30TH INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS (30TH ISEO)

September 5 – 8, 1999

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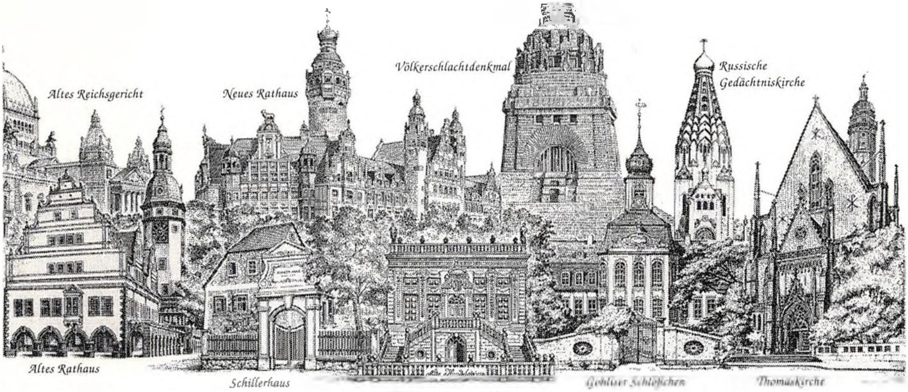

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SCIENTIFIC PROGRAM

Sunday, September 5, 1999

19.00 **Get together party** (and registration)
Leipzig-Miltitz, Schimmelstraße 1, Schimmel library

Monday, September 6, 1999
Lecture-hall building of the University, lecture-hall 11
Leipzig, Augustusplatz/Universitätsstraße

08.30 **Registration** (entrance hall)

10.00 **Opening Ceremony**

10.30 Plenary Lecture 1 Chair: P Welzel

M. H. Zenk; München, Germany
ISOPRENOID BIOSYNTHESIS IN HIGHER PLANTS

Oral Communications Chair: G. Buchbauer

11.30 1-01 **S. Fuchs, A. Mosandl**; Frankfurt, Germany
ON THE BIOGENETIC FATE OF PULEGONE IN MENTHA X PIPERITA (LABIATAE)
11.50 1-02 **K. P. Svoboda, S. K. Kyle, W. Sinclair, J. B. Hampson, Y. Asakawa**; Ayr, Scotland; Tokushima, Japan
INVESTIGATION OF ESSENTIAL OIL COMPOSITION AND DNA FINGERPRINTING OF OCIMUM BASILICUM
12.10 1-03 **M. Mucciarelli, W. Camusso, M. Maffei**; Torino, Italy
AN ENDOPHYTE OF PEPPERMINT AND ITS INTERACTION WITH HOST TERPENOIDS

12.30 Lunch

14.00 Plenary Lecture 2 Chair: K. H. Kubeczka

H. Breer; Stuttgart, Germany
THE SENSE OF SMELL: MOLECULAR BASIS OF ODOR PERCEPTION

15.00 Break

Oral Communications Chair: K. H. C. Baser

15.30 2-01 **M. Lis-Balchin, S. Hart**; London, UK
MODE OF ACTION OF JASMINE ABSOLUTE, GERANIUM OIL AND LAVENDER OIL ON GUINEA-PIG ILEUM IN VITRO
15.50 2-02 **F. Christoph, P.-M. Kaulfers, E. Stahl-Biskup**; Hamburg, Germany
A COMPARATIVE STUDY OF THE IN-VITRO ANTIMICROBIAL ACTIVITY OF TEA TREE OILS S. L. WITH SPECIAL REFERENCE TO THE ACTIVITY OF β-TRIKETONES
16.10 2-03 **J. Höfinghoff, G. Buchbauer, W. Holzer, P. Wolschann**; Vienna, Austria
STRUCTURE-ODOUR-RELATIONSHIPS OF ANALOGUES OF SANTALOL
16.30 – 18.00 Poster Session I

Tuesday, September 7, 1999

09.00 Plenary Lecture 3 Chair: P. Weyerstahl

K. Mori; Tokyo, Japan
RECENT RESULTS IN THE SYNTHESIS OF ALIPHATIC AND ALICYCLIC BIOREGULATORS

10.00 Break

Oral Communications Chair: G. Schmaus

10.30 3-01 **K. Schulze, K. Anhalt, I. Sprung, U. Wahren**; Leipzig, Germany
CHIRAL BUILDING BLOCKS FROM CAMPHOLENIC AND FENCHOLENIC COMPOUNDS

10.50 2-04 **J. Friedman, O. Barazani, A. Fait, U. Ravid, E. Putievsky**; Tel Aviv, Israel
HERITABILITY STUDIES OF CHEMICAL VARIABILITY IN INDIGENOUS POPULATIONS OF BITTER FENNEL (FOENICULUM VULGARE VAR. VULGARE) IN ISRAEL

11.10 2-05 **M. Maffei**; Turin, Italy
ALLELOPATHIC EFFECTS OF PEPPERMINT ESSENTIAL OIL IN HIGHER PLANTS

11.30 2-06 **J. Novak, Ch. Bitsch, M. Marn, Ch. Franz and C. Wender**; Wien, Austria; Villazano di Trento, Italy
INHERITANCE OF SOME ESSENTIAL OIL COMPOUNDS OF SALVIA OFFICINALIS

11.50 Lunch

13.30 Plenary Lecture 4 Chair: K. Schulze

W. A. König; Hamburg, Germany
ISOLATION AND STRUCTURE ELUCIDATION OF TERPENOID PLANT CONSTITUENTS

14.30 Break

Oral Communications Chair: W. Engewald

15.00 4-01 **A. Chaintreau, P. Pollien, L. B. Fay, M. Baumgartner**; Geneve, Lausanne, Switzerland
QUANTITATION AND GAS CHROMATOGRAPHY-OLFACTOMETRY: WHEN THE DREAM BECOMES REAL

15.30 4-02 **H. Lehmann, R. Falter**; Krefeld, Germany
AUTOMATED FAST GC/TOF-MS ANALYSIS OF MIXTURES

15.50 4-03 **C. Bicchi, S. Drigo, P. Rubiolo**; Torino, Italy
THE INFLUENCE OF TRAPPING FIBRES IN HS-SPME-GC ANALYSIS OF AROMATIC PLANTS

16.10 4-04 **M. Toyota, T. Saito, Y. Yonehara, I. Horibe, K. Minagawa, Y. Asakawa**; Tokushima, Osaka, Japan
SESQUITERPENOIDS FROM THE LIVERWORTS CHILOSCYPHUS POLYANTHOS, LEPIDOZIA VITREA AND PORELLA PERROTTETIANA

16.30 – 18.00 Poster Session II

19.00 **Congress Dinner** (Thüringer Hof, Burgstraße 19)

Wednesday, September 8, 1999

Oral Communications Chair: A. Mosandl

09.00	4-05	**H. Becker, M. Bungert, Th. Jahns**; Saarbrücken, Germany 2-METHOXY-3-(1-METHYLPROPYL)PYRAZINE, PEA ODOUR FROM THE MARINE BACTERIUM HALOMONAS VENUSTA
09.20	4-06	**Y. Holm, B. Galambosi, R. Hiltunen**; Helsinki, Mikkeli, Finland THE ESSENTIAL OIL COMPOSITION OF AGASTACHE CANA
09.40	4-07	**D. Joulain, K. B. Yaacob**; Grasse, France; Kuala Terengganu, Malaysia THE LEAF ESSENTIAL OIL OF MELALEUCA CAJEPUTI SSP. PLATIPHYLLA FROM MALAYSIA
10.00	4-08	**M. Bononi, V. Fumagalli, F. Tateo**; Milano, Italy SEASONAL VARIATION IN COMPOSITION OF A WILD POPULATION OF THYMUS VULGARIS L. FOUND IN LIGURIA (ITALY)

10.20 Break

Oral Communications Chair: Y Asakawa

10.50	4-09	**F. Tateo, M. Bononi, E. Lubian, S. Martello**; Milano, Italy MYRISTICIN, APIOL AND OTHER SAFROLE-LIKE COMPOUNDS: PROBLEMS CONCERNING THEIR PRESENCE IN FOOD AND THEIR ANALYTICAL DETERMINATION
11.10	4-10	**K. H. C. Baser, N. Kirimer, G. Tümen**; Eskisehir, Balikesir, Turkey ESSENTIAL OILS OF THE ORIGANUM SPECIES OF TURKEY
11.30	4-11	**M. D. Eggers, E. Stahl-Biskup, G. Orsini**; Hamburg, Germany; Caracas, Venezuela COMPOSITION AND VARIATION OF THE ESSENTIAL OIL OF LEPECHINIA SALVIAEFOLIA (KUNTH) EPL. (LAMIACEAE) FROM VENEZUELA
11.50	4-12	**M. M. Barazandeh**; Tehran, Iran DETERMINATION OF KOVATS RETENTION INDICES BY COMPUTER SOFTWARES

12.10 **Closing Session**

12.30 Lunch

Lectures

Lecture hall building, hall 11

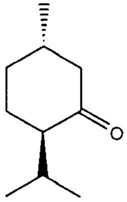
ON THE BIOGENETIC FATE OF PULEGONE IN *MENTHA X PIPERITA* (LABIATAE)

S. Fuchs, A. Mosandl, Institut für Lebensmittelchemie, Biozentrum, Johann Wolfgang Goethe-Universität, Marie-Curie-Str. 9, D-60439 Frankfurt, Germany

Peppermint oil is produced by steam distillation of the flowering herb *Mentha piperita* L. (Labiatae). The composition of the essential oil changes during plant development. In young leaves the monoterpene ketones (-)-menthone and (+)-isomenthone, in older ones monoterpene alcohols are dominant. Therefore, the main component of peppermint oil is (-)-menthol. An undesired monoterpene in peppermint oil is (+)-menthofuran.

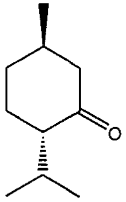
Regioselectively labelled pulegones of definite stereochemistry are suitable precursors to study stereochemical aspects on the biogenesis of the p-menthan-3-ones (-)-menthone, (+)-isomenthone, and other terpenoids during feeding experiments.

After solid-phase-microextraction the essential oil was analysed using enantio-MDGC-MS. Both enantiomers of labelled pulegones are converted into the corresponding labelled menthones and isomenthones. The generation of (4S)-and (4R)-configured p-menthan-3-ones depends on the stereoselectivity of enzymes involved. Comparing labelled pulegone enantiomers as starting material the bioconversion preferrably leads to (4S)-configured diastereomers [1,2].



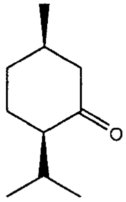
(1*S*, 4*R*)

(+)-menthone



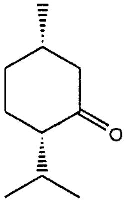
(1*R*, 4*S*)

(-)-menthone



(1*R*, 4*R*)

(+)-isomenthone



(1*S*, 4*S*)

(-)-isomenthone

[1] S. Fuchs, T. Beck, S. Burkardt, M. Sandvoß and A. Mosandl, J. Agric. Food Chem., in press

[2] S. Fuchs, T. Beck, M. Sandvoß and A. Mosandl, J. Agric. Food Chem., in press

INVESTIGATION OF ESSENTIAL OIL COMPOSITION AND DNA FINGERPRINTING OF *OCIMUM BASILICUM*

[1]K.P. Svoboda, [1]S.K. Kyle, [1]W. Sinclair, [1]J.B. Hampson, [2]Y. Asakawa, [1] Plant Biology Department, SAC Auchincruive, Ayr, Scotland, KA6 5HW, [2] Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro, Tokushima 770, Japan.

Ocimum basilicum L., sweet basil, is a member of the family Labiatae (Lamiacae), and originates from India. It has a great range of varieties due to its freely cross-pollinating nature and highly polymorphic characteristics. This creates difficulties in classification of the species and makes botanical nomenclature complex. This current study has attempted to compare the similarities in chemotaxonomical investigations with a PCR-based DNA analysis. Seeds of several different varieties of *O. basilicum* were obtained from various sources and grown under glass at Auchincruive. Plants were harvested during the vegetative stage and hydrodistilled. Volatile oil yields obtained were within the range of 0.06 - 0.83% (v/w). The samples were analysed by GC and GC-MS. The following chemotypes were described: linalol, methyl chavicol, citral and 1,8-cineole. An attempt was made to classify our collection into the chemotypes previously stated in the literature [1,2]. Further work is required to describe the wide chemotaxonomical range and more comprehensive basil collection is under investigation. Random amplified polymorphic DNA analysis (RAPDs) was carried out on several plant samples with the aim of characterising the levels of genetic variation contained therein. A random sample of DNA was chosen and forty RAPD primers were tested. PCR products were resolved using gel electrophoresis and the fingerprints obtained were statistically analysed. Binary data matrix was constructed, entered into a programme written in Genstat 5 which converted the data into a similarity matrix. The results of the analysis and their comparison with the chemotaxonomical groups was encouraging. This suggests that further research into the utilisation of such molecular approaches could be of value for classificiation and genetical studies. The description of DNA analysis and cummulative results can be obtained from the Plant Biology Department, SAC Auchincruive.

[1] B.M.Lawrence, Progress in Essential Oils (1976 – 1994)
[2] D. Bai, J. Brandle, R. Reeleder and D.P. Bai, Genome **40** (1997) 111 – 115
[3] A.L. Westman and S. Kresovich, Biotechnology in Agriculture **Series No. 19** (1997)

AN ENDOPHYTE OF PEPPERMINT AND ITS INTERACTION WITH HOST TERPENOIDS

M. Mucciarelli[1], W. Camusso[2] and M. Maffei[2]
[1]Dept. Morphophysiology, [2]Dept. Plant Biology, University of Torino, Viale Mattioli, 25
I-10125 Torino, Italy

Endophytic fungi form asymptomatic infections within aerial tissues of healthy plants for all or nearly all their life cycle, and may be described as mutualistic. Plant organ and tissue specificity by endophytic fungi has been demonstrated for many species [1]. Among the latter are those fungi, which colonize aerial tissues of aromatic plants. Essential oils are defence compounds involved in plant to plant and plant to fungus chemical interactions, and have been shown to possess growth inhibitorial and antisporulating effects [2]. The isolation of an endophytic hyaline sterile fungus (PGP-HSF) from *Mentha x piperita* L. leaves, possessing growth promoting effects both *in vivo* and *in vitro*, prompted us to study the plant-fungus interaction and the relationship between host volatile terpenoids and the endophyte. Peppermint essential oils application to PGP-HSF cultures resulted in a pronounced fungal growth inhibition (24,5% of linear growth reduction at 20 ppm). Since the inhibitory effect of some volatile monoterpens on O_2 uptake by filamentous fungi and by their spores is well documented, we studied the effects of peppermint essential oil and some of its oil components on PGP-HSF mitochondrial respiration. Total essential oil had an inhibitory activity on fungal oxygen uptake by inhibiting 50% of mitochondrial respiration (I_{50}) when used at 628 ppm. Menthol I_{50} was 5,39 mM, while menthofuran and pulegone followed menthol in their inhibitory action with a respiratory I_{50} of 5,58 and 5,96 mM respectively. Menthone was the less inhibitory compound with an I_{50} at 6,67 mM. Except for total oil and pulegone, the other three oil components showed stimulatory activity of PGP-HSF mitochondrial respiration for concentrations lower than 50 ppm. Our results suggested that peppermint essential oil components affect PGP-HSF development and reproduction in a manner, which is dose and type dependent, and probably more correlated to their effect on respiration rather than their growth inhibitory activity. The sensibility of the endophyte to essential oils could be therefore a main determinant in the plant fungus mutualistic interaction.

[1] T. N: Sieber, Mycol. Res. 92 (1989) 322-326
[2] S. Inouye, M. Watanabe, Y. Nishiyama, K. Takeo, M. Akao and H. Yamaguchi, Mycoses 41 (1998) 403-410

1 - 03

MODE OF ACTION OF JASMINE ABSOLUTE, GERANIUM OIL AND LAVENDER OIL ON GUINEA-PIG ILEUM *IN VITRO*.

[1] M. Lis-Balchin, [2]S. Hart
[1]School of Applied Science, South Bank University, Borough Road, London, SE1 OAA, UK, [2]Division of Pharmacology and Therapeutics, Kings College, Manresa Road, London, SW3 6LX, UK.

Jasmine absolute (*Jasminum grandiflorum* L.), lavender (*Lavandula angustifolia*, P. Miller) and some of its hybrids and geranium oil (*Pelargonium* cultivars) are used in Aromatherapy as relaxants. Inhalation of lavender and geranium oils has a relaxing effect on both animals and man, whilst jasmine has a stimulant effect. However, all show a spasmolytic effect on guinea-pig smooth muscle. As their exact mode of action was unknown, the spasmolytic action was studied fully.

The results showed that all these floral essential oils appeared to act postsynaptically and were not atropine-like as they inhibited the contractile responses due to exogenous acetylcholine and histamine to a similar degree. The spasmolytic action of the essential oils was unaffected by the adrenoceptor blockers phentolamine and propranolol which inhibited the antispasmodic effect of noradrenaline. Results using phosphodiesterase inhibitors, suggested that the spasmolytic action involves a rise in intracellular cyclic adenosine monophosphate, cAMP, which theoretically could be via receptor stimulation but is more likely to be a direct action on adenylate cyclase. The results of using ODQ, a selective inhibitor of soluble guanylyl cyclase, which blocked the relaxation produced by sodium nitroprusside did not indicate the involvement of the secondary messenger cyclic guanosine monophosphate, cGMP.

This mode of action via cyclic AMP has not been shown by non-floral smelling essential oils like *Melaleuca alternifolia* (tea tree) or *Eucalyptus globulus*.

A COMPARATIVE STUDY OF THE IN-VITRO ANTIMICROBIAL ACTIVITY OF TEA TREE OILS s.l. WITH SPECIAL REFERENCE TO THE ACTIVITY OF ß-TRIKETONES

[1] F. Christoph, [2] P.-M. Kaulfers, [1] E. Stahl-Biskup,
[1] Department of Pharmaceutical Biology, University of Hamburg, Bundesstrasse 43, 20146 Hamburg, [2] University Hospital Eppendorf, Institute of Medical Microbiology and Immunology, Martinistrasse 52, 20251 Hamburg

Minimum inhibitory concentrations (MICs) of five tea tree oils, **Tea tree oil**, **Manuka oil**, **Kanuka oil**, **Cajuput oil**, and **Niaouli oil** as well as of the isolated complex of three **ß-triketones** from Manuka oil, were determined applying a broth macrodilution method similar to that described by the National Committee for Clinical Laboratory Standards (NCCLS, 1990). Tween 80™ was used as detergent. This method was used to examine the susceptibility of sixteen different bacteria, yeast and fungi, namely *Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecium* ATCC 6057, *Bacillus subtilis* ATCC 6638, *Candida albicans* ATCC 10231, and the clinical isolates of *Proteus vulgaris, Staph. capitis, Staph. epidermidis*, methicillin resistant *Staph. aureus (MRSA)*, *Corynebacterium minutissimus*, *Corynebacterium spec.*, *Epidermophyton floccosum*, *Trichophyton rubrum*, *Penicillium notatum* and *Aspergillus niger*.

As antimicrobial properties are related to the chemical composition of the oils, it was not surprising that the activities of the five oils were heterogeneous. Still we could work out some remarkable details. Our results confirmed the antimicrobial activity of **Tea tree oil**. The MIC values obtained in this study varied between 0.20.-0.50 % v/v for fifteen organisms. *Pseudomonas aeruginosa* showed a higher resistance to Tea tree oil than the other species tested. **Manuka oil** was shown to be more effective against gram-positive bacteria with MICs ranging from 0.05-0.20 % v/v. Our study revealed that the **triketone-complex** is responsible for these effects. It showed excellent activity (MIC 0.005-0-04 % v/v) against gram-positive bacteria. As expected **Kanuka oil** consisting mainly of α-pinene turned out to be the less potent oil. **Cajuput oil** and **Niaouli oil** both with high contents of 1.8-cineole were also alike in their MIC values. In comparison with Tea tree oil they both showed similar activities with lower effects against gram-negative bacteria. In Tea tree oil terpinen-4-ol is generally believed to be the principal active constituent [1], the special mechanisms in which way terpinen-4-ol reacts with gram-negative bacteria are still unknown.

[1] I.A. Southwell, A.J. Hayes, J. Markham, D.N. Leach, Acta Horticulturae 334 (1993) 256-265

Structure-Odour-Relationships of Analogues of Santalol

J. Höfinghoff[1] , G. Buchbauer[1], W. Holzer, Uni Wien[1], P. Wolschann, Uni Wien [2]
[1] Institute of Pharmaceutical Chemistry, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria,
[2] Institute of Theoretical Chemistry, University of Vienna, Währinger Straße 17, A-1090 Vienna, Austria

Substitutes of East Indian Sandalwood Oil are very important ingredients in cosmetics and perfumes. Therefore it is very interesting to study stucture-odour-relationships of these fragrance compounds.

The importance of three effective contact groups (regions) in these molecules has been shown in an earlier study: the hydroxyl function, then a small anchor and/or protective group directly adjacent to it and finally a hydrophobic „bulky group" in a longer distance to these constellation [1].

The objective of this study is to obtain more information about the influence of such a hydrophobic group on the odour character „sandel" in some new santalol analogues. For the present study we chose the sterically more or the less plain and comercially available, cheap ketones isophorone and carvone as well as the tricyclic ketone tricyclo[5.2.1.$0^{2,6}$]decan-8-one as starting substances for the synthesis of new santalol analogues. The tricyclic nucleus of the last compound has been chosen especially to test the influence of a relatively long „bulky group" on the odour.

In the following the synthesis of (Z)-isophorono-β-santalol, (Z)-dihydroisophorono-β-santalol, (Z)-carvono-β-santalol [2] and tricyclodecano-β-santalol is described. The olfactorical evaluation of theses substances showed in each case the absence of the typical sandelwood note. These results could also be confirmed by CAFD -(computer aided fragrance design)-studies.

[1] G. Buchbauer, A. Hillisch, K. Mraz, P. Wolschann; Helv. Chim. Acta (1994) **77**, 2286-2296
[2] G. Buchbauer, J. Höfinghoff, A. Froese; Mh. Chem. (1998) **129**, 705-710

HERITABILITY STUDIES OF CHEMICAL VARIABILITY IN INDIGENOUS POPULATIONS OF BITTER FENNEL (*Foeniculum vulgare* var. *vulgare*) IN ISRAEL

[1]J. Friedman, [1]O. Barazani, [1]A. Fait, [2]U. Ravid and [2]E. Putievsky [1]Department of Plant Sciences Tel Aviv University, Israel
[2] Aromatic, Medicinal and Spice Crops Unit, Newe Ya'ar Research Center, Agricultural Research Organization, Ramat Yishay, Israel

The extreme gradient of annual rainfall (<100 - 1000 mm) from South to North of Israel, is characterized by both a unique richness of plant species and a remarkable variability among populations within the species level . Although, variability of indigenous plant populations, ancestors of the domesticated cereals (wheat, barley and oats) have already been approached, variability of wild spices, e.g. bitter fennel, or coriander (*Coriandrum sativum* L.), is much less known. Due to exceeding development activities, e.g. new roads, or new settlements, natural habitats in Israel are exceedingly threatened. We therefore aimed to identify unique indigenous populations for conservation. To this end, ecological *versus* genetic variability of the chemical composition of oleoresins from hexane extracted fruits (mericarps) was determined by GC-MS. Fruits were collected in natural habitats and also from cultivated plants representatives of each indigenous population, grown under irrigation in one area at the Newe Ya'ar Research Center. Based mainly on the phenylpropane derivatives i.e. *t*-anethole-estragole ratio, field data suggest that the 11 natural populations can be classified into 4 major groups[1]. When representatives from natural populations were cultivated, the chemical composition of the oleoresins of representatives of some populations was similar to that of their 'mother' natural populations, suggesting orthodoxy, or high levels of heritability. Nevertheless, in representatives of other populations, chemical composition deviated from their mother natural populations, suggesting non-orthodoxy, or a low level of heritability. Steps for *in situ* conservation of unique orthodox populations of bitter fennel are now undertaken.

[1] O. Barazani, A. Fait, Y. Cohen, S. Dimenshtein, U. Ravid, E. Putievsky, E. Lewinsohn, J. Friedman, Planta Medica 65(1999) 1-4

2 - 04

ALLELOPATHIC EFFECTS OF PEPPERMINT ESSENTIAL OIL IN HIGHER PLANTS

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email: maffeima@bioveg.unito.it

Monoterpenes inhibit seed germination and plant growth in the chemical interaction among plants known as allelopathy. Peppermint (*Mentha piperita* L.) essential oil and some of its oil components affected root and mitochondrial respiration as well as membrane permeability and ion uptake of cucumber (*Cucumis sativus* L.). Total essential oil had an inhibitory action by inhibiting 50% of root and mitochondrial respiration (I_{50}) when used at 324 and 593 ppm, respectively. Pulegone had an I_{50} on root and mitochondrial respiration at the concentration of 0.08 mM and 0.12 mM, respectively. Menthone followed pulegone in its inhibitory action with a root respiration $I_{50}$of 1.11 mM and mitochondrial I_{50} of 2.30 mM, whereas menthol was the less inhibitory compound, with root respiration I_{50} of 1.85 mM and mitochondrial I_{50} of 3.80 mM. A direct correlation between water solubility and respiratory inhibition was found for pulegone, menthone and menthol. The possible interaction of pulegone, menthone and menthol on mitochondrial respiration will be discussed.

Cucumber root membrane potentials were depolarized up to Goldman's potential after perfusion with 300 ppm menthone, 300 ppm menthol and 150 ppm of menthone + 150 ppm menthol. Pulegone, neomenthol and menthofuran were also found to strongly depolarize root membrane potentials, whereas no significant effects were measured after limonene, 1,8-cineole and menthyl acetate perfusion. Total essential oil perfusion was found to specifically block potassium uptake, as demonstrated with the use of the potassium inward channel-blocker cesium. Washing roots with a fresh buffered solution annulled total oil, menthol, pulegone, menthofuran and neomenthol depolarization, whereas menthone depolarization remained after washing. The interaction of monoterpenoids with membrane permeability and ion channel-blocking will be discussed.

The physiological, biochemical, metabolic and allelopathic effects of peppermint oil and oil components on cucumber root system are discussed along with general considerations on other higher plants.

2 - 05

Inheritance of Some Essential Oil Compounds of *Salvia officinalis*

[1]J. Novak, [1]Ch. Bitsch, [1]M. Marn, [1]Ch. Franz and [2]C. Vender
Institut für Angewandte Botanik, Veterinärmedizinische Universität Wien,
Veterinärplatz 1, A-1210 Wien, Austria
Istituto Sperimentale per l'Assestamento Forestale e per l'Alpicoltura, piazza Nicolini 6, I-38050 Villazzano di Trento

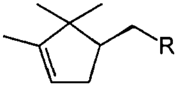
Selfing of plants leads (especially in allogamous plants like sage) to increasing genetical homogeneity, but gives also valuable information on the inheritance of traits. In sage, *Salvia officinalis*, the descendants of a selfing generation of individuals of several populations revealed a segregation in several compounds of the essential oil. Especially of interest was the segregation of α-thujone, with the presence of this compound in some individuals and the absence in a part of the descendants of the selfings. The data of the segregation of a selfing generation is compared to published enzymatic studies of sage essential oil biogenesis [1].

[1] H.-R. Schütte, Progress in Botany **59** (1998) 570-596

CHIRAL BUILDING BLOCKS FROM CAMPHOLENIC AND FENCHOLENIC COMPOUNDS

K. Schulze, K. Anhalt, I. Sprung, C. Prehn, U. Wahren
Institut für Organische Chemie der Universität Leipzig, Talstraße 35, D-04103 Leipzig, Germany

α-Pinene which is available in both enantiomeric forms is being used more frequently in the total syntheses of complex chiral biologically active products [1]. Little is known about the uses of campholenic and fencholenic derivatives which are obtainable from α-pinene oxide via camphane or fenchane rearrangement.



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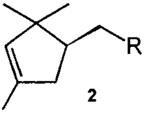

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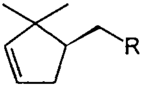

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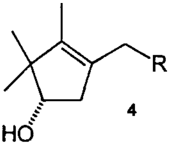
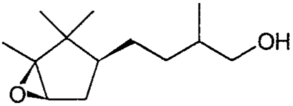


4

The cyclopentenes of type **1** (α-campholenic), **2** (fencholenic), and **3** (apocampholenic) possess an stereogenic centre which results from the C-5 centre of α-pinene. The ß-campholenic system (**4**) bears a chiral information also resulting from that of the C-5 centre of α–pinene via anti-epoxy-α-campholenic products.



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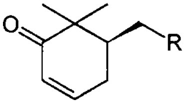

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We report the preparation of chiral cyclopentenes [2], anti-[3] and syn-Epoxides (e.g. **5**)[4] and also various enantiomeric cyclohexenones (e.g. **6**) which may be suitable starting materials for natural substances by ozonolysis via stable ozonides or by $KMnO_4$-Oxidation of campholenic and fencholenic derivatives.

[1] Tse-Lok Ho, Enantioselective Synthesis, Wiley, New York, **1992** p. 205
[2] C. Prehn, I. Sprung, K. Anhalt, K. Schulze, Mh. Chem. **129** (1998) 89-97;
I. Sprung, , K. Anhalt, U. Wahren, K. Schulze, Mh. Chem. **130** (1999) 341-354
[3] K. Schulze, A.-K. Habermann, H. Uhlig, K. Wyßuwa, U. Himmelreich, J. Prakt. Chem./Chemiker-Ztg. **335** (1993) 363-367
[4] U. Wahren, I. Sprung, K. Schulze, M. Findeisen, G. Buchbauer, Tetrahedron Lett. **40** (1999) 5991-5992

QUANTITATION AND GAS CHROMATOGRAPHY-OLFACTOMETRY: WHEN THE DREAM BECOMES REAL

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ABSTRACT

Up to now, quantifying an odorant using GC-Olfactometry was never achieved and did not seem to be realistic. However, the data treatment of the recently developed GC-„SNIF" method, showed a relationship between the concentration of the odorant and its detection frequency by a panel[1].

Starting from this approach, the method was first optimized using 8-12 judges, and then applied to quantify a model solution of 1-octen-3-one. Standard additions were not adapted to calibrate the panel response. External calibration gave excellent results in the ppt range. GC-O was then challenged using one of the most sensitive and selective methods, GC/MS/MS, to quantitate 1-octen-3-one in coffee, a complex aroma. Results showed performances comparable to GC/MS/MS for this odorant, or even better as the latter required 75-500 times more sample to perform the quantitation. Overestimation remained possible because of the extremely low concentration of odorant that may coelute with other aroma compounds. However the same limitation was found to be valid in GC/MS/MS due to the presence of isobaric ions.

Since there is no need for the synthesis of labeled compounds, and the sample preparation is limited, GC-Olfactometry can compete with sensitive and selective techniques, like MS/MS, for determination of extremely intense odorants.

1. Pollien, P.; Ott, A.; Montigon, F.; Baumgartner, M.; Munoz-Box, R.; Chaintreau, A. *J. Agric. Food Chem.* **1997**, *45*, 2630-2637.

4 - 01

AUTOMATED FAST GC/TOF-MS ANALYSIS OF MIXTURES

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The mass spectrometric analysis of different analytes or mixtures with different GC/MS systems is an established technique in instrumental analysis. Due to the timing behaviour of scanning MS detectors in the range of about 5 full mass scans per second there are some restrictions for the improvement of the applied chromatographic conditions. The shortening of the analysis time is restricted from the appearence of coeluting peaks and peakgroups which can not be resolved with slow scanning detectors. The more complex the analyte mixture is the slower chromatographic conditions should be applied in order to avoid the manual processing and interpretation of mass spectra deriving from coeluting peaks. Thus the amount of time necessary for data aquision and data processing increases significantly.

Based on the description of the design and function of an GC/MS TOF system with an extremely high data aquision rate of up to 500 full scan mass spectra per second different applications for those systems are described.

Data are provided from different applications in Food/ Flavour analysis, like automatic analysis of multicomponent mixtures. The possibility of the fully automated data processing of the aquired data gives access to additional information as automatic peak finding and deconvolution till completely automated sample comparison of two samples.

4 - 02

THE INFLUENCE OF TRAPPING FIBRES IN HS-SPME-GC ANALYSIS OF AROMATIC PLANTS

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Solid phase microextraction (SPME) is a sampling technique based on absorption, which was first introduced by Pawliszyn's group at Waterloo University (Canada). With SPME, the analytes are absorbed from the liquid or gaseous sample onto an absorbent coated fused-silica fibre, which is part of the syringe needle, for a fixed time. The fibre is then inserted directly into a GC injection port for thermal desorption, or into the HPLC injection valve for solvent desorption.

SPME is a solvent free technique, which is sensitive because of the concentration factor achieved by the fibres, and selective because of different coating materials which can be used.

Several fibres with different absorption and polarity characteristics have been made commercially available over the last few years. This communication aims to compare the efficiency of eight different fibres in sampling the headspace of aromatic or medicinal plants. The performance of each fibre is evaluated on the basis of sampling efficiency of a series of marker components with different volatility, polarity and structure characteristics from *Rosmarinus officinalis*, *Salvia officinalis*, *Thymus vulgaris* and *Valeriana officinalis*..

limits of SPE: variable recovery
plugging of the cartridge
blocking of the pores in the sorbent
by solid or oil components
batch-to batch variation on
sorbent performance

SESQUITERPENOIDS FROM THE LIVERWORTS *CHILOSCYPHUS POLYANTHOS, LEPIDOZIA VITREA* AND *PORELLA PERROTTETIANA*

[1]M. Toyota, [1]T. Saito, [1]Y. Yonehara, [2]I. Horibe, [2]K. Minagawa, [1]Y Asakawa

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The ether extract of the liverwort *Chiloscyphus polyanthos* gave sesquiterpenoids, which are enantiomeric to those found in another liverwort *Lepidozia vitrea*. The absolute configurations of the sesquiterpenoids found in *C. polyanthos* were determined by spectroscopic evidence, chemical derivatization and/or x-ray crystallographic analysis [1].

The ether extract of the liverwort *Porella perrottetiana* afforded (-)-α-eudesmol, which showed an opposite sign of the optical rotation to that found in higher plants. Present work on the absolute configuration suggested that the positive values described in many previous papers should be revised. Since the absolute configuration of (-)-α-eudesmol was identical to that of (+)-β-eudesmol found in the higher plans, it was apparent that the expression of the positive sign of α-eudesmol might be revised to that of (-)-α-eudesmol [2].

[1] M. Toyota, T. Saito and Y. Asakawa, Phytochemistry (in press).

[2] M. Toyota, Y. Yonehara, I. Horibe, K. Minagawa and Y. Asakawa, Phytochemistry (in press).

2-Methoxy-3-(1´-methylpropyl)pyrazine, pea odour from the marine bacterium *Halomonas venusta*

[1] Hans Becker, [1] Michael Bungert, [2] Thomas Jahns, [1] FR 12.3, Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, D-66041 Saarbrücken, Germany
[2] FR 13.3, Mikrobiologie, Universität des Saarlandes, D-66041 Saarbrücken, Germany

During the study of urea uptake in the marine bacterium *Halomonas venusta* (*Deleya venusta*) [1] a strong odour of fresh peas was observed. Bacteria were cultivated in ZoBell medium with the addition of artificial sea water [2]. 250 ml air was purged through the culture vessel (2 l) and then through a column filled with RP 18 silica gel. The column was eluted with $CDCl_3$. ^{1}H-NMR and GC/MS studies of the $CDCl_3$ extract revealed 2-methoxy-3-(1´-methylpropyl)pyrazine (MMPP, **4**), as the odourous substance. A similar procedure was used for the quantification of the pyrazine. Variation of the culture media and the feeding of proposed precursors was performed to optimize growth and production and to get information about the biosynthesis of the pyrazine.

Although, feeding with isoleucine caused the inhibation of the acetohydroxy acid synthetase, the addition of high amounts of glycine (**1**) and isoleucine (**2**) to the ZoBell medium doubled the cell dry weight (1,92 g/l) and raised the yield of MMPP (9,8 mg/l).

The presumed intermediate 2,5-dioxo-3-(1-methylpropyl)piperazine (**3**) was synthesized from the dipeptide Gly-L-Ile [3]. GC/MS results revealed, that the diketopiperazine was found in the cells growing on agar plates. Thus, the possible pathway may be formulated as depicted in Fig. 1.

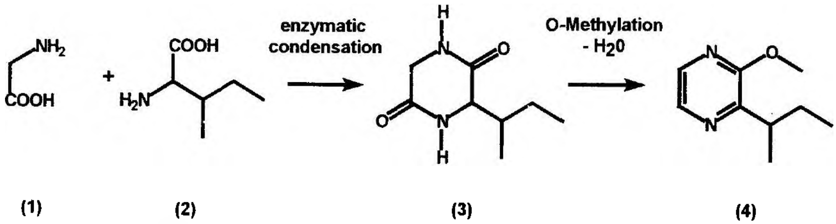

Fig. 1 Possible pathway of MMPP (**4**)

[1] T. Jahns, J. Gen. Microbiol. 138, 1815 – 1820 (1992)
[2] C. E. ZoBell and C. B. Feltham, Science 81, 234 – 236 (1935)
[3] K. D. Kopple and H. G. Ghazarian, J. Org. Chem. 33, 862 – 864 (1968)

THE ESSENTIAL OIL COMPOSITION OF AGASTACHE CANA

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[1]Department of Pharmacy, P.O.Box 56, FIN-00014 University of Helsinki
[2]Agricultural Research Centre of Finland, Karila Research Station for Ecological Agriculture, Karilantie 2 A, FIN-50600 Mikkeli

Agastache cana Wooton & Standley (Laimaceae) is a perennial plant native to the arid regions of the Southwestern United States and New Mexico [1]. It is noted for good ornamental characteristics [2]. It contains an essential oil of unknown composition.

Seeds of *Agastache cana* cv. Heather Queen were purchased from Richters seed company (Ontario, Canada) and were field cultivated in Mikkeli, south east Finland in the summer 1998. The seeds were sown 4.5.1998, transferred to the field a month later and the herbs were collected in the middle of August. At the collection time the plants were about 10 cm high and gave a fresh yield of 1.9 kg/m^2. The summer 1998 was exceptionally rainy in Finland. The plants were dried and the dried leaves used for hydrodistillation in a Karlsruher-Stahl apparatus. The oil yield was 0.13%. The oil composition was investigated using GC-MS on three different phases: a polar NB-351 and two chiral ones, β- and γ-cyclodextrin. Altogether 69 components (incl. some enantiomers) were identified from the oil. The main component was caryophyllene epoxide (about 30%). Characteristic for the oil was the presence of oxygenated terpenes of which the oxygenated sesquiterpenes comprised the main part (about 50%). They included caryophyllene epoxide isomers, hydroxy-caryophyllene isomers and humulene epoxide II. The oxygenated monoterpenes (about 35%) included carvacrol (16%) and α-terpinyl acetate (9%). (+)- and (-)-linalol, pinocarvone, menthol, iso-menthone, thymol and pulegone were identified as trace components. In addition, the oil contained 5% monoterpene hydrocarbons, 4% sesquiterpene hydrocarbons, 2% phenylpropanes and 4% other components (furanes, phtalides).

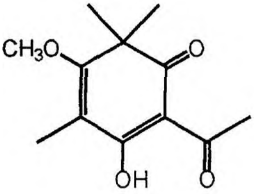
[1] H. Lint and C. Epling, Am. Midl. Nat. **33** (1945) 207-230.
[2] R.G. Fuentes-Granados, M.P. Widrlechner and L.A. Wilson, J. of herbs, Spices & Medicinal Plants **6** (1998) 69-97.

4 - 06

THE LEAF ESSENTIAL OIL OF *MELALEUCA CAJEPUTI* SSP. *PLATIPHYLLA* FROM MALAYSIA.

[1] D. Joulain, [2] K. B. Yaacob, [1] Research Division, Robertet S. A., B. P. 100, F-06333 Grasse, France,
[2] Faculty of Sciences and Professional Arts, Universiti Putra Malaysia Terengganu, 21030 Meng. Talipot, Kuala Terengganu, Malaysia.

Melaleuca cajeputi Powell (Myrtaceae) is a tree found in tropical freshwater swamps and seasonally flooded areas from northwestern Australia to Indochina. Barlow tentatively recognized three subspecies : *M. cajeputi* « ssp. *platiphylla* », *M. cajeputi* « ssp. *cajeputi* » and *M. cajeputi* « ssp. *cumingiana* » [1]. Several stands of *M. cajeputi* ssp. *platiphylla* trees were located in Peninsular Malaysia, and the leaf essential oils from a number of individual trees were prepared and investigated. Some samples were found to contain both platiphyllol 1 and 2-acetyl-3,5-dimethoxy-4,6-dimethylphenol 2 [2] in varying proportions, while other samples showed the presence of 1 as the main constituent, but the absence of 2.



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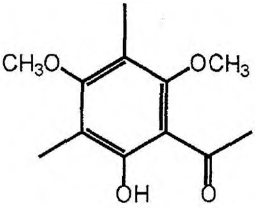

2

The chemotaxonomic relevance of these observations will be briefly discussed, together with some applications of platiphyllol in cosmetic preparations.

[1] B. A. Barlow, Proceedings of the Ecological Society of Australia (1987) 239-247
[2] J. B.Lowry, Nature 241 (1973) 61-62

4 - 07

SEASONAL VARIATION IN COMPOSITION OF A WILD POPULATION OF *THYMUS VULGARIS* L. FOUND IN LIGURIA (ITALY).

M. Bononi, V. Fumagalli, F.Tateo
DIFCA – Università degli Studi di Milano, via Celoria 2, 20133 Milano (Italy)

The seasonal variation of the yield and composition of the essential oil from a wild population of *Thymus vulgaris* L. collected in Monte Gazzo (Liguria- Italy) is reported. Analyses were performed by GC, Chiral-GC and GC-MS.
The variation of the content of two of the compounds that could characterise the chemotype (carvacrol and sabinene hydrate) has been evaluated during the vegetative cycle. The work permits to evidence sensible variations in the ratio between carvacrol and (cis- and trans-) sabinene hydrate.
The enantiomeric distribution of 4-terpineol, α-terpineol and linalool was also evaluated and seems to be sufficiently constant and characteristic; at the contrary, linalool shows a fluctuation of the R(-) enantiomeric excess.
The work reports interesting results concerning chemotype characterisation.

MYRISTICIN, APIOL AND OTHER SAFROLE-LIKE COMPOUNDS: PROBLEMS CONCERNING THEIR PRESENCE IN FOOD AND THEIR ANALYTICAL DETERMINATION.

F. Tateo, M. Bononi, E. Lubian, S. Martello
DIFCA – Università degli Studi di Milano, via Celoria 2, 20133 Milano (Italy)

The presence of myristicin, apiol and other safrole-like compounds allows toxicological problems not considered yet by European Community Directives 83/388 and 91/71 relating to flavours destined to be employed in foodstuffs as well as in basic materials for their preparation.

This work considers the foods most commonly used, in order to identify and determine the mentioned compounds.The work demonstrates that mace and nutmeg oils and oleoresins, in flavouring formulations, are destined to be employed in a unthinkable large variety of foods. That is demonstrated by HPLC and GC/MS analytical researches on food found on the market, where the presence of mace/nutmeg derived compounds cannot be taken for granted.

In this paper the results concerning safrole content are also reported and produced with analytical optimised methods.

The results suggest an appropriate modification of the limited natural compounds list mentioned in EC Directives on flavourings.

4 - 09

ESSENTIAL OILS OF THE *ORIGANUM* SPECIES OF TURKEY

[1] K. H. C. Başer, [1] N. Kirimer, [2] G. Tümen, [1] Medicinal and Aromatic Plant and Drug Research Center (TBAM), Anadolu University 26470 Eskişehir Turkey,
[2] Faculty of Education, Balıkesir University 10100 Balıkesir Turkey

The genus *Origanum* is represented by 22 species and altogether 32 taxa in which the rate of endemism is 68%. 24 taxa of *Origanum* growing wild in Turkey have been analyzed by our group for essential oils. Five Carvacrol-rich *Origanum* species top the list of Oregano exports from Turkey. In 1998, Turkey exported orer 7000 tons of dried Oregano for a return of US$ 16 million. Several *Origanum* species are used locally as herbal tea or as condiment in Turkey. The paper will summarize and discuss the results of essential oil yield and analysis of *Origanum* species so far studied at TBAM.

4 - 10

COMPOSITION AND VARIATION OF THE ESSENTIAL OIL OF *LEPECHINIA SALVIAEFOLIA* (KUNTH) EPL. (LAMIACEAE) FROM VENEZUELA

[1]M. D. Eggers, [1]E. Stahl-Biskup, [2]G. Orsini, [1]University of Hamburg, Department of Pharmaceutical Biology, Bundesstr. 43, D-20146 Hamburg,
[2]Fundación Instituto Botánico de Venezuela, Apto. 2156, 1010-A Caracas

Lepechinia is a New World genus of about 37 species, ranging from Chile to California. The leaves of *L. salviaefolia*, a shrub endemic in Venezuela and Colombia, were collected at four different sites in the Venezuelan Andes. Plant material of 60 plants was hydrodistilled separately and the variations of the composition were analysed by statistical methods. The components of highest interest are (-)-palustrol (0-28.1 %) and (-)-premnaspirodiene (0-17 %). Further components are β-phellandrene (1.0-20.6 %), borneol (5.6-16.8 %), bornyl acetate (1.6-12.9 %) and δ-3-carene (0.2-20 %) etc.. Both palustrol and premnaspirodiene are exceptional components of essential oils. Palustrol was isolated in the fifties from *Ledum palustre* (Ericaceae) and was later found in *Baccharis genistelloides* (Asteraceae). In some essential oils it occurs only in small percentages. Premnaspirodiene has been found in the essential oils of two *Premna* species (Verbenaceae) but has never turned up in further species.

As can be derived from the percentages given, a high variation of the main components is obvious. Probably we deal with real chemotypes, a phenomenon which is quite common within the Lamiaceae. Nevertheless, the plants with a high content of the spirosesquiterpene premnasprirodiene turned out to co-contain a second spirosesquiterpene which was recently described for *L. bullata* (Briq.) Epl. [1]. The latter, (-)- spirolepechinene, is a new compound of a rare skeletal class.

[1] M.D. Eggers, V. Sinnwell, E. Stahl-Biskup, Phytochemistry (1999), in press

4 - 11

DETERMINATION OF KOVATS RETENTION INDICES BY COMPUTER SOFTWARES

M.M. Barazandeh,Research Institute of Forests & Rangelands,P.O.Box:13185-116

Essential oils of Iranian aromatic plants are being analysed by a chemist team in the phytochemistry lab of Research Institute of Forests and Rangelands. So far about 80 species have been analysed and their main constituents identified by the aim of G.C. and G.C./M.S. methods.In this way we had a problem and it was the lack of an accurate method for calculating Kovats retention indices which are useful for qualitative analyses.For solving this problem, a computer program was written in basic language and applied to some species which had been previously analysed by G.C. and G.G./M.S.For example this method was applied to evaluating Kovats retention indices of essential oil constituents of two species of *Lavandula*(L.angustifolia P.Miller and L.spica L.),Three species of *Mentha*(M.longifolia L., M. x piperita L. and M. aquatica L.),five species of *Salvia*(S. officinalis L., S. sclarea L, S. santolinifolia Boiss., S. syriaca L. and S. virgata Jacq.),two species of *Calamintha*(C. officinalis Moench and C. grandiflora(L.) Moench),two species of *Thymus*(T. kotchyanus Boiss. & Hohen. and T. serphyllum L.) and also *Matricaria chamomilla* L., *Pimpinella anisum* L. and *Ferula assa-feotida* L.These species had been previously steam distillated in the lab and their essential oils injected to G.C. on two columns of different polarities(i.e. DB-1 and DB-WAX, 60m. x 0.25mm. ID, 0.25 micron film thick.).Using this method,main constituents of these species identified and then confirmed by their mass spectra.

Two basic programs which have been written for both columns as well as table of percentage composition for some species will be presented.

Poster Session I

Monday, September 6, 1999, 16.30 – 18.00
Lecture hall building, first floor

COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *LIPPIA GRAVEOLENS*

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Rua do Norte 3000 Coimbra, Portugal

Lippia graveolens HBK (Verbenaceae) is a widespread species in Central America used in folk medicine for treatment of several gastrointestinal diseases.

In order to contribute for the validation of this traditional remedy, we now report on the composition of the essential oil of *Lippia graveolens* collected in Guatemala, and its antimicrobial activity against several microorganisms.

Analysis of the volatile oil, isolated by hydrodistillation from the aerial parts of the plant, was carried out by GC and GC-MS, using two fused silica capillary columns with different stationary phases (polyethylenoglycol and polymethylsiloxane). Components were identified by comparison GC retention indexes, relative to a series of n-alkanes, and mass spectra with corresponding data of authentic compounds or components from reference oils.

Antibacterial studies were carried out by disc diffusion method against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* CECT 795 e *Proteus vulgaris* CECT 484. Antifungal studies were conducted by de same method using *Candida albicans* CECT 1394, *Cryptococcus neoformans* CECT 1078, *Cladosporium cladosporioides* CECT 2111, *Aspergillus niger* CECT 2574 e *Aspergillus fumigatus* CECT 2071.

More than 98 % of the volatile oil was identified. It was characterised by high percentage of hydrocarbons and oxygenated monoterpenes, being γ-terpinene (10%), p-cymene (7%), linalool (5%), thymol (18%) 1,8-cineol (5%) and trans-caryophyllene (6%) the major compounds.

The volatile oil showed antibacterial activity against all bacteria tested. The activity was in the order: *P. vulgaris* > *Staph. epidermidis* > *E. coli* > *Staph. aureus* > *St. faecalis*. An interesting antifungal activity, mainly against *A. fumigatus* and *Cl. Cladosporioides* was also showed.

These activities, clearly justifiable by the major oxygenated monoterpene compounds, can explain the folk use of the *Lippia graveolens* for treatment of gastrointestinal disorders.

A - 01

EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF SWEET GALE, *MYRICA GALE*

[1]M. Lis-Balchin, [2]S. Deans, [3]S. Hoffner, [1]School of Applied Science, South Bank University, Borough Road, London, SE1 OAA, UK
[2]Department of Food Science and Technology, Scottish Agricultural College, Auchincruive, Ayr, KA6 5HW, UK
[3]Institute of Pharmaceutical Chemistry, Pharmaziecentrum, University of Vienna, Althanstrasse 14, 1090 Wien, Austria

Evaluation of the antibacterial activity of Sweet gale, *Myrica Gale*, a local Scottish plant, was carried out against 50 bacteria, which included 30 different species of Gram positive and Gram negative bacteria and 20 strains of *Listeria monocytogenes.* The activity was compared with commercial thyme oil, clove bud oil and oregano oil.
10μl of each essential oil was pipetted into wells in Iso-sensitest agar seeded with each bacterium and zones of Inhibition were measured after incubation of the plates in the dark for 24h at 25^{0}C.

Myrica Gale proved very ineffective as an antimicrobial agent: it was mildly effective against only nine of the different bacterial species and five *L. monocytogenes* varieties. The three commercial oils were effective against all the 50 bacteria. The bioactivity of the essential oils was directly correlated with their chemical composition.

PHARMACOLOGICAL ACTION OF SELECTED SOUTH AFRICAN ESSENTIAL OILS ON SMOOTH MUSCLE IN VITRO

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[2] Division of Pharmacology and Therapeutics, King's College, Manresa Road, London SW3 6LX, UK

The mode of action of essential oils from five South African species from the Cape region was studied on the smooth muscle *in vitro* using guinea-pig ileum. The species included: *Agathosma betulina, A.crenulata, Coleonema album, Eriocephalus punctulatus* and *E. africanus.*

All the oils, at high concentratiion, had an initial spasmogenic activity followed by a spasmolysis. The spasmolytic action was post-synaptic and not atropine-like, as shown by an equal inhibition of exogenous acetylcholine and histamine. The spasmolytic effect was unaffected by the adrenoceptor blockers phentolamine and propranolol, which inhibited the antispasmodic effect of noradrenaline.

Using ODQ, a selective inhibitor of guanylyl cyclase, which blocked the relaxation produced by sodium nitroprusside, no evidence was obtained that the five essential oils were blocked, thus showing that cyclic guanosine monophosphate,GMP, was unlikely to be the secondary messenger involved. The possiblility that cyclic adenosine monophosphate, cAMP, is involved is now under investigation. On the basis of experiments using calcium-free buffers, there is no evidence as yet that any of the oils are calcium channel blockers at low concentrations.

A - 03

THE EFFECT OF SOME PLANT-GROWTH REGULATORS ON THE PEPPERMINT PRODUCTIVITY AND ESSENTIAL OIL COMPOSITION

[1] *T. Stoeva,* [2] *S. Zlatev,* [3] *G. Georgiev,* [3] *L. Iliev*

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Foliar application of different plant-growth regulators: phenylurea cytokinins (4-PU-30 and thidiazuron) and 2,3,5-trijodbenzoic acid (2,3,5-TB), pacrobutrazol and jasmonic acid, was examined for their influence on the productivity and composition of the peppermint essential oil. The comparative study was carried out using the concentartions of the investigated plant-growth regulators with an already established positive effect on mint productivity [1, 2, 3, 4, 5, 6].

The following regulators has increased the total yield of fresh above-ground mass (two harvests a year): 4-PU-30 by 48.09%, thidiazuron by 40.49%, jasmonic acid by 37.96% and 2,3,5-TB by 34.42%. The stimulating effect of jasmonic acid became more obvious in the second harvest. Only pacrobutrazol caused a slight decrease in the fresh mass.

All investigated regulators improved the essential oil productivity. The content of essential oil from air-dried plant material varied from 1.60 to 2.20%, as compared to the control 1.00%. As a result, the essential oil yield compared to the control significantly increased in all variants by: 187.08% (4-PU-30), 186.30% (jasmonic acid), 177.00% (thidiazuron), 126.49% (2,3,5-TB), and 35.53% (pacrobutrazol). GC analyses of essential oil composition demonstrated that qualitative oil alterations relate only to changes in the proportions of the main components: menthol, menthone, cineole, menthyl acetate, isomenthone and menthofuran. Compared to European Pharmacopeia (1997), the content of each compound varied within the permissible limits.

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A - 04

BENEFICIAL IMPACT OF THYME OIL ON AGE-RELATED CHANGES IN PHOSPHOLIPID POLYUNSATURATED FATTY ACID COMPOSITION IN A RAT MODEL

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The aim of this study was to determine any age-related changes in phospholipid polyunsaturated fatty acid composition in particular C_{20} and C_{22} fatty acids in rat liver, brain, kidney and heart and to assess and compare the effects of dietary supplementation (42.5mg/kg body weight/d) of the natural antioxidant thyme [*Thymus vulgaris* L.] oil and its major component thymol throughout the rat life span. The fatty acid composition in the various tissues from young (7-month) and aged (28-months) rats was determined by GC analyses and compared. Livers from aged control, thyme oil and thymol treated rats exhibited an increase in 22:6n-3 fatty acid levels. In contrast the 22:6n-3 content of brain, kidney and heart declined in aged rats in all three dietary groups. However, aged rats treated with thyme oil and thymol displayed significantly higher levels of 22:6n-3 than the respective age-matched controls. Tissue compositions of 20:4n-6 were found to be significantly lower in the liver and kidney from aged control rats but not those fed either thyme oil or thymol. In aged rats, the composition of 20:4n-6 in all tissues was highest in rats fed either thyme oil or thymol. These results show that dietary supplementation with thyme oil resulted in maintaining higher PUFA levels in all tissues studied. The majority of protection provided by thyme oil was by virtue of its thymol component, which comprises 49% of the whole oil. Thymol administered alone did not provide significantly higher protection than the whole oil, suggesting that other components within thyme oil are also contributing antioxidant activity through synergism [1,2].

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A - 05

IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF PLANT VOLATILE OILS AND THEIR PHYTOCONSTITUENTS

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Clove and nutmeg essential oils were analysed by GC and GC-MS. These oils together with 22 components found to be present were tested for antioxidant properties at final concentrations of 0.05 to 2.5×10^4 ppm in an egg yolk-based thiobarbituric acid reactive species (TBARS) assay and also undiluted in a β-carotene agar diffusion assay. Both the essential oils and the components tested in the TBARS assay demonstrated some degree of antioxidant activity. Only the clove oil, nutmeg oil, eugenol and terpinolene demonstrated any ability to inhibit the oxidative bleaching of the β-carotene agar. The ability of the oil components to inhibit malondialdehyde formation and therefore lipid peroxidation in the TBARS assay yet appear to possess no activity in the β-carotene agar diffusion assay, demonstrates the importance in the screening of plant material for bioactivity to use a bank of assays *in vitro* before assigning bioactivities. By the use of a number of assays not only the number of false positives and negatives should be greatly reduced, but evidence pertaining to the mechanism of action may be obtained [1,2,3].

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A - 06

ANTIBACTERIAL ACTIVITY OF PLANT VOLATILE OILS AND THEIR PHYTOCONSTITUENTS

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The volatile oils of black pepper [*Piper nigrum*], clove [*Syzygium aromaticum*], geranium [*Pelargonium graveolens*], nutmeg [*Myristica fragrans*], oregano [*Origanum vulgare* subsp. *hirtum*] and thyme [*Thymus vulgaris*] were assessed for antibacterial activity against twenty five different genera of bacteria. These included animal and plant pathogens, food poisoning and spoilage bacteria as well as opportunist pathogens. The volatile oils exhibited considerable inhibitory effects against all the organisms under test while their major components demonstrated various degrees of growth inhibition. The most active oil was found to be *Thymus vulgaris* followed by *Origanum vulgare* subsp. *hirtum* > *Syzygium aromaticum* > *Myristica fragrans* > *Piper nigrum* > *Pelargonium graveolens*. The antibacterial activity of the oil components showed the most active component to be thymol followed by carvacrol > α-terpineol > terpinen-4-ol > eugenol > (±)-linalool > (-)-thujone > δ-3-carene > *cis*-hex-3-an-1-ol > geranyl acetate > (*cis* + *trans*) citral > nerol > geraniol > menthone > β-pinene > R(+)-limonene > α-pinene > α-terpinene > borneol > (+)-sabinene > γ-terpinene > citronellal ~ terpinolene > 1,8-cineole > bornyl acetate > carvacrol methyl ether > myrcene > β-caryophyllene > α-bisabolol > α-phellandrene > α-humulene > β-ocimene > aromadendrene > *p*-cymene [1,2]. The components with phenolic structures, such as carvacrol, eugenol and thymol, were highly active against the test microorganisms. These compounds were strongly active despite their relatively low capacity to dissolve in water, which is in agreement with published data [3].

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A - 07

INVESTIGATION OF *CITRUS* ESSENTIAL OILS ON THE BRITISH AROMATHERAPY MARKET AND RELATED FRAGRANCE INDUSTRIES

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The uses and recovery of *Citrus* volatile oils, as well as their market importance, have already been discussed in great depth [1,2,3]. Recently, distillation techniques have been developed in the UK to re-use spent peel from the juicing industry, primarily as a source of winter income for traditional herb crop producers. Characteristics from a range of *Citrus* sp. vary widely depending on sites of production and cultivars. In addition, adulteration and blending is widespread within the industry. Our investigation had four aims: to summarize and clarify the taxonomy and economic importance of *Citrus* species in cultivation; to consider the main chemotaxonomical characteristics of various *Citrus* species (including fruit peel group, leaves and flowers); to distill and analyse essential oils from a variety of *Citrus* fruits on the market; to analyse commercial oil samples and compare these to both available references and our results. 23 samples of fresh *Citrus* fruits and 92 oil samples from wholesale and retail suppliers were distilled in the laboratory and analysed by GC. Two large samples of orange peel were field distilled and analysed. The volatile oil yield from fresh material ranged between 0.13% in grapefruit (*C. paradisi*) to 0.83% in clementine (*C. reticutata*). Commercial oil samples varied widely in labelling regarding geographic or botanical sources. GC analysis can serve as a quick screen for possible adulteration or mislabelling, using the components greater than 1% of total volume, with the exception of sweet orange (*C. sinensis*) and grapefruit (*C. paradisi*). However, aroma and fragrance characteristics within the esters, aldehydes and alcohol fractions of the samples typically found as trace elements [4], remained unidentified in this study. Extensive results and summary tables can be obtained from the main author.

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A - 08

EFFECTS OF FRAGRANCES ON ATTENTIONAL AND PHYSIOLOGICAL PROCESSES: EVIDENCE FOR A PHARMACOLOGICAL MECHANISM

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Scientific knowledge about the effects of fragrances on human behavior has grown considerably during the last decade; however, the underlying processes are largely unknown. Recently, several mechanisms for the effects of odors have been proposed [1] which can be roughly divided into psychological (based on subjective odor experience) and pharmacological processes. These processes are likely to be effective simultaneously when the olfactory system is exposed to fragrances. The aim of the present study was to seperate psychological from pharmacological mechanisms by excluding olfactory processing and to examine whether effects of fragrances on neuropsychological and physiological parameters can be attributed to a pharmacological mechanism.

In the present investigation 1,8-cineole (activating [2]), linalool (sedative [3]) and a placebo substance (pure peanut oil) were administered percutaneously for 20 minutes while physiological data were recorded; inhalation of the substances was prevented by breathing masks. Subjects gave ratings on their mental and emotional condition at the beginning and at the end of the application period. Afterwards subjects performed a visual vigilance task for 30 minutes.

Statistical analysis revealed a trend towards shorter reaction times for the cineole group as compared with the linalool group as well as significant group differences for breathing and blink rates. As self-evaluation was not affected by the substances and intra-group correlational analysis showed no differences between groups, it is highly likely that a pharmacological factor is involved in the effects of fragrances on neuropsychological and physiological processes in man.

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A - 09

ANTIMYCOTIC ACTIVITY OF PLANT VOLATILE OILS

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Volatile oils from aromatic and medicinal plants have traditionally been used as a means of treating systemic and superficial mycoses and as preservatives against fungal growth and mycotoxin production in human foods and animal feeds in many non-western societies and by itinerant European populations. In this study, black pepper, clove, melissa, oregano and thyme volatile oils were screened for antimycotic activity against the spoilage fungus *Aspergillus niger*, the economically significant and mycotoxigenic *Aspergillus flavus* and the plant pathogen *Fusarium culmorum*. These volatile oils strongly inhibited the growth of each of the three fungal species in a concentration-dependent fashion, from the lowest volatile oil concentration level (1 $\mu L\ mL^{-1}$ broth) through to the highest concentration level (100 $\mu L\ mL^{-1}$ broth). Black pepper and clove oils were particularly effective inhibitors of the *Aspergillus* species whilst thyme oil was highly active against all the fungal cultures [*Aspergillus* species being most susceptible with *Fusarium culmorum* growth being marginally less inhibited across the concentration range]. Melissa and oregano oils were significantly active against the *Aspergillus niger* species. Chemical analyses of oils by GC and GC-MS [1] revealed that the high levels of phenolic terpenoids, such as eugenol and thymol, were present in the most active oils, namely clove and thyme, which may explain these oils' high inhibitory activities [2]. The experimental data clearly demonstrate that the volatile oils extracted by hydrodistillation from aromatic and medicinal plants used in this study have value as plant-derived antifungal agents with potential application in the cosmetic, food and pharmaceutical industries and may provide novel lead compounds to combat mycoses and mycotoxicoses, which pose an increasingly significant threat to public health.

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EFFECT OF CLOVE AND NUTMEG VOLATILE OILS UPON TISSUE PHOSPHOLIPID POLYUNSATURATED FATTY ACID COMPOSITION DURING PREGNANCY IN A MAMMALIAN MODEL

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The nature of the acyl-chain region of the hydrophobic domains of lipids plays a cardinal role in membrane structure, fluidity and permeability, in addition to protein association and function. Furthermore, the *n-3* and *n-6* series C_{20} polyunsaturated fatty acids [PUFA] are the precursors of biologically active metabolites such as eicosanoids.

The effects upon tissue lipid fatty acid composition of plant volatile oils isolated from a variety of aromatic and medicinal plants have been carried out in this laboratory, with particular emphasis upon age-related degradation of C_{20} and C_{22} long-chain PUFA and during foetal/neonatal development. This study investigated the effect of clove [Table 1] and nutmeg [Table 2] volatile oils as dietary supplements on PUFA composition in various tissues in a pregnant rat model, with particular emphasis on arachidonate [AA; C20:4*n-6*] and docosahexænoate [DHA; C22:6*n-3*], when administered prior to and throughout the gestation period.

Table 1. Effect of clove oil on lipid composition in tissues from heart, liver and brain tissues from pregnant rats. Values expressed as % total fraction. *** P<0.001

TISSUE	FATTY ACID	
	Arachidonate	Docosahexænoate
Heart Control	17.9±0.6	16.2±0.6
Heart Treated	17.2±1.2	20.6±1.4***
Liver Control	15.7±0.5	21.4±1.2
Liver Treated	15.9±0.9	22.1±1.7
Brain Control	9.5±0.4	17.0±0.1
Brain Treated	12.4±0.2***	20.6±0.7***

Table 2. Effect of nutmeg oil on lipid composition in tissues from heart, liver and brain tissues from pregnant rats. Values expressed as % total fraction. ** P<0.01

TISSUE	FATTY ACID	
	Arachidonate	Docosahexænoate
Heart Control	17.9±0.6	16.2±0.6
Heart Treated	17.3±0.5	13.1±0.3***
Liver Control	15.7±0.5	21.4±1.2
Liver Treated	15.4±1.1	21.5±1.5
Brain Control	9.5±0.4	17.0±0.1
Brain Treated	9.4±0.4	14.1±0.7**

H.J.D. Dorman, Phytochemistry and Bioactive Properties of Plant Volatile Oils : Antibacterial, Antifungal and Antioxidant Activities, PhD Thesis, University of Strathclyde, [1999].

BIOTRANSFORMATION OF WASTE PRODUCTS OF FRAGRANCE PRODUCTION

[1] A. Görig, [1] B. Arendt, [1] A. Biermann, [1] K. Girke, [1] B. Sander, [1] W.Schmidt,
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One result of manufacturing of fragrance materials from essential oils are waste products like terpenes and sesquiterpenes. The utilization of these multi-component mixtures in the fragrance production is of great interest concerning costs and environmental reasons. The procedures for the use of these raw materials as low cost substances for fragrance compositions are limited up to now. To dump these products is destroying a raw material source and seems not to be economical and is an enviromental pollution fact. The objective of the project is in the biotransformation of the terpene and sesquiterpene mixture to high fragrance substances, with the assumption that the waste product contains no toxic compounds for the microorganisms. Higher fungi, like Basidiomycetes and Ascomycetes as well as yeasts were selected after a screening with 44 strains for the biotransformation. The biotechnological process development was started on the level of shaken cultures. Especially experiments were focused on the enzyme induction for the transformation process, which can be influenced significantly by the medium composition. After the development of the biotransformation process with selected strains in the 5-L-fermenter lab scale the process principle was successfully transfered to the 300-L-pilot scale. The biotransformations led to novel fragrance mixtures with interesting odour and fixating properties.

A - 12

USE OF *OCIMUM* ESSENTIAL OILS IN COWPEA PROTECTION IN STORAGE AGAINST BRUCHIDS IN GUINEA

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In their fight against the pests that attack stored products, farmers have used mineral powder, ash and various kinds of plant extracts. Among the plants most often used, those with essential oils are particulary important. Thus, essential oils have gradualy become a remarkable tool in the fight against insect pests in general and against insects attaking stored grains in particular. Cowpea, *Vigna unguiculata*, is a rich source of proteins for African peasants. The cowpea weevil, *Callosobruchus maculatus*, attack the stocks and synthetic insecticides are expensive and potentially harmful. In Guinea, *Ocimum basilicum* and *Ocimum gratissimum* are used in cosmetic, medicine and agriculture for crop protection (Bélanger et al. 1995, Nianga et al. 1993).

Plants of Ocimum were collected, dried and the essential oil was obtained by steam distillation in Guinea (Kéita et al. 1999). Essential oils were analyzed by gas chromatography and identification of the compounds was made by comparing Kovats indices on two opposite-polarity columns and by mass spectrometry. Kaolin was extracted, purified and refined to be used as an adjuvant of the essential oils by aromatization. Fumigation and powdering of the aromatized powder were used to apply the essential oils in laboratory conditions.

After 24h, 48% and 37% adults died when exposed to vapours of 20 µl *of Ocimum basilicum* and O. *gratissimum* respectively. Dusting gave significant results during 48h, the mortality being consistently higher with O. basilicum. No adults emerged from insect-free grains dusted with 100 or 200 µl oil, while adult emergence was observed one month later in untreated grains. A complete protection was achieved for 6 months with insect-free grains treated with either Ocimum spp.essential oil.

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A - 13

A FOUR-ARM OLFACTOMETER TO EVALUATE THE REPELLENT PROPERTIES OF ESSENTIAL OILS AGAINST INSECTS

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Many plants have the potential to protect crops against insect damage and essential oils of those plants could be used in plant protection (Grainge and Ahmed 1988). The repellent properties of some essential oils make them a useful tool to control insect pests in greenhouses. In the laboratory, using a four-armed olfactometer, we tested the repellent effects of essential oil of tansy, *Tanacetum vulgare*, white cedar, *Thuya occidentalis*, balsam fir, *Abies balsamea*, and rosemary, *Rosmarinus officinalis*, and the *d*-limonene against two greenhouse pests: the two-spotted spider mites, *Tetranychus urticae* and the greenhouse whitefly, *Trialeurodes vaporariorum*.

The two treated fields contained the volatile components of 100 µL of essential oil, the two remaining fields had only humidified air. The degree of repellency was evaluated by recording the amount of time an insect pest spent in each field.

Results indicate that the five essential oils or components could be classified in three categories depending on their properties to repel insects. The essential oil of tansy and white cedar showed a high level of repellency against the two pests. The essential oil volatile of these two plants reduced the time the insects spent in the field by as much as 17 times. α- and β-thujone probably showed high level of repellency against the two pests. These compounds have been shown to repell mosquitos and Colorado potato beetles (Hwang et al. 1985, Panasiuk 1984).

The essential oil of balsam fir and the *d*-limonene repulsed only the two-spotted spider mites which spent up to 10 times less time in the treated than the control fields. The essential oil of rosemary showed less repellency on the two-spotted spider mites and did not affect the greenhouse whitefly. The essential oil of the tansy or the white cedar could be used in plant protection to control simultaneously the two-spotted spider mites and the greenhouse whitefly.

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CYTOEMBRYOLOGICAL STUDY ON THE MALE GENERATIVE ORGANS IN *SALVIA NEMOROSA* L. (LAMIACEAE)

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Salvia nemorosa is distributed on dry grassy places and shrubs throughout Bulgaria. During flowering stage this plant contains 0.01 - 0.04% essential oil with pleasant odour like this of *S. sclarea*. In the Bulgarian folk medicine it is used against blood expectoration, abdomen ache and treatment against wound and furuncles.

The cytoembryological study on this plant is of particular interest because of scantiness of relevant information in the literature. Male sterility of unestablished nature is known in *S. nemorosa* and *S. officinalis*. That is why the present study is aimed to determine if in the Bulgarian representatives of this species male sterility is presented, which can be used for the heterosis selection of this medicinal and aromatic plant.

In *S. nemorosa* disporangiate dithecal anthers built of four layered wall - epidermis, fibrous endothecium, ephemeral middle layer and secretory tapetum - were presented. In the most studied flowers microsporogenesis runs normally. Predominantly tetrahedral tetrads were simultaneously formed.

The formation of the anthers, the microsporo- and microgametogenesis and the development of the male gametophyte in the most studied flowers were accompanied by distinct marked degeneration processes which at last coased male sterility in the flowers of *S. nemorosa*.

A - 15

SEED PRODUCTION IN *SALVIA PRATENSIS* L. (LAMIACEAE)

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Salvia pratensis is a well known plant in the Bulgarian folk medicine. It has a wide application mainly against cough, stomach and abdomen ache due to bearing of 1.15% essential oil containing phenolen and cholin.

The cultivation of this plant on the experimental field of the Institute of Botany suggested also a cytoembryological investigation on the female generative organs with the aim to clarity the low seed production. In this aspect they exist some publication on *S. pratensis*.

During our investigation following normal for Lamiaceae embryological characteristics were found: anatropous tenuinucellate unitegmic ovule, unicellular archeosporium, absence of covering cells, Polygonum-type of female gametophyte, Prunella-type of endosperm development, Onagrad-type embryogenesis and Investing-type embryo.

All these features are considered as advanced.

Seed development in *S. pratensis* is accompanied by a great number of sterilization processes starting at macrosporogenesis, which account for its low seed production.

These processes are in accordance with the founded by us sterilization of the male gametophyte in the flowers of *S. pratensis*. This accordance supports also the adopted concept for the consensual obligation of sterilization between the male and female generative organs in the Angiosperm's flowers.

DEVELOPING A *SAR* - MODEL OF SANDALWOOD ODOUR COMPOUNDS WITH MOLECULAR SIMILARITY METHODS

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East Indian sandalwood oil is being used for many years as an important part in many perfumes. Due to the limited availability and the resulting high price many scientific work has been done to clarify the connection between the molecular structure of the main constituents of this oil (ß-Santalol and analogues) and their odour impression. During the years many compounds have been both isolated or newly synthesized. Most of the models which tried to predict the odour impression of these structures were sooner or later outdated after new compounds have been found [1]. Therefore we concentrated on a few similar structure and generated a SAR - model by superposing these structures with a carefully selected lead-molecule and calculated the molecular similarity of the molecular shape and the ab-initio electrostatic potential through the well known spearman correlation coefficient. The results were able to correctly reproduce the tested biological activity of these molecules and even a novel synthesized structure [2].

[1]: Brunke, E. J.; Fahlbusch, K. G.; Schmaus, G.; Vollhardt, J.; "The Chemistry of Sandalwood Odour – A Review of the Last 10 Years", Proceedings 15 Journ. Int. Huiles Essentielles DIGNE-LES-BAINS Sept. 1996
[2]: Klinsky, A.; Weiß-Greiler, P.; Buchbauer, G.; Wolschann, P.; Journal of Molecular Modelling 1999, submitted

A - 17

MICROBIOLOGICAL TRANSFORMATION OF SESQUITERPENOIDS AND RELATED COMPOUND

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We are continuing to investigate the biotransformation of plant secondary metabolites obtained as a constituents from higer plants and liverworts by various kinds of microorganisms from a pharmacological point of view[1-3]. Now we report the microbiological transformation of several sesquterpenoids and related compounds such as α-(-)- and β-(+)-eudesmol(**1** and **2**), (-)-hinesol(**3**), (-)-ambrox(**4**), dehydrocostuslactone(**5**), α-, β- and γ-cyclocostunolides(**6**-**8**), α-santonin(**9**) and 3-oxo-α-ionone(**10**).

1 is biotransformed via 2α-OH-**1** to 2-oxo-**1** by *A.niger* and *A. cellulosae*. On the other hand, **2** is converted to 2α-OH-**2** with a small amount of 2β-OH-**2** (**2-1**), 2-oxo-**1**, 12-OH-**2-1**, 12-OH-2-oxo-**1** by *A.niger* and 9β-OH-**2** by *A. cellulosae* and 4α-OH-**2** by *A. usami*. **3** is converted to 2α-OH-**3**, 2β-OH-**3**, 2-oxo-**3**, 1α, 2β-diOH, 10, 14-ene-**3**, 1β, 2α-diOH, 10, 14-ene-**3**, 3α-OH, 1,2-ene-10α-methyl, 11β-hydroxymethyl,10, 11-oxido-**3**, 3α-OH, 1,2-ene-10α-methyl, 11α-hydroxymethyl-10, 11-oxido-**3**, 3α-OH, 11-hydroxymethyl,1,2-epoxy,10α-methyl, 10, 11-oxido-**3**, 1,2-epoxy, 3α,13α-diOH, 6α-methyl,10,11-oxido-**3** by *A.niger* and 14-OH-**3**, 7α,14-diOH-**3** by *A. cellulosae* **4** is converted to 3β-OH-**4**, 3-oxo-**4**, 3β-OH,15α-carboxy-**4**, 3β,8-diOH, 11-hydroxymethyl-(-)-eudesmane, 3-oxo, 8-OH, 11-hydroxymethyleudesmane by *A.niger* and 1β-OH-**4** and 1-oxo-**4** by *A.cellulosae*. **5** is converted to 11β,13-dihydro-**5**(**5-1**) by ten kinds of *Aspergillus* spp. and 3α-OH-**5-1** and 10β,14-epoxy-**5-1** by *A.niger* and 3α-OH,10α, 14-epoxy-**5-1**, 10α,14-di OH-**5-1** and 10β,14-diOH-**5-1** by *A.niger*. **6** is converted to 11β,13-dihydro-**6**(**6-1**) by *A. cellulosae* and *A.niger* and 2-OH-**6**, 2-oxo-**6-1**(**6-2**), 1β-OH- **6-2**(**6-3**) and 13-(2-ethoxy-carbonyl, 2-hydroxyethylthio)-**6-2** by *A.niger*. **7** is converted to 11β,13-dihydro-**7**(**7-1**) by *A.niger* and *A. cellulosae* and 2α-OH-**7-1**(**7-2**), **6-2** and **6-3** and 13-(2-ethoxycarbonyl, 2-hydroxyethylthio)-**7-2** by *A.niger*. **8** is converted to 11β,13-dihydro-**8** by *A. cellulosae* . **9** is converted to 4,5-epoxy-**9**, 11β-OH-**9** and 13-OH-**9** and 4-(3-OH,6-methylphenyl)-2α-methyl-3β–hydroxyethyl-γ-lactone by *A.niger* . **10** was converted to 7,11-oxido-3-oxo-α-ionone and 3-oxo-α-ionol by *A.niger*.

Stereospecific and regiospecific hydroxylation, epoxidation and hydrogenation were observed in the biotransformation of above mentioned sesquiterpenoids.

On the basis of the above results, we will discuss the reaction pattern on the microbiological transformation of sesquiterpenoids together with the metabolic pathways. It is of very interest to examine the biotransformation of sesquiterpenoids and related compounds to see whether or not the metabolites show a more intensely enhanced biological activity than the original compounds.

[1]Noma.Y. and Asakawa.Y.(1994) Biotechnology in Agriculture and Forestry, Springer-Verlag Berlin Heidelberg **28**, 185-202.

[2]Noma.Y. and Asakawa.Y.(1995) Biotechnology in Agriculture and Forestry, Springer-Verlag Berlin Heidelberg **33**, 62-96.

[3]Noma.Y. and Asakawa.Y.(1998) Biotechnology in Agriculture and Forestry, Springer-Verlag Berlin Heidelberg **41**, 194-237.

SYNTHESIS AND ODOR OF A NEW ANALOGOUE OF β-SANTALOL

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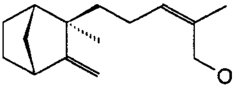
East Indian Sandalwood Oil, the essential oil of the wood of *Santalum album* (L.), is widely used in perfume industry. The bicyclic sesquiterpene alcohol β-santalol (**1**) is one of the most important compounds responsible for the sweet woody character of the essential oil. **1** is also responsible for the urinaceous, animalic tonality.
In connection with our studies on structure-activity-relationship of fragrance compounds emitting the sandalwood odor it could be shown that modification of the side chain of β-santalol (**1**) can lead to weakening or loss of the typical odor. Guided by results obtained by means of computer aided drug design the influence of the ring-system on the odor is now to be examined. B.D. Mookherjee proved a woody, amber odor for tricyclic ketone **2** <1>, so we wished to analyse the odor quality of β-santalol analogoue **3**.



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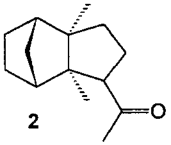

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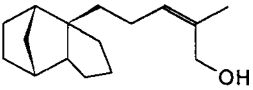

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Synthesis of the tricyclic ring-system was started by Diels-Alder reaction of p-benzochinone and 1,3-cyclopentadiene followed by various addition- and rearrangement-reactions finally affording the corresponding tricyclic aldehyde. Chain elongation at the CHO position was attempted under various conditions using Wittig reaction, but failed because of steric hindrance <2>. However, successful conversion of the aldehyde to target compound **3** was effected via a six-step reaction series in excellent yield, followed by treatment with the corresponding Wittig-reagent and reduction of the ester after separation of the Z/E-isomeric mixture via TLC.
Both isomeres of **3** as well as a number of intermediates exhibited an interesting odor profile.

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<2> Takakura, H., Toyoda, K., Yamamura, S., Tetrahedron Lett. **37**, 4043-4046 (1996)

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SYNTHESIS AND ODOR CHARACTERISTIC OF NEW COMPOUNDS WITH THE CARANE SYSTEM

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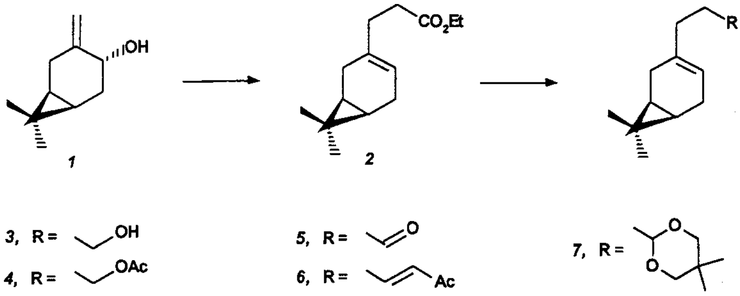
Our interest in synthesis of compounds with the carane system is connected with a possibility of their application as an odoriferous substances for perfumery using easy available component of polish turpentine as a starting material for our syntheses. Known allilic alcohol **1** obtained in four steps modified by us procedure from monoterpene hydrocarbon - (+)-3-carene was utilized for synthesis of derivatives with olfactory properties. The orthoacetate modification of the Claisen rearrangement of **1** afforded γ,δ-unsaturated ester **2**, which was reduced with $LiAlH_4$ to alcohol **3**.



Reaction of esterification of **3** with acetyl chloride gave acetate **4**. Alcohol **3** in the reaction of oxidation with PCC was transformed to unstable aldehyde **5**, which as a raw material afforded two very interesting odoriferous carane derivatives. Wittig-Horrner reaction gave analogue of ionone - derivative **6**. Reaction with appropriate diol afforded dioxane 7. All new obtained compounds possess interesting olfactory properties. Scheme of synthesis and odor characteristics are presented.

FORSKOLIN STUDIES

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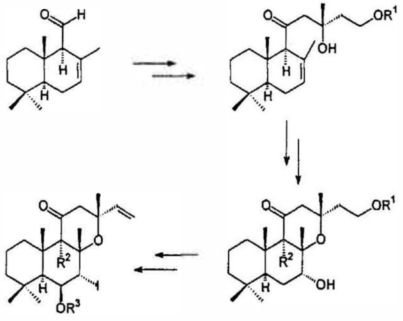
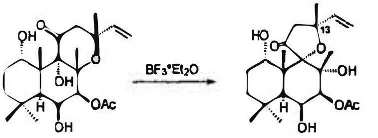
Forskolin is a labdane diterpene that has been shown to interact with different membrane proteins including adenylyl cyclase, the glucose transporter, the voltage-gated potassium channel, and ligand-gated ion channels.[1] The mode of binding to the adenylyl cyclase has been studied in detail.[2] Binding of forskolin in a complex with a soluble active form of adenylyl cyclase and its stimulatory heterotrimeric G protein α subunit has recently been determined by a crystal structure analysis (2.3 Å resolution).[3]

Synthetic and mechanistic studies along the lines depicted in the Scheme[4] and new structure-activity relationships (collaboration with the groups of H.-G. Glitsch (Bochum), J. Arnhold (Leipzig), and M. Koksch (Leipzig)) will be reported. Docking of different forskolin derivatives with the program AutoDock will be discussed.





FORSKOLIN (13R)-SPIROFORSKOLIN

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[1] For leading references, see Morris, D.I.; Greenberger, L.M.; Bruggemann, E.P.; Cardarelli, C.; Gottesman, M.M.; Pastan, I.; Seamon, K.B. *Mol.Pharmacol.* **1994**, *46*, 329-337.

[2] Zhang, G.; Liu, Y.; Ruoho, A.E.; Hurley, J.H. *Nature* **1997**, *386*, 247-253, and references therein.

[3] Tesmer, J.J.G.; Sunahara, R.K.; Gilman, A.G.; Sprang, S.R. *Science* **1997**, *278*, 1907-1916.

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SYNTHESES OF SYN- AND ANTI-EPOXIDES OF α-CAMPHOLENIC AND FENCHOLENIC DERIVATIVES

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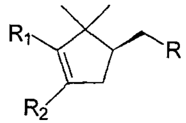
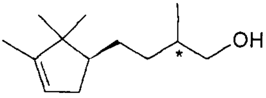
In the course of investigations of structure-odour-relationships of sandalwood odourants, substances of the α-campholenic (**1**) and fencholenic type (**2**) are of special interest [1].

A lot of α-campholenic side chain derivatives having sandalwood odour are known, Brahmanol® (**3**) is one of the most famous of these.



	R_1	R_2
1	CH_3	H
2	H	CH_3



3

We have been concerned with variations at the electronegative centre of the 5-membered ring. The epoxidation itself and selective oxirane ring opening reactions promised a variety of functionalised derivatives.

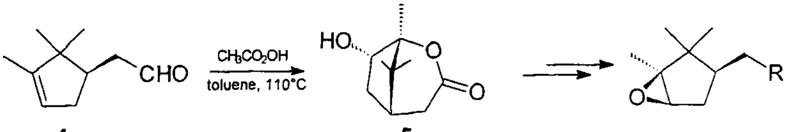
So far the anti-epoxides are the synthetically preferred products of epoxidations with various reagents under usual conditions because of steric hindrance of the double bond by the side chain. The syn-epoxides can be formed as side products with a maximum ratio of 20% by drastic epoxidation conditions (CH_3CO_2OH, toluene, 110°C) [2].

The syn-epoxide of Brahmanol® have a much stronger sandalwood odour compared to the anti-isomere.

Syn-epoxides of fencholenic compounds can be obtained easily by epoxide conversion via acidic ring opening of the anti-epoxide, monotosylation and finally ring closure to the isomeric epoxide.

On these acidic conditions α-campholenic epoxides undergo the Nametkin rearrangement to β-campholenic compounds [3].

We report a procedure to synthesise also the syn-epoxides of α-campholenic compounds via a 7-hydroxy substituted campher lactone (**5**) which we got from the α-campholenic aldehyd (**4**).



4 **5**
(60%

[1] K. Schulze, H. Uhlig, L. Weber, R. Kempe, *Liebigs Ann. Chem.* **1993**, 987
[2] U. Wahren, I. Sprung, K. Schulze, G. Buchbauer, M. Findeisen *Tetrahedron Lett.* **40**, (1999), 5991
[3] K. Schulze, H. Uhlig, K. Wyßuwa, A.K. Habermann, *J. Prakt. Chem.* **335**, (1993), 363

OXIDATION OF LIMONENE AND ORANGE OIL CATALYZED BY ZEOLITE-ENCAPSULATED IRON COMPLEXES

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Various heterogeneous catalysts were evaluated in the oxidation of limonene by either oxygen or iodosobenzene. These catalysts consisted of zeolite Y containing Fe^{+2} as extra-framework cation, either free or coordinated to N,N-bis(salicylidene)ethylenediamine derivatives. 1,2-Epoxylimonenes were the main reaction products, obtained in *cis/trans* ratios between 1.5 and 2.2. Limonene oxides, *cis-* and *trans-*carveols, and carvone were also present in the product mixture. Two-dimensional high resolution gas chromatography (2D-HRGC) was applied to the analysis of the oxidation product carvone. The optical purity of carvone was determined by 2D-HRGC using a γ-cyclodextrine/methyl silicone capillary column as the second separation step. Starting from R-(+)-limonene, carvone was formed mainly as the R-isomer (R : S = 2:1). The effects of the type of ligand, oxygen donor and reaction conditions (stirring, batch, microwave heating) on conversion, yield, selectivity, and turnover number were studied. Among the catalysts tested, those containing the coordination complexes with the bulkier substituents showed the highest activity. The microwave-assisted reactions exhibited a 70-fold increase in reaction rate.

BIOCHEMICAL PROPERTIES OF ESSENTIAL OILS

[1]J. Graßmann, [1]S. Hippeli, [1]K. Domisch, [2]N. Beuscher. [3]D. Vollmann and [1]E.F. Elstner, [1]Lehrstuhl für Phytopathologie, Labor für Angewandte Biochemie
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Essential oils are supposed to exhibit defence- or communicatory (signal transducing) functions in higher plants thus possibly involved in host-pathogen interaction („phytoalexins"), plant-animal communication („attractants"; „repellants") or plant-plant interactions („allelopathy") [1, 2]. In traditional medicine oils derived from different plants are used as antiinflammatory or immune-adjuvant drugs with increasing importance. In order to study potential mechanisms of their activities in this respect we tested several biochemical model reactions mimicing metabolic situations involved in these pathoprocesses. We can show that myrtol or terpene oil derived from *Pinus mugo* inhibit lipid peroxidation, gaschromatographically measured as ethene and ethane formation from α-linolenic acid, photodynamically induced by the dye „rose bengal" [3] or ethene formation from 1-aminocyclopropane-1-carboxylate (ACC) in whole blood after stimulation of neutrophilic granulocytes by zymosan [4]. As reaction mechanism interference with free unsaturated fatty-acid-radicals is suggested in the case of linolenic acid photoperoxidation. In the case of ethene release from ACC in whole blood interference of oils with the signal transduction chain in neutrophil plasma membranes is most likely since the oils are not inhibitory in this reaction if isolated myeloperoxidase in the presence of hydrogen peroxide and chloride ions or hypochlorite are used as oxidants for ACC [5].

[1] D. Schlee, Ökologische Biochemie, G. Fischer Verlag, Jena, Stuttgart, New York (1992)
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[3] I. Heiser, W.F. Osswald and E.F. Elstner, J. Plant Physiol. 152 (1998) 230-234
[4] E. Albrecht-Goepfert, H. Schempp and E.F. Elstner, Biochem. Pharmacol. 56 (1998) 141-152
[5] S. Kruedener, H. Schempp and E.F. Elstner, Free Radic. Biol. Med. 19 (1995) 141-146

ANTIMICROBIAL VOLATILES AND FLAVONOIDES FROM *ADHATODA VASICA* NEES.

[1]*Esawi, S.A.*, [1]*Hashem,F.A.* and [2]*Ali, A.M*
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[2]Microbial Chemistry Department, National Research Centre, Dokki, Cairo, Egypt.

Volatiles of leaves and flowers of *Adhatoda vasica* Nees were investigated using GC-MS analysis. Flower volatiles composed of 36 compounds, of which 27 compounds [1] were identified amounting to 95% of the total volatiles. Ketons were the most abundant chemical class in flower volatiles with the major compound was identified as heptanone, 3-methyl-4- (23.81%). Leaves volatiles were a complex mixture with more than 50 compounds, of which 27 compounds were identified comprising 82% of the total volatiles. Hydrocarbones constituted the most abundant chemical class in leaves (43%) and the major component was Decane (31%). Both volatiles showed higher antimicrobial activity [2] against Gram +ve, Gram -ve bacteria and yeasts more than fungi.

Ethyl acetate extract of Adhatoda vasica Nees leaves and fowers was rich in flavonides, of which 5 glycosides and an aglycone were isolated and identified [3].These are vitexin, isovitexin, 2"-O-xylosylvitexin, rhamnoxylvitexin, pendulin and the aglycone apigenin.

[1] R.P. Adams, Identification of Essential Oils by Ion Trap Mass Spect-roscopy, Academic Press, London (1989)
[2] S.M. Hammond and P.A. Lambert, Antibiotic and Antimicrobial Action, Edward Arnold, London (1978) 12-30.
[3] T.J. Mabry, K.R. Markham and M.B. Thomas, The Systematic Identi-fication of Flovonoides, Springer, New York (1970).

EFFECT OF UV-IRRADIATION ON THE ESSENTIAL OIL CONTENT AND CHEMICAL COMPOSITION OF *Ocimum basilicum* L.

[1] F. Sefidkon, [1] M.B. Rezaee, [2] A. Majd, [2] A. Mirzatooni,
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[2] Azad Islamic University, Science and research Branch, Tehran, Iran.

The influence of UV-irradiation on the essential oil content and chemical composition of *Ocimum basilicum* by 6, 20 and 40 W radiation sources was studied. Plant material was cultivated at flower vases (in greenhouse) and collected at 3 stage after UV-irradiation, i) before flowering after two weeks irradiation, ii) at flowering stage after two weeks irradiation and iii) at flowering stage while they had been irradiated from the beginning of plant growth. There are some testifier flower vases in all stages that were not irradiated and supposed as indicators. The results showed stronger effect of irradiation on the essential oil content and chemical composition of *Ocimum basilicum* at flowering stage. Irradiation from the beginning of plant growth increase the oil yield and percentage of stragol, neral, geranial and some other monoterpenoides, but decrease the percentage of some sesquiterpenoides. The effect of UV-irradiation on the essential oil, before flowering was decreasing the oil content and increasing the percentage of sesquiterpenoides like β-caryophyllene, α-humulene and γ-cadinene.
Irradiation for two weeks from the beginning of flowering stage cause different and sharp influence on chemical composition of basil oil.

ANTIMICROBIAL ACTIVITY AND CHEMICAL COMPOSITION OF THE BARK OIL OF *CROTON STELLULIFER*, AN ENDEMIC SPECIES FROM S.TOMÉ E PRÍNCIPE

[1]A. P. Martins, [1]L. Salgueiro, [1]M. J. Gonçalves, [2]R. Vila, [3]F. Tomi, [2]S. Cañigueral, [3]J. Casanova, [1]A. Proença da Cunha and [2]T. Adzet

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[2]Unitat de Farmacologia i Farmacognòsia, Fac. de Farmàcia, Universitat de Barcelona. Av. Diagonal, 643, E - 08028 Barcelona, Spain.

[3]Equipe Chimie et Biomasse, URA CNRS 2053, Université de Corse. Route des Sanguinaires, 20000 Ajaccio, France.

Medicinal plants represent the main source of health related products in the Republic of S. Tomé and Príncipe. Little information was available about the essential oils from plants of this country. For this reason, we have undertaken a study on the aromatic medicinal plants of S. Tomé.

The bark of *Croton stellulifer* is widely used in these islands as disinfectant, specially inside the houses. The bark is well dried and then burned. Since the pharmacological activity of many essential oils on a large number of human and animal pathogens has been confirmed world-wide by several laboratory investigations and these genus has a widespread use (several *Croton* sp. are referred as medicinal in local pharmacopoeias), we have performed the qualitative and quantitative determination of the essential oil of the bark of the endemic species from S. Tomé, together with the study of their antimicrobial activity.

To our knowledge, this is the first report of a detailed composition of the essential oil of *Croton stellulifer* as well as of its biological activity.

The essential oil was obtained by hydrodestillation of the bark, and analysed by GC and GC-MS, using two fused silica capillary columns of different stationary phases. Identification of the compounds with a percentage higher than 1% was also performed by ^{13}C-NMR. The major constituents were *p*-cymene (17.7%), α-phellandrene (17.4%), linalool (12.4%) and α-pinene (8.1%).

The microbial growth inhibitory properties of *Croton stellulifer* was determined using the disc diffusion method. Five bacterial strains, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* CECT 795 and *Proteus vulgaris* CECT 484; two yeasts, *Candida albicans* CECT 1394 and *Cryptococcus neoformans* CECT 1078; and three filamentous fungi, *Cladosporium cladosporioides* CECT 2111, *Aspergillus niger* CECT 2574 and *Aspergillus fumigatus* CECT 2071, were used to evaluate the essential oils inhibitory capacities.

Results showed that the essential oil of *Croton stellulifer* was active against both bacterial and fungal strains, except *Aspergillus niger*. This activity may justify the popular use of this plant.

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ANTIMUTAGENIC ACTIVITY OF MONOTERPENOIDS

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Abstract : In our search for novel naturally occurring antimutagenic compounds derived from plants that have a history of safe use as crude drug in Japan, we reported the antimutagenic activity of β-eudesmol from *Dioscorea japonica*[1]. Suppression of furylfuramide-induced SOS response by monoterpenoids were studied in this presentation. Suppression of the SOS-inducing activity by monoterpenoids were determind in the *umu* test using *Salmonella typhimurium* TA1535/pSK1002. Terpen alcohols, ketones, and aldehydes have a potent suppressive effect, but hydrocarbons have not been showed suppressive effect. Particularly, menthol, pulegone, piperitenone were showed most potent suppressive effects, the ID50 was more or less 0.5 μ mol/ml respectively.

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MEASUREMENTS ON MARJORAM USING A GAS SENSOR ARRAY („ELECTRONIC NOSE")

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Although marjoram (*Origanum majorana* L.) has already been cultivated and used as spice and medical plant for centuries a package of questions concerning that spice remains to be answered. From the chemical point of view there is the original headspace constitution of the dried marjoram samples which has to be analysed without adulteration by the analysing procedure itself. On the basis of this knowledge the reasons for a higher or lower hedonic rating of a given marjoram sample could be found out. Then, the breeders' interests are focussed on creating hybride varieties showing optimal sensory properties (aroma, colour). [1]

Here, the complex output of a special gas sensor array (Fox 4000, Alpha M.O.S., Toulouse; 17 semiconducting metal oxide sensors) acting as a "fingerprint" of the headspace under study will be shown in relation to

- sensory evaluations (taste, smell, colour)
- the genotype (different populations)
- the plot of land the plant came from
- the oil composition determined by GC/MS after solvent extraction [2].

Samples of 20 different populations of marjoram were cultivated in the experimental garden of the Federal Centre for Breeding Research on Cultivated Plants in Quedlinburg, Germany, following a randomised block design with four replicates. Dried samples were ground and then measured using the FOX 4000 gas sensor array (Alpha M.O.S, Toulouse). The sensor signals generated by the sensor array were subjected to Discriminant Analysis. The obtained discriminant functions were tested by classification of "unknown" samples not having been included in the data matrix used for their calculation. On the basis of marjoram samples of 2 years (1997 and 1998) relations between the output of the gas sensor array and sensory evaluations done by an expert panel at the Majoranwerk Aschersleben GmbH were established.

It will be shown that the sensor array is better able to detect stronger deviations from a high quality marjoram standard than only marginal differences in smell and taste. Hence, the investigated gas sensor array could be used for a rough selection of marjoram to sort out shipments showing serious deviations in quality. So the expert tasters have to examine the marginal differences only.

Additionally, the results will be discussed in relation to the long-term stability of the metal oxide sensors which was investigated by measurement of chemically stable substances. These measurements done within a four month period showed rather day-to-day-fluctuations than a linear degradation (drift) of the sensor signals.

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HRGC/FID AND HRGC/MSD STUDY OF COLOMBIAN *LIPPIA ALBA* (MILL) OILS OBTAINED BY DIFFERENT EXTRACTION TECHNIQUES

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Hydrodistillation (HD), simultaneous distillation-solvent extraction (SDE), microwave-assisted extraction (MWE), and supercritical fluid extraction (SFE), were employed to isolate secondary metabolites from leaves and stalks of colombian *Lippia alba* (Mill.), a plant from the *Verbenaceae* family which is distributed over Latin America. MWE used dichloromethane and hexane as solvents and SFE used CO_2, in a high-pressure Soxhlet extractor (J&W Scientific, Folsom, CA). The oils were analyzed by means of high-resolution gas chromatography using flame ionization detection and a mass selective detector, over fused silica capillary columns (50 m) coated with either poly(dimethylsiloxane) (DB-1), poly(ethyleneglycol) (INNOWAX), or 5% phenyl-poly(methylsiloxane) (HP-5). Kováts indices, mass spectra or standard compounds were used to identify a total of 49 individual components in the various extracts. 47, 40,10 and 22 different compounds were found in the HD, DES, SFE, and MWE *Lippia alba* oils, respectively. Carvone (45.8%) was the most abundant component, followed by limonene (37.9%), and γ-cadinene (6.1%). These results correspond to averages from three independent extractions with each method.

POTENTIAL APLICATIONS OF CONDUCTOMETRIC SENSOR SYSTEMS IN THE WOLRD OF FRAGRANCES

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The study of essentilal oils and related natural products which are applied to and released from the human body is of rising interest for the cosmetic industry anf the medical world. The potentials of conductometric sensors with regard to differentiation between essential oils as well as their quantitation will be discussed.

Medical research is primarily focused on the analysis of essential oils in the human breath and at the skin surface. A breath analysis system applicable to essential oils will be commercially available by SENSOBI Sensororen GmbH in the nearest future. It consists of several conductivity sensors with specific polymer coatings that are combined in a breathing mask. Successful detection of constituents of essential oils in the human breath will be demonstrated of the example of thymol. The difference between the orally applied amount of thymol and the exhaled thymol concentration could be measured due to the low detection limits of the system in the ppb range. In addition information about the time period between an oral intake of thymol containing pharmaca and ist appearance in the breath were obtained.

A future outlook of the expansion of SENSOBI 's product line will be given .Setups for skin surface studies that are in development will be described. The possibility of integrating specific conductometric sensors in instruments that are of interest in the quality control of the cosmetic industry will be dicussed too.

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GC/MS ANALYSIS AND OLFACTOMETRY OF THE ESSENTIAL OILS OF LEAVES AND FLOWERS OF *HYPTIS PECTINATA* (L.) Poit. FROM CAMEROON

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Hyptis pectinata (L.) Poit. is one of more than 400 species of the pantropical genus *Hyptis* (Lamiaceae), native in tropical America, but now widespread in tropical Africa (1). It is a tall, erect, aromatic, woody herb with a dense, much branched inflorescence of very small flowers, corolla pale yellowish-green with mauve mark on the lips.
The plant is locally used in folk medicine, e.g. against roundworm, fever and cough (2).
The chemical composition of *H. pectinata* materials from Asian areas (Fiji and India) has been studied by Brophy et al (3) and Pant et al. (4). The oil from Western Africa (Yvory Coast) has been examined by Lalan et al. (5). To the best of our knowledge no data on the essential oil of this Cameroonian species have been given until now. Therefore the aim of this study was to analyse the volatiles of the oils responsible for the significant odor impressions and to give information for a possible use in medicinal and cosmetic applications.
The essential oils of fresh leaves and fresh flowers of *Hyptis pectinata* were subject of this investigation.
The essential oils of leaves and flowers of *Hyptis pectinata* (L.) Poit. (Lamiaceae) from Cameroon were analyzed by GC/FID, GC/MS and olfactometry. The oils are characterized by a high percentage of sesquiterpene hydrocarbons (17-47%) and oxygenated sesquiterpenes (32-42%), represented mainly by β-caryophyllene (2-21%) and caryophyllene oxide (18-20%). Additionally, many monoterpenes could be identified, but only in a total concentration of about 5%.

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HIGH-SPEED GAS CHROMATOGRAPHY OF ESSENTIAL OILS

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Shorter analysis times, the most important aim of high-speed gas chromatography, are significant for the quality control and the routine analytic in a production company. This aim may be supported by high programming rates of column temperature and/or a high carrier gas velocity. However, if separation conditions in fast GC become strongly different from conditions for optimum of column efficiency, the resulting peak resolution will be much smaller than usually. Therefore, fast GC is successful only if columns of outstanding separation power are used. For this reason, narrow-bore open tubular columns with internal diameters of 100 or 50 µm or multicapillary columns having about 919 microchannels of 40 µm internal diameter [1] are preferred in fast GC. Such columns have been commercially available for quite a time.

In the paper possibilities and limits of the fast GC in analysis of essential oils extracted from different calamus roots (german, russian, indian) and chamomile blossoms by several examples will be demonstrated. The retention time and the peak resolution of the main components (for example β-asarone in the calamus oil and α-bisabolol in the chamomile oil) on the column DB-1 (60m long, ID = 0,25 mm) which used up to now and on the "non-classical" columns like narrow-bore or multicapillary columns will be compared.

Some problems at the handling of such columns also will be discussed. That take the difficulties during the sample introduction caused by high inlet pressures, the external contributions to peak broadening and not least the use of special data acquisition systems.

Nevertheless the given apparative circumstances offer possibilties for fast GC, which should be used in practical work.

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COMPARATIVE GC-MS ANALYSIS OF THE ESSENTIAL OIL OF WILDLY GROWING AND CULTIVATED *ARTEMISIA JUDAICA* L. IN EGYPT

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The genus *Artemisia* L.(Asteraceae) comprises about 280 species of which *A.judaica* grows wildly in Sinai peninsula as well as in other localities in the Arab region. It is an aromatic plant commonly used by natives in gastrointestinal disorders, as cardiotonic, antispasmodic, anthelmintic, expectorant and analgesic. In addition to investigations dealing with the analysis of essential oil[1-3], studies on sesquiterpene lactones[4,5] and flavonoids[6] were reported.

In this study, comparative CGC-MS analysis of the hydrodistilled essential oils of wildly growing as well as cultivated *A.judaica* L. was carried out. Qualitative and quantitative variations in the components of the two oils were observed. Seventy-one components representing 96.5% of the oil from the wildly growing plant were identified , compared to 45 components representing 89.5% of the oil from the cultivated. Out of the compounds identified in the oil from the wild plant, 33 compounds comprising alcohols, esters, ketones and sesquiterpene hydrocarbons were not traced in the oil from the cultivated. On the other hand, six of the compounds identified in the oil from the cultivated plant were not traced in that from the wild.

In both oils, oxygenated compounds predominate, being 90.5% and 81.1% in wild and cultivated plants, respectively; while unoxygenated compounds constitute 5.9% and 8.4%, respectively. The oil from the cultivated plant contained appreciable amounts of camphor(21.2%), piperitone(26.8%) and terpinene-4-ol(5.1%); being similar to the oil from the wild plant(20.4%, 26.9% and 5.1%, respectively). Certain compounds,e.g. umbellulone(20.3%), camphene(1.8%), cedrene(2.2%) were found distinctly higher in cultivated plant than in the wild(3.3%, 0.2% and 0.3%, respectively). In the oil of the wild plant, allo-aromadendrene, cuminyl acetate, cinnamic acid methyl and ethyl esters and nerodiol were significantly higher than in oil of the cultivated plant.

The study is valuable in justifying the authenticity as well as source of essential oil of *Artemisia judaica* L.

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THE SLOVAK LARGE-SCALE DISTILLATION APPARATUS

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The CALENDULA, a.s. in Nova Lubovna has been establishing to medicinal and aromatic plant proceeding (the essential oil and extract production) since the 1st January 1999. Essential oils are distilled in the large-scale distillation apparatus specifically designed for aromatic and medicinal plants. There are two types: Type HV-3000 and Type HV-300. Both types were developed between 1986 - 1993 and proved to be very successful. Five equipments of these types have been instalated recently for the field production of medicinal and aromatic plants.

This large-scale technology of essential oil distilation in this Slovak company consists from a main distillatory apparatus, a steam condenser, and additional apparates (steam boiler and apparatus for improving of a used water).

The shape of the main distillatory apparatus is a funnel. It is thermal-isolated and made from a stainless-steels. An inside screw-plate is driven by an electric engine, which is installed on the apparatus. This screw-plate works as an excelent stirrer. In regard to this system the container has a mixing apparatus, which is not a usual feature in many other types of commercial equipments. This is extremely useful for the complete distillation procedure and high yield of essential oil.

Source of steam flow is a boiler (heated by oil, gas or electricity), and the flow is controlled mechanically, according the plant mass and colling requirement. The length of distillation depends on medicinal plant species, which are used to isolation of essential oils. Essential oil collector has volume of 75 l.

GC-IRMS ANALYSIS OF *MELISSA OFFICINALIS* ESSENTIAL OILJS : FURTHER RESULTS

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The essential oil *Melissa officinalis* is a highly valuable natural product which mainly finds applications in aromatherapy. Due to its high price, adulteration of this essential oil is frequent. However, recent analytical techniques have been applied with success to the detection of adulteration with terpenic compounds taken from other cheaper sources. These techniques are chirospecific GC and GC-IRMS [1] [2].
The aim of the present study was to detect a possible variability of the carbon stable isotope ratio ($\delta^{13}C$) of some constituents of *M. officinalis* essential oils during a varietal selection study. Thus, thirty-three essential oils were prepared from plant material at different stages of a varietal creation program : these included populations from various origins, individual cloned plants and elaborated varieties. Four essential oil constituents were arbitrarily selected for the GC-IRMS (triplicate) measurements : citronellal, neral, geranial and caryophyllene.
The results thus obtained allow an evaluation of the variability that exists between populations, and within a given population as well. Moreover, the variation magnitude between time of harvest for a given variety is comparable to that observed between varieties.
The results also demonstrate that the ranges of $\delta^{13}C$ values for each of the four selected components are greater than those previously observed for five genuine essential oils [2].

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GC/MS analysis of wine bouquet compounds using SPME - Contributions to a classification of wines

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The main goal of our work is the development of a fast, easy, sensitive, cheap and environmental-friendly method, appropriated for routine quality control of wines.

An account of more than one thousand different organic compounds contained in wine, is registered in the bibliography [1], a large part of which has been analysed through gas chromatography.

According to the work of A. Rapp [1] the terpenes are wine components which enable us to characterize different grape varieties. They are unchanged by yeast metabolism during fermentation and contribute to the wine flavours.

In the last 20 years, wine analyses have been mainly carried out by means of liquid-liquid extraction [1], and more recently by solid phase microextraction (SPME) [2].

For our investigation we used a 85 µm polyacrylate SPME fibre in headspace mode, which was found to be the best available fibre for extraction of terpenes in wine. The method has been optimized as well as for the extraction conditions as for the detection. Identification of single wine bouquet compounds has been carried out by means of SCAN mode quadrupole GC/MS. SIM mode quadrupole GC/MS was used to get lower detection limit to detect trace compounds in all wine samples. Aromagrams obtained in single ion monitoring (SIM) and in full scan detection mode (SCAN) of quadrupole mass spectrometer were compared. Detection limits, reproducibility and linearity for some aroma relevant substances were estimated over a wide concentration range.

About one hundred different wines originating from different grape varieties, vintages and growing areas were investigated. Analysis series in SCAN detection mode was repeated in SIM detection mode keeping all other conditions constant. We were able to detect some variety depending trace compounds in all wine samples only by using SIM detection mode.

We could recognize the different kinds of wines by means of chemometric methods using both analysis results. However results obtained from SIM detection mode enter in a more stable and precise data set for discriminant analysis (because of it's better analytical information) compared to those created from SCAN detection mode. So the quality of classification has been increased [3].

The classification of these wine standards should be a first step for assignment of unknown wines to a variety of grapes.

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Microwave and Essential Oils

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Since 1988 the potential of microwave technology for expediting routine laboratory chemistry and large-scale chemical processing is under development.
It is believed that if one is able to resolve technical problems, particularly those associated with the development of microwave equipments for heating organic reactions, greater efficiency and new technologies could result. Therefore our aim together with MLS Ltd. (Leutkirch) was to develop and to handle with microwave reactors which could be capable of reliable and safe operation with volatile organic solvents at elevated temperatures and pressures. At this time we can work with labsystems for batchwise and continuous operations.

In this paper we describe selected microwave experiments to

- isolation (steam destillation, extraction, reactive extraction)
- reactions (acetalization, oxidation, Maillard reaction) and
- purification (evaporation, destillation)

of essential oils and model compounds.
In all cases it is to remark that the reaction time is smaller and the yields (selectivities) are higher in comparison with conventional reaction regime.
New strategies of development of essential oils, as the combinatorial chemistry, for example, have enhanced chances to be successfully in combination with microwave-assisted procedures in syntheses and material separation. First steps are in work.

SUPERCRITICAL EXTRACTION: THE ESSENTIAL OIL EXTRACTION FROM CORIANDER BY MEANS OF CARBON DIOXIDE, PROPANE-CARBONE DIOXIDE AND PROPANE SOLVENTS.

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Oil extraction from coriander seed was studied with carbon dioxide, carbon dioxide-propane and propane as solvents, under sub- and supercritical conditions.

The rates of solvent to seed required to achive a complete oil extraction were found to be 40 and 20 by using CO_2 at a temperature of 35°C and at a pressures of 200 and 300 bar. A complete oil recovery could be attained with propane or solvents rich in propane at 25°C and at pressures of 50, 80 and 100 bar. The rates of solvent to seed needed were 1.3 and 1.5 using propane at 80 and 50 bar, while using solvents rich in propane the rate of solvent to seed required was 2.5 at 100 bar.

The solvent power of propane and solvents rich in propane have proved to be much higher than that of CO_2 [1].

The fractions trapped in the initial period of extraction with CO_2 have found to be rich in essential oils [2].

It was stated that the composition of essential oils extracted depends in a high extent on the solvent and parameters of extraction applied, while the dependence was negligible with respect to the fatty acid composition of triglycerides extracted.

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IDENTIFICATION OF INDIVIDUAL COMPONENTS OF ESSENTIAL OILS USING ^{13}C NMR SPECTROSCOPY. COMPARISON OF 4.7 AND 7.0 TESLA SPECTROMETERS

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Essential oil analysis is carried out by several analytical techniques, among which GC/MS/IK, GC/MS/IRFT and Carbon-13 NMR spectroscopy. Following the pioneering work of Formácek and Kubezcka (1), we developed and improved a computerised methodology only based on carbon-13 NMR (2). This technique allows the unequivocal identification of the main components of essential oils, without previous separation, from the carbon-13 NMR spectrum of the mixture. In this procedure, an individual component is identified by comparison of the values of the carbon chemical shifts of the mixture spectrum with those of reference spectra compiled in a computerized data bank. Each compound is identified taking into account three parameters directly available from the computer program : i) the number of observed carbons, ii) the number of overlapped signals, iii) the difference of the chemical shift of each signal in the mixture spectrum and in the reference. The aim of this study was to compare the results obtained for the analysis of essential oils using two spectrometers operating respectively at 75 and 50 MHz for Carbon-13. We will report in details the analysis of a sample of rosemary essential oil (number of identified compounds, number of overlapped signals, detection level, chemical shift variations).

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Ratio of Linalool Enantiomers in Coriander Investigated by SPME Peak Transfer

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The distribution of enantiomers in essential oils is usually analysed by an on-line-column-switching, a preseparation on an achiral column is followed by the separation of the enantiomers on a chiral column. Since this technique is very expensive, we have developed an easy-to-perform off-line method for the enantiomer analysis.

A 100 µm Polydimethylsiloxane coated SPME fiber was used for the transfer from the achiral to the chiral column A GC-liner was mounted in the sniffing port of an olfactometry-gas chromatograph. Exactly at that moment when linalool of coriander oil passes the liner, the fiber is introduced into the gas flow adsorbing both enantiomers. In a following step the volatile material can be desorbed from the fiber by injection on the second column, packed with an enantioselective cyclodextrin.

In this way the variability of D(+)- and L(-)-linalool of 45 accessions of a coriander gene bank collection was investigated. We could show, that the composition of the essential oils varies a lot but the ratio of D(+)- and L(-)-linalool in all cases is absolutely constant (87:13).

For the authenticity control of coriander oil this ratio is a very characteristical feature

The background of our work was also to answer the question, whether the enantiomer relation can be influenced by breeding. At least for linalool in coriander this is probably a lost cause, because the material's variability is an important condition for the success of a breeding process which is orientated to active substances.

Keywords SPME, two-dimensional GC, natural enantiomeric distribution, authenticity of essential oils

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TERT-BUTYL HYDROPEROXIDE INDUCED INJURY IN ISOLATED RAT HEPATOCYTES: A MODEL FOR STUYDING ANTI-OXIDANT ACTIVITY OF CRUDE DRUG

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Biomembranes, such as hepatocytes, microsomes and erythrocytes, are rich in polyunsaturated fatty acids which are very sensitive to peroxidative damage induced by oxygen free radicals.
Lipid peroxidation leads to a disorganization of membranses which alters the activities of membrane bounded enzymes and other proteins. In this study some medicinal plants (essential oils and flavonoids extract) and some pure essential oil were used.
Rat hepatocytes were isolated and tert-butyl hydroperoxide (tBH) was used because tBH induces in freshly isolated rat hepatocytes malondialdehyde (MDA) formation and lactatdehydrogenase (LDH) and aspartate amino transferase (AST) leakage.
For investigation the membrane protective effects of these medicinal plant in the presense of tBH varied compound were used in three concentrations (for essential oils 2.5, 5, 10 μl/ml except for thymol and azulene that they were used as 5, 10, 50 μg/ml and for flavonoid 5, 10, 50 μg/ml) and the MDA formation, AST and LDH leakage were measured by spectrophotometric method. Among the investigated compound the highest level of inhibition for MDA formation as antioxidant effects were recpectively:

Matricariachamomilla(essential oil)(58%)>Azulene(35.4%)>Achillea millefolium (Flavonoid extract)(32.8%) > Cichorium intybus(dichlorometan extract)(22.5%)>Thymol(19.4%)>Matricaria chamomilla(flavonoid extract)(16%)>Cichorium intybus(Flavonoid extract)(15%)>Achillea millefolium(essential oil)(10%).

Some of natural product showed pro-oxidant activity that the highest levels for pro-oxidant activity were respectively:

Calendula officinalis (essential oil)(53.9%)>Citronella oils (20.4%)>Geraniol (14.1%)>
Calendula officinalis (flavonoid extract)(12.2%)

For the all compounds the antioxidant and pro-oxidant activity were dose dependent. The result were statistically significant for all compound except flavonoid extract of Cichorium intybus and essential oil of Achillea millefolium ($p<0.05$). There is a direct relationship between MDA level with enzymatic activities of LDH and AST. Surely, it is required to wide spreadly study in vivo on these compounds to determine side-effects and appropriate doses for the best efficacy and clinical usages.

COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL FROM THE LIVERWORT *MARCHESINIA MACKAII* (HOOK.) S. F. GRAY GROWN IN PORTUGAL

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Marchesinia mackaii (Hook.) S. F. Gray (Lejeuneaceae) is the only European species of the genus. This Atlantic-Mediterranean species, which shows thermophilous trends, forms olive-green to blackish-green patches that can be found on dry sheltered places in woods. *M. mackaii* is a quite rare taxon, listed as vulnerable [1], that can be found in only one region of the mainland of Portugal, but it is more widely distributed in the Azorean and Madeiran archipelagos [2,3].
The essential oil from *M. mackaii* was isolated by hydrodistillation to estimate the oil yield, and by distillation-extraction to determine the percentage composition of the oil using GC and GC-MS. The identity of one of the main components of the oil, namely 2,4,5-trimethoxyallylbenzene, was confirmed by ^{1}H-NMR and ^{13}C-NMR.
The essential oil was obtained in a yield of 0.5%; sixteen of its components could be identified, amounting to 91% of the total oil. The phenylpropanoid fraction was the most representative one (79%), being dominated by 2,4,5-trimethoxyallylbenzene (23%), α-asarone (23%), *trans*-methylisoeugenol (15%), and β-asarone (12%). The sesquiterpenes amounted to 12%, β-caryophyllene attaining 10% of the total oil. The fraction of monoterpenes, and a fourth fraction of other components were only found in trace amounts, limonene and nonanal, respectively, being the only components detected in these fractions.
The essential oil of *M. mackaii* was evaluated for antibacterial activity by the agar diffusion method, and showed only a modest activity against one (*Bacillus subtilis*) out of the five bacteria used as test organism: *B. subtilis*, *Escherichia coli*, *Salmonella pullorum*, *Staphylococcus aureus* and *Yersinia enterocolitica*.

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Volatile Components gained with Headspaceanalysis of *Melissa officinalis* L. (30th ISEO)

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Using headspaceanalysis we were able to detect several saturated and single unsaturated C_6-aldehydes, -alcohols and -acetate esters [1]. Normally only tiny amounts of these substances can be extracted by watersteam distillation and only traces of these compounds are emited by living plants. In difference to mono- and sesquiterpenes, the accumulation of C_6-components is rising with time of analysis and the amount of these substances is also rising by a second headspaceanalysis. Due to the accumulation pattern, C_6-aldehydes, -alcohols and -acetate esters are presumably generated during headspace-analyses by an enzyme system from linolenic and linoleic acid and therefore are not genuine constituents of essential oil. Some glycosidically bound alcohols like citronellol, nerol, geraniol, and 1-octen-3-ol are released in great amounts under conditions of headspaceanalysis, and therefore can be detected in much higher percentage values than in analyses with watersteam distillation.

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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF THAI *BLUMEA LACERA*

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Blumea lacera DC. (Asteraceae) is a strong-smelling herb, with thin variable leaves and yellow flowers. It commonly distributes in India, China and Southeast Asian countries. It is known to cure bronchitis, blood diseases and fevers and to alleviate burning sensations[1]. Until now, very little is known about its major and minor essential oil components responsible for the strong smell of the plant although it has been reported that the leaves contain cineole, fenchone, citral [2] and camphor [1,3] in the essential oil. In this study, the essential oil isolated by hydrodistillation from the leaves of *B. lacera* was analyzed by GC/MS. Twenty-seven components of the oil were identified, including 6 monoterpenes, 5 oxygenated monoterpenes, 11 sesquiterpenes and 5 oxygenated sesquiterpenes. Among these, the main components appeared to be 4-terpineol (11.9 %), α-terpinen-7-al (10.5 %), β-phellandrene (8.9 %) and chrysanthenone (8.3 %). Scanning electron microscopic obsenation revealed that there were a number of glandular trichomes on both sides of the leaves for the sites of oil accumulation. The leaf oil showed antimicrobial activities against *Staphylococcus aureus* and *Bacillus subtilis*.

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D-CAMPHOR - A SOLID ESSENTIAL OIL

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D-Camphor (DAB 1998) is a dextrorotatory ketone, formed from different members of the Lauraceae family i. e. *Cinnamomum camphora* L. SIEB. and respective varieties such as ssp. *japonicum* or *formosanum* indicating that the genuine Camphora is widely grown in the South of China, Taiwan, Japan, but also produced in India and Georgia (USA). The best yield of natural D-Camphor is obtained from old trees, however, other parts of the relevant plants produce the terpen. The wood is cut into chops and treated with steam in order to obtain a crude volatile camphor oil, which is fractionated and resublimated into an almost pure D-Camphor quality corresponding to the relevant Pharmacopoeia monographs. The different steps of D-Camphor production are strictly controlled and the enrichment of D-Camphor can be clearly demonstrated by an adequate GLC-analysis.

COMPOSITION AND ANTIMICROBIAL ACTIVITY OF VOLATILE OILS FROM *AMOORA ROHITUKA* Wigth & Arn LEAF AND FRUIT

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Amoora rohituka Wigth & Arn (Meliaceae) is indigenous to India and cultivated in Egypt for shade and ornamental purposes. The bark of the plant is used in the treatment of spleen and liver disorders as well as in tumors and abdominal complaints. Amooranin isolated from the ethyl acetate extract of stem bark exhibited antitumor activity. Significant insecticidal activity of the plant was reported. Studies on the isolation and identification of alkaloids[1], limonoids[2], saponins[3], flavonoids[4], anthraquinone glycosides[5] and seed oil[6] were reported. However, nothing could be traced concerning volatile constituents from the plant.

The essential oil obtained by hydrodistillation of the fresh leaf(~2%) and fruit(~0.5%) of the plant was analyzed by CGC-MS. Qualitative and quantitative variations in the components of the two oils were observed. Fourty-one components representing 88.12% of the oil were identified in the fruit, while twenty components representing 90.4% of the oil were identified in the leaf. The oils were found poor in monoterpenoids (0.19% and 0.38% in the leaf and fruit, respectively); while being rich in sesquiterpenoids (90.22% in the leaf and 86.84% in the fruit). Sesquiterpene hydrocarbons represent 65.54% of the leaf essential oil, the major components being:cayophyllene(36.16%),α-humulene(8.09%), β-elemene(8.04%), γ-gurjunene(4.03%), α -selinene(3.59%),α -gurjunene(2.53%) and γ - elemene(1.7%). In the fruit oil, sesquiterpene hydrocarbons represent 47.14% of the oil, γ -cadinene(11.74%), caryophyllene(7.5%), β -elemene(4.08%), α -humulene(3.45%), viridiflorene(3.29%), δ -elemene(2.92%), α -ylangene(2.33%) and δ -cadinene(2.26%) being the major compounds. On the other hand, oxygenated sesquiterpenoids represent 24.7% and 39.7% in the essential oils of the leaves and fruits, respectively. In the leaf oil, β -eudesmol(11.41%), elemol(6.3%), caryophyllene oxide(3.46%) and nerolidol(1.86%) represent the major oxygenated sesquiterpenoids; while muurolol(7.2%), β -eudesmol(6%) , *cis*-lanceol(3.6%), α -bisabolol(3.55%), spathulenol(3.5%), allo-aromadendrene-7-ol(2.92%), γ -eudesmol(2.5%), caryophyllene oxide(2.5%), α -cadinol(2.16%), ledol(1.59%) and globulol(1.53%) are the major ones in the fruit oil.

Antimicrobial activity of the oils was tested using standard strains of organisms known to be among the common pathogens in many topical and internal diseases in comparison with Rifamycin SV(antibacterial) and amphotericin B (antifungal). The two oils exhibited significant antibacterial activity but no antifungal activity.

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BILATERAL PROJECT 'UTILIZATION OF TRADITIONAL MEDICINAL PLANT PREPARATIONS IN INDONESIA': ETHNOMEDICINAL, ANALYTICAL AND PHARMACOLOGICAL INVESTIGATIONS ON ESSENTIAL OIL DRUGS *BLUMEA BALSAMIFERA* AND *KAEMPFERIA GALANGA*

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From the bilateral project [1] on Indonesian medicinal plants between German GTZ (Department of Education and Culture) and Indonesian Ministries of Health, Education and Culture, Agriculture and Forestry, we here present two essential oil containing drugs, namely *Blumea balsamifera* DC leaves (Asteraceae) and *Kaempferia galanga* L. rhizomes (Zingiberaceae), both frequently used in traditional Indonesian "Jamu"-medicine. On the basis of studies carried out in the test region of the island Flores [1,2], ethnobotanical / -medicinal aspects, proposals for cultivating, proposals for pharmacopeial monographs according to German Commission E [1] on the basis of analytical data (GC-MS and enantioselective GC analyses of essential oils, e.g. borneol, campher in *B.b.*) [2], and results of antibacterial / antiviral screening [2] are reported.

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INVESTIGATIONS INTO THE BIOSYNTHESIS OF THE ANTIBIOTIC MOENOMYCIN A

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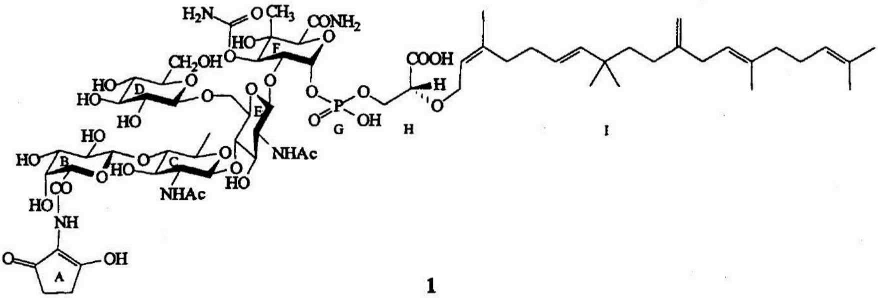

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The moenomycin antibiotics (exemplified by moenomycin A (**1**)) are unique inhibitors of the transglycosylation reaction a main step in the biosynthesis of the bacterial cell wall peptidoglycan. Moenomycin is composed of an oligosaccharide part, linked via phosphoglycerate to a C_{25} lipid moiety. The other terminus is 2-aminocyclopentane-1,3-dione (the chromophore of moenomycin). Some of the moenomycin building blocks are common, others like the lipid moiety (I) and the moenuronamide unit (F) are characteristic for the moenomycin-type antibiotics. 2-Aminocyclopentane-1,3-dione is a constituent of other microbial metabolites, too.[3] Until recently, practically nothing was known on the origin of the unusual structural elements and how the complex array of building blocks is assembled in the course of the biosynthesis.

The poster will summarise feeding experiments using [1-^{13}C]-acetate, [1-^{13}C]-glucose, (S)-[$^{13}CH_3$]-methionine and ($^{15}NH_4$)SO_4. The experiments clarify the origin of the unit A, the branching methylgroup in the uronamid unit F and the lipid moiety I.[4]

The aim of the work is to prepare labeled moenomycin derivatives for binding studies with the enzyme that catalyzes the transglycosylation reaction.

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TOPICAL TREATMENT OF INFECTED AND DEHISCENT POSTOPERATIVE WOUNDS USING NATURAL ESSENTIAL OIL PRODUCTS

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Recent experimental and clinical studies demonstrated that natural essential oil ointment (containing essential oil blends, plant fatty acids and Vit. E) have antibacterial and antioxidant activities, aid tissue repair and promote tissue regeneration, thus enhancing the wound healing process. Further experimental studies performed by us on induced infected wounds of a rat model revealed prompt and complete wound healing, either macroscopically as well as histologically, using topical essential oil ointment when compared to conventional wound care procedures. In the last 18 months, 89 patients (age 45-89) following various operative procedures and massive infected of dehiscent postoperative wounds were treated in our outpatient clinic. All patients had previously undergone conventional treatments without any improvement or wound healing failure. The wounds were treated topically twice daily with essential oil blend ointment, after washing the treated area with soap and water. massive neurotic of infected tissues were vaporized (laser debridement) by using the Kaplan Pendulaser 115 in order to achieve a clean wound. In all patients, the wounds healed completely forming a mature scar (up to 3 weeks, according to the severity of the wound), with no side effects and excellent cosmetic results. We, therefore, advocet the use of natural essential oil products for the treatment of dehiscent of infected postoperative wounds. The experience of the animal study, the method of treatment and some of the more complicated cases will be demonstrated.

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MELISSA OFFITINALIS L. IN CULTURE IN VITRO: AN ESTIMATION OF BIOSYNTHETIC ACTIVITY OF THE OBTAINED LINES

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The use of Melissa officinalis L. and its subspecies in traditional and official medicine is explained by the fact that in shoots and leaves of these plants significant amounts of ether oils, ascorbic acid, carotene, resins, tanning and bitter substances, organic acids (caffeic, oleic, ursolic, succinic, chlorogenic, oleanolic), resins and saponins are synthesised [1].

The purpose of the investigation has been obtaining stable callus and suspension cultures of Melissa officinalis L. and estimating their biosynthetic activity.

It has been estimated that callusogenesis best occurs on stem explants of M. officinalis in B5 medium [2] supplemented with 0.1 mg/l Kin and 1 mg/l 2.4-D. B5 medium with half content of macro- and microsalts (B5/2, 10 mg/l of sucrose, 300 mg/l of casein hydrolysate, pH = 5.5), supplemented with 0.2 mg/l Kin and 1.2 mg/l 2.4-D is the most effective for proliferation of the obtained M. officinalis callus. The suspension culture of M. officinalis on liquid medium B5/2 with 0.2 mg/l Kin and 1.2 mg/l 2.4-D has been obtained. It has been found that callus and suspension cultures extracts of M. officinalis are characterized by antimicrobial action on bacteria cultures of Staphylococcus albus and Bacillus mycoides. Chromatographic analysis has proved that components of ether oils (citral, linalool, α-pinene and some substances not identified by us), characteristic for intact plants, are synthesised in the callus and suspension cultures obtained by us.

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Poster Session II

Tuesday, September 7, 1999, 16.30 – 18.00
Lecture hall building, first floor

New advanced hybrids of mentha plants for medicine

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The main aim of our investigation is receiving new hybrids with the characteristic features such as rich yield of pharmaceutics leaves, the essential oil with large amount of menthol. In our Research Laboratory of chemistry and biochemistry of essential oils this kind of investigations have been carried out during the last 35 years.

Interspecific hybridisation is the method of choice. The essential oil was investigated by a combination of common techniques such as absorption column chromatography, TLC, GLC, UV-spectroscopy etc.

As a result of difficult selection we found a number of new hybrids with the principal characteristic features:

ID	oil content, %	menthol total, %	yield of		
			herbage, kg/hec	oil, kg/hec	menthol, kg/hec
11P295	2,5	72,5	200,0	50,0	36,3
11P310	3,3	73,3	160,0	52,8	38,7
11-151	2,8	69,0	185,0	51,8	35,7
M-119	3,2	70,0	167,0	53,4	37,4
M-136	3,5	76,1	150,0	52,5	39,9

We developed a more effective method of giving off crystal menthol from high-menthol oils according to the Certificate. After giving off menthol the rest of the oil has a pleasant scent and may be used in different fields thanks to its composition as follows: l-menthol 40-50%,menthon up to 15%, limonen 5-10%, menthofuran 4-7% and minor components less than 1% .

ABSTRACT FORM

ESSENTIAL OIL OF *Satureja khuzistanica* Jamzad

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Satureja khuzistanica is an endemic plant which has been introduced as a new species from Iran [1]. This species is close to *S. edmondi* Briquet, but differ from it in stem, infloresence and leaves.

The essential oil isolated by steam distillation from the aerial parts of S*atureja khuzistanica* Jamzad was analyzed by capillary GC and GC/MS. Among 18 compounds identified, the major components were p-cymene (39.6%), carvacrol (29.6%), and γ-terpinene (18.9%).

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CONSTITUENTS OF THE ESSENTIAL OILS FROM THE HULLS OF *PISTACIA ATLANTICA SUBSP. MUTICA DURING* GROWTH TIME

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Pistacia atlantica subsp. Mutica (Beneh) is a dominant native pistacia species throughout the Iran plateau. About two million hectars of Iran, mostly on west and northwest, are suitable for beneh cultivation and rehabitation. Fruit sampling was done in every two weeks for three times at fruit growth to maturity. The essential oils obtained from fresh fruits were water distilled for 4 h. They yielded a relatively high percentage of oil (sample-1 and 2 , 0.14% and sample-3, 0.20% , V\W), calculated on the fresh weight. The essential oils, were analyzed by capillary GC and GC\MS. The gall volatiles contained 14 components of which 90.69% were found to be monoterpenes. The major constituents were α-pinene (73.60%), β-pinene (5.30%), myrcene (3.26%) and limonene (2.52%). This is a first investigation in Iran on essential oils components of *Pistacia atlantica subsp. Mutica*.

Volatile Constituents of *Nepeta glomerulosa* Boiss. subsp. *carmanica*

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Nepeta glomerulosa Boiss. subsp. *carmanica* (Lamiaceae) is a herbaceous wild plant native to Iran[1]which is used in Iranian folk medicine to treat pneumonia[2]. However, no phytochemical studies about this plants have yet been done. In the course of systematic investigation on the essential oils of *Nepeta* species we have studied the composition of the essential oil of *N. glomerulosa* Boiss. subsp. *carmanica*. The constituents of the essential oil obtained by hydrodistillation of *Nepeta glomerulosa* Boiss. subsp. *carmanica* were investigated by GC and GC-MS analysis. The oil contains over 35 components. The major components were α-pinene (18.3%),1,8 cineole (13.9%), limonene (9.7%), linalool (4.8%), *trans-β*-ocimene (4.7%), humulene epoxide (4.2%), *trans-α*-bergamotene (3.5%), *α*-humulene (3.2%) and camphene (3.1%). Identification of the compounds was based on retention indices, MS data and comparison with authentic samples.

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COMPOSITION OF THE ESSENTIAL OIL OF *TEUCRIUM SALVIASTRUM*, AN ENDEMIC SPECIES FROM PORTUGAL

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Continuing the characterisation of the chemical composition of essential oils from spontaneous plants from Portugal we undertook the study of species of *Teucrium* genus (Lamiaceae).

We now report on the composition of the essential oils of *Teucrium salviastrum* Schreber, an endemic species from the highlands of the centre and north of Portugal (above 1000 m high). No reports were found about the essential oil of this taxon.

The essential oils from leaves and flowers were isolated by hydrodistillation in a Clevenger type apparatus and analyzed by GC and GC-MS, using two fused silica capillary columns with different stationary phases (polyethylenoglycol and polymethylsiloxane). Components were identified by comparison GC retention indexes, relative to a series of n-alkanes, and mass spectra with corresponding data of authentic compounds or components from reference oils.

More than 97 % (forty-one compounds) of the volatile oils was identified in each sample. Sesquiterpene hydrocarbons were the major compounds in both, leaves and flowers samples (75 and 81%), being the major ones *E*-*β*-farnesene (24 and 25%), *β*-caryophyllene (21 and 25%) and germacrene D (19 and 20 %). Among monoterpenes, *E*-*β*-ocimene was the major compound in both samples (7 and 5%).

VOLATILE AROMA CONSTITUENTS OF SUPERCRITICAL CARBON DIOXIDE EXTRACTS OF BLACKCURRANT BUDS *(RIBES NIGRUM L.)*

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Supercritical carbon dioxide extracts of dormant blackcurrant buds obtained at different pressures, 8.0, 20.0 and 29.5 MPa, and for comparison the hydrodistilled essential oil, were analysed for volatile constituents employing gas chromatography-mass spectrometry. As oxygenated compounds, like mono- and sesquiterpene alcohols and oxides and monoterpene acetates, are considered responsible for characteristic blackcurrant odour [1,2], their relative content was compared in investigated isolates. The highest content of the volatile oxygenated compounds was found in supercritical carbon dioxide extract obtained at pressure 29.5 MPa.

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ESSENTIAL OILS FROM LEAVES AND FROM UNRIPE BERRIES OF TEN POPULATIONS OF *LAURUS AZORICA*

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Laurus azorica (Seub.) Franco (= *L. canariensis* Webb & Berth., non Willd.), most commonly known as 'loireiro', 'loureiro', or 'louro', is an endemic species of the Azores, Madeira and the Canary Islands. The tree is an important constituent of the laurel-juniper forest (cloud-zone forest), that occurs on all islands of the Azorean archipelago, except on Graciosa, generally above 500 m, but occasionally down to 175 m and, on Pico, rarely above 1400 m [1]. The berries and the oil are used as an anti-infective skin preparation; the oil is applied externally as an anti-rheumatic and for wound-healing, and is taken orally for treatment of apoplexy; the oil is also used for treating boils, as a hemostatic and for troubles of the respiratory system [2].

As part of a screening programme of the aromatic flora of Macaronesia, we analysed, by GC and GC-MS, the composition of the essential oils isolated from leaves and from unripe berries of ten populations of *L. azorica* collected on five Azorean islands. The oil samples isolated from the leaves were obtained in yields ranging from 0.3% to 0.7% (v/w), while the yields of the oils isolated from the berries ranged from 0.4% to 0.9% (v/w). All the oil samples were dominated by their monoterpene fraction (61-85% for the leaves; 80-94% for the berries). α-Pinene (15-37%), 1,8-cineole (12-31%) and β-pinene (9-18%) were the main components of the oils from the leaves, whereas *trans*-β-ocimene (27-45%), α-pinene (12-22%) and *cis*-β-ocimene (9-16%) were the major components of the oils from the berries. The sesquiterpene fraction occurred in a concentration ranging from 5% to 17% in the leaf oils, and from 3% to 10% in the oils from the berries. Phenylpropane derivatives also occurred in the oils (2-17% for the leaves; traces-2% for the berries). *trans*-Cinnamyl acetate, the main component of this fraction, amounted up to 15% in the oils from the leaves.

Cluster analysis of the essential oils revealed two distinct groups, one from the leaves and the other from the berries. Regarding the enantiomeric composition of α- and β-pinene in the leaf oils, there is a separation between the two populations from the island S. Jorge and the populations from the other four islands.

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COMPOSITION OF THE ESSENTIAL OILS FROM THIRTEEN POPULATIONS OF *THYMUS CAESPITITIUS* BROT. GROWN ON THE ISLAND S. JORGE (AZORES)

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Thymus caespititius Brot. is a species that is characteristic of Atlantic wet areas; it is an endemic plant of the NW Iberian Peninsula, and of the Madeiran and Azorean archipelagos. 'Erva-úrsula', as it is commonly known in the Azores, is the only representative of the genus in this archipelago.

Samples of the aerial parts of the plant, collected during its flowering period on S. Jorge (Azores), were submitted to hydrodistillation to estimate the oil yields, and to distillation-extraction to determine the percentage composition of the oils. The sites of collection of the plant material, the corresponding altitudes, the oil yields, the total percentage of the oil components identified, and the main components (>10% in at least one sample) of each oil sample are given in Table 1.

Table 1- Main components identified in the essential oil of *Thymus caespititius* grown on S. Jorge.

Collection site		Pico Verde				Serre Topo			Cume Arrebentões		Pico Ponta Furada		Fajã de S. João	
Population		1	2	3	4	5	6	7	8	9	10	11	12	13
Altitude (m)		900				750		600	580		600		350	
Components (>10%)	Sabinene	74.3	26.8	8.7	66.1	1.0	2.2	6.3	3.5	2.5	0.3	0.1	1.5	1.1
	γ-Terpinene	2.0	3.7	3.7	1.9	15.8	1.8	2.0	4.1	3.0	4.0	2.1	1.8	0.9
	α-Terpineol	0.3	0.1	0.1	0.2	34.3	50.1	39.9	51.6	38.7	4.8	1.5	54.9	55.9
	Thymol	0.5	30.2	39.0	0.4	12.2	10.0	9.9	1.6	15.9	12.0	20.9	6.2	6.3
	Carvacrol	1	2.7	11.7	1.2	1.6	1.0	1.1	0.7	1.8	43.4	36.3	0.8	0.9
Total % identified		98.7	92.0	88.8	89.0	92.5	87.9	86.5	85.7	84.1	92.2	92.9	88.0	84.7
Oil yield (%)		0.6	0.6	0.5	0.5	0.4	1.1	0.7	0.5	0.5	0.4	0.7	0.6	0.7

A clear chemical variability was observed for the composition of the oils studied: seven populations (5-9, 12, 13) yielded oils with the same main component, α-terpineol, as populations from the mainland studied previously, two populations (10, 11) showed carvacrol as dominant oil component (as oils from the islands Pico and Faial), the oils from another two populations (1, 4) showed a high sabinene content, and the oils from the last two populations (2, 3) showed a high thymol content. The latter two oil types (with high sabinene or thymol contents) which have not been reported previously for this species, were found for populations growing 50m apart on the same slope of Pico Verde. Cluster analysis of all identified components grouped the oils into four clusters, which corresponded to four of the main components of the oils: sabinene, α-terpineol, thymol and carvacrol.

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INVESTIGATION OF AROMATIC PLANTS FROM CAMEROON : GC/MS ANALYSIS OF ESSENTIAL OILS OF PLECTRANTHUS GLANDULOSUS (LAMIACEA)

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Plectranthus glandulosus Hook f. (syn. *Coleus laxiflorus* (Benth.) Roberty) is one of the 14 species of the genus *Plectranthus* found in the flora of West Africa [1]. The plant is used for the treatment of colds and sore throat in folk medicine
The essential oils of other *Plectranthus* species *(P. tenuifolorus, P. incanus, P. defoliatus)* have been investigated [2-4], but not the essential oil of *P. glandulosus*. The objectives of this study were to analyse both oils from fresh and dried leaves by GC and GC/MS using conditions reported by *Jirovetz et al.* [5], and to correlate the chemical composition with the odour impression.

The olfactory evaluation of both investigated essential oils was given by professional perfumers as follows: Minty (menthol), fir and eucalyptus (pinene and terpinene-notes), thujone-notes (cedarwood leaves), thymol-notes, pleasant fresh, later herbal. The yields of essential oil obtained by hydrodistillation is: 0.3% from fresh leaves, 0.8% from dried leaves.

More than sixty compounds have been identified in *P. glandulosus* oil from leaves, the major components belong to the oxygenated monoterpenes (57.5-67.6%) represented by piperitone oxide (10-36%), fenchone (16-28%) and cis-piperitone oxide. The monoterpene hydrocarbones (27-36.9%) are represented mainly by terpinolene (20-26%) and myrcene (1-2%). The sesquiterpene derivatives are in a very low percentage (less than 3.5%), represented mainly by germacrene D (1.3-0,9%).

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SOURCES OF ORGANICALLY PRODUCED LAVENDER *(LAVANDULA ANGUSTIFOLIA)*, LAVANDIN *(LAVANDULA INTERMEDIA)* AND SPIKE LAVENDER *(LAVANDULA LATIFOLIA)* FOR ESSENTIAL OIL QUALITY

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This study was conducted to compare essential oil quantity and quality of twelve varieties of organically produced *Lavandula spp.* The study was conducted at the Frontier Organic Research Farm in Norway, Iowa on a Dinsdale silty clay loam soil. All lines were monitored for date of flowering, height at flowering, plant vigor, and plant uniformity. Dried flowers were evaluated for essential oil content and quality, color, aroma, and size. Lavandin *(Lavandula intermedia)* cv. 'Super' gave the highest yield of oil (10%) on a dry weight basis, while the lavender *(Lavandula angustifolia)* varieties 'Hidcote' and 'Munstead' produced the lowest (2.3%). The lavandin cv. 'Abrialli' produced the highest percentage of camphor (7.8%), while the lavandin cv. 'Alba' produced the lowest (0.6%). All of the lines that flowered in the first year produced acceptable quality ratings (3-5 on a 5 point scale) for color and aroma, with all lavender lines being rated 5, and the lavandin lines ranged in quality from 3-4. The lavandin cv. 'Alba' was one of the earliest flowering plants with 100% open bloom by June 30 and the lavender 'Stoechas' *(Lavandula stoechas)* being the latest to bloom at October 20. Lavandin cv. 'Super' and lavender 'Stoechas' were the tallest plants at bloom, 41 and 40 cm, respectively. In contrast, the lavenders 'Hidcote' and 'Lady' produced the shortest plants at bloom, 29cm. The lavandin cvs. 'Provence' and 'Alba' produced the most vigorous lines, 3.8 and 3.5 respectively, while 'Alba' produced the most uniform plants, rating 4. The 'English' lavender was both the least vigorous (1.3) and the least uniform (2). The quality of oil produced from each line will be discussed.

VOLATILE CONSTITUENTS OF *CITRUS AURANTIFOLIA* (CHRISTM.) SWINGLE FROM IRAN

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Citrus aurantifolia (Christm.) Swingle is a culinary and medicinal plant from Rutaceae family that cultivating wide-spread in the south of Iran. The dry fruits of *C. aurantifolia* are used as spice in cookery and as an appetizer and stomachic in Iranian folk medicine[1]. In this study, the chemical composition of the volatile oil of the fruits of *C. aurantifolia* collected in May 1997 near Minab in Hormozgan province was examined. The amount of the oil was determined after hydrodistillation and it's composition was investigated by TLC,GC and GC/MS [2-4].

The essential oil yield was 0.5% . The major components of the oil were limonene, α-terpineol and linalool. In contrast to the literature [4], no γ-terpinene and 1,8-cineole were detected in the volatile oil of fruits ot the Iranian *C. aurantifolia*.

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ESSENTIAL OILS OF THE LEAVES OF SILVER FIR (Abies alba Mill.) FROM BULGARIA

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The objective of this work is to investigate the quantitative and the qualitative composition of essential oils from silver fir forest plantations of first class of age (0-20 years old). The samples from foliage (the young branches with needles having diameter of the cut 0.8 cm) are collected during the winter period from the southern part of the crowns. The qualitative content of essential oil is determined with the help of micro-apparatus of Klevenger, with double repetition of each sample, during the beginning of the growing – in March. Physico-chemical indices of essential oil are determined by the analytical methods, according to the ISO 279,280,592,1242 and 709, while the chemical composition – by ISO – 7609 from the gas-chromatographic profile using „Perking-Elmer" apparatus, model 8500. The results of the investigations show that the essential oil from young silver fir plantations has a fine fresh-balsamic fragrance with characteristic notes close to the essential oil extracts from nature stands. It is an easy mobile coulorless or lightly yellow-green liquid. The essential oil from silver fir has a higher content of α-pinene – about 25%, which is bigger than β-pinene (20%). The ratio between α : β is 1.2 - 1.5, which is different from the other pine essential oils (from Scots pine and Australian black pine) where the quantity of ?-pinene prevail. Other terpen carbons established in the content of essential oil from silver fir are camphene (9.94%), myrcene (1.5-2.5%) and ocimene. From oxygen containing compounds, borneol, α-Terpineol and mostly acetates of presenting alcohols are established, as well as some alifat aldehydes as aldehyd C12 and C10. There were also identified sesquiterpen carbons as caryophylene, humulene, etc. Essential oil from young silver fir plantations possess physico-chemical indices close to these from essential oil extracted from nature stands. It is valuable from a cosmetical point of view with its fine fresh-balsamic fragrance and is extremely suitable for using in a large scale of aromatic compositions for the perfumery and cosmetics, for air-refreshing and disinfecting.

About the Composition of Essential Oils of Some Wild Growing *Mentha* L. Species from Serbia

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The quantity and chemical composition of volatile oils from the aerial parts of four wild growing *Mentha* L. species: *Mentha aquatica* L., *Mentha arvensis* L., *Mentha longifolia* (L.) Huds. and *Mentha spicata* L. *ssp. tomentosa* (Briq.) Harl. collected from scattered sites in Serbia were investigated. Plant parts were collected in pre-blooming or full-flowering stage, then dried and water-distilled in accordance with official Proceedings I *(M. aquatica, M. arvensis* and *M. spicata ssp. tomentosa)* or III *(M. longifolia)* of the Yugoslav Pharmacopoeia IV [1]. Obtained essential oils were analyzed using GC and GC/MS. In the essential oil of *M. aquatica* 31 constituents were identified, with a mixture of piperitone and *trans*-piperitone-oxide as the principal one (43.9 %), along with *cis*-piperitone-oxide (33.2 %). Among the other constituents, only isopiperitenone was present in remarkable quantity (4.3 %). In *M. arvensis* oil 31 components were identified too, with isomenthone (63.4 %) and a mixture of *cis*-piperitone-oxide and germacrene-D (16.6 %) as major constituents. Piperitone was also present, but in significantly lower quantity (3.1%). Piperitone was the most abundant constituent (72.1 % of total oil) out of 30 identified in essential oil of *M. longifolia.* The essential oil of *M. longifolia* was significantly richer in content of 1,8-cineole than any other oil reached in this study (4.6 %). In the essential oil of *M. spicata ssp. tomentosa,* 26 components were identified. The principal ones were linalool (71.5 %), and carvone (7.8 %). All the other components were present in quantities of about 3 %: limonene (3.4 %), (E)-tagetone (3.3 %), 1,8-ocimene (2.9 %), myrcene (2.1 %), or less.

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The Quality of Essential Oil of *Abies alba* Mill., *Pinaceae*, From the Pilot Production in Montenegro

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The analyses were performed on the commercial essential oil of *Abies alba*, obtained from fresh summits of young branches with conifer needles collected in Montenegro during falling of trees either for the reason of forest caretaking or wood exploitation. The plant material was chopped and subjected to a steam distillation in a self-constructed, semi-industrial stainless steel device equipped with a separate steam generator, under the pressure of 50-80 kPa and the temperature below 90 ^{0}C. The condensate was captured in a florentine bottle and left aside long enough to achieve a complete separation of layers. After decantation, essential oil was treated with anhydrous sodium sulfate to remove water and packed into metal barrels of different sizes. For this research, we handled a 10 ml randomly sampled quantity of essential oil. Composition of essential oil was elucidated by GC and GC/MS. Some physical characteristics (specific gravity, refractive index and optical rotation) of the oil were also determined. In our essential oil 37 constituents were identified. The prominent ones were β-pinene (32.80 %), α-pinene (17.30 %) and camphene (16.70 %), as well as bornyl-acetate (9.00 %), limonene (6.10 %) and β-phellandrene (4.90 %). In Montenegrin essential oil, compared to those of French provenience [1], 17 out of 27 components were found identical. The main components of French oil were α-pinene, but twice more and limonene, six times more than in Montenegrin oil. The quantity of β-pinene, the principal component of our essential oil is almost ten times less in French, while the content of camphene is twice higher in our oil. Our oil is also eight times richer in bornyl-acetate, the only and valuable oxidized component present in a significant amount.

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Composition of Essential Oil from the Fruits of *Ligusticum seguieri* (Jacq.) Koch, *Apiaceae* from Yugoslavia

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The genus *Ligusticum* L. comprises 40-50 species spread over the Northern hemisphere, Chile and New Zealand. Only three grow wild in Serbia: *Ligusticum seguieri* (Jacq.) Koch, *L. mutellina* (L.) Crantz. and *L. albanicum* Jav. [1]. In traditional medicine of Serbs, the use of *L. seguieri* and other species of this genus, is uncertain. Like a number of plants belonging to the *Apiaceae* family, its fruits were seldom used to improve lactation, where it was necessary [2]. The mature fruits of *Ligusticum seguieri* (Jacq.) Koch, *Apiaceae,* was collected from hillsides of Durmitor mountain (Montenegro) in August 1996. The extraction of essential oil was performed by steam distillation in a Clevenger-type apparatus, according to the Proceeding I of the Yugoslav Pharmacopoeia IV [3]. Its quantity (4 %, in ml of essential oil per 100 g of plant material) was determined during the same experiment. The obtained essential oil was a yellow, slightly bitter liquid, with distinctive, mildly celery-like and intensive odor. Its composition and relations between constituents were revealed using GC and GC/MS. In analyzed essential oil, 19 constituents (91.89 % of total amount) were identified. The major constituent was a mixture of (Z)β- and (E)β-farnesene (37,80 %), while the other important were 2,4,6-trimethylbenzaldehyde (16,40 %), α-pinene (11,84 %) and β-caryophyllene (10,5 %).

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A Comparative Study on Essential Oils of *Geranium macrorrhizum* L. and *Geranium phaeum* L., *Geraniaceae* From Serbia

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Wild *Geranium macrorrhizum* L. and *Geranium phaeum* L. are very appreciated in traditional medicine of Serbs and other Slavic nations from Balkan Peninsula: its common name in Serbian is „zdravats" that could be translated as „health" or „to be healthy" [1]. Aerial parts of both investigated species were collected from Topli Do locality at Streselj mountain in Southeastern Serbia, during the period of full flowering. The extraction of essential oils was carried out by steam distillation in a Clevenger-type apparatus, according to Proceedings I *(G. phaeum)* and III *(G. macrorrhizum)* of the Yugoslav Pharmacopoeia IV [2]. The aerial parts in full flowering stage (herb) of *G. phaeum* contained only 0.025 % of essential oil, expressed in ml per 100 g of dried plant material. Obtained essential oil was green-yellowish liquid with intensive but pleasant, somewhat balsamic odor. On the other hand, the herb of *G. macrorrhizum* yielded 0.080 % (in g per 100 g of dried plant material) of gold-yellowish, easy solidifying essential oil with mild and pleasant scent. Using GC and GC/MS, 16 constituents (55.6 % of total oil) were detected in essential oil of *G. macrorrhizum*. The dominant component was germacrone (37.4 %), while trans-β-elemenone, piperitone, geranylmethyl ether and phenylethyl isovalerate were present in significantly lower quantities. In essential oil of *G. phaeum,* 20 constituents were identified. The most abundant one was piperitone (12.3 %), while the other important were trans-caryophyllene oxide, borneol, linalool and 2-methyl tridecane. Essential oil of *G. phaeum* contained β-bisabolene, bornyl acetate, terpinen-4-ol, nonalol and nerolidol in quantities close to 1 %.

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Essential Oils of *Stachys recta* L. and *Stachys officinalis* (L.) Trevis., *Lamiaceae*

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In the Flora of Serbia, genus *Stachys* L. is represented with 16 species [1]. We investigated the essential oils of two wild species: *Stachys recta* L. from the vicinity of Nis (Gornja Toponica location, Southern Serbia) and *Stachys officinalis* (L.) Trevis., from Durmitor mountain (Velika Greda locality, Montenegro). The aerial parts of these plant species were collected during the period of full flowering. Once harvested, the plant material was dried at room temperature and steam distilled to produce an essential oil, according to Yugoslav Pharmacopoeia IV [2]. We established that the essential oils yields in dried aerial parts from *S. recta* and S. *officinalis* were 0.014 % (in g of essential oil per 100 g of plant material) and 0.50 % (in ml of essential oil per 100 g of plant material), respectively. Using GC and GC/MS, thirty-two components were identified, in each oil. In the essential oil of *S. recta*, the major constituent was oct-1-en-3-ol (19.70 %). The other important components were caryophyllene-trans-oxide, humulene oxide and nerolidol (4.60, 3.80 and 3.60 %, respectively). The principal component in the essential oil of *S. officinalis*, was a mixture of epi- and β-caryophyllene (22.9 %). The most abundant class of components were hydrocarbons, which make 62.5 % of total oil. Among the oxygenated compounds, oxides of epi- and γ-caryophyllene (0.4 and 6.5 % respectively) and humulene epoxide (1.6 %) were the most prominent. The oil could be characterized with a low content of alcohols - 4 % of total oil. Only the content of linalool reached a value above 1 %.

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COMPOSITION OF THE ESSENTIAL OIL OF *ROSMARINUS OFFICINALIS* L. FROM JORDAN AND LIBYA

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Rosmarinus officinalis L. is an aromatic Lamiaceae plant of common use in Arabian countries. In addition to studies on the composition of rosmary oil from different sources, other constituents,*viz.*, antioxidants, triterpenes, diterpenes, flavonoids, organic acids, etc.. were investigated[1-4]. Topical formulations for rheumatism,eczema, ulcers and wounds usually contain rosmary oil. Inhalation or oral administration of the oil stimulates the CNS[5]. Antimicrobial activity of the oil was reported[6]. Analysis of commercial samples of rosmary oil revealed qualitative and quantitative differences[7]. Moreover, the effects of soil properties on yield and composition of the oil were reported[8].

In this study, the hydrodistilled essential oils prepared from the leaves of *Rosmarinus officinalis* L. collected in Jordan and Libya were analysed by CGC/MS. The different mono- and sesquiterpene hydrocarbons and oxygenated compounds were identified by their retention times and mass spectra. Qualitative and quantitative differences were observed in the two oils investigated. The following compounds were identified in the oil from the plant grown in Jordan, while being absent in the oil of the plant growing in Libya: myrcene, camphor, linalyl acetate, dihydrocarvone, borneol, verbenone, carvone, geraniol, *trans*-myrtenol, bornyl acetate, geranyl acetate, α-copaene and eugenol methylester. Conversely, the following compounds were identified in the oil from the plant growing in Libya, while being absent in the oil from the plant grown in Jordan: β-phellandrene, α-terpinene, terpinolene, β-terpenene, santalene, *cis*-ocimene, fenchene, *o*-cymene, *p*-cymene, menthatriene, *cis*-sabinenehydrate, 1-terpineol, carvacrol, verbenyl acetate, carvacryl acetate, thymol acetate, α-muurolene, γ-cadinene, δ-cadinene, caryophyllene oxide, cederene-13-ol, cederane diol, beyrene, abitariane and abital. The composition of the oils investigated was found different from those of the plants cultivated and wildly growing in Egypt[6].

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COMPARISON OF THE ESSENTIAL OIL COMPOSITION OF WILD MINTS FROM MOLDOVA.

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The comparison of essential oil of some wild mint species from different localities of Moldova and mint introduced in collection of mint of Moldavian State University have been carried out.

M. piperita L. from Russia, Brazil and local populations of Moldova have the most stable essential oil content and its chemical composition. But chemotype with citral (up to 20%), geraniol (10%), geranilacetat (up to 15%) and mentol (up to 30%) have been found in seed generation of the cultivated mint.

The chemotypes of *M. longifolia L.* from Crimea, Caucase and some Moldavian areas differs to a great extent in essential oil content (0,5–2,5 percent) and chemical composition. The main components are menton–isomenton, linalool and linalilacitat, oxides of piperiton and piperitenon. Like that and *M. spicata L.* has the same chemotypes and also carvon–dehidrocarvon one.

Oxides of piperiton and piperitenon are the main components of essential oil of the species–*M. silvestris L.* (local population), *M. incana L.* (wild, from Bulgaria and Moldova), *M. royleana* Bent, *M. citrata* Ehrh from Cube.

M. arvensis L. from Vietnam and Guinea have the similar characteristics: oil content 2.9–3.2%, mentol up to 72%, ketones 11–20%. *M. arvensis L.* from the Ukraine has the other chemorases: the first with linalool (up to 85%) as the main component and the other with geraniol and geranilacetat. Chemotype of *M. arvensis L.* from the Far East is of special interest because synthesizes menthol and cytrale simultaneously.

Crossing experiments permited to obtain a wide variety of strains with prominent oil composition.

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THE INFLUENCE OF DIFFERENT HEIGHTS OF CHAMOMILE PLANTS TO YIELD QUANTITY& ESSENTIAL OIL QUALITY

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German Chamomile, *Chamomilla recutita* (L.)Rauschert, is the most favoured and most used medicinal plants. Accordingly, the Chamomile drug belongs to the most important and profitable in the the world market of medicinal plants. Not surprisingly, several thousand tons has been recently consumed in the world per year. This consuption is, of course, covered predominantly by large scale cultivation. This cultivation and production of a high quality and quantity chamomile drug poses a series of complex questions.

This contribution is aimed at the study of the different heights of Chamomile productional stands and their influence upon flower drug quantity and essential oil quality.

A comparison of Chamomile flower biomass, number of flower anthodia and average weights of one chamomile flower from the 5 different stand heights indicates the highest quantities into these productional parameters from 450 mm to 650 mm of this stand height . Any way the comparison of essential oil yields from these heights shows the highest quantity of essential oil in the stand height from 350 mm to 450 mm .

In general, the highest stand heights yielded the highest flower quantities than the lower heights, which were accumulated more essential oil.Though the qualitative characteristics of Chamomile essential oil were influenced by various stand heights. Compositional trend for increases in the /-/- α - Bisabolol and En-yn-dicycloethers from 250 mm to 650 mm of height was determinated. It was quite different from compositional trend for decreases in decreases in the Chamazulene and Farnesene content.

External morphological characteristics (flower biomass, number of flower anthodia and average weights of one chamomile flower) of Chamomile stands were not constant and different heights of plants were influenced upon quantity production. The quantitative and qualitative composition of Chamomile essential oil was modified by the growing conditions too. The similar plant demography studies are very important.

COMPOSITION OF THE ESSENTIAL OILS OF THE CALLUS TISSUE OF *SOLIDAGO VIRGAUREA* L.

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Two lines of callus tissue of *Solidago virgaurea* L. were obtained from cotyledons and roots of seedlings on MS medium with 2,4-dichlorophenoxyacetic acid (2,4-D, 2 mg/l) and benzyladenin (BA, 0.2 mg/l). The essential oils of the callus were obtained by hydrodistillation and analysed by GC and GC-MS methods.
Both lines of callus produced very small amounts of essential oil. Great variety of components was found in these essential oils in contrast to the essential oil of intact plant [1] and plant from in vitro micropropagation.
The qualitative composition of the researched essential oils was similar but there were significant differences in quantitative composition. Callus tissues of *S. virgaurea* produced the same monoterpene hydrocarbons and partly the same oxygenated monoterpenes as intact plant and they produced many sesquiterpene compounds that have not been found in intact plant.

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VOLATILE CONSTITUENTS OF *NEPETA ISPAHANICA* BOISS. FROM IRAN

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Nepeta ispahanica Boiss. is a herbaceous wild plant from Labiatae family native to Iran[1]. However, no phytochemical studies about this plants have yet been done. In the course of systematic investigation on the essential oils of *Nepeta* species we have studied the composition of the essential oil of *N. ispahanica* Boiss. The constituents of the essential oil obtained by hydrodistillation were investigated by GC and GC-MS analysis[2]. The oil contains over 32 components. The major components were 1,8- cineole (45.8%), β-pinene (8.9%),α-terpineol (4.3%),trans-β-ocimene (3.3%), α-pinene (2.8%) and sabinene (2.1%). Identification of the compounds was based on retention indices, MS data and comparison with authentic samples.

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COMPOSITIN OF THE ESSENTIAL OIL FROM SEED OF FERULA GUMOSA BOISS.

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Among 31 Ferula species in Iran, Ferula gumosa is growing in many habits[1].The gum of this plant is valuable in perfumery industry[2,3]. Seed of aforementioned plant was gathered from Tehran province(Lar dam) on July 1998.The essential oil was isolated from crushed seed by Clevenger apparatus for 4 hours. A colorless essential oil was obtained in 7.5% w\w yield and The oil analysed by GC\MS. Twenty components were identified representing 99% of the oil with β-Pinene(79%), α-Pinene(5.2%) and δ-3-Carene(3.96%) as major components.

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EFFECT OF DRY FARMING DATE ON YIELD AND CHEMICAL COMPOSITION OF CUMIN OIL

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The seed of Cuminium cyminium was produced by dry farming at 4 separate planting dates (Feb. to Mar. with 10 day intervals),in Kerman province(Sirch region).Then the seeds were harvested in June.

The essential oils of seeds was isolated by Clevenger apparatus to produce the oil in A=1.5%, B=3.4%, c=3.4% and D=3.1% w/w yield and analysed by GC\MS. Among thirteen identified components, Non-oxygenated monoterpene consist 57.3, 44.7, 41.6 and 40.2 percent, and oxygenated monoterpens(aldehyde) consist 42.7, 55.3, 58.4 and 59.8 percent of the essential oil respectively.Cuminyl aldehyde and P-Mentha-1,4-dien-7-al were the major consyituents in the oil.

This survey revealed that the planting date has a strong influence on the quantitative and qualitative aspects of cumin oil.

COMPARATIVE CHEMICAL COMPOSITION OF THE LEAF AND FLOWER OILS OF *THYMUS ALBICANS* HOFFMANNS & LINK FROM PORTUGAL

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Thymus albicans Hoffmanns & Link is a straight shrub with nearly 80 cm high, growing on sandy soils of Iberian Peninsula Southwest. In Portugal, this plant can only be found at Algarve and even so in very diminute quantities.

Aerial parts of *T. albicans* belonging to the 1,8-cineole chemotype were collected at different growth stages during last year since April 1998 up to April 1999.

Fresh leaves and flowers were hydrodistilled, for 4 hours, using a Clevenger-type apparatus. Essential oils were analysed by gas chromatography and gas chromatography-mass spectrometry.

The qualitative composition of the oils of both leaves and flowers were very similar but with some quantitative differences.

Higher concentrations of α-pinene, linalool, δ-terpineol+borneol and α-terpineol were registered in flowers' essential oils, in contrast to the camphor that is present in smaller amounts when compared with those of the leaves.

Some quantitative differences were also registered in leaves' essential oils depending of the growth stage of plant

The highest amounts of linalool and camphor from leaves were detected in Mars and September, respectively.

The amounts of borneol+δ-terpineol reached the lowest levels in leaves' essential oils during the months of February, April and December, while α-terpineol reached the lowest concentration in September.

ALTERNATIVE SOURCES FOR ESSENTIAL OILS OBTAINED BY EXTRACTIVISM: LINALOOL-RICH OIL FROM LEAVES OF *CROTON CAJUCARA* BENTH.

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Linalool and its esters are largely used in the perfumery industry. In the past, Cayenne Bois de Rose oil (from *Aniba rosaeodora* Ducke) and mainly Rosewood oil (from *Aniba duckei* Kost.) were the main sources of linalool. Brazil was the dominant producer and exporter, reaching 500 tons of oil in the early 60's [1]. After decades of unsustainable extractivism, the Brazilian production fell down to 59 tons in 1994. Nowadays, the linalool market is supplied by synthesis [2]. Looking for renewable sources of essential oils, an Amazonian shrub called sacaca (*Croton cajucara* Benth.), already used in folk medicine against stomach and intestine diseases, was found to produce an oil rich in linalool. The hydrodistillation of fresh leaves of sacaca yielded (0,64%) an oil containing linalool (47,5%), β-caryophyllene (7,0%), β-bourbonene (1,8%) and other minoritary constituents, identified by mass spectrometry and retention indices. The optical rotation of the oil (-22,34°) suggested a high content of (3R)-(-)-linalool, the enantiomer associated with woody and lavender-like notes [1]. A programme is currently under development at Embrapa concerning agronomic studies and trials on *Croton cajucara* plants collected in different localities of Amazon and Acre states. Other Amazonian *Croton* species are also being investigated.

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A NEW SOURCE OF SARISAN FROM LEAVES OF "PIMENTA-LONGA"

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Safrole, an important raw material for the pharmaceutical, perfumery and food industry was obtained by fractional distillation of the essential oil present in the bark of Brazilian "sassafras" (*Ocotea pretiosa* Mez). Since 1991, after decades of deleterious extractivism, the harvesting of "sassafras" was forbidden in Brazil. Prompted by the necessity of a renewable source of safrole, many native species of the Amazon region were investigated. *Piper hispidinervium*, a native shrub from Acre State and locally known as "pimenta-longa", is known to produce an essential oil very rich in safrole [1]. Other *Piper* species found in Acre, also called "pimenta-longa", are *Piper aduncum*, *Piper hispidum* and *Piper sp.* In an agronomic and chemical study, the safrole content of 370 samples of essential oils from wild and cultivated plants was determinedd by gas chromatography . The oils could be grouped in four distinct categories: one rich in safrole (over 95%), corresponding to *Piper hispidinervium*; the second, rich in dillapiole (up to 79%) corresponding to *P. aduncum*; the third, associated with *P. hispidum*, with no major component and the fourth, *Piper sp.*, which produced an oil rich (up to 90%) in a substance with mass spectrum related to myristicin, but different retention index. The oil was fractionated by column chromatography over silica and the pure substance was analyzed by ^{1}H and ^{13}C NMR spectroscopy. From NMR and literature data it became clear that the unknow compound was 1-allyl-3,4-(methylenedioxy)-6-methoxybenzene, sarisan. It had been found in roots of *Piper auritum* from Jamaica [2]. Plant material of this group was collected and sent to a botanic specialist. It is not clear whether it is a chemotype or constitutes a new species.

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ANALYSIS OF YLANG-YLANG (*CANANGA ODORATA*) OIL OBTAINED BY SIMULTANEOUS DISTILLATION-SOLVENT EXTRACTION AND BY SOLID-PHASE MICROEXTRACTION

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A micro-scale apparatus for simultaneous distillation-solvent extraction (SDE) was used to obtain essential oil from ylang-ylang (*Cananga odorata*) flowers collected every 6 hours over a 5 day period from 4 trees separated more than 100 m from each other. Volatiles from the same group of flowers were sampled by solid-phase microextraction (SPME), in which a silica fiber coated with PDMS/DVB (65 µm thickness) was utilized. A Hewlett-Packard 5890 gas chromatograph equipped with an HP-5972 mass selective detector and a flame ionization detector, and a 50 m HP-5 capillary column were used to analyze the composition of the various flower extracts. 69 different compounds at concentrations above 0.01 ppm were detected in the DES extracts. Low molecular weight oxygenated compounds (LOC), (38.7%) and sesquiterpenes (38.8%), were the most abundant components, followed by monoterpenes (6.9%), high molecular weight oxygenated compounds (HOC) (6.2%), and nitrogen-containing (NC) compounds (0.7%). 24 hours after their collection, ylang-ylang flowers afforded a lower yield of volatiles (15% drop) and the corresponding oils showed a large decrease in sesquiterpene content (from 38.8 to 22.2%), while the remaining component families showed smaller variations in concentration (LOC, 42.9%; monoterpenes, 5.4%; HOC, 7.0%; NC, 0.7%). SPME extracts were composed mostly of LOC (92%), followed by monoterpenes (5.3%), sesquiterpenes (1.8%) and HOC (0.1%). The oils from flowers collected at noon contained higher concentration of low-molecular weight oxygenated compounds.

ANALYSIS OF SECONDARY METABOLITES OBTAINED FROM DIFFERENT PARTS OF RUE (*RUTA GRAVEOLENS*) BY SUPERCRITICAL FLUID EXTRACTION

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A high-pressure stainless steel vessel fitted with either a Soxhlet extractor (J&W Scientific, Folsom, California, U.S.A.) or a percolating system was used to extract secondary metabolites from leaves, flowers, stems and roots of rue (*Ruta graveolens*) by means of supercritical CO_2. The oil yields represented around 5% of the starting material. Triplicate extractions with each type of plant material showed that the percolating system and the Soxhlet extractor afforded very similar oil yields, except when stems were used (5% versus 3.9%, respectively). High resolution gas chromatography-mass spectrometry was used to analyze the composition of the various extracts. 67, 45, 25 and 24 different components were detected in the extracts from leaves, flowers, stems and roots of rue, respectively. The main compound families in these extracts were furancoumarines (28-34%), ketones (19-38%), and furanquinolines (14-21%). 2-undecanone was the most abundant compound in all extracts, at a concentration between 13 and 24%, followed by chalepensin (13-14.5%), 2-nonanone (9-11%) and rutamarin (9.1- 11.7%). Benzodioxol derivatives were present at concentrations between 9.5 and 12% in the oils from leaves and between 4.5 and 8% in the oils from flowers, stems and roots. Coumarins were present at concentrations below 1% in the oils from leaves, flowers and stems, but were much more abundant (15%) in the oils extracted from rue roots.

The Essential Oil and Polyacetylenes From *Eryngium campestre* L. (Apiaceae)

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Eryngium campestre L. (Field Eryngo) is a pale green, glaucous perennial plant belonging to the apiaceae family with coriaceous, pinnately-divided spiny leaves growing on dry places in South and Central Europe extending to Central Russia, North Africa and South West Asia.

In course of our investigations of essential oils from Apiaceae, especially from the genus Eryngium we have analyzed the essential oils obtained from different parts of *Eryngium campestre* collected in Borg El-Arab near Alexandria (Egypt) by GC, GC/MS, TLC, HPLC and NMR spectroscopic techniques.

In the herb of the plant mono- and sesquiterpene hydrocarbons (23.4% and 44.9%, respectively) were the most abundant constituents with myrcene (11.2%), germacrene-d (11.9%) and α-pinene (8.1%) being the most prominent constituents. Differentiation of the plant material into leaves, stalks and fruits yielded divergent results. In the leaves and the stalks relatively high amounts of ß-elemene (10.4% and 14.1%) were found in addition to the before mentioned components. Germacrene-d exhibited the highest proportion in the essential oil from stalks (22.4%) and from the fruits (20.7%). Worth to mention is in addition a high proportion of palmitic acid in the oils from fruits (6.2%) and from leaves (8.5%).

The root oil from *Eryngium campestre* differs significantly from the oils of the above ground green parts and contains above all the polyacetylene derivative falcarinol with 60-83%. We, therefore, decided to investigate this type of constituents in more detail with TLC and HPLC and spectroscopic techniques. Besides the known components falcarinol and falcarinone, which are common constituents of many species of the apiaceae plant family [1], a hitherto unknown constituent was isolated and the structure determined as heptadeca-1,8-dien-5,6-diyn-3,9-diol by MS and NMR-spectroscopy including 2-dimensional techniques.

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PRELIMINARY STUDIES ON GENETIC POLYMORPHISMS BETWEEN TWO CHEMOTYPES OF *THYMUS MASTICHINA* SUBSP. *MASTICHINA*.

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Three main chemotypes of *Thymus mastichina* subsp. *mastichina*: 1,8-cineole, linalool and 1,8-cineole/linalool, have been identified in Portugal [1]. The 1,8-cineole plants were found all over the country, whereas the linalool chemotype was restricted to the Serra da Arrabida. In the present study we intend to determine whether differences in essential oil composition in *T. mastichina* correlate to genetic differences between populations. Bulks of equal quantities of genomic DNA of 12 individual plants of each population were used to assess genetic distances between populations by RAPD analysis. Stem apices of plants, found at least 25 m distance from each other, were collected from two locations: Fonte Férrea (Algarve) and Sesimbra (Arrabida). Essential oils were analysed in plant distillates by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). In all samples, independent of the geographic origin, oxygenated monoterpenes were the main group of essential oil constituents. Plants collected at Fonte Férrea, Algarve exhibited a characteristic 1,8-cineole chemotype since the concentration of this compound ranged from 32.4 % to 60 % and linalool was only 0.6 %-2 %. Samples collected at Sesimbra showed an opposite pattern, 43.9 % -72.1 % of linalool and 0.2-3,2% 1,8-cineole, characteristic of the linalool chemotype. Genetic differences were found between the two populations. Among the 56 RAPD 10-mer primers, which showed amplification products, 31 primers revealed 83 polymorphisms among the 440 amplification products generated. A coefficient of genetic distance of 0,104 between both populations was estimated. 1,8-Cineole type plants have recently been found in Arrabida. Such a population will provide a very useful control to clarify the question whether the 1,8-cineole and linalool chemotypes are of genetic or environmental origin.

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INTRASPECIFIC CHEMICAL VARIABILITY OF THE ESSENTIAL OIL OF *PISTACIA LENTISCUS* L. FROM CORSICA

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In the course of our ongoing work on the chemical variability of essential oils of plants growing wild in Corsica [1,2], the composition of 105 samples of oil obtained from individual plants of *Pistacia lentiscus* L. was investigated. The identification of the main components of each sample was lead by ^{13}C NMR, without previous separation, using a computerised method developed in our laboratory and their quantitation was carried out by GC [3]. In this procedure, an individual component is identified by comparison of the values of the carbon chemical shifts in the mixture spectrum with those of reference spectra compiled in a computerised data bank. Myrcene, limonene, terpinen-4-ol, α-pinene, β-pinene, α-phellandrene, sabinene, p-cymene and γ-terpinene, were found to be the main constituents. The results were submitted to Cluster Analysis and Discriminant Analysis which allowed three groups (I-III) of essential oils to be distinguished with respect to the content of terpinen-4-ol/α-pinene, limonene and myrcene. The chemical composition of samples of group I (74% of the samples) was characterised by high contents of terpinen-4-ol and α-pinene, the former was predominant in subgroup IA (mean content 25.6%, SD = 8) while the later was the major component of the samples of subgroup IB (31.9%, SD = 8.4). The essential oils which belonged to cluster II (7% of the samples) and III (19% of the samples) were characterised by a high content of limonene (47%, SD = 4.9) and myrcene (76.9%, SD = 10.8) respectively.

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INTRASPECIFIC CHEMICAL VARIABILITY OF THE NEEDLE ESSENTIAL OIL OF *PINUS NIGRA* SSP. *LARICIO* FROM CORSICA

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In Corsica, *Pinus nigra* ssp. *laricio*, represents an valuable species for timber production (35000 m^3/year) and generates an important quantity of residual biomass. It would be interesting to valorize this biomass by producing essential oil from needles. In the course of our ongoing work on the chemical variability of plants growing wild in Corsica, we carried out a study on individual essential oils obtained from needles of 123 adult pines growing wild in the most important forests of Corsica. The identification of components has been performed by ^{13}C NMR spectroscopy without previous separation of the compounds, following the pioneering work done by Formàcek and Kubeczka [1] and according to an experimental procedure and a computerized method developed in our laboratory [2]. In this procedure, the components are identified by comparison of the carbon chemical shift values in the mixture spectrum with those of reference spectra compiled in a computerised data bank. The main constituents were found to be α-pinene, germacrene-D, manoyl oxide, myrcene, E-caryophyllene, limonene, β-phellandrene and β-pinene. The results were submitted to Cluster Analysis and Discriminant Analysis which allowed two groups of essential oils to be distinguished with respect to the content of α-pinene and manoyl oxide.

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COMPARISON OF THE ESSENTIAL OILS OF *ORIGANUM MAJORANA* L. AND *ORIGANUM X MAJORICUM* CAMBESS.

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Origanum majorana (Lamiaceae) is a native plant of Turkey [1]. It is naturally distributed in an area stretching from Antalya to İçel provinces along the south Anatolian coastal line. This species is characterized by high carvacrol content in its essential oil. Therefore, it differs from the European *O. majorana* (Sweet marjoram, Mercanköşk in Turkish). The latter is grown in gardens in western Anatolia. During our field studies we discovered the cultivation not only of *O. majorana* but also of a hybrid *Origanum x majoricum*. This sterile hybrid morphologically resembles *O. majorana* however, its calyx and corolla characteristics are close to the other parent *O. vulgare* L. subsp. *virens* (Hoffmanns et Link) Ietswaart. The latter is not a native plant of Turkey. Therefore, we could only compare the essential oil compositions of *O. majorana* L. and *O. x majoricum* Cambess.

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COMPARATIVE MORPHOLOGICAL AND PHYTOCHEMICAL CHARACTERIZATION OF *SALVIA CADMICA AND S. SMYRNEA*

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Salvia cadmica Boiss. and *S. smyrnea* Boiss. (Lamiaceae) differ from each other by the colour of their corolla in that the former is white and the latter is violet-blue, and that calyx widens in fruit in the latter [1, 2].

Both species are characterized by having cryptone in their essential oils as main constituent. Both are endemic. While *S. cadmica* grows in central and western Anatolia, *S. smyrnea* has a narrower distribution growing in an area adjoining İzmir and Aydın provinces.

The paper compares morphological and chemical features of the two species with a view to confirm their taxonomical identity.

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THE ESSENTIAL OIL OF TWO *THYMUS* SPECIES FROM TURKEY: *T. MIGRICUS* AND *T. FEDTSCHENKOI* VAR. *HANDELII*

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The genus *Thymus* (Lamiaceae) is represented in Turkey by 38 species and 64 taxa, 24 of which are endemic [1, 2]. Dried herbal parts of *Thymus* species are used as spice and herbal tea in Turkey. *Thymus* is an important genus due to presence in the oils high concentration of carvacrol and thymol which are isomeric phenolic monoterpenes. However, there are *Thymus* species poor in phenolic compounds and some may not contain phenolic compounds at all. During our cotinuing study, we have come across with all these three types [3]

This paper compares compositions of the essential oils of *Thymus migricus* Klokov et Des.-Shost. and *T. fedtschonkoi* Ronniger var. *handelii* (Ronniger) Jalas. The latter is an endemic species and the morphological characteristics of the two species are similar. The main components in the essential oils were found as carvacrol and linalool, respectively.

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THE COMPOSITION OF ESSENTIAL OILS FROM THREE ENDEMIC *SIDERITIS* SPECIES

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The genus *Sideritis* (Lamiaceae) is represented by 48 species [1-3] and most *Sideritis* species are used as herbal tea in Turkey. Those used as herbal tea generally contain α and β-pinenes [4]. This paper describes the composition of the essential oils of three endemic *Sideritis* species: *S. argyrea* P.H.Davis, *S. hololeuca* Boiss. & Heldr. apud Bentham and *S. stricta* Boiss. &Heldr. apud Bentham. The water distilled oils were analysed by GC/MS. Main compounds characterized in both oils were β-pinene (20%, 35% and 30%) and α-pinene (14%, 16% and 13%), respectively.

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CONSTITUENTS OF VOLATILE OIL FROM GINKGO BILOBA L.

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Abstract : Up to now a great number of apolar and polar compounds have been isolated from Ginkgo leaves : long-chain hydrocarbons and derivatives, alicyclic acid, cyclic compounds, carbohydrates and derivatives, flavonoids, isoprenoids (sterols, terpenoids), various compounds like (*Z,Z*)-4,4`-(1,4-pentadiene-1,5-diyl)diphenol, 6-hydroxykynurenic acid, cytokinins, β-lectins, carotenoids, and others[1]. So far, there are no detail report about volatile oil from leaves of *Ginkgo Biloba*. In this poster, the constituents of volatile oil from *Ginkgo biloba* was examinated. The constituents of the volatole oil from leaves of *Ginkgo biloba* were found to be 1-hexadecene, 3-eicosene, 6,10,14-trimethyl-2-pentadecanone , tricosane, heptacosane, docosane, hexadecanoic acid, ethyl formate, ethyl acetate, ethyl alcohol, 1-hexanal, *cis*-3-hexenal, 1-pentene-3-ol, *trans*-2-hexenal, 6-methyl-5-heptene-2-one, 1-hexanol, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, 1-nonanal, 2,4-hexadienal, acetic acid, β-ionone, linalool, 1-octanol and 4-ethylphenol, There components were compared to flesh and change of colour leaves.

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PROVENCE-RELATED COMPARISON OF THE CONSTITUENTS OF THE ESSENTIAL OIL OF ARTEMISIA MARITIMA L.

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The name of *Artemisia maritima L.* designates an officinal plant which in former times was of medicinal significance and in Germany also of economic relevance.

In 1923 the area around Artern (northern Thuringia) and the neighbouring villages of Kachstedt and Schönfeld, under a contract with Schering AG in Berlin started growing *Artemisia maritima* L. for the extraction of santonin as a worm expellent: this cultivation continued for over 30 years and was ceased only after it was possible to produce anthelmintics by synthetic methods.

In 1997 and 1998 comparitive investigations were conducted in order to check the constituents of essential oils from different sources. Typical qualtitative and quantitative differences were found to exist between the essential oils of plants originating

from maritime locations - North Sea coast
and continental location - upcountry salt patches in Central Germany.

While in the essential oils originating e.g. from the salt-water ditch near Artern or from the salt meadows at Kachstedt the two thujone isomers cis-and trans-thujone are predominant up to 80%, the sources from North Sea habitats are characteristically showing different constituents. Apart from small amounts of thujone these plant oils contain mainly chrysanthenone, filifolone, ascaridol, davanone and hydroxyperox-davanone. Davanone is the characteristic constituent (up to 60%) of davana oil obtained from *Artemisia pallens* Wall. ex DC.

Artemisia maritima oils originate e.g. from the hercynic arid basin in northern Thuringia are characterized by a spicy-aromatic sage-like odour and a sage-like herbaceous-bitter taste.

On the other hand oils from North Sea have an acrid, radish-like, less thujonic and somewhat fruity odour and a sage-like herbaceous-bitter taste.

These different ranges of constitutens influence the various applications of the respective essential oils.

Essential Oil Composition of *Peucedanum cervaria* and *Laserpitium latifolium* from Eastern Austria.

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Peucedanum cervaria (L.)Lap. and *Laserpitium latifolium* L. are two perennial umbelliferous plants growing in Eastern Austria in dry meadow slopes and forest edges. The plants were collected at two sites in Niederösterreich and Burgenland in spring and autumn. The volatile components of watersteam distillates and dichloromethane extracts from leaves and fruits were analysed by GC/MS. The main components identified were α-pinene, β-pinene and sabinene in *Peucedanum*, where the oil content was very low in the leaves. Sabinene, α-pinene, limonene and germacrane D were foud in the leaves of *Laserpitium*. Ripening fruits of the latter species became rapidly dry; their main compouds were α-pinene and sabinene. In both species the same pattern of oil components were found in plants from both sites.

Composition, Content of THC and Antimicrobial Activity of the Essential Oil of Some Cultivars of *Cannabis sativa* L.

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The essential oils of some cultivars of *Cannabis sativa* L. were analysed by GC/MS. These cultivars are low in the content of the halloucinogenous Δ9-tetrahydrocannabinol and in many countries of the European Union allowed for the production of fibre, fatty oils and many new applications. The THC-content was analysed by TLC and GC/MS. The main compounds identified were α-pinene, myrcene, α-terpinolene, trans-cayophyllene and α-humulene. The genotypes differed in their composition of the essential oil. No tetrahydrocannabinol could be found in the essential oil of these cultivars in contrast to the essential oil of drug varieties [1]. The antibacterial activity can generally be described as weak.

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Composition of the Essential Oil and Extracts of Two Subspontaneous Populations of *Cannabis sativa* L. from Austria

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The essential oil and extracts of fresh plant parts of two populations of *Cannabis sativa* L. subsp. *spontanea* [1], occuring at ruderal sites in 'Seewinkel', Burgenland (Eastern Austria, pannonic climate) were analysed by GC/MS. The main compounds were α-pinene, myrcene, trans-beta ocimene, trans-caryophyllene and α-humulene. The main difference of the populations were found especially in the content of trans-beta ocimene (12.5% in the essential oil of population 1 and 3% in the essential oil of population 2, respectively). The essential oil and extracts of the fresh parts of these populations contained no Δ9-tetrahydrocannabinol.

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VARIABILITY IN ESSENTIAL OILS FROM THE GENUS *MENTHA* CULTIVATED IN DEPARTMENT OF BRASOV, ROMANIA (II)

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By crossing experiments in the genus *Mentha*, starting from *Mentha aquatica* L., *Mentha spicata* Huds, *Mentha crispa* L., *Mentha viridis* L., *Mentha* × *piperita* L we obtained four groups of hybrids, classified according to the **modern biochemical criterion**, of a certain, preferred pathway in essential oil components biosynthesis and less of principal component analysis (PCA) [1]-[3] as it follows :

- **Group A** (10 hybrids), characterized by the preferential biosynthesis of the C_3 oxygenated p-menthane pathway, with menthol/menthone and derivatives. The Hybrid H 617 is highly valuable, mostly because of its resistance to stress factors, a high productivity and a great percentage of menthol (45-50%), during the period of maximum vegetal productivity;
- **Group B** (7 hybrids), that biosynthesizes mainly the C_6 oxygenated p-menthane compounds, with carvone, dihydrocarvone, dihydrocarveols and their esters. The Hybrid H 611 has a high content of dihydrocarvyl acetates.
- **Group C** (7 hybrids), biosynthesizes preferentially linalool (65-90%), that represents a cheap and important source of natural linalool.
- **Group D** (one hybrid), presents an intense activity of trans sabinene hydrate and the corresponding acetate biosynthesis pathway.

This work presents new results in composition of essential oils and extracts from some performant hybrids belonging to the first three groups. Essential oils obtained from these hybrids present some differences from those mentioned in the literature of the genus *Mentha* [4]-[7].

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EFFECT OF ENHANCED ULTRAVIOLET RADIATIONON *MENTHA SPICATA L.* ESSENTIAL OILS IN IRAN.

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The effect of enhanced different (8, 20, 40 Watt) ultraviolet radiation on *Mentha spicata L.* which are grown on field and in a greenhouse condition was studied. The essential oil obtained in during flower time by steam distillation was examined by GC and GC/MS. In this research carvone is major constituent of *Mentha spicata L.* degradation significantly in all samples in compare with control, except sample under 40 Watt (in greenhouse), altthough carvone has decrease in sample control from greenhouse (4.6%), in comparison with field (47.39%). Furthermore,piperitone oxide have increased from field sample compared greenhouse samples. In all treatment, sesquiterpenoides are increased in greenhouse compared to field. This is a first investigation report on essential oils compounent of *Mentha spicata L.* under UV-radiation on field and in a greenhouse condition.

COMPARATIVE STUDY OF THE ESSENTIAL OILS OF THREE ACHILLEA SPECIES FROM IRAN.

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The composition of the oils from flowers of three Iranian Achillea species (A. albicaulis C.A.Mey, A.bibersheinii, A.fillpendula Lam.) were collected during the flowering period. The essential oils obtaned by steam distillation. The percentage of all three oils were 0.20% v\w calculated on the dry weight ,analyzed by GC and GC/MS, all three oils consisted mainly of monoterpenes. The majore constituents of A.albicaulis C.A.Mey were γ-Muurolene(13.29%), α-Pinene(9.99%), Camphor(9.35%), p-Cymene(8.48%), and trans-Carveol (8.41%), and A.bibersheinii were Piperitone (45.92%), 1,8-Cineole(17.64%), limonene(5.63%) and p-Cymone(5.17%), A.fillpendula Lam were Limonene (26.70%), Carvacrol(9.26%), 1,8-Cineole(8.66%), Borneol(7.82%) and Germacrene D(5.62%).

Essential Oil Composition of Myrtus communis L. from Iran

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Plant material was collected at flowering stage from National Botanical Garden of Iran in Tehran.The essential oils isolated by steam distillation from the fresh and dry leaves of *Myrtus communis* L. were analyzed by GC and GC/MS.Among the 32 compounds identified,the major components were α-pinene (29.1,22.4%),limonene(21.5,19.2%),1,8-cineole(17.9,23.4%), linalool(10.4,11.7%) and linalyl acetate(4.8,6.1%).

ESSENTIAL OIL COMPOSITION OF LAURUS NOBILIS L. FROM IRAN

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Plant material(cult.) was collected at flowering stage from National Botanical Garden of Iran in Tehran and dried at room temperature.The essential oil isolated by steam distillation from the leaves of plant was analyzed by capillary GC and GC/MS.Among 35 compounds identified,the major components were 1,8- cineole(49.4%), α-terpinyl acetate(13.2%) and sabinene(10.0%).

RAW FRANKINCENSE FROM SOMALIA AND QUEST FOR QUALITY STANDARDS

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Progressive Interventions is an Irish development organisation who has worked with collectors, graders and brokers of frankincense in Somalia for over a year, attempting to increase the income they receive. The main areas of production are in the Bari and the Saanag regions in the north east and north west of Somalia respectively. Frankincense is a gum-resin, harvested from trees of the genus *Boswellia*. Inferior forms of frankincense come from *B. papyrifera* found in Ethiopia, Sudan and East Africa, and from the Indian species, *B. serrata*. In addition, the quality of gum-resin differs between regions (coastal plains or mountainous areas), and is affected by the age of the trees, time of harvest, storage and further processing, which includes grading according size, shape and colour. Original material was brought directly from Somalia. *B. carterii* was graded into mixed, red and white, and *B. thurifera* graded into six grades according to size, shape and colour. They were all distilled using the BP distillation apparatus and analysed by GC, and several representative samples by GC-MS. The oil yield (v/w) was very high; 8.9 % for *B. carterii* red and 7.7 % for *B. carterii* white; from 18.9 % to 11.1 % for *B. thurifera* from grades 1 through to 6. Requests were sent to 40 commercial oil suppliers asking for a sample of their oil. The results were compared with the authentic samples. An extensive reference search was undertaken, and a concise summary of geographical distribution and taxonomical descriptions of the species was produced. GC results and other data are available on request from SAC. Recommendations were made to Progressive Interventions and interesting questions were raised about oil quality.

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ON THE COMPOSITION OF ESSENTIAL OIL OF WILD MELISSA

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10 provenances of wild melissa were collected in south-east Turkey in the years 1991 and 1992 and cultivated in Adana since 1993 together with the provenance Münster, having been cultivated already in Germany for a long period for comparison of purpose. From these cultivars the essential oil was seperated by means of water-steam distillation (Neo-Clevenger) from the dried material of plants that were harvested in 1997 and analyzed by means of gaschromatography and mass spectrometry. Neral and geranial, the two components characteristic of melissa , were detected in only three provenances however. In the essential oil of the stored dried material that was analyzed after more than 6 months later, these compounds were missing in these provenances, too. In the next cut of the melissa plants in June 1998, neral and geranial were missing entirely in the essential oil of all provenances. The reason for the dissapearance in the case of the 3 provenances should be decomposition by storage, but it seems, that these components cease to be produced by plants of a certain age or even harvesting date.

Separation of trans sabinene hydrate rich essential oils by hydrodistillation and supercritical CO_2 extraction from *Mentha* hybrids

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Trans sabinene hydrate (1R,2S,5R)-2-methyl-5-(methyl-ethyl) biciclo [3.1.0] hexan-2-ol was identified as a component of the odor bouquet emitted by several bark beetle species and also in sunflower aroma, being considered as one of the components stimulating the honeybee workers antennal receptors; its precise role in chemical communication systems is yet to be investigated [1].

We are interested to obtain this substance from a natural source-a valuable mint hybrid, codified as H 616, caryologically characterised, identified from those resulted in a complex mint breeding research, starting from *Mentha aquatica* L., *Mentha spicata* Huds, *Mentha crispa* L., *Mentha viridis* L., *Mentha* × *piperita.*

The use of hydrodistillation in the case of sabinene hydrates rich essential oils extraction does not give a correct picture of the system present in the genuine plant. A prolonged water contact time at high temperature and the decrease of pH during the hydrodistillation favorise isomerisations and hydrolysis [2], [3]. .Supercritical CO_2 extraction avoid thermal degradation and solvent pollution, giving compositions closer to vegetable matrix then other methods do [4].

Partial results of some intrinsic and extrinsic factors influence on essential oil composition have been previously reported [5].

Essential oils and supercritical CO_2 extracts were obtained by modifying process parameters such particle size, extraction time, flow rate, charge, extraction and separation parameters. The kinetic extraction study and monitorization of extraction using GC analysis permited us to estabish optimal conditions for extracts with a flavour similar to plant material [6]-[8].

This study presents other results in order to

- a better characterisation of the complex terpenoidic system present in the plant
- a comparison between the kinetic of hydrodistilation and SC- CO_2 extraction
- separation of sabinene hydrates by flash chromatography

The fractions were analysed by GC-MS. Identification of trans/cis sabinene hydrates by GC-MS and Kovats indices was also confirmed by ^1H and ^{13}C-NMR, two dimensional HETCOR and COSY techniques.

SC-CO_2 extract (dried leaves 0,6 mm, in CO_2 at 92-94 bar, 50°C, 70 min extr. time), contained 43.51% trans sabinene hydrate and 19.15 % the corresponding acetate. We established that it is possible to obtain an essential oil with a high content of trans sabinene hydrate (37,3%), after 70 min of hydrodistillation, at pH = 6-7, but never a high content of its acetate could be reached (0.42%).

This study reveals also the importance of SC-CO_2 extraction in chemotaxonomy, specially in the case of plants with thermolabile compounds rich essential oils.

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[8] D. Barth, E. Pop, L. Phillipon, "Supercritical Carbon Dioxide Extraction of Essential Oil: the Case of two Romanian *Lamiaceae*" Proceedings of "High Pressure Chemical Engineering" International Meeting, March3-5,1999, pag. 197, Karlsruhe, Germany.

Solid Phase Microextraction (SPME) an Efficient Tool to Analyze Flavor and Fragrance

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SPME is an efficient method for analysis of essential oils for quick screening prior to a detailed analysis as well as applicable with good precision in quantitative analysis under consistent sampling conditions. SPME is comparable in accuracy to other techniques -like static headspace, purge and trap, liquid-liquid extraction, solid phase extraction or simultaneous distillation/extraction- but easy to handle without costly organic solvents. It can be used to concentrate volatile and nonvolatile compounds in liquid samples or headspace, and provides linear results over a wide concentration of analytes – often down to parts per trillion range. Headspace SPME coupled with capillary GC is an ideal approach for characterizing quality and composition of flavor oils. Especially the short extraction and desorption times allow a rapid sample preparation with the elimination of matrix. The benefit of SPME for investigation of essential oils is pointed out. Furthermore the optimation of the technique is described.

[1] Solid Phase Microextraction: Theory and Optimization of Conditions; Supelco Technical Bulletin 923.

[2] Solid Phase Microextraction: Solventless Sample Preparation for Monitoring Flavor and Fragrance Compounds by Capillary Gas Chromatography; Supelco Technical Bulletin 869.

[3] Yang and Peppard; Solid Phase Microextration for Flavor Analysis; (1994) J. Agric. Food Chem., 42, 1925 – 1930.

Chiral DEX™-Columns with Versatile Selectivity for Quality Controll of Essential Oils

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The determination of enantiomeric distribution of ingredients is routinely used in quality controll of essential oils. For that purpose highly selective chiral GC columns are needed. Supelco offers a broad range of stable derivatized cyclodextrin (CD) stationary phases for high resolution of optical isomers. α-Dex™, β-Dex™, and γ-Dex™ columns which differ in enantioselectivity due to differences in CD derivative and size of CD cavity enables reliable quality controll of complex mixture. The separation of caraway-, citrus-, spearmint-, lavender- and peppermint oils are given as examples to show the versatile Dex™-columns for enantiomeric investigation of essential oils.

Column	Cyclodextrinderivative
α–DEX™ -120	20% permethyliertes α-CD in SPB-35
β–DEX™ -110	10% permethyliertes β-CD in SPB-35
β–DEX™ -120	20% permethyliertes β-CD in SPB-35
γ-DEX™ -120	20% permethyliertes γ-CD in SPB-35
α-DEX™ -225	25% 2,3-Di-O-acetyl-6-O-TBDMS-α-CD in SPB-20
β-DEX™ -225	25% 2,3-Di-O-acetyl-6-O-TBDMS-β-CD in SPB-20
γ-DEX™ -225	25% 2,3-Di-O-acetyl-6-O-TBDMS-γ-CD in SPB-20
α-DEX™ -325	25% 2,3-Di-O-methyl-6-O-TBDMS-α-CD in SPB-20
β-DEX™ -325	25% 2,3-Di-O-methyl-6-O-TBDMS-β-CD in SPB-20
γ-DEX™ -325	25% 2,3-Di-O-methyl-6-O-TBDMS-γ-CD in SPB-20

Dimensionen: 30m, 60m x 0,25mmID, 0,53mm ID; 0,25µm u. 0,5µm D_f
[TBDMS] tert-butyldimethylsilyl, [CD] Cyclodextrin

[1] Chiral Cyclodextrin Capillary GC Columns; Supelco Technical Bulletin 869

[2] Mani and Woolley; Solid Phase Microextraction and Chiral Gas Chromatography Separations; (1995) LC-GC 13 (9), 734 - 740

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Lectures (L) and Posters (P)

Corrigendum in the Final Circular

L 2 – 06 Vender (instead of Wender)
PA – 42 Rafiei (instead of Rafiel)
PB – 35 additional: Satil,F.

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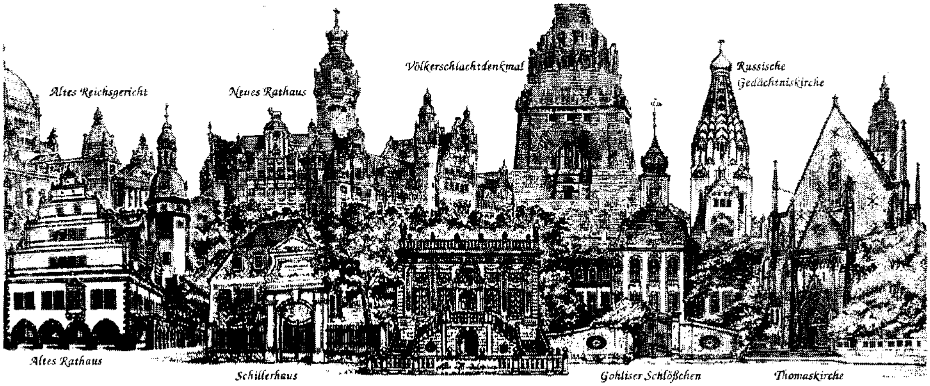

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