracts of this species have anticholinesterase, antioxidant and anti-inflammatory effects [2]. Its essential oils have been already subjected to several studies, being normally rich in 1,8-cineole, camphor, α- e β-pinene [1].
Since several reports indicate that essential oil constituents from this species are, at least in part, responsible for its anticholinesterase activity [2] we decided to study their season- and organ-dependent variations.
Spanish sage plants growing in Braga (northern Portugal) were collected every two months from April 2006 to April 2007. Small samples (around 5g) of fresh leaves were hydro-distilled and the resulting essential oils were analyzed by GC and GC/MS. In May 2007, during full bloom, samples of stems and flowers were hydro-distilled and analyzed by the same way.
1,8-Cineole, camphor and α-pinene were identified as the main compounds in the essential oils obtained from leaves over the year. Together these three compounds represented 57.5% to 69.3% of the total essential oil. 1,8-Cineole was the major constituent in all samples. Its percentage in the essential oil increased from April 2006 to February 2007 (34.6% - 52.4%), then it decreased in April 2007, reaching levels close to the initial ones (35.4%). Camphor percentages varied inversely, decreasing until February 2007 (from 17.6% to 5.7%) and rising again in April 2007 (8.6%). Globally, α-pinene percentages increased during the assay period (8.1% - 13.7%), except in June 2006, where a small drop was observed (from 8.1% to 6.0%). No thujones were detected.
During flowering (May 2007) we compared the essential oil from stems, flowers and leaves of *S. lavandulifolia*. Contrary to stems and leaves, where 1,8-cineole dominated (26.5% and 25.4%, respectively), flowers yielded high levels of α-pinene (37.6%), which was the main essential oil constituent in these organs. Camphor percentages in the essential oil from flowers were around 6-fold lower than in the essential oils from stems and leaves.

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Composition of the essential oils of *Eucalyptus camaldulensis* Dehn., and *Myrtus communis* L. growing in Northern Cyprus

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Northern Cyprus is rich in aromatic plants. The majority of the people living the countryside of the region use naturally grown plants for medicinal purposes. It is therefore, important to find out the medicinal value of those plants scientifically. The aim of our research,which is carried out for the first time for these two plants grown in Northern Cyprus,is to acquire valuable information about composition of the essential oils.The essential oil compositions of leaves of *Eucalyptus camaldulensis* Dehn., and *Myrtus communis* L., collected from Northern Cyprus, were analysed by GC-MS [1]. The composition of essential oil isolated from *E.camaldulensis* Dehn., leaves were analysed by GC-MS, and 22 constituents were identified, representing 82.09 % of the oil. The major components were ethanone (25.36 %), eucalyptol (13.73 %), β-caryophyllene (11.55 %) and carvacrol (9.05 %) respectively. In the oil of *M. communis* L. 16 components were identified that represented 87.86 % of the oil. Eucalyptol (50.13 %) was identified as the major constituent of the essential oil of *M. communis* L. The other important components were linalol (12.65 %), α-terpineol (7.57 %) and limonene (4.26 %) respectively.
It is well known that environmental conditions of plant can affect composition and content of essential oil.
Key Words: *Eucalyptus camaldulensis* Dehn.; *Myrtus communis* L.; Myrtaceae; essential oil composition; GC-MS

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The Determination of antibacterial effects of some plants of Labiatae growing naturally around Şırnak-Silopi, Turkey

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In this research, the essential oils and the ethanol extracts of *Thymbra spicata* var. *spicata* L., *Cyclotrichium stamineum* (Boiss. & Hohen.) Manden.& Scheng., *Teucrium polium* (Stapf Brig.), *Salvia russellii* Bentham and *Mentha longifolia* L. Hudson var. *calliantha*, plants were tested against *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 29998, *Salmonella cholerasuis* ATCC 14028, *Enterococcus faecalis* RSKK 97008, *Streptococcus mutans* NCTC 10449 and *Sarcinia lutea* ATCC 9341, by using the disc diffusion method [1,2]. The aim of our research,which is carried out for the first time for these plants grown in Şırnak, Turkey, is to acquire valuable information about antibacterial activity againts these strains of the essential oils and extracts.
The oils of *T. spicata* var. *spicata* and *C. stamineum* were effective on *S. aureus, B. cereus, E. coli* and *S. cholerasuis* strains, whereas the oils of *S. russellii* and *M. longifolia* var. *calliantha* were effective on *S. aureus, B. cereus, E. coli, S. cholerasuis* and *E. faecalis* strains, on the other hand, the oil of *T. polium* was effective on *S. aureus, B. cereus, E. coli* and *E. faecalis* strains. When the essential oil discs were used together with the control antibiotic discs (Chloramphenicol 30 µg) for the antibacteriological tests, *S. lutea* and *S. mutans* bacteria did not show any reproduction performances in their petries. The extract of *T. spicata* var. *spicata* was effective on *S. aureus, E. coli, S. mutans* and *S. lutea* strains, while the extracts of *C. stamineum, S. russellii* and *T. polium* were effective on *S. mutans* strain and than, the extract of *M. longifolia* var. *calliantha* was effective on *E. coli* strain. The essential oils were found more effective than the extracts. These results confirm the possibility of using these plants in food systems, medicine and pharmacy.

Key Words: Labiatae, essential oils, ethanol extracts, disc diffusion method, antibacterial effect

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Variation in the Essential Oil composition of *Rosmarinus officinalis* L. from differents Spanish locations from Andalucia region

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Rosemary (*Rosmarinus officinalis* L.) is a spontaneous shrub growing in the Mediterranean area. There exist several rosemary species all over the world: *R. officinalis*, *R. eriocalyx*, *R. laxiflorus* and *R. lavandulaceus*. The differences between plants and oils composition have been previously correlated to differences in the substrate [1,2,3]. In this work, plant material from spontaneous populations of *R. officinalis* was collected in different locations from the Spanish region of Andalucia: Cadiz, Cordoba, Jaen, Malaga and Sevilla, in order to select those populations with the highest essential oil yield and the best profile according to the International Standard Limits on Oil of Rosemery (2nd edition, 2000). The composition and chemical polymorphism of the essential oils were analysed by GC with a Hewlett Packard 6890 Series instrument equipped with an FID and HP-5 capillary columns (30m x 0.25mm, 0.25µm film thickness), working with the following temperature program: 70ºC for ten minutes and then ramp of 3ºC/min to 220ºC; injector and detector temperature, 250ºC; carrier gas nitrogen was adjusted to a flow of 2ml/min. The samples were injected using the split mode (split ratio 1:30) and an injection volume of 0.2µL. Identification of α-pinene, camphene, β-pinene, myrcene, limonene, 1-8 cineol, γ-terpinene, p-cimene, bornil acetate, camphor, borneol, α-terpineol and verbenone was performed by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of n-hydrocarbons.

The essential oils were obtained by hydrodistillation of the dried ground material in a Clevenger-like apparatus for 2h at atmospheric pressure on about 100g of sample. Time was measured from the falling of the first drop of distillate. For each sample two replications of each extraction were done. The essential oil yield was evaluated gravimetrically.

Our results show that only samples from Cadiz and Cordoba fulfil the demanded limits. Samples from Jaen show lower values than those required for every compound, except for myrcene, borneol and verbenone; samples from Malaga are quite close to the limits, with only camphor values being lower than the former; samples from Sevilla show that most components have lower values than the inferior limits demanded by the International Standard quality rules.

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Essential Oil Composition of *Tanacetum* L. *kotschyi* (Boiss.) Grierson from Turkey

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T. kotschyi is a small perennial herb growing on limestone slopes, crevices and screes in eastern and southeastern parts of Anatolia [1]. Previously there is no report on the chemical composition and essential oil composition of *T. kotschyi.* Plant material is collected from eastern province of Turkey Güzeldere-Van at 2800 m altitude. Yellowish essential oils are obtained by 4h hydrodistillation from the flowers and stems of *T. kotschyi* with the yields 0.25% and 0.15% (v/w) respectively. Analysis were performed on Agilent 5975 GC-MSD and Agilent 6890N GC-FID systems equipped with Innowax FSC coloumn simultaneously. Relative percentages of the compounds were calculated from FID chromatograms. Identification of the essential oil components were perfomed by comparision of their RRI and with computer matching against commercial GC/MS libraries and in- house "Başer Library of Essential Oil Constituents". 90.5% and 81.3% of the flower and stem oils were identified respectively. Main composition of essential oils are artemisia ketone (54.6%, 26.5%), longiverbenone (9.2%, 8.9%), artemisia alcohol (4.6%, 5.2%), yomogi alcohol (4.6%, 2.4%), intermedol (only in stem oil 9.0%) in flower and stem oils of *T. kotschyi* respectively . Also an unknown compound with M+280 and bp 108 (5.5%, 9.3%) is present in both oils. Previously some chemotypes of *T.vulgare* was reported to have high content of artemisia ketone in their essential oils however there are no other reports on other *Tanacetum* species with high content of this compound is reported [2-4].

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Intraspecific and developmental variability of wormwood (*Artemisia absinthium* L.) in respect of the essential oil content and composition

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Ex situ study on accumulation of essential oil in five populations of wormwood (*Artemisia absinthium* L.) was carried out. Two populations originated from the natural sites in the north-eastern Poland (Siemiatycze and Wandalin) and three from Belgium, Hungary, and France. Wormwood herb was harvested from three-year-old plants at three stages of their development: vegetative stage (beginning of June), flower bud formation (mid July), and full blooming (August). The herb was dried at 35°C. The content of essential oil in the raw material was determined by hydrodistillation method [1]. For the qualitative analysis of essential oil gas chromatography was used, with the parameters as follows: gas chromatograph HP 6890, capillary column Carbowax 20M (25 m × 0.32 mm; 0.3 µm film thickness), detector temperature 250°C, injector temperature 220°C, split 1:70, programmed oven temperature: from 60°C (2 min) to 220°C (held for 5 min) at the rate of 4°C min^{-1}, helium as a carrier gas at a flow rate 2.1 ml min^{-1}. The separated constituents of essential oil were identified by comparing their retention times with the retention times of standards. Sabinyl acetate, chrysanthenyl acetate and Z-(myroxide) were identified on the basis of GC-MS.

The investigated populations differed in the content and composition of essential oil. These parameters were also affected by the stage of plant development. The highest content of essential oil in herb was characteristic for the population from France (0.63-0.76% v/w) and the lowest one for the population from Hungary (0.19-0.29% v/w). In all populations blooming plants were characterised by a slightly higher content of essential oil in comparison with those being at vegetative stage of development. A dominant constituent of the essential oil obtained from the plants of the population from France was sabinyl acetate. High content of this compound was also found in the volatile oil of the population from Hungary. However, only at vegetative stage of plant development it was definitely a dominant compound, whereas at the stage of flower bud formation its content was similar to that of caryophyllene oxide, and at the stage of full blooming to that of linalool. In the essential oil of the population from Belgium the main compounds were (Z)-myroxide and beta-myrcene. Plants of the population from Siemiatycze were characterised by high content of beta-thujone in essential oil, especially at the stage of full blooming. In the essential oil of plants of this population collected before blooming chrysanthenyl acetate and cineol were found as co-dominant compounds. The main constituents of the essential oil obtained from the plants of the population from Wandalin were sabinyl acetate and beta-thujone. The former appeared to be a dominant constituent of essential oil at the vegetative stage of plant development, whereas the latter at the stage of full blooming. In all populations at the stage of plant blooming the content of linalool in essential oil was higher than at the vegetative stage.

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Volatile organic compounds from seeds of *Picea abies* L. and *Abies alba* Mill.

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Norway spruce (*Picea abies*) and Silver fir (*Abies alba*) are one of the most common industrially important tree species in Europe. The essential oils from different tree's parts of these genus are used commercially in the cosmetic and fragrance industry, especially the oil from fir cetin.

Many studies concerning the composition of essential oils of spruce bark, wood and needles, also oils of fir needles and cetin have been reported. However there is no information about the yield and the composition of volatiles from seeds of spruce and fir.

The yield and the composition of essential oils from seeds of *P. abies* and *A. alba* differed strongly. The yield of spruce seeds was 0.08%, while the content of volatiles in fir seeds was over seven times higher (7.3%). Chemical composition of hydrodistilled oils were evaluated by GC and GC-MS. In order to carry out a detailed analysis, the oil from fir seeds was repeatedly fractionated during flash chromatography. Chosen fractions were analysed by means of NMR.

Spruce oil was characterised by very high amounts of diterpene hydrocarbons (50%) and oxygenated diterpenes (25%), the monoterpene fraction was relatively small – 15%. While in essential oil obtained from fir seeds the main group was monoterpenes, constituted almost 90% of essential oil. In total more than 50 and more than 80 constituents were identified in hydrodistilled oil of spruce and fir seed, respectively. The main volatiles of spruce seeds essential oil were kaur-6-ene (11.9%), abieta-7,13-diene (7.8%), cembrene (4.2%), α- and β-pinene (5.4% and 6.7%), tricyclene (5.3%) and limonene (3.7%). The oil of *Abies alba* seeds was characterised by large amount of (-)-limonene (73.4%), and also α-pinene (9.3%), mircene (2.8), β-pinene (1.6%). It may be concluded that seeds of Silver fir are rich source of essential oil and pure (-)-limonene.

Chemical composition of essential oil and superctitical carbon dioxide extract of savory (*Satureja spicata* L.)

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Savory *(Satureja spicata* L. subsp. *montana)* is an aromatic herb, growing wild on barren and sunny places along the submediterranean, rather rocky, part of Montenegro as endemic species. It is recognized among domestic population as a honey plant which is used in folk remedies [1].

The essential oil of aromatic herbs has been usually isolated by hydrodistillation as traditional spice-processing methods. This technique presents serious drawbacks: low extraction efficiency, long extraction time, deterioration of the thermally sensitive materials etc.
Because of that, in recent times supercritical carbon dioxide extraction has gained increasing attention in the recovery of essential oils, since the use of CO_2 as solvent protects extract from thermal degradation and solvent contamination [2].

In this study hydrodistillation and supercritical extraction of savory herba were carried out to evaluate the essential oil and extract yield and chemical composition, as well as essential oil composition from different herb sizes.

For hydrodistillation experiments Clevenger apparatus was used while for the supercritical extraction experiments conditions were: temperature 40°C, pressure 100 bar, extraction time 360 min and CO_2 flow rate 0.3 kg CO_2/h.
Herb was pretreated mechanically in the blender while sieving was the technique employed for obtaining the desired particle sizes. The yield and the composition of the gained essential oil were evaluated by GC-FID using a VARIAN 3400 Gas Chromatograph with a DB-5 (30m × 0.32mm, film thickness 25μm).

The constituents of extracts were identified by comparing their retention times with those of available standards and their mass was calculated from a predetermined peak area response factor.
The yield of obtained essential oil from hydrodistillation and supercritical extraction was 0,7% (w/w) and 0,5% (w/w), on dry herb basis.
In essential oil, 16 compounds were identified: α-pinene was the most abundant component within investigated herb while β-cubebene and γ-cadinene also gave high yields.
In supercritical extract, 18 compounds were identified: α-pinene and γ-cadinene were the most abundant components.

From the hydrodistillation od different herb particle size, it was difficult to pick out a specific trend, but there is an indication that the quantity of essential oils increases with a decrease in herb particle size.

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Essential oil composition and antimicrobial activity of *Rosmarinus tournefortii* De Noé an endemic species in Morocco

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This study was designed to investigate for the first time the essential oil of the leaves of wild growing *Rosmarinus tournefortii* De Noe endemic to Morocco, in order to find new bioactive natural products. The essential oil obtained by hydrodistillation was characterized by capillary gas chromatography/ mass spectrometry. The yield of the oil was 0.65% (w/w), based on dry weight. Ten components, representing 70% of the total essential oil, were identified. Camphor (17.3%), tricyclene (14%) and, α-pinene (13.9%) were found to be the main components. The oil was screened for antimicrobial activity against both Gram positive (*Staphylococcus aureus*, *Enterococcus hirae*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and a pathogenic fungus *Candida albicans*. Except for *P. aeruginosa*, which showed tolerance, the oil had pronounced antibacterial and antifungal activities.

Keywords: *Rosmarinus tournefortii*; essential oil; antibacterial activity; antifungal activity

Inhibitory Effects of Thai Herb Essential Oils and Their compositions on the Formation of *N*-Nitrosodimethylamine

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More than a hundred compounds were found in 9 essential oils extracted from Thai herbs by using steam distillation and identified by GC/MS. It was found that β-caryophyllene were main compound in black pepper and holy basil while citral occupied a large proportion in lemongrass. Methyl chavicol was predominant compound found in sweet basil. Lesser galanga had main part with β-ocimene, camphor and 1,8-cineole. Kaffir lime leaves composed of high proportion in citronellal and linalool. Ginger contained citral as predominant component. Turmeric composed of high amount of turmerone and β-sesquiphellandrene. 1,8-Cineole was the major component in galanga. α-Pinene, β-pinene, myrcene, α-terpinene, γ-terpinene, α-terpinolene, linalool, citral and α-terpineol were reported that inhibited on the formation of *N*-Nitrosodimethylamine. Those volatiles were found various amounts in these essential oils. Thus, this paper was proved the inhibitory effect of some essential oils from popular Thai herbs in the aspect of the functional property of flavor. The data displayed the inhibitory activity of 9 essential oils on the formation of *N*- Nitrosodimethylamine from 55.3 to 96.9% in sweet basil(55.3%), holy basil(65.9%), lemongrass(67.5%), Kaffir lime leaves(68.9%), black pepper(73.8%), galanga(75.9%), ginger(89.4%), turmeric(93.4%) and lesser galangal(96.9%).

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Antimycobacterial Extracts from *Telekia speciosa* (Schreb.) Baumg

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Mycobacterium tuberculosis is one of the most important human pathogens which causes worldwide a greater than 8 million new infections annually and more than 3 million people die of this disease each year. Thus there is an urgent need for new effective antimycobacterial agents.

In this instance, the **aim** of this study was to evaluate the *in vitro* antimycobacterial activity of two crude plant extracts; the root extract (**Tr**) and the leaf extract (**Tf**) of ***Telekia speciosa*** (Schreb.) Baumg. For each type of extract, the dried material plant was extracted with hexan, the solvent was evaporated and then the residue was washed with an ethanol-water (2:1) mixture. The test of antimycobacterial activity was performed by the conventional method on Lowenstein-Jensen medium, using eleven *M.tuberculosis* strains.

The samples (**Tr, Tf**) were chemically investigated for their active constituents by gas-chromatographic analysis. The MIC values (μg/ml) for **Tf** ranged from 64 to 128 μg/ml, and for **Tr** is 32 μg/ml. The gas-chromatographic study of leaf extract of ***Telekia speciosa***, which exhibited significant activity against *M. tuberculosis*, provided the known eudesmanolides alantolactone and isoalantolactone (1, 2).

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The Chemical Composition and the Antimicrobial Activity of the Volatile Oil Extracted from the Aerial Parts of two *Origanum vulgare* Natural Populations from Romania

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In a phytochemical and biochemical study on some spontaneous populations of *Origanum vulgare* L. harvested in Romania, in which we monitored the influence of some secondary mertabolites bioaccumulation, we analyzed, by means of GC/MS, and tested the antimicrobial action of volatile fractions separated from two samples of *Origani herba* originated from neighbouring geographical locations.

Although the plants have grown in close geographical areals, with similar climate, light and soil conditions, there was an evident bioaccumulation difference, the volatile oil content being two times and a half greater than in the case of the product originating from Petru Voda, compared to the one from Baltatesti (1.0:0.40). In its turn, the chemical composition of two volatile oils also proved different, from both the qualitative point of view and, especially, from the quantitative one.

Observing the reflection of the chemical differences between the two samples of *Origani aetheroleum* in their biological action, we investigated their antimicrobial activity, compared to the positive controls, chloramphenicol (30 μg) and nistatine (100 μg).

Though the volatile oils of *Origanum* are well known for their antimicrobial potential (1, 2, 3), in case of the investigated Romanian samples, we noticed that the antifungic action is confirmed by the data from scientific literature (table 1).

Table 1. The antimicrobial activity of volatile oils obtained from the aerial plant parts harvested from two samples of *Origanum vulgare*

Microorganisms test	Diametre of the inhibition area (mm)			
	Samples		Standards	
	PV	B	Chloramphenicol	Nistatine
Sarcina lutea	7	8	29	-
Staphylococcus aureus	10	9	20	-
Bacillus cereus	16	16	24	-
Bacillus subtilis	15	15	30	-
Escherichia coli	0	0	20	-
Pseudomonas aeruginosa	0	0	30	-
Candida albicans	30	30	-	30

Concluding, we may assert that the results obtained show the fact that the two oils are chemically different, moderately active to the sporulated bacillus, very active to *Candida*, but developing a reduced activity to the other micro-organisms.

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The Chemical and Microbiological Profile of the Volatile Oil Isolated from Lavender Species Cultivated in Romania

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Lavandula angustifolia Mill., is a fragrant perennial herb with small, blue-violet flowers, which grows in the Mediterranean areas. Lavender is largely employed for its sedative, anxiolytic, diuretic properties and to relieve insomnia. In this paper we describe the *in vitro* antimicrobial activity of volatile oils from lavender and the possibility of their synergistic interactions with other antibacterial agents has also been investigated (1, 2).

The essential oils of five samples of *Lavandula*, grown in Romania, obtained by hydrodistillation of aerial parts were studied for their antimicrobial activity. Material plants were five varieties of different lavender species: *L. angustifolia* spp *angustifolia*, *L. hybrida* ssp. *pyrenaica*, *L. hybrida* var. *Fundulea*, *L. angustifolia* var. *Hidicote Blue*, *L. angustifolia* var. *Munstead*. Chemical compositions of the essential oils were also determined by GC-MS analysis.

The qualitative antimicrobial activity was evaluated by the agar diffusion method. The results obtained in the antimicrobial assay showed that all oil samples have activity against *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus subtilis*, *Bacillus cereus* while Gram-negative bacteria were not affected. The oils exhibited the strongest activity against *Candida albicans* and *Candida sake*. A standard antibiotic Chloramphenicol, 30 µg/disc (Oxoid) and Nystatin 100 µg/disc (Hi Media, Laboratories) were used as a standard for comparison. The major component of the oil samples from different species of Lavandula was found to be linalool (24.22-36.33 followed by 4-terpineol (3.53-9.68%). The antimicrobial activity was attributed to monocyclic terpene (as terpinen-4-ol or linalool).

Evaluation of synergistic interactions of oil samples has explored in association with 10 µg ampicillin disc (Bio-Rad), 30 µg chloramphenicol disc (Oxoid), 5 µg ofloxacin disc (Oxoid), 30 µg amikacin disk (Oxoid) and 100 µg Nystatin disc (Hi Media Laboratories) respectively. The reason for using the combination of antimicrobial agents is based in part on to their possible synergistic effect.

The greatest action was observed with all lavender oil samples against yeasts and *B. cereus*.

The lavender oils exhibited moderate or pronounced activity against the Gram-positive bacteria but *E. coli* are insensitive.

Comparing the activity of all oils tested, our results show that oil from *L. angustifolia* var. *Hidicote Blue* was the most active against tested organisms.

A synergistic interaction was observed with all organisms for each of the oil-antimicrobial agent combinations. The synergism was greatest with the combination of oil sample and Nystatin. The results confirm the antimicrobial effects of lavender oil, especially their antifungal action.

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Chemical composition of *Nigella nigellastrum* essential oil and its chemotaxonomical aspect

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Until recently, family Ranunculaceae was generally considered as a taxon not containing essential oils [1]. However, during last years several works describing essential oils in seeds of four species of genus *Nigella* have appeared in literature [2,3,4,5]. Moreover, the most recent papers describe presence of volatile oils also in genera *Aquilegia* [6] and *Delphinium* [7]. Therefore, we have carried out analysis of *Nigella nigellastrum* Willk. seeds essential oil by GC and GC-MS with aim to contribute to understanding the chemotaxonomical distribution of terpenoid compounds within the family Ranunculaceae.
The oil was isolated from the seeds of the plants grown in the Czech Republic. The distillation yielded 0.028% w/w of essential oil of pale yellow color. In total, 23 components were identified, accounting for 92.7% of total oil content. Monoterpene hydrocarbons, mainly α-pinene (43.0%), and β-pinene (45.78%) were the main constituents accounting together for almost 89% of the essential oil. Among other constituents were diterpenes pimaradiene (1.22%) and neocembrene A (0.90%), followed by sesquiterpene hydrocarbons (0.66%) and oxygenated monoterpenes (0.37%). Our findings are in correspondence with previous works reporting presence of essential oils in Ranunculaceae. Moreover, it appears that *N. nigellastrum* contains an essential oil related in its composition rather to *N. sativa* and *N. arvensis* than to *N. damascena* or *N. orientalis* which were shown to contain predominantly sesquiterpenes, such as β-elemene [3,4], whereas essential oils from *N. sativa* and *N. arvensis* seem to be similar in prevalence of monoterpenes [2,5].

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Tahitian biodiversity as new sources of fragrances and bioactive compounds

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In the last decade, there has been a growing demand for new natural products in the areas of perfumes, cosmetics, and medicine. Considering the large and very rich biodiversity of French Polynesia, essential oils of indigenous Tahitian plants might represent a valuable source of natural bioactive compounds and original fragrances. Therefore, considerable biotechnological attention has been given to these plants, particularly to the chemical composition of the aromatic volatile fraction, which in turn will determine their potential applications in various industrial sectors.

Hydrodistillation of plant material and analyses by gas chromatography and mass spectrometry of essential oils have permitten to select the most interesting aromatic profiles among all the chemotypes designated. Biotests have then been carried out on the essential oils to assess the antioxidant, antimicrobial and repellency activities, which could be of interest for agro-food and cosmetic industry. In the near future, other biological tests will be developed for medical applications. The experimental procedure and results will be presented on one of our model plants : *Psidium cattleyanum* L.

Key words : essential oils, French Polynesia, antioxidant, antimicrobial, repellency

Effect of Portuguese *Thymus* chemical variability on antiacetylcholinesterase activity

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Eleven *Thymus* species have been recorded for Portugal included in five sections of this genus. The large majority of Portuguese *Thymus* taxa are characterized by a major chemical polymorphism [1]. Although both the antimicrobial and antioxidant activities of Portuguese *Thymus* taxa essential oils have been subject to several studies [2,3], much less is known on their acetylcholinesterase (AChE) ability, only one species having been evaluated for this activity [4]. Acetylcholinesterase (AChE) is a human enzyme that hydrolyzes the acetylcholine neurotransmitter, thereby terminating signal transmission at the synapses. An acetylcholinesterase inhibitor will increase the level as well as the extent of action of the acetylcholine neurotransmitter. Compounds with this property will be interesting in the treatment of Alzheimer's disease, several types of plant extracts and essential oils now being assayed for their antiacetylcholinesterase activity [5,6].

In the present work, the antiacetylcholinesterase activity of five Portuguese *Thymus* species, collected in different regions of Portugal, mainland and Azores islands, was screened. The activity was assessed as previously reported [4].

Th. zygis subsp. *zygis* essential oil was the best acetylcholinesterase inhibitor, with an IC_{50}=1±0.02mg/L. This value was significantly lower than that obtained with the remaining *Thymus* taxa essential oils. For example, and still considering the same species, *Th. zygis* subsp. *sylvestris*, the oils isolated from plants collected at two other different locations, showed poorer acetylcholinesterase inhibiting capacity, since IC_{50} ranged from 980±14 to 1766±49mg/L.

The IC_{50} obtained with the essential oils of *Thymus carnosus* and *Th. camphoratus* ranged from 87±3 to 159mg/L±4 and from 115±9 to 195±8mg/L, respectively.

Th. capitellatus essential oils, isolated from plants collected in two different locations, showed again significantly different IC_{50}, 125±2 and 561±59mg/L, respectively.

Th. caespititius oils were among those that showed the highest IC_{50} differences. Whereas the oils isolated from plants collected in the mainland showed an IC_{50} = 12448±52mg/L, plants collected in Terceira (Azores Island), yielded an oil with an IC_{50} = 139±3mg/L.

The differences in acetylcholinesterase inhibiting capacity are probably mainly due to the high chemical polymorphism of Portuguese *Thymus* taxa.

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Chemical Composition and Antimicrobial Activity of the Essential Oils of three Chemotypes of *Lippia graveolens* HBK from Guatemala.

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The variability of the essential oils of *Lippia graveolens* HBK (Family: Verbenaceae) from eight populations cultivated in one experimental field and from 50 individuals from nine populations of Guatemala was evaluated. *L. graveolens*, a shrub to 2 m tall known as Mexican oregano grows wild in arid regions in the east of Guatemala. The leaves of *L. graveolens*, are used for treatment of colds, bronchitis and as seasoning for food preparations.

The aim of the study was to apply the Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) multivariate techniques to differentiate the chemotypes of *L. graveolens* based on their essential oil composition and to evaluate the antimicrobial activity of the essential oil of the three chemotypes. Essential oils were extracted by hydrodistillation using a Clevenger apparatus and analyzed by Gas Chromatography with Mass and Flame Ionization Detectors. The compounds were identified by their mass spectra, external standards as reference and retention indices. The results of chemical composition were submitted to PCA and HCA. Essential oils were tested for antimicrobial activity (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*).

Three chemotypes have been described previously for *L. graveolens* from Guatemala (thymol, carvacrol and mixed chemotypes), showing differences in yield and composition among the essential oils from experimental cultivation of *L. graveolens* from five different populations with thymol (6.8-80.6%), carvacrol (1.1-44.2%), β-caryophyllene (2.8-8.7%) and p-cymene (2.7-6.9%) as major components [1].

The three chemotypes of *L. graveolens* were found in this study. There was coincidence between the results obtained by PCA and HCA with the major compounds in the essential oils. The samples from the experimental cultivation were classified as six corresponding to thymol chemotype, one corresponding to carvacrol chemotype and one corresponding to mixed chemotype. Previously, it had been assumed that populations of *L. graveolens* from the same region would correspond to chemotypes differentiated due to the existence of various climatic regions [2]. In this study more than one chemotype was found in individuals of the same populations. The explanation for this difference lies on the fact that in previous studies only composite samples coming from a large number of plant individuals were analyzed, thus, yielding essential oil average compositions. Samples of populations located to the west of the region of study showed higher proportion of mixed chemotype, central populatons showed predominance of thymol chemotype and samples from the east showed predominance of carvacrol chemotype.

It had been reported previously that the essential oil of two samples of *L. graveolens* from Guatemala showed activity against Gram-positive and Gram-negative bacteria and fungi [2]. In the present work the essential oils of the three chemotypes showed antimicrobial and antifungal activities. The thymol and carvacrol chemotypes showed higher activity than the mixed chemotype. Bioautographic analyses showed that the activity is caused mainly by thymol and carvacrol.

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The role of the essential oils from three South African *Salvia* species on the biological activities of their solvent extracts

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Many South African *Salvia* species are used in traditional medicine to treat various diseases such as malaria and microbial infections. As the solvent extracts of aromatic plants contain both volatile (e.g. essential oils) and non-volatile compounds the purpose of the study was to explore the specific contribution of the two fractions to the biological activities. The antibacterial and antiplasmodial activity of the solvent extracts of three South African *Salvia* species (*Salvia africana-caerulea*, *S. africana-lutea* and *S. lanceolata*) were evaluated in the presence and absence of the essential oils. The solvent extract of *S. africana-caerulea* free of essential oil exhibited the best activity against Gram-positive bacteria (MIC value: 0.4 mg/mL), while the solvent extract containing essential oil of *S. lanceolata* was the most active against Gram-negative bacteria (MIC value: 2.0 mg/mL). No significant difference was observed in the antiplasmodial activity for the solvent extract with or without the essential oils of *S. africana-caerulea* and *S. lanceolata*, while the activity of the solvent extract without essential oil was significantly higher than that of the solvent extract containing the essential oil in *S. africana-lutea* ($p < 0.05$). The toxicity profile of all three species was significantly higher ($p < 0.05$) with the solvent extracts containing essential oils than when assessed in the absence of the essential oil. The major compounds of the essential oil include spathulenol (29.1%), β-caryophyllene oxide (14.6%) followed by ledol (6.5%) and α-pinene (4.1%) for *S. africa-caerulea*, while myrcene (11.5%), *p*-cymene (7.6%), α-pinene (6.0%) and *cis*-β-ocimene (5.4%) being the major components in *S. africana-luta*. The major compounds in *S. lanceolata* include spathulenol (18.3%), β-caryophyllene oxide (14.3%) followed by β-caryophyllene (5.7%) and ledol (5.2%).

The effect of five common essential oil components on *Listeria monocytogenes* biofilms

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Many essential oils have been used since antiquity to control pathogenic diseases. Previous investigations on the essential oil components as antimicrobial agents have acutely focused on the planktonic bacteria with less emphasis on the biofilms which are more resistant to antimicrobial agents. In this study, the effect of five essential oil components (EOC's) (α-pinene, 1,8-cineole, (+)-limonene, linalool and geranyl acetate) on biofilm growth and development of two pathogenic *Listeria monocytogenes* isolates were investigated. Growth of the biofilm was assessed using the crystal violet (CV) staining assay and the XTT (a tetrazolium derivative) reduction assay. Scanning electron microscopy (SEM) was used to assess the architectural changes of the biofilm. Treatment of a 6 h preformed biofilm with each of the EOC's at a concentration of 1 mg/ml enhanced growth of the biofilm which was confirmed by SEM. A complex, multilayered biofilm showing more cell-to-cell attachment and less surface adhesion was observed after treatment with the EOC's. In the presence of the EOC's, the metabolic activity of the biofilms was distinctly reduced after 6 h of incubation as noted for the XTT reduction assay. These preliminary results justify further research to better understand the possible mechanism by which essential oils (and specific constituents) affect biofilm development.

Extraction of volatile oil from *Vitex agnus castus* by supercritical CO_2

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Introduction: Supercritical fluid extraction (SFE) from vegetable matter, of compounds responsible for the fragrance and aroma, is an industry in expansion. Indeed, there is considerable interest in replacing the water or steam distillation and solvent extraction processes traditionally used to obtain these products. Supercritical CO_2 is the solvent of choice in this field. Supercritical CO_2 behaves like a lipophilic solvent but, compared to common liquids, it has the advantage that its selectivity and solvent power is adjustable and can be set to values ranging from gas-like to liquid-like. Correct reproduction of the natural fragrance in a concentrated extract, devoid of traces of organic solvents, is obtained because the mild conditions adopted avoid thermal degradation and limits hydrolysis and of hydrosolubilization. Chaste tree (*Vitex agnus castus* L., Verbenaceae) is a shrub of up to 5 m in height and can often be encountered in humid and mild regions of central Europe and Asia. The brown or nearly black coloured seeds of *Vitex agnus castus* L. ripen in stony berries and give a pepper-like taste an smell [1]. The fruits as well as leaves and flowers contain an essential oil, which is reported to vary in content and composition according to geographical origin [2].
Materials: Leaves, flowers and berries of *Vitex agnus castus* have been collected in Ogliastra, Sardinia-Italy on May, July and August 2007, respectively. Plant materials were air-dried at room temperature in the shade, for three weeks. Before SFE, the vegetable matter was ground and sieved (300–800 μm).
Hydrodistillation: Hydrodistillation of the leaves, flowers and berries of *Vitex agnus castus* were separately done using a Clevenger-type apparatus. In all cases, about 100 g of material were treated.
Apparatus for SFE: Supercritical CO_2 extraction were performed in a laboratory apparatus equipped with a 320 cm^3 extraction vessel and two separators connected in series.
GC/MS analysis: A gas chromatograph, GC, AGILENT Technologies Inc. (Santa Clara, CA, USA) model 6890N coupled with a quadrupole mass spectrometer, MS, AGILENT model 5973 was employed for analysis of the extracts. It was equipped with a split-splitless injector, an autosampler AGILENT model 7683 and an AGILENT HP5 fused silica column. Compounds were identified by matching their mass spectra and retention times with those reported in the literature [3-4]. Moreover, whenever possible, identification has been confirmed by injection of authentic compound.
Results: The volatile concentrate of leaves, flowers and berries of *Vitex agnus castus* were obtained by SFE at 90 bar and 50 °C (CO_2 density, ρ_{CO2} = 0.287 g cm^{-3}). The volatile concentrate content, relative to the SFE process, varied greatly according to the plant part extracted; the maximum oil yield was obtained from the berries (2.2%), followed by leaves and flowers (0.5%). The three oils shared the same main constituents but differences were noticeable in percentages. These compounds were: bicyclogermacrene (13.6, 13.7 and 15.7% in leaves, flowers and berries respectively), (E)-β-farnesene (9.2, 8.3 and 16.9%), 1,8-cineole (11.2, 4.1 and 9.1%),) and manool (5.3, 8.2 and 5.9%). The oils obtained by hydrodistillation showed quite similar composition

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Chemical composition and potential uses of essential oils from leaves and flowers of *Tephrosia vogelii* and *Bidens tripartite* for curing of dermatophytoses.

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The chemical composition and the antidermatophytic properties of essential oils from leaves and flowers of *Tephrosia vogelii* (Leguminosea) and *Bidens tripartite* (Asteraceae) were studied with the aim to find out an essential oil that can be readily used to cure dermatophytoses. These two plant species are used in traditional medicine in Cameroon to cure skin diseases. Hydrodistillation using a Clevenger-type apparatus was the method used for the essential oils preparation. The flowers oil from *Tephrosia vogelii* (the most antidermatophitic among the oils) was fractionated by column liquid chromatography. The crude oils and the fractions obtained after column chromatography were subjected to GC/MS analyses. The Agar dilution method[1] was used to investigate the *in vitro* antidermatophytic activities of oils and fractions against three fungal species (*Microsporum gypseum, Trichophyton mentagrophytes and trichophyton terretre*). The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were determined by the macrodilution method[2]. Microscopic examination of the resulting culture enabled us to evaluate the effects of the essential oils and fractions on conidia production and fungal morphology. Young guinea pigs (two month of age) infected with *Trichophyton mentagrophytes* were used to evaluate the *in vivo* antidermatophytic activity of *Tephrosia vogelii* flowers essential oil[3]. The treatment period was 21 days.

Essential oils were obtained with a yield of 0.6 (flowers of *T. vogelii*) and 0.1% (leaves of *T. vogelii*, leaves and lowers of *B. tripartite*). The flower oil contained α-pinene (11.7%), limonene (6.1 %), (E)-β-ocimene (7.7 %), (Z)-β-ocimene (8.9 %), and germacrene D (7.2 %) as major components while the main components of the leaves oil were: α – terpinene (38.1 %), p-cymene (20.3 %), α -pinene (12.0 %) and germacrene D (8.1 %). The essential oil from *B. tripartita* where mainly made up of α-pinène (7.0 % and 16.7 %), sabinene (16.0 % and 21.0 %), limonene (13.6 % and 23.7 %) respectively for flowers and leaves plus α-curcumene (5.4 %) in flowers and 4-terpineol (6.7%) in leaves. All the oils and fractions exhibited antifungal activities against the three dermatophyte species. The flower oil of *T. vogelii* (MIC = MFC = 2.5mg/ml) appeared to be more active than the others (MIC = 5 – 10 mg/ml, MFC = 10 – 15mg/ml). The six fractions obtained from the flower oil of *T. vogelii* showed qualitative and quantitative differences in their chemical composition and in their antidermatophytic activities. Fractions F_C, F_D and F_E (MIC = 0.156 – 0.625 mg/ml, MFC = 0.312 – 1.25 mg/ml) were more efficient when compared to crude oil. Some of the oils and fractions at low concentrations induced an increase in the production of macroconidia and resistance structures like chlamydospores. But at higher concentrations, these substances induced morphological changes on mycelia and conidia and in some cases their destruction.

The ointment of the oil (5%) prepared in kernel palm oil control established ringworm infection in guinea-pigs in 21 days against 15 days for reference drug griseofulvin, showing no culture recovery on treated animals. These results suggest that the essential oil from flowers of *T. vogelii* can be use as ointment to cure dermatophytoses although toxicity tests should be carried out before.

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Effects of volatile oil of extracts of *Artemisia sieberi (A. herba-alba)* growing in Jordan on the fertility in normal and alloxan induced diabetic male rats

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Artemisia herba-alba Asso. (Syn: A. inculta Del.), commonly known as the desert wormwood (Arabic name: Sheeh), is a dwarf shrub (or semi shrub) growing widely in Jordan and in the Middle East. Our study carried out to investigate the effects of volatile oil of extracts of *Artemisia sieberi (herba-alba)* growing in Jordan on the fertility in normal and alloxan induced diabetic male rats. The oil (average yield: 1.3% v/w of dried material) about 11.2% (mL/kg dried aerial parts) was collected and stored at 4°C. After the separation of volatile oil, the oil was diluted by DMSO (Dimethyl sulfoxide) in different ratio. The effective dose (Therapeutic dose) which was given orally was either 1/10 or 1/20 of LD50 (1.37 ml/kg). A total of 50 rats were used in the experiment. Twenty rats were used as control and thirty rats were used as alloxan induced diabetic received 10 mL/kg tap water and were served as control and orally diluted volatile oil of *Artemisia sieberi.* The results show that the oil of *Artemisia herba alba* produced a significant hypoglycemic effect in diabetic rats within 4 weeks, indicating that the plant oil stimulates the glucose utilization by peripheral tissue, In treated diabetic rats free testosterone level increased within 8 weeks compared to the control ($p < 0.05$). Testes sperm count were significantly reduced ($p < 0.05$) in diabetic male rats compared with control. However, daily production of the sperm in treated group were increased ($p < 0.05$) compared to the control. The weight of the testes and epididemly in treated group with diluted volatile oil of *Artemisia herba alba* were increased compared to the control, but no change in weight observed in diabetic rats treated by *Artemisia herba alba* compared with the control within 8 weeks.
The histological studies of the islets cells of pancreas of the daily treated diabetic group with diluted oil showed irregularity in size, with few small islets which were atrophied as a result of their activity. Most of the islets in diabetic rats showed hyperplasia while all in treated group appeared normal compared to the control islets. Sections through the testis of control showed normal circular or oral seminiferons tubules with normal spermatogenesis process and without any signs of damages of the section. The diabetic rats significantly showed a total loss of germinal cells with severely damaged seminiferons tubules. Also treated group with oil showed that the seminiferons tubules consist of germinal epithelium with all stages of maturation including spermatozoa, compared to the control group.

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Chemical and biological variation of essential oils from *Cyperus papyrus* L. growing wild in Mid-Coast Part of KwaZulu-Natal, South Africa.

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The essential oils were isolated from the rhizomes of Cyperus papyrus L. (Asteraceae) growing wild in two different localities: KwaDlangezwa and Richards bay of KwaZulu-Natal Province, South Africa, by hydrodistillation method. A range of 33 – 38 constituents were identified from the GC-MS spectra of the oils accounting for 71.3 – 83.7 % of the total oil compositions. The major components of the Richards Bay oil sample were caryophyllene oxide (24.4%), humulene epoxide II (13.1%) aristolene (9.0%) and aromadendrene expoxide (7.2%). The oil isolated from KwaDlangezwa plant sample had caryophyllene oxide (10.6%), cyperene (10.2%), 1,8-cineole (8.3%) and humulene epoxide II (5.4%).

Chemical composition, antibacterial and antioxidant activities of the essential oils isolated from *Senecio pterophorus* DC.

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The chemical composition, antibacterial and antioxidant activities of essential oils isolated from the leaves of *Senecio pterophorus* DC. during the flowering stage from three different locations in KwaZulu – Natal were analyzed by GC and GC/MS. The main components of the essential oils were limonene, myrcene, sabinene, α-phellandrene and *p*- cymene. The antibacterial activities of the oils were evaluated against 12 bacterial strains including two different strains of *Proteus vulgaris* (ATCC 6830 and CSIR 0030) using agar disk diffusion and broth microdilution methods. The antibacterial test reveals the oils to have moderately to strong inhibitory activity against all the bacterial strains with MIC value ranging from 0.078 – 5.000 mg/ml. Furthermore, antioxidant activities of the oils and synthetic antioxidants showed no metal chelating activity, while, in other methodologies, there was a general increase in the antioxidant activity with increasing oil concentration.

The Variability of the Essential Oil Composition in the Sage Collection of the Genebank Gatersleben

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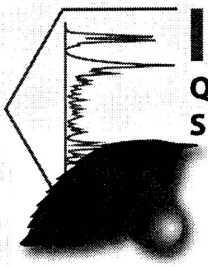
Genebanks are valuable resources for ex-situ conservation and breeding of economic plants and wild plant relatives. Analysing plant secondary compounds of such collections delivers useful information about the variability (heterogeneity) in the collection and enables plant breeders to pre-select accessions when breeding for specific plant secondary compounds like essential oil content and/or composition.
For garden sage (*Salvia officinalis* L.), 10 individual plants of each of the 19 accessions available in the genebank were analysed for their essential oil content and composition.
The essential oil content was in the range of 0.8% to 2.4%. The essential oil composition was comparable to results already published with the exception of two accessions, which were very low in α-thujone (3%) and β-thujone (<1%), representing a new chemotype in *Salvia officinalis*.



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Breeding and cultivation strategies

Essential oil composition of *Thymus daenensis* Celak subsp. *daenensis* growing wild and cultivated in Iran

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Genus Thymus is widespread in Iran. *Thymus daenensis* Celak subsp. *daenensis* is a perennial sub-shrub medicinal plant belonging to the Lamiaceae (Labiatae) family. This species only distribution in Iran and recognized by narrow leaves from other Thyme species. [2] The composition of its oils *T. daenensis* subsp. *daenensis* from natural sites in Iran has been reported [1] This is the first detailed report of the oil yield and essential oil composition of *T.daenensis* subsp. *daenensis* collected from natural site in comparison with cultivation this subspecies in farm.

The root stock of *Thymus daenensis* provided from growing samples as wild in nature with elevation 1450m (in 2004) cultivated in farm. The herb of 3-year-old plants were harvested flowering phase in May in cultivated condition and at the end of May in wild condition(2006). Aerial parts of 20 plants of *T. daenensis* Celak subsp. *daenensis* were randomly collected at natural site for the oil isolation. The same procedure was carried out with the samples growing in farms. Hydro-distilled essential oil from the herb of thyme (*Thymus daenensis* Celak subsp. *daenensis*.) was analyzed by a combination of GC and GC/MS. [3]

The hydrodistillation of the aerial parts of *T. daenensis* subsp. *Daenensis* in cultivated and wild condition gave oils with a yield of 2.8 ± 0.1% (v/w) and 2.4± 0.1% (v/w) on dry weight basis respectively. Twenty six components were identified. The main constituents of the essential oil in cultivated samples were thymol (74.6%), *p*-cymene (4.6%), γ-terpinene (4.5%) carvacrol methyl ether (4.3%) borneol (1.7%), 1,8 cineol (1.6%), and carvacerol(1.4%). The main constituents of the essential oil in wild samples were thymol (73.8%), *p*-cymene (4.06%), γ-terpinene (2.8%) carvacrol nethyl ether (2.4%), borneol (2.36%), 1,8 cineol (2%), carvacerol (1.7%) β – bisabolene (1.6%) β –caryophyllene (1.5%) and myrcene(1%). The results of this study indicate that both oils are rich in monoterpene phenols, especially thymol in cultivated condition.

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The effects of sowing date and nitrogen levels on yield and some morphological characteristics of black cumin (*Nigella sativa L.*)

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Iran plains have different climates, so different plant species grow in these areas. Some of these species are useful as medicinal herbs. Black cumin (*Nigella sativa L.*) is a medicinal herb that its grains have too much uses. For the agronomy of a new crop to be refined successfully, there must be a detailed understanding of its interaction with major agronomic, climate and other factors. In order to study of planting date and nitrogen effects on yield and yield components of black cumin an experiment was conducted at Agricultural Research Centre of Islamic Azad University, Birjand Branch in 2006. Four sowing dates (21 March, 4 April, 21 April and 5 May) and three levels of nitrogen (40, 80 and 120 kg/ha) were compared in a split- plot design based on randomized complete blocks with three replications. The results show that delayed sowing date caused to decrease traits such as plant height, number of branch and follicles per plant and seed and biological yields. However, sowing date didn't has significant effects on number of follicle per branch, seed number per follicle and 1000 seeds weight. Nitrogen utilization had not significant effect on biological and seed yield (probably because black cumin is a small plant and soil nitrogen content had been adequate for it). Nitrogen utilization above 40 kg/ha reduced harvest index, significantly. Totally, the results show that at the first sowing date by using 40 kg/ha nitrogen has the highest seed yield.

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The effects of sowing date and plant density on yield and yield components of fenugreek (*Trigonella foenum- graccum L.*)

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According to harmful effects of chemical and synthetic medicines in human, using medicinal products of plants has increased. Medicinal herbs have been used to promote health for centuries, and have increased in popularity and sales in the last 10-20 years. Because their natural growth area is not enough for growing human needs, it is important to increase the medicinal plants cultivated area. Fenugreek (*Trigonella foenum- graccum L.*) is a medicinal herb that has too much uses. Some scientists believed that at first this plant was grown in Iran. In order to understand the effects of sowing dates and plant density on fenugreek, an experiment was conducted at Torbat-e-Jam city in 2005. Four sowing dates (28 February, 19 March, 9 April and 29 April) and four plant densities (10, 20, 30 and 40 plants/m^2) were compared in a split- plot design based on randomized complete blocks with 4 replications. Sowing date had significant effects on seed yield and its components. The earliest and the latest sowing dates had the greatest and the lowest seed yield, respectively. Also, these treatments had the greatest and the lowest 1000 seeds weight and sheath number per plant. Harvest index was the lowest in 29 April , because in late sowing date, flowering and seed formation was been contemporary with high temperature. Also, delay in sowing time reduced plant height, significantly. Plant density had significant effect on seed yield. Seed yield was the highest at 40 plants/m^2. There was not any significant interaction on the measured different characteristics.

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Effects of sowing date and plant density on yield and morphological traits of fennel (*Foeniculum vulgare L.*)

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Fennel (*Foeniculum vulgare L.*) is a medicinal herb that its grains have too much uses. For the agronomy of a new crop to be refined successfully, there must be a detailed understanding of its interaction with major agronomic, climate and other factors. In order to assess the effects of sowing date and plant density on yield and morphological traits of fennel, a field experiment was arranged in spring of 2007. The main objective of this research was to find out effect of sowing date and plant density on the seed yield. This experiment was conducted as split plot in the base of randomized complete blocks design with three replication in agricultural research station of Azad University-branch Birjand. In this experiment, the effects of three levels of sowing date (20 March, 9 and 30 April) and three levels of plants density (6.7, 10 and 20 plant/m^2) were studied. The measured characteristics in this investigation were: seed and essential oil yield per hectare, height, diameter stem, branch number of main stem and umbelle number in plant. The results showed that sowing date and plant density had a significant effect on seed yield per hectare and other traits. According to the results of this investigation the best sowing time is 20 March and the most suitable plant density is 20 plant/m^2.

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The effect of the weather conditions on the essential-oil and total phenol content of different *Thymus vulgaris* L. cultivars

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Thymus vulgaris L. is a well-known aromatic and medicinal plant. The drug of the plant has been used as a spice and healing agent since ancient times. The antioxidant effect of garden thyme has been investigated several times; it is proved that both volatile and non volatile phenol compounds are responsible for the free-radical scavenging activity [1]. The main conditions (spacing, harvesting time, age) having an effect on the quantity and quality parameters of the essential oil were analysed before [2], however, there is no data referring to the non volatile phenol constituents.
In our research work a German garden thyme cultivar (Deutscher Winter) and a cultivated Hungarian population (Kalocsai) were analysed in 2006-2007 in different collection times (May and September). We investigated the effect of the harvesting time and the different weather conditions of the two years. According to the results the Hungarian selected population has significantly higher essential oil amount, however, the ratio of thymol was higher in the German cultivar in both years. With the exception of the second collection in 2006 the plant origin had no effect on the total-phenol content and the total antioxidant capacity as well as on the production biological characteristics.
In 2006 the second cutting (in September) while in 2007 the first cutting (in May) was characterised by higher total phenol content and total antioxidant activity, while in the ratio of thymol significant differences were not detected. It is obvious that these two chemical characteristics are mainly determined by non volatile phenol components. Higher total phenol content and stronger antioxidant activity were connected to the more sunny, dry and warmer weather conditions in both years.
The predicted weather changes have a great effect on medicinal plants too. According to our result in the case of garden thyme the more dry, sunny and warm conditions have no effect on the production-biological and essential oil characteristics, however, the non-volatile phenol components as well as the total antioxidant capacity of the plant extracts can increase significantly.

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Color shade nets effects on growth and essential oil contents and composition of *Origanum vulgare* L.

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Shading with coloured netting modifies the quality of natural radiation and may be employed as a means of manipulating physiological responses in plants. This study was performed to establish the essential oil composition and correlation between the growth and essential oil contents of *Origanum vulgare* L. cultivated under color shade nets. The treatment was carried out in 10 L pots containing soil, cattle manure and sand. The pots were distributed completely randomized design with 6 repetitions and 4 pots per repetition as the following treatments: 1) Black shade net; 2) Red shade net; 3) Blue shade net and 4) Full sunlight. The essential oils obtained by hydrodistillation of the leaves were analyzed by GC and GC/MS. It was observed that the color shade nets were not influenced in total dry biomass and foliar area, but the full sunlight decrease these parameters. The content of essential oil changed under color nets but yield did not. *Trans*-sabinene was the principal constituent. The major changes in the essential oil content and composition were found in blue and red shade nets. The content of *trans*-sabinene compound increased in red and blue color nets but some constituents identified in full sunlight condition missed (Table 1).

Table 1. Chemical composition and % (peak area) of essential oil compounds from *Origanum vulgare* cultivated under color shade nets.

Compounds	IK	Enviroment culture			
		Sunlight	Blue net	Red net	Black net
Cis-sabinene	1070	3,61	3,32	6,18	2,57
Trans-sabinene	1098	36,81	51,87	60,18	44,22
Terpinen-4-ol	1177	15,08	9,37	11,88	9,23
α-Terpineol	1189	5,74	3,98	3,58	4,36
Lynalil acetate	1257	5,00	4,48	3,52	5,19
Thymol	1290	23,19	23,54	12,93	27,67
β-Cariofilene	1419	1,70	1,22	-	1,48
γ-Muurolene	1480	2,04	1,75	-	2,39
p-Cymene	1025	-	-	1.08	-
Methyl thymol	1235	1,01	0,46	-	0,39
Methyl carvacrol	1245	0,87	-	-	0,45
Carvacrol	1299	0,48	-	-	-
Geranyl acetate	1376	0,17	-	-	
Biclyclogermacrene	1493	1,31	-	-	1,11
Spathulenol	1575	0,83	-	-	-

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Composition and anti-microbial activity of essential oil of *Aloysia gratissima* cultivated under color shade nets

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Aloysia gratissima (Brazilian lavender) is a 1 to 3.0 meter tall shrub with aromatic leaves. Its main ethnopharmacological effects are bronchial, lungs and bladder infections. The present study evaluated the essential oil composition and antimicrobial activity of *Aloysia gratissima* (Gill. et Hook) Troncoso (Verbenaceae) cultivated under color shade nets. The vegetable material was propagated through cuttings and cultivated for five months in four types of environment, under full sunlight and three colored nets (black, red and blue). The essential oils were obtained by hydrodistillation of the leaves and flower. The chemical analyses were analyzed by GC and GC/MS. The microorganism used in the antimicrobial assay were *Bacillus subtilis* (CCT2576), *Staphylococcus aureus* (CCT2740), *Salmonella choterasuis* (CCT4296), *Pseudomonas aeruginosa* (ATCC13388), *Streptococcus pneumoniae* (ATCC11733) and *Candida albicans* (ATCC10231).The antimicrobial activity was determined by minimum inhibitory concentration (MIC). For MIC determination the starting inoculums was standardized according to the McFarland scale (0.5), with final concentration of cells at 10^5/mL. The higher content of essential oil was obtained under full sunlight. Twenty-nine and twenty-one constituents were identified in oil leaves and flowers of *A. gratissima*, respectively. The main constituents in oil leaves obtained by plants cultivated under all mentioned conditions were *trans*-pinocamphone (12.4 to 20.9%), *trans*pinocarvyl acetate (14.5 to 17.6%) and guaiol (11.5 to 17.3%) and in the flowers were guaiol (19.5%), germacrene-B (10.5%) and bulnesol (10,0%). The composition of the oils showed mostly quantitatively than qualitatively different in plants cultivated under four types' environments. The oil of blossoming was the most effective in inhibiting the growth of the bacterial strains tested with MIC values between 20 µg/mL and 250 µg/mL, except for *Salmonella choleraesuis*. The data indicate that the biologically active compounds are present in oil of blossoming, which showed remarkable inhibitory activity against *Streptococcus pneumonia* (25 µg/mL) and *Candida albicans* (20 µg/mL).

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B 008

Constituents of the essential oil of *Matricaria* recutita L. As affected by altitude

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An experiment were carried out in Zagroz in 2002/03 to study the responses of chamomile (M. recutita [Chamomilla recutita]) cv. Goral to differents altitudes(500, and more 1000 m). The constituents of the essential oil of Matricaria recutita L. (Asteraceae) were investigated by GC-MS. components in camomile oil in the flower heads required a reinvestigation of the essential oil's content and composition during flowering period of camomile. The development of flowers and yields as well as the changes in the essential oil were investigated over two vegetation periods. The volatile constituents of Matricaria recutita L. cultivated in the lower region of the Zagroz were analysed by GC and GC-MS. Forty-one components, representing 97.5% of the oil, were identified. The main constituents were α-bisabolol oxide A (36.5%) and B (8.6%), (E)-β-farnesene (14.0%), α-bisabolol (16%) and chamazulene (5.6%). The variation in oil composition and oil yield during hydrodistillation was studied, and it has been found that the yield of the main components in chamomile oil increased from 0.5 to 2 h of distillation. The main constituents, except for α-bisabolol oxide B, were found in higher concentration in oil from the foothills of the Zagroz than in the oil from the plains. The oil showed a dark blue colour and a strong characteristic odour.

Keywords: essential oil, Matricaria recutita L., altitude

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Response of black cumin to nitrogen application in different plant density with logistic model

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The use of a mathematical model on crop response to nutrient has been extended. The logistic model has highest application in this study. To investigate black cumin (*Nigella sativa* L.) to nitrogen application on different plant density with the logistic model, an experiment was conducted by the Ferdowsi University of Mashhad, agricultural college experiment station. This study had a factorial arrangement with the two factors "plant density" (60, 120, 180, 240 plants/m^2) and "nitrogen supply" with four different dosages (0, 50, 100, 150 kg/ha) based on a completely randomized block design with four replications. The result of this experiment indicated that the applied logistic model gives a best fit for yield of black cumin. Response of dry matter to nitrogen application has a logistic response. Increasing of nitrogen application increased yield components and increased yield. The result of this study showed that 240 plants/m^2 and 50 kg/ha nitrogen application resulted in a maximum yield in Mashhad area based on the applied logistic model.

Keywords: black cumin, plant density, nitrogen, logistic model

Phenology study of the essential oil of cultivated yarrow in Tehran-Iran

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Flowering tops of yarrow *Achillea millefolium* L. were collected from the cultivated plants with code No.83001 in the herboratum (Educational and research medicinal plants garden) faculty of pharmacy, Tehran University of medical sciences in several seasonal stages, through 2006-2007.
Volatile oil of the flowers were obtained by using steam distillation and the chemical components were analysis via GC with a flame ionization detector(FID)and DB-5 column and GC/MS by using a theroquest-finging Gas chromatograph Varian 3400,equipped with above mentioned column and couple trace Mass quadrupled detector and identified in comparison to authentic compounds. The yield of essential oil was 0.7 to 1.3 v/w % and the major components were:
Sabinene,Cineol<1,8->,Chrysanthenol <cis->,Cumin aldehyed,Chrysant-henyl acetate<Cis->,Caryophyllen<(E)->,Germacrene D,Cadinol<epi-alp-ha->,Eudesmol<alpha>,Intermedeol <neo->.The results showed that ,July was the best time for harvesting the cultivated yarrow in Tehran area, because of its interesting major compounds.

Seasonal variation of *Salvia lavandulifolia* subsp. *lavandulifolia* essential oil yield and composition

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Among the four subspecies of *Salvia lavandulifolia* that can be located in the Iberian Peninsula, the subsp. *lavandulifolia* is the most widely distributed in the whole territory. These subspecies are characterized by a large chemical variability at intra and inter- population levels. This fact makes it difficult to detect real changes occurring in their essential oil yield and composition during the vegetative cycle. Based on this, clones of *Salvia lavandulifolia* Vahl. subsp. *lavandulifolia* were used in this present work to monitor seasonal variations in the yield and composition of the essential oil. A total of 20 shrubs were harvested at four different phenological stages during the vegetative cycle: Full bloom (FB) in April; fruit maturation (FM) in May; advance fruit maturation (AFM) in June, and vegetation (VEG) in July. For the essential oil extraction, aerial parts of individual plants were steam distilled for three hours using a Clevenger-type system. Essential oil yield ranged from 0.83% at the FM stage to its maximum yield 1.84% at the VEG phenological stage, ($p<0.01$).

The volatile profile of the essential oil samples was determined by capillary GC/MS analyses. This technique identified 17 volatile components as the most abundant in these sage essential oils. Major components quantified were 1,8-cineol, followed by camphor and viridoflorol. With respect to the concentrations of some of the most abundant components, the AFM stage seems to be the most appropriate harvesting time for this species. Cineol, bornyl acetate, and viridoflorol exhibited their maximum concentration ($p<0.01$) at this phenological stage; although, camphor reduced its concentration in this period, which can be beneficial for the essential oil quality.

In contrast, α–terpineol, camphene, and myrcene, were mostly concentrated ($p<0.01$) at the vegetative stage in summer. Terpenic hydrocarbons including α-pinene, β-pinene, p-cymene, γ-terpinene and α - terpinene reached their highest concentration between the phenoloical stages of FB and FM.

As a major conclusion, the selection of the most adequate phonological stage for the harvesting of this subspecies depends on the preferences of the producers. Essential yield has its maximum production in summer, but the quality of the oil, attending to the most abundant components is reached at the phonological stage of AFM.

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Agronomical evaluation and identification of chemotypes of *Lippia alba* in Distrito Federal, Brazil.

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The main objective of this work was to evaluate the volatile profile of 16 genotypes of *Lippia alba* from the germplasm collection of the Universidade de Brasília and to analyze its potential of production. A field assay was installed in the rural area of Distrito Federal, and the following parameters were analyzed: flowering period, growing habit, foliar area, length of the main branch, fresh and dry weight of the biomass (leaves and branches), essential oil content and oil constituents profile. The essential oil was extracted using a modified Clevenger apparatus and essential oil constituents were analyzed by gas chromatography and GC/MS. The experimental design was randomized blocks with 3 plants by plot. Five chemical types were reported: limonene-citral; myrcene-citral, myrcene-neral, citral, carvone and linalool. Higher levels of linalool were found in genotype, L.16 (89.8%); myrcene in L.37 (47.6%); limonene in L.27 (36.0%); carvone in L.27 (46.9%) and citral in L.17 (56.8%). The genotypes with higher foliar leaf area and leaf length of the main branch seem to be correlated with the best yields of essential oil, the higher level of linalool and lower levels of geranial and neral. The yield of essential oil was inversely proportional to the foliar area. The accessions that presented the best average yields were: L.29 (0.56 $g.pl^{-1}$), L.28 (0.53 $g.pl^{-1}$) and L.24 (0.51 $g.pl^{-1}$) for linalool chemotype; L.37 (0.05 $g.pl^{-1}$), L.02 (0.05 $g.pl^{-1}$) and L.05 (0.06 $g.pl^{-1}$) for myrcene; L.38 (0.13 $g.pl^{-1}$), L. 02 (0.12 $g.pl^{-1}$) and L. 17 (0.10 $g.pl^{-1}$) for geranial and accession L. 27 for limonene (0.17 $g.pl^{-1}$) and carvone (0.21 $g.pl^{-1}$).

The effect of GA3 and IAA 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]. The present report is a part of a project aimed at the enhancement and development of research activities on medicinal and aromatic plants in Iran. Previous investigations have been carried out on chemical composition [2-4,8,9], biological activity of essential oil [2,6,8], anticonvulsant activity [3], antioxidant activity [4], loss of essential oil due to drying [5] and effect of NAA on the accumulation of volatile oil components in cell-cultures of tarragon (*Artemisia dracunculus*) [7].
Aerial parts of tarragon plants treated under field condition were sprayed with IAA (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 essential oils of two treatments and their control (water sprayed) were analyzed by GC/MS .The main components of the essential oil were α-pinene (0.61%), limonene (1.83%), *cis*-ocimene (5.15%), *trans*-ocimene (4.01%), methylchavicol (82.31%), methyleugenol (2.78%) for control plants. It was seen that IAA 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.

Keywords: Tarragon; *Artemisia dracunculus*; GA3;IAA; essential oil; GC/MS

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The effect of naphthalene acetic acid (NAA) on composition the essential oil of *Lavandula angustifulia Mill*

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The genus Lavandula belongs to the family Lamiaceae (Labiatae), and it comprises of more than 30 species of which only very few are commercially used: *Lavandula angustifolia* Mill. (syn. *L.vera, L.officinalis)* .Lavender is native to the western Mediterranean region, primarily in the Pyrenees and other mountains in northern Spain and is cultivated extensively (1).*Lavandula angustifolia* is a strongly aromatic shrub growing up to 0,5 -0,8 m tall.

The present report is a part of a project aimed at the enhancement and development of research activities on medicinal and aromatic plants in Iran. Previous investigations have been carried out on effect of NAA on the accumulation of volatile oil components in cell-cultures of tarragon (Artemisia-dracunculus) [2]. It has been observed that linalool and linalylacetate can increase intracellular cAMP (3).

Aerial parts of lavender plants treated with NAA(50,100,150 µm) three time 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 and investigated by GC/MS.The essential oil content was increased in all treatments. The percentage of linalool in lavender essential oil showed a increase by treatments.

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Influence of Phytohormones in the Volatile Profile of *in vitro* Plantlets of *Thymus vulgaris* L.

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Thymus vulgaris L., a member of the Lamiaceae family, is native to the Mediterranean region. In the South and Southeast of Brazil it is well cultivated and widely used in culinary. The plant has been used in folk medicine for its stimulant, antispasmodic and expectorant properties, and also for gastrointestinal and respiratory disorders. The essential oil, which is used in food, perfumery and cosmetic industries, also possesses antimicrobial and antioxidant properties and some of its components, such as thymol and carvacrol, are known to be biologically active. Climatic conditions and genetically heterogeneous plants are associated with a high degree of variability in the amount of active constituents. Besides, food-processing operations may cause loss of aroma that calls for subsequent supplementation and the growing market of flavour and bioactive compounds forces the search for alternatives sources. The use of biotechnological tools as plant tissue culture for up-regulated metabolism pathways may create a source of homogeneous, well defined product. The aim of this work was to investigate the influence of growth regulators on the production of volatile compounds by *in vitro* plantlets of *Thymus vulgaris* L.
Plantlets cultivated on MS (Murashige and Skoog) medium solidified with 0.8% agar and containing 3% sucrose were separately supplemented with IAA (indole-3-acetic acid), BA (benzyladenine), Kin (kinetin) and Zea (zeatin) in concentrations of 5 and 10µmol.L^{-1}. A control experiment without growth regulators was also run. The cultures were incubated at 30 ± 2ºC under a 16/8 hours light/dark photoperiod (daylight fluorescent lamps, 23 µmol.m^{-2}.s^{-1}) for 60 days. Solid phase microextraction was used for the analyses as follows: 200mg of plant leaves were placed in 4mL vials and kept at 60ºC for 60min. A 65µm polydimethylsiloxane-divinylbenzene (PDMS/DVB) fiber was exposed to the headspace of the vials for 15 min and immediately transferred to the heated injector of an Agilent 6890N gas chromatograph (splitless, 3min at 250ºC) equipped with a HP5 (30m X 0.32mm X 0.25 µm) capillary column. Oven temperature was kept at 40ºC for 3min then rised to 180ºC at 3ºC.min^{-1}. Normalized area from a flame ionization detector was used for quantitative comparison. Identification was performed in the same way but using an Agilent 5973N mass detector (EIMS, 70eV). All experiments were conducted in triplicate.
The major volatiles components in all treatments were thymol (16.6-29.0%), γ-terpinene (25.0-34.9%) and p-cymene (13.8-18.6%). IAA (5 µmol.L^{-1}) in the medium resulted in a 16.0% increase in thymol content, while addition of BA (10 µmol.L^{-1}) induced an increase in p-cymene of about 15.5%. In relation to γ-terpinene, IAA (5µmol.L^{-1}) and Zea (10µmol.L^{-1}) concentrations induced an increase of 20.0%, while BA (10µmol.L^{-1}) lead to an increase of 39.6% in relation to control plants. For *in vitro* plantlets of *Thymus vulgaris* L., the phytohormones tested induced only quantitative modifications in the production of volatile compounds.

Acknowledgements: CAPES

Essential oil composition of cultivated *Thymus caramanicus* from Iran

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Thymus caramanicus Jalas is an endemic species grown in very limited populations in some parts of Iran. Our previous study showed that essential oil of this species contains high concentration of carvacrol. For commercial production of this species we have started some experiments. Rootstock of *T. caramanicus* were collected from natural habitat in Kerman province of Iran and planted in field station of our Institute in north of Tehran. Here essential oil content and composition of two year's old cultivated plants are reported. Aerial parts of cultivated stocks were collected at flowering stage. The essential oil of air dried samples isolated by hydrodistillation and analyzed by GC and GC-MS. Oil yield of cultivated sample was 2.6%. Twenty four components were identified in analyzed sample. Carvacrol identified as major component of the oil (65.1%). Other minor components were thymol (6.5%), *p*-cymene (4.7%), γ-terpinene (4.0%) and borneol (4.9%). The results showed that there is no considerable variation between wild and cultivated stocks which confirmed that *Thymus caramanicus* can be used as stable carvacrol rich chemotype in agricultural systems.

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Nejad Ebrahimi, S. et al., (2008) Food Chemistry. doi:10.1016/j.foodchem.2008.02.083

Variability of oil content and composition of different Iranian accessions of *Satureja hortensis* L.

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Summer savory (*Satureja hortensis* L.) is an aromatic plant usually used in kitchen, food and pharmaceutical industries. It is cultivated and used as a culinary herb for a long time by local people in Iran. An experiment was conducted during 2006, to investigate variability of drug yield, essential oil content and compositions of 30 Iranian accessions of *S. hortensis* collected from different parts of Iran. The results revealed great variability among accessions. Mean yield of essential oil was varied between 0.57 - 2.9 % among accessions and highest oil content was belonging to accession no. 25. Cluster analysis according to main components of the oils, revealed three different groups. Accessions no. 30, 28, 24 and 27 with carvacrol contents of 83.3, 83.3, 76.9 and 67 %, respectively, are categorized as carvarcrol rich group in the cluster. With regard to the three most important traits "drug yield", "essential oil content" and "carvacrol content", accessions numbers 8, 28 and 29 showed the best performance and selection among the analysed accessions.

Chemical constituents of the essential oils of mint genotypes growing in Southern Brazil

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Mint species (*Lamiaceae*) are widely cultivated in the world because of the economic importance of the terpenoids menthol and menthone. This work had as main objective to investigate the essential oil composition of mint species collected from different regions of Brazil to select genetic materials for menthol production. Thirty-days-old rooted cuttings of ten genotypes, including *M. arvensis* L. (5), *M.* x *piperita* L. (3), *M. campestris* Schur (1) and *M. sylvestris* L. (1) were transferred to the field and harvested approximately 90 days after planting. The essential oil was extracted from 100 g of fresh leaves by hydrodistillation during two hours using a Clevenger-type apparatus. The essential oil constituents were identified by gas chromatography coupled to mass spectrometry (GC-MS) and quantified by gas chromatography equipped with a flame ionization detection (GC-FID). The highest menthol (87.1%) content was observed in *M. campestris* Schur., followed by *M. arvensis* L. (61.1 to 66.2%), *M.* x *piperita* L. (33.6 to 38.5%) and *M. sylvestris* L. (33.4%). A great variation of menthone levels was observed in *M.* x *piperita* genotypes (14.5 to 31.1%) compared to *M. arvensis* (16.8 to 18.9%). This constituent was also identified on *M. sylvestris* L (12.8%) and *M. campestris* Schur (2.2%). Menthofuran was identified in *M.* x *piperita* L. (6.5-16.5%) and *M. sylvestris* L. (16.7%), but not in the essential oils of *M. arvensis* L. and *M. campestris* Schur. Isomenthone and neomenthol levels varied from 0.63 to 6.17 and 1.53 to 3.95%, respectively, according to the plant material. Technologies are now being developed to increase essential oil yield of *M. campestris* Schur and *M. arvensis* L. genotypes to make this crop an economic option for farmers at Southern Brazil.

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Influence of gibberellic acid and seaweed extract on vegetative development and essential oil yield and composition of *Pogostemon cablin* Benth

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Patchouli (*Pogostemon cablin* Benth.) essential oil is used by the perfumary industry to give a base and lasting character to fragrances. A greenhouse experiment was carried out to investigate the effect of gibberellic acid (200 mg L^{-1}) and seaweed extract (0.15 and 30 mg L^{-1}) on vegetative development and essential oil yield and composition of patchouli. Thirty-days-old healthy rooted cuttings were planted in pots containing fertilized soil as substrate. The treatments were applied at 30 days after planting and the plant material was harvested at 45 days after treatment application. The evaluated plant growth parameters included leaf and stem dry mass, leaf and stem number and leaf area. The essential oil was obtained from leaves by hydrodestilation during five hours using a Clevenger-type apparatus. The constituents were identified by gas chromatography coupled to mass spectrometry (GC-MS) and quantified by gas chromatography equipped with flame ionization detection (GC-FID). Although leaf dry mass significantly increased after gibberellic acid and seaweed extract (15 mg L^{-1}) application, stem dry mass was affected only by gibberellic acid. Similar results were also observed for plant height when plants were treated only with gibberellic acid. The stem and leaf number increased when 15 mg.L^{-1} of seaweed extract was applied in absence of gibberellic acid. Leaf area also significantly increased when seaweed extract (30 mg L^{-1}) was applied. When gibberellic acid was applied, leaf area increased using the lowest dose of seaweed extract (15 mg L^{-1}). A significant effect of both regulators was observed for essential oil yield. Gibberellic acid, in absence or presence of seaweed extract (15 mg L^{-1}) increased essential oil yield but did not change the level of its major constituents. The obtained results indicate that gibberellic acid and seaweed extract present a promotory effect on plant development and essential oil yield of patchouli.

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Analytical methods and active principles

Antioxidant activity of essential oils in various environments

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The purpose of this work was to evaluate antioxidant properties of essential oils of 11 spice and medicinal plants of the *Lamiaceae* family: *Salvia officinalis* L., *Satureja montana* L., *Mentha piperita* L., *Mentha piperita var. citrata* (Ehrh.) Briq, *Origanum vulgare* L., *Hyssopus officinalis* L., *Agastache rugosa* (Fisch. et Mey), *Monarda fistulosa* L., *Salvia sclarea* L., *Ocimum basilicum* L. as well as those of some terpene and phenol compounds composing them in the reaction with cation-radicals of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$) [1] in aqueous and aqueous-ethanolic media.

For all the species, antiradical activity (ARA) of essential oils expressed as quantity of Trolox having the same effect as 1 ml of an essential oil under investigation was higher in water than in ethanol 80 % (v/v). In the last media, *S. montana* and *M. fistulosa* oils reduced their activity towards $ABTS^{+\bullet}$ to a smaller extent than carvacrol and thymol which are dominating phenolic compounds in these oils. It is an evidence of complex character of $ABTS^{+\bullet}$ interaction with the essential oils, which are multicomponent systems of interacting to each other compounds with properties essentially different from a simple additive mixture.

It has been found, that a positive correlation exists between total phenolic compounds contents in the essential oils and ARA shown by them in aqueous and aqueous-ethanolic media.

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Essential oil composition in leaf and peel of *Citrus sinensis* (L.) Osbeck cultivated in Iran

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Hydrodistilled leaf oils and cold expressed peel oils of *Citrus sinensis* cv. Shahsavari (local variety), *Citrus sinensis* cv. Moro, *Citrus sinensis* cv. Thomson Navel and *Citrus sinenesis* cv. Valencia, which were cultivated in Ramsar, a province of Mazandaran (North of Iran) were analyzed by means of GC and GC-MS. Identification of components were done by comparison of the relative retention indices and mass spectra with authentic references [1]. Among the characterized compounds in the leaf oil of *C. sinensis* cv. Shahsavari (35 compounds), *C. sinensis* cv. Moro (23 compounds), *C. sinensis* cv. Thomson Navel (24 compounds) and *C. sinenesis* cv. Valencia (23 compounds), δ-elemene (5.5%, 20.0%, 27.2% and 29.5%), sabinene (32.5%, 25.9%, 12.4% and 9.4%) and linalool (14.9%, 7.7%, 10.9% and 23.7%) were found to be the main components in the leaf oil of *C. sinensis* varieties, respectively. The main constituents among the identified constituents in the peel oils of *C. sinenesis* varieties (17, 12, 14 and 22 compounds, respectively) were limonene (92.3%, 90.8%, 90.4% and 9.5%) , myrcene (1.9%, 2.4%, 1.1% and 2.5%) and pinene (0.7%, 1.8%, 1.6% and 1.4%), respectively.

Acknowledgements: The authors are grateful to the Institute of Citrus of Iran, Ramsar, Mazandaran, Iran.

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Volatile compounds in leaf and peel of *Citrus limon* (L.) Burm. F. and *Citrus medica* L. grown in Iran

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Essential oils obtained by hydrodistillation of air-dried leaves and cold expression of peels obtained from *Citrus limon* and *Citrus medica* (collected in Ramsar, Province of Mazndaran, North of Iran) were analyzed by means of GC and GC-MS. The components were characterized by comparison of relative retention indices and mass spectra with authentic references [1]. Among the 34 characterized compounds in the leaf oil of *Citrus limon*, pinene (22.6%), limonene (18.4%) and geraniol (10.3%) were the main constituents. Twenty one compounds were identified in the peel oil of *Citrus limon* with limonene (61.4%), α-pinene (13.1%) and γ-terpinene (11.3%) as the main constituents.
Neryl formate (22.3%), limonene (21.7%) and neral, (20.2%) were found to the major components among 24 identified compounds in the leaf oil of *Citrus medica*. In 16 characterized compounds in the peel oil of *Citrus medica*, limonene (46.9%) and γ-terpinene (34.3%) had the highest percentage.

Acknowledgements: The authors are grateful to institute of Citrus of Iran, Ramsar, Mazandaran, Iran.

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Composition of essential oil obtained from *Hyperyicum hyssopifolium* growing wild in Iran

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The water distilled essential oil leaves, stems and flowers of *Hypericum hyssopifolium* (Hyperricaceae) were analyzed by means of GC and GC/MS. Identification of components had been done by comparison of the relative retention indices and mass spectra with authentic references [1]. α-Pinene (44.0%), limonene (6.3%) and β-pinene (5.3%) were the major compounds among the 34 components detected in the oil of leaves. Among the 15 compounds in the oil of stems nonane (36.9%), α-pinene (32.8%) and undecane (14.0%) were found to be the main constituents. The high percentage constituents between the 28 components in the oil of flower were α-pinene (47.6%), β-pinene (8.2%) and γ-cadinene (5.2%).

Acknowledgements: The authors are grateful to the Institute of Forest and Rangelands, Dr Mozaffarian for identifying of plant material.

References: 1. Adamz, R. P., (2004) Identification of Essential Oil Components by Gas Chromatography/quadrupole Mass spectroscopy, Allured Publ. Corp., Carol Stream I1 USA.

Chemical composition of the essential oil from flower, stem and leaves of *Tanacetum parthenium* from Iran

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The essential oils obtained by hydrodistillation of the flowers, stems and leaves of *Tanacetum parthenium*, endemic in Iran, were analyzed by GC and GC/MS. The components were identified by comparison of mass spectra and relative retention indices with authentic reference [1]. The oils yields were found 0.51%, 0.62% and 0.42% in leaves, flowers and stems, respectively. α-pinene (8.5%), linalool (28.1%), nerolidol (14.2%) and 1, 8- cineole (7.1%) were the major components in the leaves oil.
Furthermore, the main compounds in the flowers oil were to be α-pinene (9.1%), trans-verbenol (12.1%), iso cyclocitral (24.0%) and 1, 8- cineole (16.8%).
Subsequently, iso cyclocitral (12.1%), linalool (15.5%), nerolidol (10.1%) and 1, 8- cineole (10.6%) were the main constituents in the stems oil.

Acknowledgements: The authors are grateful to V. Mozaffarian (Research Institute of Forests and Rangelands, Tehran) for identifing the plant materials.
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Chemical composition and comparison of rosemary oils with BHA and BHT for relative antioxidant effectiveness in fats, oils and foods

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The antioxidants have been widely used in processed foods to prevent or retard oxidation of fats or oils, because lipid oxidation can cause changes in colour, odour, and loss of food quality. Lipid oxidation has been considered to be initiated by active oxygen species such as super oxide anion, singlet oxygen, hydroxyl radical and hypochlorite ion. Thus, it is very important to study inhibition effect of different antioxidants [1]. Synthetic antioxidant; such as tert-butyl-4-hydroxy- anisol (BHA) and tert-butyl-4-hydroxy-tolune (BHT), have been widely used. However, special attention has been recently focused on natural antioxidants in food as alternatives to synthetic antioxidants [2]. Among herbs and spices, rosemary is a common household plant grown in many parts of the world. It is used for flavoring food, in cosmetics and traditional medicine and also known to exhibits antimicrobial activities [3].
The aims of this work were to investigate the effect of the addition of natural antioxidant (rosemary essential oils). In this work, the rosemary leaves were collected from Kerman in September and the essential oils of rosemary were isolated by hydrodistillation in a Clevenger apparatus and by n-hexane solvent extraction in a Soxhlet apparatus; then the antioxidant effect of non-water soluble and water soluble rosemary extract was studied in fats, oils and foods. The results obtained were compared with the known antioxidant activity of the common commercial antioxidants BHA and BHT.

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Rapid evaluation of basil accessions applying micro-hydrodistillation combined with ATR-infrared and Raman spectroscopy

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Due to its aromatic and antimicrobial properties sweet basil (*Ocimum basilicum* L.) is widely used in the food, phytopharmaceutical and cosmetic industries. Today, it is well-known that the composition of basil essential oil is largely influenced by the individual chemotype, the plant development stage and various environmental conditions [1-4]. Gas chromatography in combination with mass spectroscopy detection is typically used to identify the individual basil types. However, this method requires highly qualified technicians and is not applicable for field measurements. The aim of this study was to develop an easy method for rapid evaluation of single basil plants regarding the profile of main volatile substances. Isolation of basil essential oils was performed by means of a micro-distillation device (Micro-Distiller Eppendorf, Hamburg, Germany). After hydro-distillation the n-hexane layer containing the main part of volatiles was separated from the aqueous phase and the solvent was allowed to evaporate at room temperature. The remaining essential oil was then placed on the surface of a diamond zinc selenide ATR crystal of a Bruker Equinox 55 FT-IR spectrometer. For Raman measurements the same samples were analyzed on a Bruker RFS 100 equipped with Nd:YAG laser and Germanium detector without application of any pretreatments. Our study demonstrated that both vibrational spectroscopy methods presented in this study can be applied to rapidly monitor wild basil populations (including hybrids) or progenies of crossing experiments regarding their individual essential oil composition. Each basil chemotype shows a characteristic fingerprint and most of the distinctive signals can be assigned to specific vibrational modes of specific essential oil substances (Figure 1). High correlation between the spectroscopic and the GC data set was found applying chemometrics. Based on individual calibration calculations the content of most valuable volatile substances (e.g. 1,8-cineole, linalool, estragole, eugenol, methyleugenol) can be successfully predicted in a very short time. Micro-distillates can be obtained even from very small sample amounts and approx. 5-10 uL of the distillate is sufficient to perform reliable IR and Raman spectroscopic measurements.

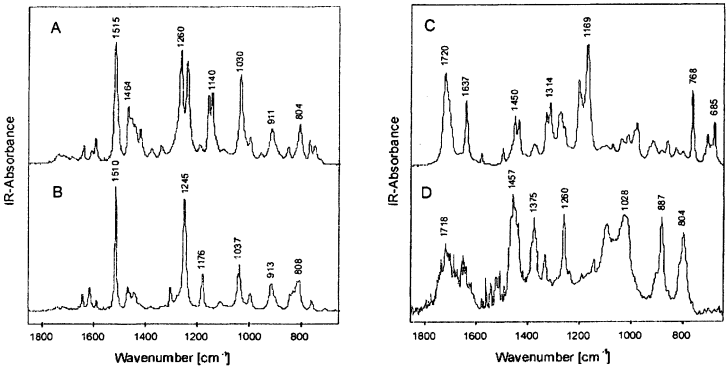

Figure 1:

ATR-IR spectra of essential oils obtained from different basil chemotypes.

A: methyleugenol type
B: estragole type
C: geraniol type
D: linalool type

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Microwave-assisted hydrodistillation of *Eryngium caeruleum* M.B. and comparison with conventional hydrodistillation

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The volatile composition of *Eryngium caeruleum* M.B has been studied and the obtained essential oil from aerial parts was analyzed by GC and GC/MS [1]. Microwave–assisted hydrodistillation (MAHD) and hydrodistillation (HD) were compared in terms of extraction time, yield, chemical composition and quality of the essential oil, efficiency and cost of process. Twenty seven components were identified by HD representing 97.5% of the total oil content with limonene (61.7%) as the main constituent. Thirty six components were identified by MAHD representing 95.5% of the total essential oil content also with limonene (42.9%) as the main constituent. The MAHD method yields an essential oil composition with higher amounts of more valuable oxygenated compounds and allows substantial savings of cost in terms of time and energy.

Acknowledgements: The authors are grateful to the Institute of Forest and Rangelands, Dr. Mozaffarian for identifying the essential oil in the plant material.

References: 1. Adamz R. P. (2004) Identification of Essential Oil Components by Gas Chromatography/quadrupole Mass spectroscopy, Allured Publ. Corp., Carol Stream I1 USA.

Composition of essential oil from *Thymus kotschyanus* Boiss. & Hohen growing wild in Iran

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Water distilled essential oils obtained from leaves, stems and roots of *Thymus kotschyanus* Boiss.& Hohen (*Labiatae*) were analyzed by means of GC and GC/MS. Identification of components were done by comparison of the relative retention indices and mass spectra with authentic references [1]. Carvacrol (69.5%), borneol (8.9%) and p-cymene (7.3%) were the major compounds among the nineteen components in the oil of leaves. Among the twenty two compounds in the oil of stems carvacrol (71.6%), borneol (11.7.8%) and p-cymene (4.2%) were found to be the main constituents. The high percentage constituents between the twenty-seven components in the oil of root were carvacrol (71.3%), pinene (5.3%) and borneol (4.8%). The identified compounds in the oil of leaves, stems and roots represent (98.5%, 99.2% and 98.3%) respectively of the essential oils.

Acknowledgements: The authors are grateful to the Institute of Forest and Rangelands, Dr. Mozaffarian for identifying the essential oil in the plant material.

References: 1. Adamz R. P. (2004) Identification of Essential Oil Components by Gas Chromatography/quadrupole Mass spectroscopy, Allured Publ. Corp., Carol Stream I1 USA.

Comparative study on chemical compositions of cultivated and wild carrot seed oils

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Carrot (*Daucus carota* L., *Apiaceae*) is an herbaceous biennial plant which is cultivated worldwide for its edible root. Carrot seeds contain about 0.8%-1.6% (w/w) essential oil reported to be endowed with medicinal properties, i. e. diuretic, carminative and stomachic as well as non-medicinal applications concerning the formulation of beverages and aromatic and fragrance composition [1]. The aim of this study was to compare the chemical composition of the oils obtained from the seeds of cultivated carrot (*Daucus carota* var. *sativus*) and wild carrot (*Daucus carota* ssp. *carota*) growing in Iran. Because of the high content of sesquiterpene alcohols in carrot seed oil, the hydrodistillation method did not result in any measurable oil content. So, we decided to try the steam distillation method. Hundred grams of carrot seeds were subjected to steam distillation for 4 h and the oil yield was 0.7% and 0.4% (w/w) for cultivated and wild carrot seeds, respectively. The chemical composition of the individual essential oils was analysed by GC-MS. In the oil obtained from cultivated carrot seed, the oxygenated sesquiterpenes accounted for 79.07% of the whole composition due to the high percentage of carotol (76.95%). The other major components were β-pinene (5.66%), α-pinene (4.84%), geranyl acetate (3.33%) and daucol (2.12%). Monoterpene hydrocarbons (68.50%) were the most important substances identified in the oil of wild carrot seed, mainly due to the high percentage of α-pinene (22.74%), sabinene (22.34%) and β-pinene (13.11%). Other main components were carotol (26.32%) and myrcene (3.90%). Contents of carotol and daucol as the most specific sesquiterpenes of carrot seed oil varied significantly in cultivated and wild seeds. The dominant component of both oils was carotol; in cultivated seeds its content was found to be three times higher than in the seeds of wild carrots (daucol content in seeds of cultivated carrots: 2.12%; daucol content in seeds of wild carrots: 0.041% related to the whole oil content).

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Chemical Composition of Essential Oil of *Achillea wilhelmsii* From Iran, Hydrodistillation and Microwave Methods.

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Hydrodistillation (HD), Free-Solvent microwave extraction (FSME) and microwave a hexane as solvent were employed to isolate volatile oils from *Achillea wilhelmsii*. Oils were analyzed by GC and GC/MS. Identification of components had been done by comparison of the relative retention indices and mass spectra with authentic reference [1]. Eleven components representing 92.57% were identified in the oil which isolated by hydrodistillation.The main components of the oil were Comphor (45.78%), Comphene (21.24%), 1,8-cineol (18.5%), α-pinene (3.56%) and Thymol (3.19%). Fifteen components representing 85.88% were characterized in the oil that achieved by free-solvent microwave extraction. The main components of the oil were Comphor (49.7%), γ-terpinene (14.83%), p-cymene (8.91%), 1, 8-cineol (6.25%) and Thymol (6.19%). Eight components representing 97.35% were characterized in the oil that obtained by microwave a hexane as solvent. The main components of the oil were Comphor (82.12%), Comphene (6.36%), 1,8-cineol (5.50%), Borneol (2.31%) and P-cymene (1.06%).

Acknowledgements: the authors are grateful to institute of Forest and Rangelands, Dr Mozaffarian for identifying of plant material.
References: 1Adamz R. P., (2004), Identification of Essential Oil Components by Gas Chromatography/quadrupole Mass spectroscopy, Allured Publ. Corp., Carol Stream I1 USA.

A search for well suited fragance material for shampoos

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It would be a mistake to think that the work of perfumers relates only to the development of perfume oils for the "fine fragrances" that are offered so elegantly on the shelves of perfume shops. Almost every time they use a personal cleansing product or a toiletry or a household cleaner or care product, consumers encounter the fragrance that these products contain. Fragrance is not a singale material of clearly defined properties, but rather a mixture of individual chemicals, each behaving according to its own unique attributes, characterizing these chemicals seperately & then combining their effects allows the behaviour of the complete fragrance composition in diverse media to be understood.
However, the job of perfumer does not stop there, even once the perfume has been successfully dosed into the product. A number of other factor have to be studied to ensure that the perfume remains evenly dispersed within the product and does not cause its physical and chemical characteristicsto change significantly over time. The integrity of the product needs to be checked over a period of accelerated storage at different conditions. The latter are often stipulated by the product manufacturer, who wants to ensure that the product's characteristic or activity are unaltered by the fragrance, even if exported to tropical climates or sub-Arctic conditions. However equally important to the perfumer who is designing a new perfume, or an evaluator who is selecting a perfume from a repertoire or 'shelf',is to monitor any changes in the fragrance odour once in the poduct base.

Shampoo is a common hair care product used for the removal of oils, dirt, skin particles, dandruff, environmental pollutants and other contaminant particles that gradually build up in hair.The present paper deals to evaluate the stability study of various flavorings and fragrances materials in the shampoo formulation containing active ingredient. For stability study 110 ingredients of fragrance materials have been incorporated in to shampoo at 1%. These fragrant shampoos have been stored under ambient conditions and at 48^{0}C in oven for stability. These samples have been evaluated periodically for the odor profile by sensory panel of 7 people. During study it was found that most of the fragrant materials that were tested at ambient temperature as well as 48^{0}C show good stability, whereas few of chemicals are unstable and give off odor during storage under ambient conditions and 48^{0}c temperature.

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GC quantitation using a database of response factors and prediction of missing values

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Usual Gas Chromatography (GC) methods commonly applied to report the quantitative composition of flavors, fragrances and essential oils, *e.g.* semi-quantitation with Mass and Flame Ionization Detectors (MS-SQ and FID-SQ), or Internal Standardization (MS-ISTD and FID-ISTD), are compared.
The FID-SQ determination is less inaccurate than the MS-SQ, but still unsatisfactory. The MS-ISTD and FID-ISTD provide the best results. However, the determination of Relative Response Coefficients (RRFs) is time-consuming for mixtures composed of many constituents. The use of a response coefficient database is thus proposed and validated by testing the dependence of RRFs on GC-FID parameters using an experimental design *e.g.* injection and detector temperature, the split ratio, the column phase and proportion of hydrogen in the flame. The use of the database requires that identical instrumental settings are applied. Under standard GC-FID conditions, very low time variations of the RRFs are observed. If a quantitation using RRFs from a previously built database is a bit less accurate than a true ISTD, it nevertheless provides better results than semi-quantitation. The quantitation of a model mixture with instruments from four different suppliers with RRFs from the database exhibits a mean bias of less than 3.4%. The database saves time, but it never exhaustively lists all possible compounds. This can be solved by predicting the missing RRFs using a theoretical model. The present one is based on combustion enthalpies, which is simpler than previously published approaches. The learning set of 300 experimental RRFs was used to generate the model and calculate the RRFs of 75 compounds as a validation set.

Gaschromatography – Olfactometry for identification of volatile aroma-important compounds in oregano

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The investigations report the suitability of Gaschromatography - Olfactometry (GC-O) in terms of analyzing and characterizing the volatile fraction of aroma active components in oregano. The air dried plant material was obtained from the Dr. Junghanns GmbH, Groß Schierstedt, Germany. The samples of Origanum vulgare ssp. hirtum cultivated for the investigations have been analyzed with previously optimized methods for sample preparation and gas chromatographic separation. For extraction the volatile compounds a magnetic stir bar (SBSE) was used, which is coated with a specific layer (PDMS) whereupon volatile aroma components accumulate. Previous analysis [1] showed that SBSE has low detection limits for minor components which is especially important for establishing aroma profiles due to the sensory affectivity of secondary components. The precision of experimental procedure was evaluated by calculating a series of six analyses. Variation coefficients of the total area were found in the range of 4.37% and 6.16%, which is acceptable. Furthermore the recovery of the method was studied by using herb sample spiked with defined quantities of volatile standards under analysis conditions. The recoveries for each standard using the developed SBSE method were satisfactorily and ranged from 76.18 % to 86.23 %.

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Volatile Constituents of Fruit,Stem & Leaf of *Xanthium spinosum* L.Growing Wild in Iran

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The composition of the essential oils from fruit, stem & leaf of *X.spinosum* L.obtained by hydrodistillation were analyzed by GC/MS method. Identification of components had been done by comparison of the relative retention indices and mass spectra with authentic reference [1]. Limonene(32.87%), borneol (15.41%)and δ-cadinene (12.11%) were the main component among the 10 constituents characterized in the oil of fruit of *X.spinosum* L.representing 91.10%of the total components detected.eleven compounds were identified in the oil of stem of *X.spinosum* L.representing 94.35 % of the total oil,with limonene(47.54%),sabinene(17.75%) and bornyl acetate(6.21%) as the major constituents . The oil of the leaf of *X.spinosum* L.were characterized by limonene(36.80%) , sabinene (23.12%) and alloaromadendrene(6.82%) among the 8 components comprising 91.38% of the total oil detected.

Acknowledgements: the authors are grateful to institute of Forest and Rangelands, Dr Mozaffarian for identifying of plant material.

References: 1Adamz R. P., (2004), Identificati on of Essential Oil Components by Gas Chromatography/quadrupole Mass spectroscopy, Allured Publ. Corp., Carol Stream I1 USA.

Volatile Constituents of Flower, Stem & Leaf of *Achillea callichroa* (Boiss.) Growing Wild in Iran

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The composition of the essential oils from flower, stem & leaf of *A .callichroa*(Boiss.) obtained by hydrodistillation were analyzed by GC/MS method. Identification of components had been done by comparison of the relative retention indices and mass spectra with authentic reference [1]. 1,8cineole(22.44%), terpinene-4-ol (19.68%)and α-pinene(19.54%) were the main component among the 9 constituents characterized in the oil of flower of *A.callichroa*(Boiss.) representing 91.90%of the total components detected.eleven compounds were identified in the oil of stem of *A. callichroa*(Boiss.) representing 92.34 % of the total oil,with linalool(15.94%),α-pinene(15.85%) and terpinene-4-ol(15.35%) as the major constituents . The oil of the leaf of *A. callichroa*(Boiss.) were characterized by linalool(31.44%) , α-pinene(21.13%) and caryophyllene oxide(14.35%) among the nine components comprising (93.29)of the total oil detected.

Acknowledgements: the authors are grateful to institute of Forest and Rangelands, Dr Mozaffarian for identifying of plant material.

References: 1Adamz R. P., (2004), Identificati on of Essential Oil Components by Gas Chromatography/quadrupole Mass spectroscopy, Allured Publ. Corp., Carol Stream I1 USA.

Volatile Constituents of aerial parts of *Phlomis pachyphilla*(Rech.f.)Growing Wild in Iran

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The composition of the essential oils from aerial parts of *Ph.pachyphylla(Rech.f.)* obtained by hydrodistillation were analyzed by GC/MS method. Identification of components had been done by comparison of the relative retention indices and mass spectra with authentic reference [1]. Germacrene-D(32.09%),α-pinene(13.5%), β-caryophyllene(11.45%), pentadecanol(7.62%), trans-β-farnesene(7.47%) and bicyclogermacrene (7.45%) were the main component among the 12 constituents characterized in the oil of aerial parts of *Ph.pachyphylla(Rech.f.)* representing 94.99%of the total components detected.

Acknowledgements: the authors are grateful to institute of Forest and Rangelands, Dr Mozaffarian for identifying of plant material.

References: 1Adamz R. P., (2004), Identificati on of Essential Oil Components by Gas Chromatography/quadrupole Mass spectroscopy, Allured Publ. Corp., Carol Stream I1 USA.

Haplophyllum stapfianum(Hand.Mzt.) Growing Wild in Iran

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The composition of the essential oils from flower, stem & leaf of *H. stapfianum*(Hand.Mzt.) obtained by hydrodistillation were analyzed by GC/MS method. Identification of components had been done by comparison of the relative retention indices and mass spectra with authentic reference [1]. α-pinene (23.56%), limonene (23.57%)and β-pinene(13.68%) were the main component among the 10 constituents characterized in the oil of flower of *H. stapfianum*(Hand.Mzt.) representing 93.75%.of the total components detected.8 compounds were identified in the oil of stem of *H. stapfianum*(Hand.Mzt.)representing 93.75 % of the total oil,with caryophyllene oxide (32.00%),α-pinene(28.00%) and limonene(15.00%) as the major constituents . The oil of the leaf of *H. stapfianum*(Hand.Mzt.) were characterized by caryophyllene oxide(22.22%),β-caryophyllene(19.34%) and limonene(11.99%) among the ten components comprising 93.08% of the total oil detected.

Acknowledgements: the authors are grateful to institute of Forest and Rangelands, Dr Mozaffarian for identifying of plant material.

References: 1Adamz R. P., (2004), Identificati on of Essential Oil Components by Gas Chromatography/quadrupole Mass spectroscopy, Allured Publ. Corp., Carol Stream I1 USA.

Comparison of essential oil composition of *Carum copticum* obtained by hydrodistillation and microwave free solvent methods

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Essential oil of *Carum copticum* cultivated in Iran was obtained by hydrodistillation and microwave free solvent. Oils were analyzed by GC and GC/MS .Identification of Components had been done by comparison of the relative retention indices and mass spectra with authentic reference [1]. Twelve components 99.31% were identified in oil which isolated by hydrodistillation. The major components of oil of *C.copticum* were γ-terpinene (37.40%) *p*-cymene (28%) thymol (24.96%) β-pinene (5.78 %.) myrcene (1.31%). Twelve components representing 96.91% were characterized in the oil that achieved by free-solvent microwave extraction. The major components of the oil were thymol (35.28), γ-terpinene (32.49%) *p*-cymene (23.51%) β-pinene (3.47%)

Acknowledgements: the authors are grateful to institute of Forest and Rangelands, Dr Mozaffarian for identifying of plant material.
References: 1Adamz R. P., (2004), Identification of Essential Oil Components by Gas Chromatography/quadrupole Mass spectroscopy, Allured Publ. Corp., Carol Stream I1 USA.

Vibrational spectroscopy studies on rose concrete, rose absolute and rose water

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Rose concrete obtained by extraction of fresh harvested rose blossoms (*Rosa damascene* Mill.) using n-pentane, n-hexane, or petroleum ether as extraction solvent contains mainly paraffins, fatty acids, methyl esters, di- and triperpenes and pigments [1]. Rose absolute is produced by extracting rose concrete with aqueous ethanol. The resulting product presents a typical rose odour which is mainly referred/related to the comparatively high content of phenylethyl alcohol. Usually the chemical composition of rose absolute, rose concrete and rose water (obtained from industrial hydrodistillation of rose blossoms) is analysed by GC-MS applying headspace trapping and SPME techniques [2-4]. Especially headspace trapping on a condenser is very time-consuming and therefore not useful for routine measurements to be performed in the context of quality control. Furthermore, both analytical techniques do not allow determining the real amounts of phenylethyl alcohol in the individual rose samples. Also non-volatile adulterants cannot be identified on the basis of GC-MS measurements. This is the reason why the present study was to elucidate the special advantages of vibrational spectroscopy methods (IR and Raman) for rapid characterisation of the above mentioned rose products. Mid-infrared spectroscopy (MIR) has until recently been of little use for cosmetic and perfumery industry owing to the difficulties of sample handling and time necessary for data acquisition. However, today the development of Fourier transform spectroscopy in combination with the development of powerful chemometric algorithms supplies a very efficient approach for fast and reliable characterisation of various plant extracts [5,6]. Contrary to MIR the selection rule for Raman spectroscopy is that during vibration of a bond, there must be a change in the electronic polarizability. Thus, both spectroscopy techniques are complementary and together provide a complete description of the vibrational modes of a molecule. Raman spectra of rose absolute and rose concrete show significant vibrational modes at 1605 cm^{-1} and 1202 cm^{-1} which are related to the aromatic ring system. The most intense signal, observed at 1003 cm^{-1}, is assigned as deformation vibration (-C-C-OH) of the ethyl alcohol substituent. Based on calibration equations developed on the basis of samples containing different amounts of phenylethyl alcohol a reliable and fast determination of the analyte in rose absolute and rose concrete can be easily performed. Because Raman is not sensitive for/to changes of the dipolmoment during vibration of a bond even high amounts of water in the sample do not interfere with the analytical results. The IR and Raman calibrations presented in this paper are especially useful for quality control purposes in perfumery and flavour industry.

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Chemical compositions of the essential oil from flowers of *Astragalus schahrudensis* Bge. from Northeast Iran

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The genus *Astragalus* (Papilionaceae) contains about 800 species of perennial and annual in Iran, and most of these are endemic [1]. Investigation on the chemical composition of essential oils from the genus *Astragalus* has been subjects of few reports [2-4]. The aim of our study was to identify the constituents of the essential oil of the flowers of *A. Schahrudensis* Bge. endemic in Iran growing wild at the flowering stage in Yam valley, Northeast Iran. Colorless oil were obtained by hydrodistillation, in a Clevenger-type apparatus for 3 hours, in yield of 0.14% (w/w), from flowers of *A. Schahrudensis*. The essential oil was analyzed by GC and GC/MS. The flowers oil of *A. schahrudensis* was characterized by higher amount of ethyl octadecanoate (57.2%) and hexadecanoic acid (27.8%) among the five components comprising 96.1% of the total oil detected. The flowers oil of *A. schahrudensis* contain only nonterpene hydrocarbons.

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

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The genus *Calycanthus* belongs to Calycanthaceae family that includes two to four species depending on taxonomic interpretation and two are accepted by the *Flora of North America* [1] . In Iran two planted shrubs from *calycanthus* genus were found as ornamental plant and those are *Calycanthus floridus* and *Calycanthus fertilis* [2]. To the best of our knowledge this is the first report on the essential oil from leaves of *Calycanthus floridus* L. var. oblongifolius. The aim of our study is to identify the constituents of the essential oil of leaves of *Calycanthus floridus* L. var. oblongifolius at the flowering stage. The essential oil obtained by hydrodistillation of leaves of Calycanthus floridus L. var. oblongifolius (Nutt.) D.E. Boufford & S.A. Spongberg , planted in Sabzevar, Iran, was analyzed by GC and GC/MS. Thirteen compounds representing 93.4% of leaf oil of Calycanthus floridus L. var. oblongifolius were identified among them pregeijerene B (18.3%), isobornyl 2-methyl butanoate (15.3%), bornyl acetate (14.8%), elemodiol<8-α-11> (14.4%) and camphene (10.3%) were the major ones. Another major component of the oil was α-pinene (5.6%). In this essential oil monoterpenes (55.8%) predominated over sesquiterpenes (19.3%) and nonterpene hydrocarbon (18.3%).

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Chemical composition of the essential oil from threshed aerial parts of *Cuminum cyminum* L. from Northeast Iran

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The genus Cuminum belongs to family Apiaceae. *Cuminum cyminum* L. is annual herb and the only species from the genus *Cuminum* that is found in Iran [1]. Several investigations on chemical composition, biological activity and medicinal applications of essential oil of Cuminum cyminum L. have been done recently [2,3], of which three were reported in 2007. Our study deals with the analysis of the oil isolated from threshed aerial parts of *Cuminum cyminum* planted in Northeast of Iran. The aim of our study is to identify the constituents of the essential oil of threshed aerial parts of planted *C. cyminum* L. at harvesting stage. The threshed aerial parts was separated from desirable seeds of the plant. Pale yellowish colored oil was obtained by 3-hour hydrodistillation, using a Clevenger-type apparatus, of the threshed aerial parts, which gave yield of 0.52%. GC and GC-MS analysis identified fifteen compounds that accounted for 99.4% of the threshed aerial parts of the plant. Of these, cuminaldehyde (33.0%) and γ-terpinene-7-al (28.3%) were the major components; other main constituents were p-cymene (12.4%), γ-terpinene (9.7%) and β-pinene (7.0%). Threshed aerial parts oil of the plant consist of monoterpenes mainly, of which oxygenated monoterpenes were the predominant ones.

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Chemical composition of the essential oil from aerial parts of *Eremostachys macrophylla* Montbr. & Auch. from Iran

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The genus *Eremostachys* belong to labiatae family and 15 perennial species from this genus are found in Iran among them 5 species are endemic and *Eremostachys labiosa* grows in Pakistan, Iraq, Afghanistan and Turkmenistan besides of Iran [1]. Three investigations has been carried out on the chemical composition of the essential oils of the genus *Eremostachys*, and deals with the aerial parts of *Eremostachys macrophylla* from Central Iran [2], aerial parts of *Eremostachys laevigata* Bge. [3] and aerial parts of *Eremostachys laciniata* (L.) Bge. [4]. The aim of our study is to identify the constituents of the essential oils from aerial parts of *E.* macrophylla Montbr. & Auch. growing wild at flowering stage in Northeast of Iran. The colorless oils were obtained by hydrodistillation, using a Clevenger-type apparatus for 3 hours, from aerial parts in 0.036% yield (w/w) and analyzed by GC and GC-MS. Six compounds representing 96.9% of the aerial parts oil of the plant were identified. The constitiuents were germacrene D (36.1%), bicyclogermacrene (11.0%), δ-cadinene (16.1%), trans-dauca-4(11),7-diene, α-muurolol (15.4%) and 1-octadecene (8.1%). As can be seen from the above information the aerial parts oil contain four sesquiterpene hydrocarbons (73.4%), one oxygen containing sesquiterpene (15.4%) and one aliphatic hydrocarbon (8.1%).

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Chemical composition of the essential oil from aerial parts of *Phlomis cancellata* Bge. and *Hymenocrater platystegius* Rech. f. two labiate herbs indigenous from Northeast Iran

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Phlomis cancellata is one of 17 perennial species from genus *Phlomis which* belongs to family Labiatae Ten of these species are endemic in Iran [1]. *Hymenocrater platystegius* is one of 9 species of *Hymenocrater* (family Labiatae) found in Iran, of which 4 are endemic [1].Several investigations of the chemical composition of essential oils from the genus *Phlomis* have been done recently [2], of which three were reported in 2007; one report deals with aerial parts of *Phlomis cancellata* from North of Iran in 2006 [3]. Although some investigations on the chemical composition of essential oils from the genus *Hymenocrater* were done a few years ago [4] to the best of our knowledge this is the first report on essential oils from aerial parts of *Hymenocrater platystegius*. Our study deals with the analysis of the oil isolated from aerial parts of *Phlomis cancellata* and *Hymenocrater platystegius* growing wild in Northeast Iran. The aim of our study is to identify the constituents of the essential oil of aerial parts of *P. cancellata* Bge. growing wild at flowering stage and *Hymenocrater platystegius* Rech. f. at non-flowering stage in Northeast of Iran. Pale yellowish colored oil of *P. Cancellata* and Colorless oil of *H. Platystegius* were obtained by 3-hour hydrodistillation, using a Clevenger-type apparatus, of the aerial parts, which gave yields of 0.026% and 0.060% (w/w), respectively. GC and GC-MS analysis identified 18 compounds that accounted for 90.4% of the aerial parts of the *p. cancellata*. Of these, germacrene D (26.4%), caryophyllene oxide (10.4%), α-humulene (6.3%), α-thujene (6.0%) and bicyclogermacrene (5.0%) were the major components. Aerial parts oil of the plant consist of sesquiterpenes mainly, of which sesquiterpene hydrocarbons were the major constituents. Analysis by GC and GC-MS revealed seventeen compounds representing 90.7% of the aerial parts oil of *H. platystegius*. This oil was particularly rich in α-pinene (23.4%) and limonene (23.2%); another major component of the oil was β-pinene (11.7%) Aerial parts oil of the plant consist of sesquiterpenes mainly, of which sesquiterpene hydrocarbons were the major constituents.

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Chemical composition of the essential oil from aerial parts of *Ferulago angulata* (Schlecht.) Boiss. From Iran

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The genus *Ferulago* consist of 35 species, seven of which are found in Iran, including two endemics: *F. contracta* Boiss. Et Hausskn. And *F. phialocarpa* Rech. f. et H. Riedl [1,2]. Our study deals with the analysis of the oil isolated from aerial parts of *Ferulago angulata* growing wild in Northeast of Iran. The aim of our study is to identify the constituents of the essential oil of aerial parts of *Ferulago angulata* growing wild at flowering stage. Light yellow oil was obtained by hydrodistillation, in a Clevenger-type apparatus for 3 hours, in yield of 0.57% (w/w), from the aerial parts of *Ferulago angulata* (Schlecht.) Boiss. (Umbelliferae), growing wild in Khorasan province (Iran). Analysis by GC and GC-MS revealed twenty compounds representing 98.1% of aerial parts oil of *Ferulago angulata.* Among them α-phellandrene (24.2%), β-phellandrene (14.9%), α-pinene (14.7%) and p-cymene (10.3%) were the major ones. Other major component of the oil were δ-3-carene (6.7%) and (Z)-β-ocimene (5.8%). Aerial parts oil of the plant consist of monoterpenes (97.6%) and one oxygen containing sesquiterpene (0.5%). Monoterpene hydrocarbons (90.8%) were the major constituents.

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Chemical composition of the essential oil from fruits of *Vitex pseudo-negundo* (Hausskn.) Hand-Mzt. From Iran

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The genus *Vitex* belongs to family Verbenaceae. Three shrubs from this genus are found in river-beds and Southern regions of Iran [1]. Several investigations of the chemical composition of essential oils from the genus *Vitex* have been done recently [2,3], of which one report deals with chemical composition of leaf, flower and fruit oils of *Vitex pseudo-negundo* from Iran in 2006 [4]. Our study deals with the analysis of the oil isolated from fruits of *Vitex pseudo-negundo* growing wild in river-beds near of Sabzevar, Khorasan province (Iran). The aim of our study is to identify the constituents of the essential oil of fruits of *Vitex pseudo-negundo* growing wild at fructifying stage. Light yellow oil was obtained by hydrodistillation, in a Clevenger-type apparatus for 3 hours, in yield of 0.48% (w/w), from the fruits of *Vitex pseudo-negundo* growing wild in Khorasan province (Iran). Analysis by GC and GC-MS revealed twenty-two compounds representing 98.5% of fruits oil of *Vitex pseudo-negundo.* Among them 1,8-cineole (33.6%), α-pinene (22.0%) and sabinene (15.7%) were the major ones; other major components of the oil were iso-verbanol acetate (7.1%) and trans-β-farnesene (4.0%). In fruits oil of the plant monoterpenes (88.7%) predominated over sesquiterpenes (9.8%).

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The essential oil composition of *Ajuga chamaecistus.Ging.subsp.scoparia*(Lamiaceae)

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One of the most important families in Iran is Lamiaceae that includes several medicinal, ornamental,aromatic and perfume plants [1- 2]. *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 [3-4]. There are about 40 known species belonging to this genus [5]. The Iranian flora comprises 5 species of *Ajuga* and one of them is *Ajuga orientalis* L. [4]. *Ajuga* species are used in folk medicine of different parts of the world for the treatment of rheumatism, gout, asthma, diabetes, malaria, ulcers and diarrhea and have antibacterial, antitumor, antifeedant, and vulnerary properties [6-8].The essential oil composition of the aerial parts of *Ajuga chamaecistus.Ging.subsp.scoparia*. (Lamiaceae) grown in northern parts of Semnan (Shahmirzad). parts of plants were dried in a dark place so that the continuous air stream flows through. The essential oil were obtained by hydrodistillation and have been analyzed by GC/MS. The main compounds were ρ-cymene (34.4%),β-pinene (18.1%), α-phellandrene (17.7%) and α-pinene(15.2%).

Key words: Ajuga chamaecistus.Ging.subsp.scoparia; essential oil; p-cymene

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A Comparative investigation on Chemical composition of the essential oil of *the aerial parts of Prangos latiloba and Prangos ferulaceae* (L.) Lindl.

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The genus *Prangos* (Umbelliferae) involves about 30 species [1]. In Iran, fifteen species of the Umbelliferae family plant exist among which five are endemic [2]. Various species of this plant have been widely distributed in the brackish regions of Iran. The common pharmaceutical and clinical applications of *Prangos* species are as emulient, carminative, tonic antiflatulent, anthelmintic, antibacterial agents, cytokinerelease inhibitor, antifungal, antioxidant and anti HIV [4-9]. A literature survey reveals that steam distilled from aerial parts and seeds [10] and hydrodistilled fruits [11] of *Prangos ferulacea(Pf)* have been the subjects of some reports. Nonetheless, recognition and contrasting the constituent components of hydrodistilled essential oil of the aerial parts of *Prangos latiloba (Pl)* and *Prangos feulacea is* the main goal of the present work. Aerial parts of the *Prangos latiloba and Prangos ferulacea* were collected during the flowering stage near Sheshtamad river of Sabzevar in north Khorasan province and Shahmirzad mountains located in the east of Semnan Province, respectively. The altitudes of sampling were 1400 and 1550 msl for *Pl* and *Pf*, sequentially. A Voucher specimen was deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

The homogeneous crushed powders of air-dried aerial parts (100 g) of the plants were subjected to hydrodistillation for 3 h, dried over anhydrous sodium sulfate and kept under liquid nitrogen in brown sealed vials at -12°C. The oil of *Pl* and *Pf* were clear yellowish and pale yellowish comprising 0.25 and 0.6 w/w%. Afterwards, injection a 0.2 microliter portions of the volatile oils of *Pl* and *Pf* to a GC/MS instrument led to a total of 12 and 27 compounds comprising 95.23% and 96.23% of the oil composition.The main components which were characterized in the essential oil of *Pl were germacrene D(27.79%),* α-pinene (17.81%), β-carryophyllene (12.75%), β-pinene (11.23%). On the other hand, The major compounds of *Pf* were β-phellandrene (20.39%), α-terpinolene (15.26%), α-pinene (11.59%), δ-3-carene (11.06%), α-phellandrene (9.09%) and trans-β-ocimene (9.67%).

Key word: *Prangos latiloba; Prangos feulacea*; Essential oil; GC/MS.

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Chemical compositions of the essential oils from flowers of *Salvia leriifolia* Bench. *and Salvia multicaulis* Vahl from Iran

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The genus *Salvia* comprises 700 herbs and shrubs, growing in the temperate and warmer zone of the world. Fifthy-eight species are found in Iran, among which 17 are endemic [1-2]. Some species of genus *Salvia* are used as medicinal aromatic and ornamental plants. *Salvia officinalis* is one of the most widespread species and since ancient times has been used in the treatment of various disorders, such as berculosis, psoriasis and seborrhiec eczema[3-4]. The odor of *Salvia leriifolia* is very complex, strong and warm-balsamic-woody. Therefore, a lot of constituents will contribute to the total sensory impression. Previous chemical investigation of different species of *Salvia* has shown the presence of flavonoides[5], diterpenoides[6-7], sesterpenes[8-9] and essential oils[10-13].

The aim of our study is identification the constituents of volatile oils of flowers of *Salvia leriifolia* Bench. and *Salvia multicaulis* Vahl. as growing wild plants in the brackish regions of Iran. The plants were collected during the flowering stage in May 2007 from Sabzevar in north Khorasan and Shahmirzad in Semnan province at an altitude of about 1450 and 1550msl, respectively. A voucher specimen has been deposited at the herbarium of Research Institute of Forests and Rangelands, Tehran, Iran.

The oils were obtained by hydrodistillation using a Clevenger-type apparatus for 3 hours from crushed air dried flowers of the plants and analyzed by GC(Shimadzu GC-9A equipped with a SE30 fused silica column) and GC-MS(Hewlett-Packard 6890/5973 fitted with a fused silica HP5MS capillary column). The flower oil of *Salvia leriifolia* Bench was characterized by compounds constituting 95.5% of the total oil which were predominately gamma terpinene (62.2%), p-cymene(11.1%), α-terpinene (7.3%) and myrcene (5%). Twenty one compounds representing 94.3% of flower oil of *Salvia multicaulis* were identified among them 1,8-cineol (25.3%), α-pinene (18.3%), camphor (12.4%), camphene (8.4%) and bornyl acetate (7.9%) were the major ones. Accordingly, In the both volatile oils, monoterpenes predominated over sesquiterpenes.

Key word: *Salvia leriifolia* Bench., *Salvia multicaulis* Vahl; Essential oil; GC/MS; Monoterpenes.

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Simultaneous Distillation-Extraction versus Simultaneous Distillation-Solid Phase Extraction

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For the isolation of steam volatile substances as well as for the characterisation of cooking flavours equipments are used which combine hydrodistillation and liquid-liquid extraction. Examples are the equipments for separation of essential oils in the European Pharmacopoeia and the extraction by the Likens-Nickerson (LN) simultaneous distillation-extraction method (SDE). For the separation of volatiles in these equipments, solvents are necessary which are to a large extent insoluble in water. The water-insolubility of these solvents is the precondition for the separation of these components. However, the nonpolarity of the water-insoluble solvents prevents frequently a complete extraction of active substances and flavours, because the oxygen-containing terpenoids are partly in considerable measure water soluble. Therefore it is tried to compensate the incomplete extraction by numerous distillation-extraction cycles. The extension of the distillation times leads, however, to a stronger thermal loading and sensitive substances are decomposed because they are constantly led back into the distillation flask. So steam volatile terpenes with relative good water-solubility are under represented in essential oils [1], the same as thermally unstable terpenes.
A method, which gives much better results, is shown in Fig.1. This can be demonstrated for chamomile as example. After hydrodistillation and condensation in the distillation-extraction apparatus according to Fig. 1, a flow inducer immediately pulls the distillate on the RP18 phase. The solid phase fixes the volatile components completely and only pure water flows back into the flask. Also thermally sensitive substances such as spiroethers do not flow back into the distilling flask. Fig. 2 shows the results of comparative studies including hydrodistillation, SDE and Simultaneous Distillation-Solid Phase Extraction (SDSPE).

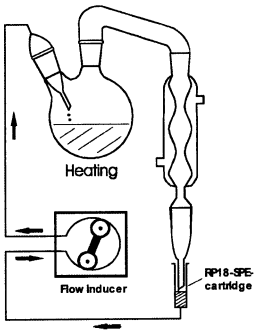

Fig. 1: Combination of hydrodistillation and solid phase extraction in a circulation equipment

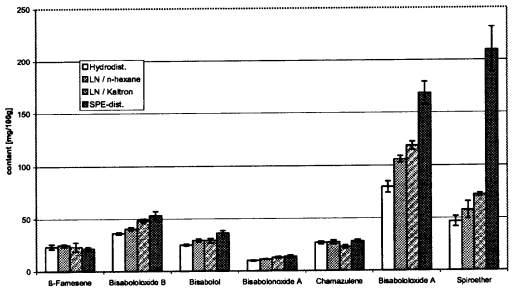

Fig. 2: The quantities of volatiles depending on the isolation method (n = 6 in each case), related to 100 g air-dried chamomile flowers

The results show, that bisabololoxide A and spiroethers occur in higher amount in the chamomile vapour space than so far assumed. Thus also the pharmaceutical activity (e.g. during inhalation) will be higher than described up to now. Generally it has been found that in comparison to SDE the vapour phase will be extracted more completely by applying SDSPE.

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Fast determination of essential oil components in parsley with vibrational spectroscopy

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In an extensive field experiment 220 accessions of parsley (*Petroselinum crispum* [Mill.] Nym.) were planted for comparison in test fields at Quedlinburg (JKI) and Gatersleben (IPK). This parsley material represents a great amount of the worldwide existing variability of the species. In this experiment a great number of characteristics and parameters were determined like morphological characters, disease index of different phytopathogens, amount and composition of distilled essential oils of leaves and seed, volatile components, molecular markers and the degree of ploidy. The preparation of essential oils was carried out by distillation according the German Pharmacopoeia from 20 grams of dried leaf material of each sample. In the oils we determined 11 components by GC analysis. The great variability of oil components suggests the possibility of developing a fast spectroscopic prediction method for the components. Therefore infrared spectra and Raman spectra were recorded from all 220 oil samples. The spectroscopic measurements were carried out with the FT-IR spectrometer EQUINOX 55, equipped with a diamond ATR unit (RESULTEC Analytic Equipment, Oberkirchberg, Germany) and the FT-Raman spectrometer RFS 100/S. The manufacturer of both spectrometers is the Bruker Optics GmbH, Ettlingen, Germany. For interpretation of spectra the Opus/Quant 2.0 software (Bruker Optics GmbH) was used. This is a quantification program that connects the spectra with the analysed concentrations of oil samples for the purpose of a multivariate statistical calibration using a PLS algorithm. If such a chemometrical calibration is successful, the prediction of components concentration is possible from measured spectra alone. The prediction of each component concentration is tested by a cross validation procedure. The quality of prediction is expressed by the determination coefficient R^2 and the root mean square error of cross validation RMSECV. The calculations lead to the result that better predictions are possible with diamond ATR-IR spectra instead of Raman spectra. For 6 components of parsley oil (from leaves) the calibration

Table 1: Range of concentrations and parameters of PLS statistics for parsley oil components, based on ATR-IR spectra.

oil component	range (%)	R^2	RMSECV (%)
apiol	0.00 - 41.01	1	0.61
a,p-dimethylstyrene	2.88 - 25.35	0.97	0.7
limonene	1.71 - 6.54	0.83	0.36
myrcene	1.36 -27.34	0.97	0.89
myristicine	0.00 - 57.97	0.99	1.14
ß-phellandrene	1.81 - 34.75	0.97	1.13
1,3,8-p-menthatriene	1.71 - 54.73	0.97	1.89

calculations supplied a very good determination coefficient (table 1). For these chemical components the diamond ATR infrared spectroscopy is a fast and cost effective way to substitute partly time consuming chromatographic analyses of parsley oils. The spectroscopic measurement and following calculation (with a working calibration model) of an oil sample were done in only 90 seconds. This is an advantage of time compared to the one-hour chromatographic analysis.

Quality control of various essential oils from Turkey by means of mid-infrared and Raman spectroscopy

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The essential oils obtained from various plant species collected in Turkey (*Carum copticum, Carum carvi, Ocimum basilicum, Ocimum minimum, Origanum vulgare, Foeniculum vulgare, Myrtus communis, Laurus nobilis, Pimpinella anisum, Echinophora tenuifolia*) were analysed by ATR-mid-infrared and Raman spectroscopy. The vibrational spectra measured with both complementary techniques show characteristic key bands of the individual main volatile components such as thymol, p-cymene, γ-terpinene, limonene, 1,8-cineole, anethole, fenchone, and carvone (Figure 1). Detailed spectral analysis of the investigated oils is based on their individual vibrational spectral modes. It has been found that hierarchical cluster analysis can be successfully applied for classification and selection of the analysed essential oil samples.
The special advantage of FT-Raman spectroscopy is the possibility of applying fibre-optics for remote measurements in a reaction vessel or a distillation apparatus to continuously control the individual refining steps. For ATR-IR studies mobile spectrometer systems can be used for rapid monitoring of wild populations with regard to the individual essential oil profiles. In comparison to the standard reference GC/MS method, no sample pre-treatments are required for the applied non-destructive vibrational spectroscopy methods. Therefore, if a rough characterization of essential oils is sufficient (e.g. to perform a rapid discrimination of different chemotypes), the described methods offer some advantages over time-consuming GC-MS measurements [1-4].

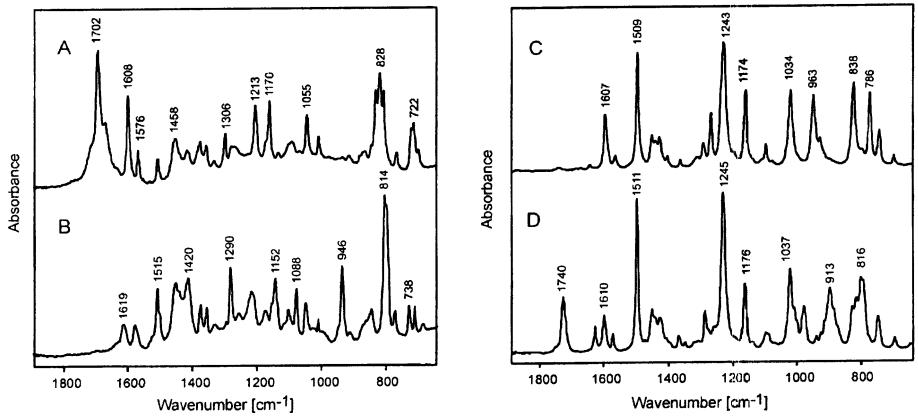

Figure 1: ATR-IR spectra of essential oils obtained from *Carum carvi* (A), *Carum copticum* (B), *Pimpinella anisum* (C) and *Foeniculum vulgare* (D).

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Application of NIR, ATR-IR and Raman spectroscopy for rapid characterisation of buchu leaf oil

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Buchu (*Agathosma betulina*, family Rutaceae) is an indigenous South African shrub with rounded leaves, dotted with oil glands. Renowned for treatment of ailments, it is used as a diuretic and mild urinary antiseptic drug [1]. In small doses it serves as an appetite stimulant and shows good digestive, carminative and antispasmodic properties. In addition, it has potential as a flavour enhancer. Buchu is mainly used in the flavour industry to enhance fruit flavours such as black currant [1]. The oil is also increasingly used in perfumery (in chypre bases and in certain types of Eau-de-Cologne). Round-leaf buchu oil is regarded as being safe in most countries in the world. The oil is hydro-distilled from the dried leaves and the quality of the oil is mainly evaluated in terms of its diosphenol (high levels required) and pulegone (low levels required) contents. Main components of the oil are limonene, menthone, iso-menthone, pulegone, iso-pulegone diosphenol and pseudo-diosphenol [1].

The composition of the oil is usually determined by gas chromatography. In order to reduce efforts and time necessary for analytical measurements, new spectroscopic methods have been developed and the potential of near-infrared (NIR), mid-infrared (MIR) and Raman spectroscopy has been evaluated on 51 buchu leaf oil samples from three seasons and a number of different localities. This was done to confirm the potential of these vibrational spectroscopy techniques as useful approaches to rapidly quantify the respective components in buchu oil.

NIR spectra were recorded, from 10 000-4000 cm^{-1}, on a Büchi NIRFlex N-500 Fourier transform NIR (FT-NIR) spectrometer (Büchi Labortechnik AG, Flawil, Switzerland). Raman spectra were recorded using an NIR-FT-Raman spectrometer RFS 100 (Bruker GmbH, Ettlingen, Germany) equipped with a diode-pumped Nd:YAG laser, emitting at 1064 nm and a Ge detector. MIR measurements were performed with a Bruker FT-NIR spectrometer system IFS 55 EQUINOX from 12 000-4000 cm^{-1} applying a diamond ATR unit (Resultec Analytical Equipment, Oberkirchberg, Germany.

Using partial least square (PLS) regression models it was possible to predict the various components accurately with a standard error of cross-validation (SECV) values ranging from 0.68 to 5.54% using NIR spectroscopy. Generally, ATR-IR and Raman spectra obtained from the individual essential oils are mostly well-structured and present characteristic key bands of the main volatile terpene substances. Based on specific marker bands produced by the individual volatile substances, spectroscopic analyses in principle allow discriminating different buchu species and even chemotypes among the same species. Combination of vibrational spectroscopy and hierarchical cluster analysis provides a fast, easy and reliable method for chemotaxonomy characterisation. In most cases determination of main oil components applying PLS regression results in comparatively high R^2 and low SECV values. The ability to rapidly monitor various buchu oils makes it possible to efficiently select high-quality single plants from wild populations as well as progenies of cross-breeding experiments. Furthermore, the vibrational spectroscopy methods applied in this study can be used in the flavour and fragrance industry in order to perform fast quality checks of incoming raw materials as well as continuous monitoring of distillation processes.

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The determination of allergens in cosmetics using GCxqGCMS

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The determination of allergens in cosmetic products like perfumes, shower gel or shampoos is regulated according to the 7th amendment of the European cosmetic directive. Cosmetic products need to be labeled when containing more than 0.01 % and 0.001% for rinse off and leave on products, respectively. When using one dimensional chromatographic techniques sometimes the measurements result in unprecise determination due to coelutions with matrix compounds specially with complex matrices like crèmes etc. In comprehensive GCxqGCMS using thermal modulation the separation power however is drastically enhanced [1]. Thermal pulses using a loop modulator (ZOEX corporation) result in base peak widths of about 230 msec. Perfumes were diluted 10:1 and subsequently injected into the split injector. Matrices like shower gel and crèmes were placed into microvials (DMI, difficult matrix introduction) which in turn were put into the optic3 (ATASGL) ptv injection system automatically by using the automatic liner exchanger (LINEX, ATASGL). The analytes were then directly thermally extracted from the matrix by using a ptv temperature program of 45 °C start temperature, then 16 °/sec ramp rate up to 200 °C. This makes sure that the high boiling matrix components are retained in the micro-vial. The column set used was a 5% phenyl phase with 30 m, 0.25 mm, 0.25 µm combined with a wax column of 1 m, 0.1mm, 0.1 mm in the second dimension. The modulation frequency was set to 8 s resulting in about three cuts per peak by using an adequate oven temperature program of 50 °C, 1 min and 2.5 °C/min to 250 °C, 10 min. The quadrupole mass spectrometric detector (GCMS-QP2010 Plus) was operated in the high speed scanning mode i.e. with maximum 50 scans/sec at 10,000 amu/sec (mass range 43 – 191 amu). Each spectrum of the modulated peaks gave similarity indices of larger than 95 indicating that the scanning speed was enough to prevent scewing. Qualitative identification was done using the FFNSC library (Flavour, Fragrance, synthetic and Natural compound library, Shimadzu Europa GmbH). As several compounds do have similar fragment patterns, linear retention indices (LRI) were used additionally to confirm the identification. After applying the LRI in automatic library search the identification result in many cases with only one hit left from the hit list which made the automatic identification procedure very reliable. Quantitative determination was done using external calibration. Calibration curves were recorded for crèmes by spiking blank crème with an allergen standard.
The target analysis for the 24 potential allergens in cosmetic products using comprehensive GCxqGCMS with the quadrupol mass spectrometric detector operated in high speed mode with an optimized mass range together with the concept of linear retention index supplies a powerful tool for qualitative and quantitative determination of the potential allergens in cosmetic products. Due to the DMI technique no sample preparation steps are necessary.

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Multidimensional gas chromatographic techniques for the quality assessment of tea tree oil

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Tea tree oil is the essential oil steam distilled from the Australian plant *Melaleuca alternifolia*. It has also been known as melaleuca oil. The *M. alternifolia* species is unique to Australia and native to Northern New South Wales. Tea tree oil contains over 100 components, mostly monoterpenes, sesquiterpenes and their alcohols. The component terpinen-4-ol is the most abundant (minimum 30%) and is said to be responsible for most of the antimicrobial activity. A small number of people experience allergic contact dermatitis as a reaction to dermal contact with tea tree oil. Allergic reactions may be due to the various oxidation products that are formed by exposure of the oil to light and/or air. Conventional gas chromatography is the most used technique for the quality assessment of essential oils but, considering the high number of components, the identification and quantification of particular compounds, responsible of allergic contact dermatitis and microbiological effects is not an easy task. Multidimensional gas chromatography provides the necessary separation power for the complete elucidation of the compounds of interest, using two different columns coated with different stationary phases. The use of a mass spectrometer detector provides an unambiguous identification obtained by comparing the pure spectra information of the resolved compounds with a MS database. Quantification is achieved using a fast quadrupole MS generating a high scan rate for a reliable reconstruction of the peak shape.

Comprehensive LCxLC-PDA-MS applied for the analyses of free carotenoids and carotenoid esters In citrus products

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The largest number of carotenoids found in any fruit are those of citrus: more than 100 different compounds and their isomers have been reported [1]. Citrus species are therefore considered as the most complex natural source of carotenoids [2]. Complex samples require analytical methods characterised by an extremely high resolving power in order to provide through analysis of the sample components.
The carotenoids composition in natural sources has usually been studied by monodimensional HPLC, which often do not provide enough separation and its identification power is limited [3]. Multidimensional chromatography is an approach capable of providing greater resolution. On-line multidimensional HPLC systems usually consist of two columns (ideally with orthogonal separation mechanisms) coupled via a switching valve used as interface, that permits the transfer of specific volumes of 1D effluent onto the secondary column. When used in comprehensive mode, with the multidimensional advantages extended to the whole sample, the MD HPLC analysis offers enhanced peak capacity and identification power due to the formation of group type patterns on the 2D plane.
The present research describes the application of comprehensive HPLC (LC x LC) for the analyses of carotenoids, both in their free and esterified forms, present in different citrus products. A micro-NP column was used in the first dimension, where the components were separated into groups of different polarities, and a monolithic C18 column was used in the second dimension separation, where carotenoids eluted according to their increasing hydrophobicity. By using this novel analytical technique together with the use of PDA and APCI-MS detectors it was possible to identify in the samples several different carotenoids, both in their free and esterified forms including mono-esters and di-esters, therefore also providing information on the native carotenoid esters composition present in the samples. The present optimized methodology could be effectively applied to the identification of the intact carotenoids pattern of other interesting natural matrices.

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Synergistic combination of quantitative GC-FID and innovative GC-MS analyses for the characterization of some *Artemisia* species from Iran

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At least 400 species are listed within the genus *Artemisia* and the interest toward it has always been strong, as testified by the impressive number of papers published in the last 50 years, which is around 50,000 [1,2]. Some Artemisia species are endemic, as in the case reported in the present study, which is dedicated to the chemical investigation of the essential oils obtained from Iranian plants of *Artemisia kopetdaghensis* Krasch ex Poljakov, *Artemisia oliveriana* J. Gay ex Besser, *Artemisia austriaca* Jacq. and *Artemisia diffusa* Krasch ex Poljakov. Accurate GC-FID and GC-MS analyses were carried out. Quantitative analysis was based on both the internal standard method and the measurement of FID response factors. For GC-MS analysis, a quadrupole mass analyzer was used, equipped with innovative library and software (*FFNSC* and *GCMSsolution*, Shimadzu, Japan); the library collects spectra derived from the fragrance and flavor field, each provided with experimental Linear Retention Index (measured on a selection of columns and against various series of standards). Indeed, LRIs were used, thanks to the versatility of the software, for getting rid of library matches that presented high similarity score but values of Retention Index far from the target ones [3].

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POSTERS

Biogenesis and identification of selected substances

D 001

Chemical composition and antimicrobial activity of the essential oil of *Hypericum scabrum* L. root from Iran.

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Hypericum is one of the important genuses in Hypericaceae family. It is represented in Iran by seventeen species including 3 endemics [1]. This genus is composed of shrubs or herbs usually with translucent glands containing essential oils and sometimes red or black glands containing hypericine [2]. *Hypericum* species are medicinal plants known as healing herbs. The whole plant extract has antidepressive effects on neurotransmitter levels in the brain [3]. The antidepressive, anticarcinogenic and antimicrobial activities of these plants are currently under investigation and some of those studies have been reported [4].

In this study, plant material was collected on 23 June 2006 in Khalkhal area (Ardabil province) at an altitude of 1900m, in Iran. The roots of plant were air- dried at ambient temperature in the shade and hydrodistilled by using a Clevenger- type apparatus for 4h. The essential oil was analyzed by GC and GC/MS. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature [5] and by comparison of their mass spectra with the Wiley library or with the published mass spectra. The main components of the oil were α- pinene(45.15%), undecane(19.48%), limonene(7.54%), α- copaene(7.0%), β- pinene(6.94%)and δ- cadinene(4.17%).The essential oil of plant root was found rich in monoterpenes(81.92%).

Antimicrobial activity of the root oil was evaluated using the micro- dilution broth method. The oil showed good inhibitory effects on *Escherichia coli, Staphylococcus aureus* and *Candida albicans*.

Acknowledgements:

We are grateful to Dr. V. Mozaffarian (Research institute of Forests and Rangelands, Tehran) for helpful assistance in botanical identification.

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Antioxidant activity and essential oil composition of root and over ground of *Chaerophyllum macropodum* L. from Iran.

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The Umbelliferae family comprising about 300 genera and 3000 species worldwide is also widespread in Iran. The genus *Chaerophyllum* L. is represented in the flora of Iran by eight species of which two are endemic [1]. Some species of *Chaerophyllum* are used as medicinal plants [2].In this work, the plant material was collected on 3 July 2005 in khalkhal area (Ardabil province) at an altitude of 1950 m near Lonbar village, in North – west of Iran.

The aerial part and root of plant were air-dried at room temperature for ten days then subjected to hydrodistillation for 3 and 4 hours respectively, using a Clevenger – type apparatus. The analysis of oils was performed by using GC and GC/MS methods. Twenty three constituents representing 97% of the essential oil of aerial parts and ten components (96.3%) of the root oil has been identified. The oil of aerial part was characterized by higher amount of α-pinene (22.3%), trans-β –ocimene (17.5%), fenchyl acetate and β-pinene (4.6%). Whereas, the main components of the root oil were myristicin (39.16%), terpinolene (23.05%), trans-β-ocimene (21.9%), and γ-terpinene (5.38%).

Evaluation of antioxidant activity included free radical scavenging activity towards 2, 2-diphenyl-1-pycrylhydrazile (DPPH) radicals, together with inhibition of Fe^{2+}/ascorbate induced lipid peroxidation in liposomes. Compounds responsible for DPPH-scavenging activity were determined by DPPH-TLC assay. Essential oils of aerial part and root of *Chaerophyllum macropodum* Showed free radical scavenging activity.

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D 003

Antimicrobial activity and composition of the essential oil of *Chrysanthemum parthenium* flowers from Iran.

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In Iran, the genus *Chrysanthemum* (syn. *Tanacetum*) is represented by twenty-six species including 12 endemics and so grows in the other regions of world such as Iraq, Turkey, Caucasia and Medial Asia [1].The present report deals with the composition and antimicrobial activity of the oil obtained from the flower of *Chrysanthemum parthenium* (L.) Bernh. Flowers of *Ch.parthenium* were collected on 14 August 2006 in Khalkhal- Asalem road northwest of Iran, at an altitude of 2200m. The air-dried flowers of plant were subjected to hydrodistillation for 3h using a Clevenger type apparatus.

The essential oil was analyzed by GC and GC/MS. Nineteen components were characterized, representing 98.97% of the total components detected. Identification of the constituents of oil was made by comparison of their mass spectra and retention indices with those given in the literature and those authentic samples [2].

The oil of the major constituents was identified as camphor (61.1%), camphene (9.2%), farnesol (4.56%), bornyl acetate (3.5%), chrysanthenon (3.1%), and borneol (2.87%). The essential oil of flowers was found rich in monoterpenes. The essential constituents from aerial parts of *Chrysanthemum parthenium* have been reported by Netherlands researchers in 1996.Camphor and chrysanthenyl acetate are found to be the major constituents [3].

Antimicrobial activity of the flowers oil was evaluated using the micro- dilution broth method. *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis* and *Candida albicans* were used as the test microorganisms. The oil showed good inhibitory effects on S.epidermidis and C. *albicans*.

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Volatile constituents and antioxidant activity of the essential oils of *Zosimia absinthifolia* (Vent.) Link. stem and root from Iran.

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Zosimia absinthifolia (Vent.) Link. is a species of Umbelliferae herb which growing in many regions of Iran and Turkey. It is represented in Iran by two species one of which is endemic [1]. The use of natural antioxidants as food additives for inactivating free radicals receives major attention nowadays, not only for their scavenging properties, but also because they are non-synthetic product and favored by the consumers.

In this work, the antioxidant activity and volatile constituents from stem and root of Z.absinthifolia has been reported. The water distilled essential oils from stems and roots of *Zosimia absinthifolia* were analyzed by GC and GC/MS. In the stem oil of *Z.absinthifolia*, germacrene-D (15.5%), chrysanthenyl acetate (12.5%), β-caryophyllene (8.5%), bicyclogermacrene (6.6%), α- pinene (6.5%), β- phellandrene (4.6%) and cycloisolongifolene (3.4%) were the predominant compounds. The oil obtained from root of the plant were rich in α- pinene (40.9%), undecane (12.8%), β- pinene(11.6%), verbenene(9.5%), α- amorphene(4.8%), δ- cadinene(3.1%) , limonene(3.0%) and γ- cadinene(1.5%) .The stem oil of *Z.absinthifolia* consisted mainly of sesquiterpenes compounds, while in root oil of the plant monoterpenes predominated over sesquiterpenes.

Evaluation of antioxidant activity included free radical scavenging activity towards 2, 2-diphenyl-1-pycrylhydrazile (DPPH) radicals, together with inhibition of Fe^{2+}/ascorbate induced lipid peroxidation in liposomes. Compounds responsible for DPPH-scavenging activity were determined by DPPH-TLC assay. Essential oil of stem of *Zosimia absinthifolia* showed free radical scavenging activity. The essential oil of root is not active on free radicals.

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D 005

Antibacterial activity and chemical composition of leaf essential oil of *Artemisia fragrans* Willd. from Iran.

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The Asteraceae is one of the largest species of plants, and more than 28000 substances have been identified in chemical studies on this family. The genus *Artemisia*, usually represented by small herbs and shrubs, is one of the important and most widely distributed genera of the Asteraceae (syn: Compositae) family. Thirty-four species of this genus are found in Iran, among which tow are endemic (1). Members of this genus have botanical and pharmaceutical interest due to their characteristic scent or taste and are used in the liqueur-making industry(2), in addition of considerable attention of the antimalarial activity of artemisinin that is present in the aerial parts of A. anuua.(3)

The large genus *Artemisia* has been studied chemically by many researchers and the presence of acetylenic compounds and terpenoids, especially sesquiterpene lactones were reported (4)

In this study, the oil obtained by hydrodistillation from the dried powdered of *Arthemisia fragrans* (Leaves) collected from Khalkhal area (Aznow) in north- west of Iran (Ardabil province) at an altitude 1850m , was analyzed by GC and GC/MS.

Nineteen constituents were characterized, representing 91.0% of the total components detected. Identification of the constituents of oil was made by comparison of their mass spectra and retention indices with those given in the literature and those authentic samples.

The main compounds presented were chrysanthenon (23.8%), 1, 8-cineole (23.7%), p-cymene (7. 7%), filifolide- A (5.6%) and filifolone (5.6%).

The antibacterial activity of the oil from leaf was assessed against 2 microorganisms *Staphylococcus aureus* and *Escherichia coli.* A significant antibacterial activity was determined with the agar diffusion method. The results indicated a moderate activity *on staphylococcus* and *Escherichia coli.*

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Biotransformation of sesquiterpen lacton by *Aspergillus niger*

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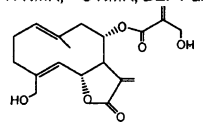
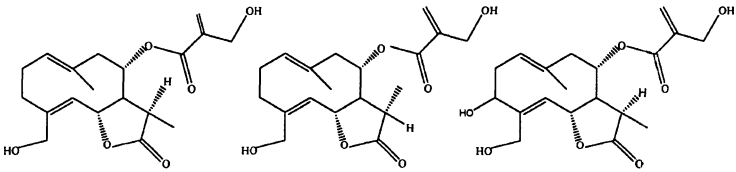
The biotransformation of sesquiterpen lacton from *Aspergillus niger* was studied previously(Hashimoto, Noma &Askawa 2001). In the course of ,our work related to extracted of the aerial parts *Onopordon Leptolopis* the Onopordopicrin (Rustaiyan,Nazarians&Bohlmann) , we studied the biotransformation Onopordopicrin by *Aspergillus niger.* Onopordopicrin compound studied by ^{1}H NMR, ^{13}C NMR, DEPT and IR Spectroscopy.



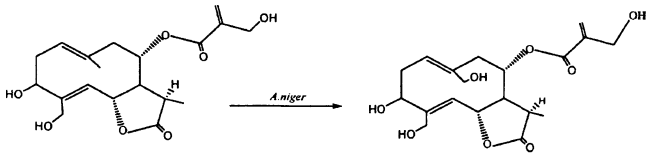
Onopordopicrin

Biotransformation onopordopicrine composition by Aspergillus niger gave 11α,13-dihydro onopordopicrin(**1**), 11β,13-dihydro onopordopicrin(**2**) and 3-Hydroxy-11α,13-dihydro onopordopicrin(**3**).



11α,13-dihydro onopordopicrin(**1**) 11β,13-dihydro onopordopicrin (**2**) 3-Hydroxy-11α1,13-dihydro onopordopicrin(**3**)

Again Biotransformation 3-Hydroxy-11α,13-dihydro onopordopicrin compound by Aspergillus niger gave 3,14-Dihydroxy - 11α,13-dihydro onopordopicrin(**4**)



3 4

3,14-Dihydroxy - 11α,13-dihydro onopordopicrin(4)

Their compounds were established by high-resolation NMR, IR,DEPT ,GCand MS spectral and chemical reaction .

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A study of the structure and thermal behaviour of eugenyl acetate from *Caryophyllus aromaticus* L.

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Aromatic plants have been used since ancient times attributed to their preservative and medicinal proprieties, to impart aroma and flavour to food, due to the essential oils present. In nature, essential oils play an important role in plant protection as antibacterials, insecticides and against herbivores. They also may attract some beneficial insects or repel undesirable ones [1]. Futher, they represent an important class of natural products, since they are often utilized in the cosmetic, food and pharmaceutical industry.
Eugenol is the main constituent of the essential oil from *Caryophyllus aromaticus* L., extracted from dry clove flower buds by hydrodistillation, with an average concentration of around 90%. Clove is widely used in many countries in traditional medicine, especially in dentistry, because of its primary proprieties, such as bactericidal, fungicidal, antiseptic, anesthesic and others [2]. However, eugenol has shown some problems in the practical sense, such as intense and spicy odor, volatility and low physicochemical stability; which can limit its clinical utilization [3].
In spite of these applications and disadvantages, a modification of the eugenol molecule was synthesized by adding an acetate group to the hydroxyl link, which results in the eugenyl acetate molecule. This chemical modification may solve or improve its psycochemical proprieties and pharmacological actions.
The purpose of this study was to determine and examine the relationship of the thermal behavior, using the Differential Scanning Calorimetry (DSC), and the molecule structure of eugenyl acetate synthesized from the raw oil of *Caryophyllus aromaticus* L. through X-Ray Diffration (XRD), and compare it with the standad eugenyl acetate properties.
The thermal analysis was performed using a Mettler DSC 822. Samples (~7.5 mg) were weighed in 100 µl aluminum pans. The DSC scans were recorded in a heating/cooling program at 2°C/min, nitrogen atmosphere (50mLmin^{-1}), and -40°C and 60°C for the initial and final temperatures.
The crystal was mounted on an Enraf-Nonius Kappa-CCD difractometer with graphite monochromated Mo Kα (λ = 0.71073 Å) radiation. The final unit cell parameters were based on all reflections. Data collections were made using the COLLECT program; integration and scaling of the reflections were performed with the HKL Denzo-Scalepack system of programs.
The eugenyl acetate DSC revealed two events, one broad and exothermic which is associated with the crystallization, and the other, a sharp and endothermic which is attributed to the melting process. The eugenyl acetate standard data showed the same events, but with some differences in the amount of energy required and the temperatures range observed. These changes are justified when using the extracted oil in the synthesis.
These impurities act together as second constituents, and when added to the eugenyl acetate synthesis process behaves as a eutectic substance. It is characterized by the inibition of the crystalline process of each substance due to the mixture of compounds, thus it remains in the liquid state. The most important characteristc of this eutectic blend is the lower melting point, rather than each compound crystallizing on its own at a definitive temperature.
The x-ray diffraction analysis confirmed the crystallization process in the standard eugenyl acetate at room temperature, which is due to its purity. The conformation and stereochemistry of the standard eugenyl acetate molecule was determined. Nevertheless, the XRD analysis for the raw eugenyl acetate was not possible because of its physical state, but the crystallization was shown by the DSC experiments at lower temperatures.

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Chemical composition, olfactory evaluation and antibacterial activity of an historical peppermint oil from Bulgaria compared to a commercial oil

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The chemical compositions of a more than 50 years old peppermint oil sample from Bulgaria as well as a commercial oil sample were investigated using GC/FID and GC/MS. Thirtyeight and thirtyfour constituents of the essential oils were identified (representing 98.0%/98.4% of the total composition of the oil, each) with menthol (40.9%/38.0%), menthone (21.8%/23.4%), neomenthol (3.3%/3.2%) and 1,8-cineole (6.5%/5.3%) found to be the major compounds. Using olfactory evaluations a characteristic odor with strong menthol and menthone-notes of the *M. x piperita* L. was found. Chemical composition of Bulgarian historical peppermint oil corresponded to all criteria stated in ISO 856:2006 [1]. The historical peppermint oil demonstrated antibacterial activities against *Bacillus cereus, Staphylococcus aureus, S. epidermidis, Escherichia coli* and *Salmonella abony* and was inactive against *Citrobacter diversus, Pseudomonas aeruginosa* and *P. fluorescens*. The obtained results are in accordance with data, showing that Gram-negative bacteria are more resistible to various antimicrobials [2]. The historical peppermint oil was probably obtained from an almost distinct old local population of the Bulgaro-Mitchum type [3]. The commercial peppermint oil was obtained from an American cultivation of the Mitchum type and its antimicrobial effects (partly investigated in a previous study, but mostly using different strains of microorganisms [4]) compared with the results of the historical oil.
On the basis of the carried out researches and the results obtained we can summarize that the studied historical peppermint oil sample from Bulgaria was chemically and microbially stable for a very long storage period. It differs somewhat from the commercial oil but is absolutely in the line of the ISO/DIS 856:2006 criteria.

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Isolation and identification of 7-hydroxy-calamenene from the essential oil of *Croton cajucara*

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Croton cajucara Benth. (Euphorbiaceae), locally known as sacaca, is a native shrub from the Amazon region used as medicinal and aromatic plant. Embrapa Easter Amazon has established a gemplasm bank for agronomic studies of this species with individuals collected from different areas of the Amazon. Two morphotypes were identified, namely white sacaca and red sacaca. From chemical studies, the essential oils of these plants could be classified in two groups: one rich in linalool (up to 45%), and other rich (up to 44%) in a hydroxylated aromatic sesquiterpene. From MS data and retention index on HP5, the sesquiterpene was assumed to be 5-hydroxy-calamenene [1]. Biological studies with linalool rich oil have demonstrated its leishmanicidal activity [2]. The aim of this work was to obtain the hydroxy-calamenene compound in pure form to be used in biological studies and for confirmation of its identity.

Croton cajucara essential oil rich in hydroxy-calamenene was fractionated over silica gel column chromatography with hexane-ethyl acetate gradient. Fractions containing the hydroxy-calamenene were re-purified by preparative thin-layer chromatography, dissolved in $CDCl_3$ and analyzed by ^1H and ^{13}C NMR in a Brucker 400MHz DRX spectrometer. Signal assignments are presented in Table 1. Hydrogen resonance at 6.56 ppm and 6.95 ppm were clearly singlets, which is compatible with the structural formula of 7-hydroxy-calamenene (Figure 1) instead of 5-hydroxy-calamenene, where a coupling of 7 and 8 hydrogens would lead to two doublets (J=8Hz). All ^1H and ^{13}C experimental data are in good agreement with literature for 7-hydroxy-calamenene [3,4]. HSQC and HMBC experiments confirmed the proposed structure.

Table 1: NMR assingmets for 7-hydroxy-calamenene

#	^1H	^{13}C
1	2.78 (m, 1H)	32.6
2	1.59-1.79 (m, 2H)	28.7
3	1.59-1.79 (m, 2H)	19.9
4	2.52 (dd, 1H, 7Hz)	43.0
4a	-	141.7
5	6.95 (s, 1H)	130.6
6	-	121.1
7	-	151.7
8	6.56 (s, 1H)	114.5
8a	-	131.8
9	1.21 (d, 3H, 7Hz)	23.3
10	2.19 (m, 1H)	31.2
11	1.01 (d, 3H, 7Hz)	21.4
12	0.75 (d, 3H, 7Hz)	17.6
13	2.20 (s, 3H)	15.7

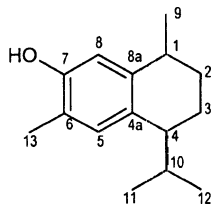

Figure 1: 7-hydroxy-calamenene

Acknowledgements: CAPES

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D 010

Antifungal activity of functional extract obtained from *Inula helenium* L. with ethyl heptanoate as co-solvent.

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Hydrodistillation is the main method used to obtain essential oils from aromatic plants. Traditionally, water is used as solvent in the hydrodistillation process, but recently, fatty acid ethyl esters have been found as an excellent co-solvent in the extraction process. Combining traditional techniques such as hydrodistillation and solvent extraction, the co-hydrodistillation process can be an option to obtain functional extracts. Using hydrodistillation and co-hydrodistillation processes, the extracts obtained from *Inula helenium* L. were analyzed by gas chromatography-mass spectrometry analysis (GC-MS) and the profile of volatile compounds were compared. Significant differences were found in the quantitative and qualitative composition of volatile compounds present. The main components of the volatiles obtained by hydrodistillation and co-hydrodistillation (with 10ml ethyl heptanoate), were *alantolactone* (56.64, 49.50%) and *isoalantolactone* (37.31%, 32.21%) respectively. The extracts were also evaluated for antifungal activity with phytopathogens, including *Phytium* ssp and *Rhizoctonia* spp. The extract obtained by co-hydrodistillation was found to be fungicidal at 500 ppm. The products obtained by co-solvent extraction can be used in the formulation of phytosanitary products.

Kewwords: Hydrodistillation, co-hydrodistillation, *Inula helenium* L., ethyl heptanoate, GC-MS.

Attempts to application of alcohol dehydrogenase from horse liver (HLADH) to synthesis of optically active isomers of whisky lactones

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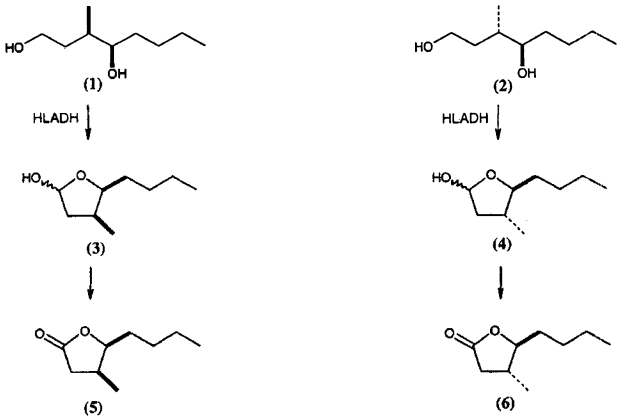
The synthesis of optically pure lactones with various biological activities was the aim of many our research projects [1]. We are especially interested in odoriferous properties of γ- butyrolactones, which are widely spread in the nature [2]. In this paper we present enzymatic synthesis of two isomers of whisky lactones. They are also called "oak lactones", because they are extracted by wine or other alcoholic beverages like whisky and brandy from oak barrels, in which they are kept for maturing [3].
The *cis* and *trans* isomers of whisky lactones (5) and (6) were obtained as products of enzymatic oxidation of racemic *erythro-* and *threo-* 3-methyl-1, 4-octandiols (1) and (2) respectively with commercially available NAD^+-dependent horse liver alcohol dehydrogenase (HLADH). The choice of this enzyme was not accidental. The application of alcohol dehydrogenases in the asymmetric and enantioselective synthesis of chiral compounds is well-documented [4].



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Biotransformations of *cis*- nerolidol by fungal strains

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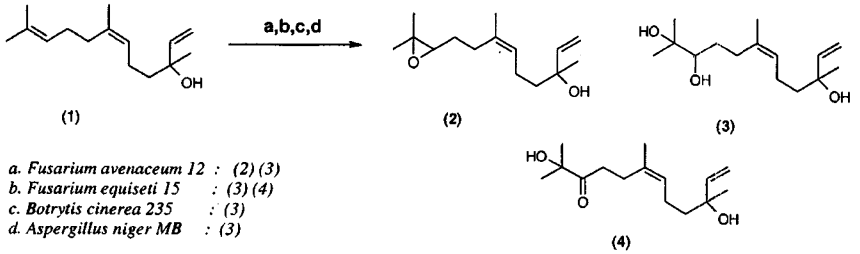
Isoprenoids are widely distributed in nature [1] and they are important biosynthetic precursors of many biologically active compounds. Some of them have also considerable industrial value in the flavour and perfumery industries [2]. Their oxyderivatives obtained in the biotransformations have the wide spectrum of activity and they are valuable blocks in the chemical synthesis. The 12-hydroxy-*trans*-nerolidol obtained from nerolidol in the microbial conversion is the precursor in the chemical synthesis of α-sinensal which possess the special sweet orange aroma and occurs in the other citrus oils. Nerolidol is a component of many essential oils (cabreuva oil, *Dalbergia parviflora* woods oils) and is used as a base note in many delicate flowery odor compositions. The aim of our study is microbial synthesis oxyderivatives of sesquiterpenoides. Recently we have published the conversion of farnesol by fungal strains [3]. Here we report the biotransformation of nerolidol **(1)** by means of four strains of microorganisms, which were selected in the screening procedure. The substrate was transformed by *Fusarium avenaceum 12, Fusarium equiseti 15, Botrytis cinerea 235* and *Aspergillus niger MB* (being the UV mutant) into three oxyderivatives of nerolidol **(2, 3, 4)**.



a. Fusarium avenaceum 12 : (2) (3)
b. Fusarium equiseti 15 : (3) (4)
c. Botrytis cinerea 235 : (3)
d. Aspergillus niger MB : (3)

In the culture of these fungal strains the regioselective and enantioselective oxidation of terminal double bond was observed. 10, 11-epoxynerolidol **(2)**, 1,2-dihydroxynerolidol **(3)** or dihydroxyketone **(4)** were obtained as the products of this oxidation.

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Chemical composition and antibacterial effects of essential oil of *Majorana syriaca* against food-borne pathogens and methicillin-resistant *Staphylococcus aureus*

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The aims of the study were to identify antibacterial effects of essential oil of *Majorana syriaca* against food-borne pathogens and against methicillin-resistant *Staphylococcus aureus* and to determine chemical composition of the essential oil.

The essential oil of *Majorana syriaca* was tested by the modified diffusion method for testing of essential oils in vapour phase [1] against 6 strains of methicillin-resistant *S. aureus* (MRSA), which were obtained from two hospitals and one standard strain of methicillin susceptible *S. aureus* ATCC 25923 (MSSA). 5 of 6 strains of MRSA and the standard strain were inhibited in concentrations 0.0083-0.033 µl/cm³ of air. Only one of the MRSA strains was not inhibited by the essential oil in these tested concentrations.

Further, the essential oil was tested by the same method against 2 gram-positive and 3 gram-negative foodborne bacteria. Gram-positive: *Listeria monocytogenes* ATCC 7644; *Staphylococcus aureus* ATCC 25923. *Gram-negative*: *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; *Salmonella enteritidis* ATCC 13076. *S. aureus* and *S. enteritidis* were inhibited by the essential oil in concentration 0.0083 µl/cm³ of air, *E. coli* and *L. monocytogenes* were inhibited in concentration 0.017 µl/cm³ of air. Only *P. aeruginosa* was not inhibited in the tested concentrations.

Chemical composition was carried out by gas chromatography/mass spectrometry (GC/MS). The identification of the chemical constituents was based on comparison of their Kováts indices, obtained using n-alkanes (C8–C22), and mass spectra of the NIST/NBS, Wiley libraries. The content of each component was estimated from the value for the respective peak area by divided by that for the total peak area. Carvacrol (81.19%) was evaluated as the main constituent of the essential oil, followed by p-Cymene (6.29%), Thymoquinone (3.90%), trans-Caryophyllene (1.85%), cis-Sabinene hydrate (0.96%) and α-Thujene (0.88%).

In conclusion we can hypothesise about high antibacterial effects of essential oil of *Majorana syriaca* from vapour phase, because based on these tests it is possible to say, that *S. aureus* is one of the most sensitive bacterium to the essential oil [2] and the essential oil of *Majorana syriaca* is highly effective in vapour phase against MRSA strains. Further, the essential oil could be used in fight against food-borne bacterial pathogens, because most of the tested bacteria were inhibited by the essential oil in very low concentrations. The high antibacterial activity against foodborne bacteria is supposedly because of the high content of carvacrol [3].

Acknowledgements: Ministry of Educacion of the Czech republic MSM 6046137305; CIGA 20082009.
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Chemical composition and antibacterial activity of *Rhaponticum carthamoides* essential oil

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Rhaponticum carthamoides (Willd.) Iljin (Asteraceae) is a perennial herbaceous plant originating in southern Siberia and Middle Asia and is currently cultivated mainly in Russia and East European countries as a medicinal plant. The roots and rhizomes of the plant have been traditionally used in folk Siberian medicine mainly as tonic, roborant and stimulant. A great number of constituents were isolated from this species, however, the composition of its essential oil remained unclear. Therefore, we have carried out a detailed analysis of *Rhaponticum carthamoides* root essential oil by GC, GC-MS, GC-FTIR techniques. The oil was isolated from the roots grown in the Czech republic. The distillation yielded 0.043% w/w of essential oil of dark yellow colour, resembling in its olfactometric properties the smell of fresh-cut roots. In total, 30 components were identified, accounting for 94.1% of total volatiles in the oil. New norsesquiterpene (22.6%), followed by known compounds aplotaxene (21.2%) and cyperene (17.9%), were isolated and structures confirmed by ^{1}H, ^{13}C and 2D NMR spectroscopy (COSY, HSQC, HMBC, INADEQUATE and NOE). Selinene type sesquiterpenes and aliphatic hydrocarbons were among minor constituents of the essential oil. The composition determined by us differed significantly from previous brief reports in the literature [1, 2]. The oil was tested against five G+ and three G- bacteria and one yeast using broth microdilution method. Antimicrobial activity was observed against five strains in the concentrations between 32–256 µg/mL. The most susceptible strain was *Staphyllococcus aureus* (MIC 32 µg/mL), followed by *Listeria monocytogenes* and *Candida albicans* (MICs 128 µg/mL), G- bacteria were not inhibited.

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Chemical Composition and Antibacterial Effect of Essential oil of *Eucalyptus camaldulensis* Dehn. Growing in Turkey Against Bacterial Seed Pathogens of Tomato

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The chemical components of the essential oils obtained by the Clevenger hydrodistillation method from leaves and fruits of *Eucalyptus camaldulensis (EC)* were analysed by GC-MS. From identified 30 constitutents of the essential oil, Spathulenol (23.5%) and Globulol (18.65%) were main components. Antibacterial activity of the essential oil of *EC* was investigated against some economicaly important bacterial seed pathogens of tomato including *Pseudomonas tomato* (syringae) pv. *tomato*, *Clavibacter michiganensis* subsp. *michiganensis* and *Xanthomonas axonopodis* pv. *vesicatoria*. Volatile phase effects of different doses of the EC were determined for the three bacterial seed pathogens of tomato plant. The volatile effect of the essential oil on seed germination were also determined. Seed treatment of EC can be a natural control measure for tomato bacterial seed pathogens, instead of agrochemical seed treatments.

Chemical Composition and Antibacterial Effect of Essential oil of *Cinnamomum zeylanicum Blume* Against Seed Bacterial Pathogens of Bean, Tomato and Pepper

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Akdeniz University, Faculty of Agriculture, Department of Plant Protection, 07070, Antalya-TURKEY

The chemical components of the essential oils obtained by the Clevenger hydrodistillation method from bark of of *Cinnamomum zeylanicum (Zc)* were analysed by GC-MS. From identified constitutents of the essential oil, Cinnamaldehyde (84.87%) was main component. Antibacterial activity of the essential oil of *Zc* was investigated against some economically important plant bacterial pathogens including *Xanthomonas axonopodis* pv. *phaseoli*, *Pseudomonas syringae* pv. *phaseolicola*, *Pseudomonas tomato* (syringae) pv. *tomato*, *Clavibacter michiganensis* subsp. *michiganensis* and *Xanthomonas axonopodis* pv. *vesicatoria*. Volatile phase effects of different doses of the *Zc* were determined for the three bacterial seed pathogens of tomato plant. The volatile effect of the essential oil on seed germination were also determined. Seed treatment of *Zc* can support on efforts for searching a natural pesticide for controlling bean, pepper and tomato bacterial seed pathogens, instead of using chemical pesticides .

Microbial Transformation of Grifolin, Neogrifolin, Sclareolide, Sclareol, Sclareodiol and Pinane-2,3-diol and Biological Activities of Metabolites against MRSA

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In the continuing studies on microbial transformation and the searching of useful compounds of terpenoids [1] the microbial transformation of grifolin (**1**) and neogrifolin (**2**) from the mushroom *Albatrellus confluens*, sclareolide (**3**), sclareol (**4**) and sclareodiol (**5**) by *Aspergillus niger* TBUYN-2 and *A. cellulosae* and the biological assay of the biotransformation metabolites together with metabolites of (+)-pinane-2,3-diol by *A. niger* against methicillin-resistant *Staphylococcus aureus* (MRSA) were investigated.
Microorganisms were cultivated rotatory (100 rpm) in the 200ml Czapek-pepton medium (500ml Erlenmeyer 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 for 4-7 days. 2 ml Aliquots of cultured broth was applied to Extrelute column every day 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.
Seven metabolites (**6~12**) from **1**, three metabolites (**13~15**) from **2**, four metabolites (**16~19**) from **3** and **20** from **4** and two metabolites (**21~22**) from **5** were obtained in the biotransformation by using *A. niger* and *A. cellulosae*.
Grifolic acid, a constituent of *A. sispansus*, (-)- and (+)-2,5-dihydroxy-3-pinanones from (+)-pinane-2,3-diol showed the strong antifungal activity against methicillin-resistant *S. aureus* (MRSA). On the other hand, **1** and **2** showed the strong cytotoxic activities against HL-60.
This time, we will discuss the metabolic pathways based on the identification of metabolites together with the biological activities against the methicillin-resistant *S. aureus*.

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Chemical Constituents and Antibacterial Activity of Essential Leaf Oil obtained from *Ferula foetida* (Bunge) Regel (Umbelliferae)

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The genus of *Ferula* which belongs to the Umbelliferae family (subfamily: Apioideae) has 133 species distributed throughout Mediterranean area and central Asia [1-3]. The chemistry of this genus has been studied by many investigators. More than 70 species of *Ferula* have already been investigated chemically [4]. Several species of this genus have been used in folk medicine [5]. The Iranian flora comprises 30 species of *Ferula*, of which some are endemic [2-6]. The popular Persian name of the most of these species is "Koma" [6]. *Ferula foetida* (Bung) Regel is one of these species which is distributed in different regions of Iran [2]. Anti-spasmodic, anticholinergic and smooth muscle relaxant activities of the aqueous extracts of some species of *Ferula* have previously been reported [7-8]. This investigation describes the constituents of the oil of *F. foetida* which has not been studied previously.
The essential oil of *Ferula foetida* leaves obtained by hydrodistillation was analyzed by GC and GC/MS. Among the 38 identified constituents accounting for 81.57% of the total oil, the major components were elemicin (34.56%), 2,5-dimethyl-4-ethylthiazole (15.56%), methyleugenol (9.32%), 2,3,4,5-tetramethylthiophene (6.57%), and hexafarnesyl acetone (5.01%). Antimicrobial activity of the essential oil was investigated against various Gram-positive and Gram-negative bacteria. The essential oil of *Ferula foetida* showed activity against Gram-positive and Gram-negative bacteria.

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Volatile components as chemosystematics markers of selected liverworts

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The Marchantiophyta (liverworts) we can find everywhere in the world except in the sea. The classification of the liverworts is often morphologically difficult since they have small gametophytes. The secondary metabolites, such as lipophilic terpenoids and aromatic compounds in their cellular oil bodies can assist in their taxonomic differentiation [1,2]. The pattern of terpenoids and aromatic compounds often depends not only on the developmental stage, season and altitudinal distribution, but also on sexual (male, female and sterile) forms of the same species and collection from different locations. The knowledge of their chemical constituents might serve to delineate not only chemical, but also evolutionary relationship within the Marchantiophyta at the genus or family level.

In this communication, we wish to report the volatile components of selected liverworts with the focus on their chemosystematics. All liverworts were extracted with diethyl ether and then each extract were analyzed by TLC and GC/MS. Several volatile mono-, sesqui- and diterpenoids, and also aromatic compounds were identified.

The Japanese and German *Pellia endiviifolia* biosynthesize a persistent pungent taste that is due to the sacculatane-type diterpene dialdehydes, among which sacculatal is the main component. The sesquiterpene lactones (4-*epi*-arbusculin A, α- and β-cyclocostunolide) that cause allergic contact dermatitis are characteristic compounds detected in the Japanese *Frullania tamarisci* subsp. *obscura*. *Wiesnerella denudata* from Borneo and Japan belongs to two different chomotypes; the Japanese species to guaianolide-type, while the Borneo liverwort to costunolide-guaianolide chemotype. *Radula perrottetii*, like other *Radula* species, is rich in bibenzyl derivatives. *Trocholejeunea sandvicensis* is one of the liverwort to produce a large amount of pinguisane-type sesquiterpenoids. It contains dehydropinguisenol as the main compound. The Japanese *Reboulia hemisphaerica* collected in two different places is characterized by totally different chemical composition. The first collection contains gymnomitranes, but in the second one we identified cyclomyltaylane and chamigrane sesquiterpenoids. Besides *Reboulia*, to Reboulioideae subfamily belongs also *Asterella*. Two investigated Mexican *Asterella* spesies are chemically different. Sesquiterpenes are characteristic of *A. echinella*, while *A. venosa* produced mainly monoterpenoids. In ether extract of German and Chinese *Monoselenium tenerum* only two compounds have been detected. The first one was a new bibenzyl derivative, 3,5,4'-trimethoxybibenzyl and the second one a phthalide, identified as 3-(4'-methoxybenzyl)-5,6-dimethoxy-phthalide The chemical constitution of *M. tenerum* is absolutely different from that of all Marchantiales species. It is quite interesting to note that the present species is closely related chemically to the *Frullania* (Jungermanniales) chemotype II, which produces bibenzyls as the major product. The most interesting species belonging to the Metzgeriales order was *Fossombronia angulosa*. This liverwort collected in Crete Island (Greece) contained exactly the same compounds as previously found in brown algae [3]. Such chemical similarity suggests that some families of liverworts and algae may have an evolutionary relationship.

In conclusion sesquiterpenoids, such as eudesmane, germacrane and guaiane sesquiterpene lactones, pinguisane and gymnomitrane sesquiterpenoids and sacculatane and cyatane diterpenoids can be used as chemosystematic markers of liverworts. In several cases, monoterpenes and aromatic compounds especially bibenzyls derivatives are valuable chemosystematic indicators.

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Antibacterial activities of essential oils extracted from fruits of *Schinus molle* and *Schinus terebinthifolius* against some plant pathogenic bacteria

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The essential oils occurring in the fruits of *Schinus molle and Schinus terebinthifolius* were obtained by steam distillation and subsequently analyzed by GC and GC-MS. The isolated oils were tested against four plant pathogenic bacteria: *Agrobacterium tumefaciens* , *Pseudomonas savastanoi* pv. *savastanoi, Pseudomonas syringae pv. syringae* and *Pectobacterium carotovorum*. All essential oils exhibited a high level of antibacterial activity against *Agrobacterium tumefaciens* strains and *Pectobaterium carotovorum*. However, they were less efficient against *Pseudomonas savastanoi* and *Pseudomonas syringae pv. syringae*.

Chemical Compositions of Seven Essential Oil Samples from 3 Nigerian Acanthaceae: *Asystasia gangetica*(L), *Brillantaisia patula* T.And. var and *Hypoestes phyllostachya* 'Rosea'

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Seven essential oil samples obtained from leaf, stem, root and seed of 3 Nigerian Acanthaceae plants were analysed by GC, GC-MS to identify the rich part in specific chemical constituents that can be utilized industrially and used for taxonomic classifications. Analyses of the essential oils from three parts [aerial, seed and root] of Nigerian *Asystasia gangetica* [AG] resulted in identifying forty-nine compounds in the aerial, 12 in the seed and four in the root parts. The 3 essential oils characterised by dominance of aromatic and saturated compounds respectively [aerial (46.90%,49.82%), seed (87.34%,3.01%), root (100%,0%)]. Derivatives of phthalate are obvious in the three essential oils of *AG* [(aerial19.57%), (seed44.10%), (root58.44%)]. Notable also are the presence of few hetero compounds. Stem and leaf volatile oils obtained from *Brillantaisia patula* [BP]. 6 compounds were identified in the stem oil makes about 99% of it and dominated by alcohols. Abundant compounds are 1-octenol (60.37%) and 3-octanol (26.05%). The usual ubiquitous terpenes are conspicuously absent in the stem oil. The ten significant compounds identified in the leaf oil is also dominated by alcohols. Most abundant compound 1-octenol accounts for 42.46% of the leaf oil. Stem and leaf oils are characterised by dominance of alcohols. 1-octenol can be taken as the taxonomic compound for identifying BP specie, which is also a good commercial source for the alcohol. Stem and leaf volatile oils were obtained differently from *Hypoestes phyllostachya 'Rosea'*[HR]. 14 main volatiles were identified in the oil of the stem part, most abundant compounds are 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-2(1H)naphthalenone(38.01%), 1ethenyl, 1-methyl-2(1-methylethenyl),4-(1-methylethylidene)cyclohexane(14.26%), tetramethyl cyclo propylidene, methylbenzene(7.38%). Ubiquitous monoterpenes are scarce in this stem oil that has appreciable amount of sesquiterpenes. Twenty-five compounds were identified in the leaf oil of HR. The most abundant compounds are 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-2(1H)naphthalenone(23.3%), and 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane(20.73%), which are same as in the stem parts. Leaf oil contains appreciable amount of sesquiterpenes, while the known ubiquitous monoterpenes are also absent. The two most abundant compounds are the same in both leaf and stem part of HR essential oils. They could be taken as taxonomic compounds for identifying HR as well as being commercial sources of the naphthalenone and cyclohexane derivatives. The oils are characterised by presence of derivatives of azulenes and naphthalenes. Natural hypoestoxide, a bicyclic diterpenoid which was isolated from HR is a potent non-steroidal anti-inflammatory drug and has antitumor activity [1]. Sesamin and sesamolin are similar simplexolin which are from the extract of *Justicia simplex* D.Don.[2]. Tryptanthrin an alkaloid-like compound is the active principle in the fresh juice from leaves of *Strobilanthes cusia* Kuntze use as remedy for athletes' foot [3]. AG proposed to have high nutritive value; its leaf extract has been reported to have anti-asthmatic properties [4]. Reports on volatile composition of the three Acanthaceae in this report are scarce in literature.

Acknowledgements:We acknowledge the assistance of Staff of the herbarium, Botany and Microbiology Dept in the identification and confirmation of each plant, and also University of Sokoto, Nigeria where the GC, GC-MS was done.

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Microbial transformation of bicyclic oxoderivative obtained from (+)-3-carene

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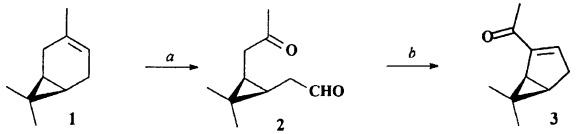
Microbial transformation is an effective tool for the structural modification of bioactive natural and synthetic compounds. Its application in asymmetric synthesis is increasing due to its versatility and ease.

In our previous papers we presented bioconversion to the acetate [1], propionate and butyrate [2] from mixture of diastereoisomers of secondary bicyclo[3.1.0]hexane alcohol obtained in three 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 terpenes the microbial transformation of 6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl-ethanone **3** was carried out by ten different microorganisms such as *Saccharomyces cerevisiae, Aspergillus sp., Penicillium sp., Pseudomonas sp...*

The key-compound, bicyclic enone **3** was synthesized in a two step procedure. Ozonolysis of **1** followed by intramolecular aldol condensation of ketoaldehyde **2**, forming during reductive decomposition of ozonide, afforded desired ketone **3** [3], which was distilled under reduced pressure and then purified by means of column chromatography on silicagel.



(a) O_3/AcOH;
(b) 5% NaOH;

Microorganisms were cultivated at 28°C for 24-48 hours in the 250 ml Erlenmeyer flasks and rotated at 200 rpm. After sufficient growth of microorganisms the substrate **3** was added (50 µl) incubated for further 10 days under the same conditions. The progress of biotransformation was monitored by TLC and evaluated by GC/MS. The biotransformation process was stopped by successive EtOAc extraction, concentrated under reduced pressure and then applied to silica gel chromatography column for isolation of the metabolites. The stereostructure of isolated metabolites were established by ^1H, ^{13}C NMR and IR spectroscopy.

Synthetic and biosynthetic details of the applied procedures will be presented with emphasis on stereochemical aspects.

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A Study of Fragrance Materials in Roll on Deodorants formulation

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Man's first attempt to control body odour probably took the form of bathing in some convenient stream or lake. We know that both the ancient Greeks & Romans undertook practices to achieve more pleasant body odour. Perfume oils & other odoriferous materials probably were the first "products" used to favorably alter body odour.The generally accepted attitude that body odour is an undesirable attribute is interesting in itself. The migration of people to metropolitan areas, the population explosion & influence of advertising have all increase the desire to change normal body odour into something which is more accepted by our society. It should be remembered, however that the odour of freshly washed healthy body is not unpleasant; it is the favourable or unfavourable associations with this conditions which have influenced the publics attitude.

The present paper deals to evaluate the stability study of various flavouring and fragrance materials in the Roll on Deodorant formulation containing active ingredient. For stability study 90 ingredients of fragrance & flavouring materials have been incorporated in to Roll on Deodorant at 1%. These fragrant Roll on Deodorant have been stored under ambient conditions and at 48^{0}C in oven for stability. These samples have been evaluated periodically for the odor profile by sensory panel of 7 people. During study it was found that most of the fragrant materials that were tested at ambient temperature as well as 48^{0}C show good stability, whereas few of chemicals are unstable and give off odor during storage under ambient conditions and 48^{0}c temperature.

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Fluidized Bed Extraction – a new technology for the extraction of volatile aromatic compounds of medical and spicy plants

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Within the optimisation of established methods and the development of new innovative procedures relating to the production of essential oils product quality, production costs and the conservation of natural resources are particularly important.
In this context it could be managed to develop a technological solution (pilot plant level) that meets these high demands. Central point of the equipment is a fluidized bed where the plant material is efficiently extracted in superheated water steam. The process-time until exhausting extraction is controlled by an adequate measuring and control system and mostly finished only within a few minutes (<10min; yield >90%). To get a proper fluidized bed for some plants – e.g. cloves – a pre-treatment is required which results in pellets. Due to the short time extraction the whole process can be evaluated being gentle, energy- and labour-saving. The products are quite similar compared to conventionally produced essential oils, whereas it has been proved that actually described degradation of sensitive compounds is in certain cases reduced. If so the product complies to come closer with the natural composition.
Further advantages of the technology regard the value of by-products – e.g. the plant residual and the condensate. First mentioned – the residual plants – are almost dry after extraction (residual moisture < 6%) and there is no further cost-intensive drying process necessary to make the material durable and therefore marketable. The condensate – the second by product – still contains sparse amounts of essential oil and is therefore suited for several applications – e.g. in the field of food flavouring.

Study on the complete extraction technique of the essential oil of niaouli – *Melaleuca quinquenervia* (Cav.) S.T. Blake – from New Caledonia

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New Caledonian niaouli oil is an item of commerce for more than hundred years. However, its detailed chemical composition was only recently established, showing also the possibility to distinguish between several chemotypes [1]. Main component of the oil is 1,8-cineole, but nevertheless, the oil is rich also in sesquiterpene alcohols such as viridiflorol, α-guaiol, ledol, α-eudesmol, β-eudesmol, 10-*epi*-γ-eudesmol etc., viridiflorol being the major one. This compound could be of interest, since viridiflorol-rich oils were found to exhibit larvicidal activity against the larvae of a mosquito (*Aedes aeqypti*) [2].
Lab-distilled niaouli oils were found to contain up to 48% of viridiflorol. However, commercial niaouli oils from New Caledonia, being produced mainly in the southern part of the island because of the higher oil content of the leaves, are usually rich in 1,8-cineol (up to 55%) and depleted in the oxygenated sesquiterpene fraction (with a viridiflorol content not exceeding 7,0%). Because of economic reasons – short distillation times of not more than 2 hours and low steam flows – , much of the valuable heavier compounds remain in the exhausted plant material. In consequence, the resulting essential oil of commerce represents only a fraction of the complete volatile matter of the plant.
The aim of this work was to found the optimum conditions for the complete extraction of the industrial essential oil, in view of the lowest possible cost increase of the process. The composition of the oil was studied at different stages of the distillation process and under different conditions of steam flow. The studies revealed that the concentration of the sesquiterpene alcohols could be substantially increased by working with a higher steam flow, in combination with prolonged distillation times. Enhancing only the distillation time and maintaining the steam flow at the usual low level had practically no increasing effect on the concentration of viridiflorol. Using a steam flow three times higher than the usual one, after 5 hours 95% of the total volatile matter and 85% of the total viridiflorol content could be recovered. Under these conditions, prolonging the distillation time for 1 additional hour, the complete volatiles were distilled. This technique could represent a viable alternative to the traditional method used in New Caledonia, in order to obtain viridiflorol-rich niaouli oils on industrial scale.

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Study of volatile chemical composition of *Myrtus communis* L. berries for quality assessment of Corsican alcoholic beverages

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Chemical compositions of essential oils obtained in laboratory from ten sampling location and collective essential oil obtained using industrial process was established by GC, GC/MS and compared. No significant difference was reported between the various samples. 39 components were identified representing 99.0% of the total amount. The major components were β-pinene (44.8±2.3%) and 1,8-cineol (26.1±1,6%). The essential oils was characterized by 15 monoterpene hydrocarbons (60.3± 4.8%), 10 oxygenated monoterpenes (34.3±4.3%), 9 sesquiterpene hydrocarbons (2.7±0.3%), 2 oxygenated sesquiterpenes (0.4±0.1%) and three other compounds (1.0±0.2%) for the ten sites of sampling.

A large part of these volatile components were also detected after extraction by HS-SPME. The major components of the HS-SPME volatile fractions obtained were always β-pinene (44.9 ± 2.3%) and 1,8-cineol (26.1 ± 1.6%). Moreover, no chemical variability in the volatile fractions of *Myrtus communis* berries was observed using HS/SPME. The volatile compositions of two commercial alcoholic beverages (myrtle liqueur and eau-de-vie) from Corsica were carried out using HS/SPME, GC and GC/MS. The volatile composition was characterized by 21 components from liquor and 18 compounds from eau-de-vie. The two alcoholic beverages were characterized by the high amounts of monoterpene hydrocarbons and oxygenated monoterpenes. The major components obtained from berries and alcoholic extracts were always β-pinene (31.9-60.0%), 1,8-cineol (13.5-29.0%), limonene (2.9-5.6%) and *para*-cymene (3.0-9.0%); except for eau-de-vie witch exhibited a relative high content of (E)-β-caryophyllene (4.2%). HS-SPME is simple, rapid, clean and environmentally friendly approach for the determination of the volatile compositions of commercial alcoholic beverages.

Keyword:

Myrtus communis; Myrtle liqueur; Eau-de-vie; SPME; GC/MS.

Chemical composition and antimicrobial activities of an historical rose oil from Bulgaria

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The chemical composition of an historical rose oil (produced in 1944) from Bulgaria was investigated by GC and GC/MS. The rose sample was found to be rich in citronellol (23.4%), geraniol (19.0%), nonadecane (11.9%) and nerol (7.5%). In general, the chemical composition of this historic rose oil corresponded exactly to the criteria stated in ISO 9842:2003 [1].
Furthermore, antimicrobial activities of the rose sample were tested against three Gram-positive and three Gram-negative bacteria as well as against 2 yeasts. This old oil still demonstrated antimicrobial effects against all of the used microorganisms, with *Bacillus cereus* found to be the most susceptible and *Pseudomonas aeruginosa* the most resistable strain among the tested microorganisms.
In addition, a comparison of these analytical and antimicrobial results with data of some confirmed varieties from the type "Bulgarian red oleaginous rose" [2] will be given.

Acknowledgements: K.W. thanks the Austrian Academy of Sciences for a DOC-fFORTE-fellowship.
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Effect of the geographical region of production on the quality of cold-pressed lemon essential oil

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In order to evaluate the differences in the qualitative and quantitative composition of lemon essential oils obtained from different regions of the world, the composition of genuine commercial samples from Argentina, Ivory Cost, Italy, Spain and Uruguay was studied [1,2]. The oils were obtained by industrial processing (FMC on line) during the 2005 season. The samples were representative of the species and its geographic area of distribution. The volatile fractions were analyzed by HRGC-FID and GC-MS The results were compared with those obtained for Uruguayan and Italian FMC oils. The results obtained are informative of the different oils quality and explain the variation of the cold pressed lemon oils from different region of the world. The results statistically elaborated have demonstrated that the oil composition allowed us to correlate the origin of the samples and, thus, their sensorial value according with their flavouring properties.

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Following up the influencing factors of drug quality of Hungarian wild chamomile during primary processing

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Higher proportion of chamomile drug of Hungary originates from wild collection. The Hungaricum quality drug collected in the Great Plain region has outstanding properties due to the unique ecological conditions of the habitat and probably to the adapted populations together. The collected inflorescence has strong, rich flavour and slightly bitter taste, during processing it does not fall apart. Considering the organoleptic and chemical characteristics of the drug coming from the saline soil of the Great Plain it constantly gives first class commodity. In order to represent the unique quality of the Hungaricum chamomile drug properly, the process of Protected Designations of Origins (PDO) is under way at the EU Comission.

Within the frameworks of project cooperation samples were taken during primary processing of chamomile in a large-scale company, Herbária Close Company; and in a small enterprise, Pusztadrog Ltd. The aim of sampling was to follow the practical steps of post-harvest processing of the high grade basic material, and to unfold their influencing effects on quality.

During the experiment fresh samples of 5 wild chamomile populations were collected and followed on a conveyor drier with 5 drying belts on 50-55 C° temperature, during natural pre-drying, and in plate-chamber drier. The sorting and purification were sampled as well. Besides the effect of purity (presence of foreign plant parts, stem) the essential oil content and its main compounds were measured as well.

The essential oil content of chamomile drug depending on the sampling phase changed between 0,30 and 0,53%. According to our data the essential oil content of the end product depends on the genetic potential of the basic material (habitat - population), on the purity of the drug (foreign plant parts, stem), does not depend on the drying technology used (conveyor drier or plate-chamber drier), and on the natural pre-drying (spreading out for 1-2 days on the sun before drying). The typical compounds of the essential oil of the Hungarian wild chamomile were found in different ratio in the samples of the populations. The main compound α-bizabolole between 7,5-50 %, and the chamazulene between 4,6-12,2 % were measured. The deciding factor for essential oil composition (chemosyndrome) is the genetic potential of basic material (habitat – population). During primary processing the ratio of the main components slightly changed however in this change no tendency related to drying was demonstrable.

Our work was supported by GVOP (3.2.1.-2004-04-0134/3.0).

Chemical composition, antifungal and antibacterial activity of the essential oil of *Chamaecyparis nootkatensis* (D. Don) Spach. from Spain

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The *Chamaecyparis* Spach. genus belongs to the *Cupressaceae* family. It is a small genus that contains only few species. C. *nootkatensis* is native from North America, but it also grows in other continents. In Spain, it has been widely cultivated and is very commonly used as ornamental tree in our gardens.

The antifungal and antibacterial activity of the essential oil of this species has been tested in the following microorganisms: *Candida albicans*, *Bacillus polymyxa*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella* sp. and *Serratia marcescens*. Most of them were sensitive to the treatment although the grow inhibition varied with the microorganism tested.

The chemical composition of the oils was also analysed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MS). The oil was richer in monoterpenes than in sesquiterpenes, being the main compounds limonene (53.2%), δ-3-carene (21%) and α-pinene (12.2%).

As far as we know, this is the first report about the chemical composition, antifungal and antibacterial activity of this species.

Composition of hydrosols of *Sambucus nigra* L. and *Rosa rugosa* Thunb. flowers

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Hydrosols (hydrolats), by-products in essential oil production, have been well known and used *e.g.* in aromatherapy and as food and cosmetic additives. Recently, hydrosols as the only products obtained during hydrodistillation of plant material become more and more popular.

Hydrosol from elder flowers (*Sambucus nigra* L.) was obtained as the only product and hydrosol from flowers of Japanese rose (*Rosa rugosa* Thunb.) was obtained together with its essential oil. The constituents of both hydrosols were isolated by extraction with diethyl ether and analysed by GC and GC-MS. The total content of constituents was 208 mg in elder and 490 mg in rose hydrolat.

The main constituents in elder hydrosol were cis- and trans-linalool oxides (pyranoid), that were accompanied by cis- and trans-linalool oxides (furanoid), linalool and thymol. In rose hydrosol 2-phenyl ethanol dominated and the minor constituents were thymol, citronellol, geraniol, nerol, eugenol and methyleugenol.

Because of their fragrances and composition resembling appropriate essential oils, both hydrosols could be new valuable cosmetic components.

E 010

Chemical Composition of Essential oil of *Scirpus lacustris* and *Cyperus longus* (Cyperaceae)

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The genus *Scirpus* and *Cyperus* belong to the sedges(Cyperaceae) family. Cyperaceae are one of the largest families of vascular plants ,with about 4000 to 5000 species in 70 to 105 genera[1,2].Many of species of sedges (Cyperus articulatus and C. prolixus, Cyperaceae) are widely utilized for various medicinal purposes, including birth control and induction of labor, Anticonvulsant properties and in hallucinogenic preparations.[3,4]
The essential oil composition of the aerial parts of *Scirpus lacustris* and *Cyperus longus* grown in Karadj regions of Tehran. The essential oil were obtained by hydrodistillation and have been analyzed GC by GC/MS. Mustakon (28.5%) and α -copaene (18.8%) were detected as major compounds in *Scirpus*. Cyperene(31.4%), rotundene(7.6%) and cyperotundene(6.8%) were found as main compounds in *Cyperus* .

Key words: Scirpus lacustris, Cyperus longus, essential oil , Mustakon, Cyperene

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Furyl and thienyl analogues of citronellol

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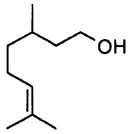
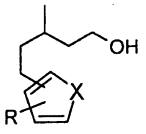
The EU Cosmetics Directive introduced considerable limitations in utilization of natural monoterpene alcohols in fragrances and cosmetics. On the other hand, compound like citronellol is well-known and highly valued component of perfume compositions, so searching of "scent-identical" analogues of these alcohols is well motivated.

In our work, we decided to replace the isobutenyl moiety in the parent monoterpene alcohol by a furyl or thienyl substituent.



CITRONELLOL



X=S - THIENYL ANALOGUE
X=O - FURYL ANALOGUE
R= CH_3 or H

The scent characteristics of obtained compounds are similar to their natural analogues. Introduction of heteroaromatic ring brought lower, relative to natural analogues, volatility factors and much higher tenacity of odor.

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Antibacterial Activity and Chemical Composition of the Essential Oils from Thai Medicinal Plants against Nosocomial Pathogens

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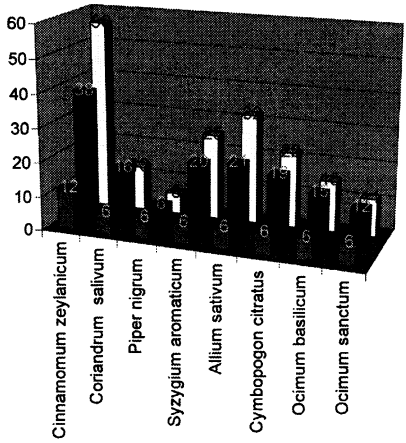
Nosocomial infections, an infection acquired in a hospital, are specifically an infection that was not present to the patient being admitted to the hospital, but occurred within 72 hours after admittance to the hospital. After antibiotics came into common usage in 20th century, bacteria resistant to antibiotics were selected for, especially in hospitals. Nosocomial infections proliferated that are resistant to antibiotics. Complementary and alternative medicines such as essential oil from many herbs have become increasingly popular in recent decades. The present study was designed to evaluate the antimicrobial activity of eight essential oils from Thai herbs. The antimicrobial activity of these commonly used Thai herbs was tested against three potent hospital acquired pathogens, namely *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. These were tested using disc diffusion method, broth dilution method. The results showed that garlic and clove oils had good inhibitory activity. MICs were determined by broth dilution in Mueller–Hinton medium supplemented with 0.12% (w/v) bacteriology agar as enhancing solubility shown susceptible to cinnamon oil (0.83 %v/v) and garlic oil (0.5 %v/v). The GC–MS analyses was determined; the main constituents of the effective essential oils were cinnamaldehyde in cinnamon oil, eugenol in clove oil and allicin in garlic oil.

Inhibition zones of eight essential oils tested against *P. aeruginosa*, *A. baumannii* and *S. maltophilia* using the disc diffusion method



MIC of eight essential oils tested against *P. aeruginosa*, *A. baumannii* and *S. maltophilia* using the broth dilution method

Bacterial strains / Essential Oils (% v/v)	*Pseudomonas aeruginosa*	*Stenotrophomonas maltophilia*	*Acinetobacter baumannii*
Cinnamomum zeylanicum	1.000	≤ 0.167	≤ 0.167
Coriandrum sativum	> 2.000	2.000	1.000
Piper nigrum	> 2.000	> 2.000	> 2.000
Syzygium aromaticum	> 2.000	0.500	0.500
Allium sativum	0.330	0.250	0.500
Cymbopogon citrates	> 2.000	1.000	> 2.000
Ocimum basilicum	> 2.000	1.00	1.000
Ocimum sanctum	> 2.000	2.000	2.000

Acknowledgements: Chulalongkorn University, Kasetsart University.
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Screening of Essential oils on *Giardia lamblia* trophozoites grow and adherence.

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Giardia lamblia is one of the most important worldwide causes of intestinal infections produced by protozoa. Current therapy against *G. lamblia* infection is unsatisfactory due to high incidence of undesirable side effects and a significant failure in clearing parasites from the gastrointestinal tract. Consequently, new alternatives are being screened viewing antigiardial chemotherapy. Now we report on a screening for anti-giardia activity of selected essential oils - *Crithmum maritimum, Distchoselium tenuifolium, Eryngium maritimum, Lavandula luisieri L. stoechas, 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. zygis* and *T. zygis subsp. sylvestris* - and some pure volatile natural compounds – borneol; iso-borneol, carvacrol, eugenol, geraniol, menthol, linalool or thymol.
Effects were evaluated on the growth trophozoites of *G. lamblia* (WB strain [ATCC 30957]) maintained in axenic culture at 37°C in 10 ml of Diamond's TYI-S-33 medium, determining the inhibitory concentration (IC50)
G. lamblia cells (5x 104) were incubated at 37 °C for 48 h with crescent concentrations of essential oils (0,01-0,4 mg/ml) or pure compounds (0,01-0,3 mg/ml) in fresh culture medium. Controls were performed in similar experimental conditions, in the presence of dimethylsulfoxide. After incubation, the total number of trophozoites was determined by using Neubauer cell-counter chamber and results were expressed as cell number (percentage of control). All experiments were performed in duplicate and in at least three independent assays (n=6). The mean and means standard error of the mean (SEM) of at least three experiments were determined. The p values of 0.05 or less were considered significant.
Excepting *Crithmum maritimum* oil phenolic essential oils revealed the most active with IC_{50} values ranging from 68,62 µg/ml for *Thymbra capitata* oil to 205 µg/ml for *Lippia graveolens* oil. Pure compounds such as carvacrol, its isomer thymol, and eugenol inhibited the growth of *G. lamblia* with an IC_{50} of 50µg/m, 50 µg/ml and 131,1 µg/ml, respectively justifying the oils activity. Oils with monoterpenic alchools, *Lavandula viridis* or *T. zygis subsp. zygis* chemotype geraniol, as well as, pure compounds borneol or geraniol are less, but also active on *G. lamblia.*
On the most active oils and pure compounds other activity parameters, such as inhibition of trophozoites adherence ($p<0,001$) or the capacity to induce cell death, were evaluated. Results show that these essentials oils and pure compounds modify the attachment ability and induce cell death factors concurring for the potential of these essential oils as giardiasis therapeutic agents.

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Antioxidant activity of eleven essential oils by two different *in vitro* assays

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Essential oils may have antioxidant properties and their consumption can be related to immune cell functions. Also, their use in food industry may serve to replace synthetic antioxidant food additives. In order to contribute to a better knowledge of their antioxidant mechanisms, eleven essential oils were investigated using two different *in vitro* models: the intracellular generation of reactive oxygen species (ROS) in human leukocytes and the scavenging of free radicals.

The essential oils investigated, obtained from commercial sources, were from: clove leaves (*Syzygium aromaticum* (L) Merr. et L.M. Perry), leaves and branches of niauli (*Melaleuca* sp.), tarragon (*Artemisia dracunculus* L.), coriander (*Coriandrum sativum* L.), juniper berries (*Juniperus communis* L.), tea tree leaves and branches (*Melaleuca alternifolia* L.), rosemary (*Rosmarinus officinalis* L.), ginger roots (*Zingiber officinale* Roscoe), cayeputi aerial parts (*Melaleuca cajeputi* Powell), lemon (*Citrus limon* (L.) Burman fil.), and Spanish oregano (*Thymbra capitata* Griseb.). In addition, eugenol and carvacrol were also tested. The essential oils and compounds studied were chemically characterised by GC-FID and GC-MS.

Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) [1]. Activity on the intracellular production of ROS in human polimorphonuclear leukocytes (PMNs) stimulated by PMA (phorbol myristate acetate, 10 µM) was determined by flow cytometry [2], using 2',7'-dichlorofluorescin diacetate (DCFH-DA) as fluorescence probe.

No radical scavenging activity was observed for the essential oils and compounds investigated between 0.1 µg/mL to 200 µg/mL, except for clove oil and its main constituent, eugenol. Their IC_{50} were 13.2 ± 2.9 µg/mL and 11.7 ± 0.6 µg/mL, respectively, as already reported by our group [3]. Clove oil and ginger oil were the most efficient inhibitors of intracellular generation of ROS after activation by PMA, (IC_{50}=7.5 ± 1.6 µg/mL and IC_{50}=8.9 ± 5.8 µg/mL, respectively). Moreover, eugenol (main component from clove oil) has also strong inhibition of ROS generation (IC_{50}=1.6 ± 0.3 µg/mL). The other essential oils assayed and carvacrol were considered inactive (IC_{50} > 45 µg/mL). Clove essential oil and eugenol displayed intense activity in both tests, suggesting that their antioxidant activity is related both, to enzymatic mechanisms and to free radical scavenging activity.

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Composition and combined antimicrobial efficacy of *Kunzea ericoides* and *Leptospermum petersonii* essential oils

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Notwithstanding the current size of the tea tree oil industry, there is still enormous potential for research and commercial growth especially in terms of value adding. Two less popular tea tree species *Leptospermum petersonii* (lemon tea tree) and *Kunzea ericoides* (kanuka) were investigated for antimicrobial activity independently and in various combinations in order to optimize antimicrobial efficacy. Using the micro-dilution assay, the minimum inhibitory concentrations (MIC's) of the oils were tested on 13 pathogens. The MIC values ranged between 0.06-12.00 mg/mL, depending on the pathogen tested. When tested in combination (fractional inhibitory concentration and isobologram determination), the interaction between *L. petersonii* with *K. ericoides* indicated synergism against all four pathogens tested (*Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis* and *Candida albicans*). A seasonal constituent analysis (gas chromatography combined with mass spectrometry) showed little annual variation in oil composition. Some major compounds identified include neral (17.9-22.5%) and geranial (30.4-40.7%) in *L.petersonii and* α-*pinene* (30.0-46.7%) in *K. ericoides*. The consistent annual composition, together with synergistic combinations supports the continued commercialization of these tea tree oils.

Fragrance analysis using Fast GC- and GC×GC-TOFMS

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Samples analysed in flavour & fragrance studies are by their very nature extremely complex. Being able to detect unknowns, allergens and/or pesticides in these matrices presents major issues for the analyst. Additionally, matrix interference and the levels at which specific analytes need to be reported compound these difficulties. This poster discusses the use of Fast GC- and GC×GC-TOFMS in this context.

The benefit of Fast GC-TOFMS is that all full range mass spectral information is always collected. By taking advantage of high data acquisition rates (up to 500 spectra sec^{-1}) chromatographic peaks do not require baseline separation for quantification and closely eluting peaks are readily resolved by deconvolution algorithms. Using this approach removes the need for single ion monitoring (SIM).

Comprehensive gas chromatography (GC×GC) enables the continuously flow to one detector from a single sample injection and offers an enormous benefit to the analyst. For quantification, an additional benefit of using GC×GC is the ability to choose a lower intensity, but higher m/z ion as a quantification mass due to the re-focusing effect of thermal modulation increasing peak height.

Analyses by GC×GC-TOFMS used a primary column (VF5 30m x0.25mm x 0.25µm) as a boiling point separation and a secondary column (VF17 2m x 0.18mm x 0.2µm) as a polar separation. Using this column set allergens were automatically identified and quantified in four different high-quality perfumes. Furthermore, issues associated with matrix effects can also be overcome by optimising peak separation in both dimensions.

Using GC- & GCxGC-TOFMS it is possible to achieve both non-target and target analysis in one sample run. The acquired raw data are simply processed according to what result is required – specific allergens or alternatively to provide a data set that can investigate 'unexpected' or 'unknown' analytes similar to the analytical approach of the metabolomic community.

Modern methods that use Time of Flight mass spectrometers (TOFMS) such as GC- & GC×GC-TOFMS vastly increase the information gleaned from a single sample injection [1]. Dallüge et al. [2] reported that only detectors able to acquire fifty or more spectra per second enable effective reconstruction of the two dimensional chromatogram and subsequent quantification. Currently, the only compatible MS is time-of-flight mass spectrometry (TOFMS) and Cochran [3] describes the advantage of GC×GC for eliminating potential quantification bias when the matrix contains the same *m/z* ions used to quantify analytes of interest. Furthermore, Dallüge et al. [4] and Shellie et al. [5] concluded that GC×GC–TOFMS provides a reliable basis for the automated analysis of complex samples.

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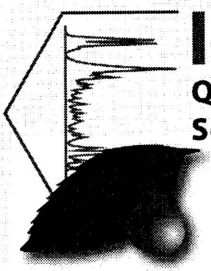

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AUTHORS INDEX

AUTHORS INDEX

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AUTHORS INDEX

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NOTES

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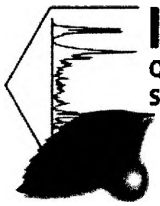

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