ISEO 2000
31st INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

Hamburg / Germany
September 10 - 13, 2000

Chairmen:
Prof. Dr. K.-H. Kubeczka / Prof. Dr. W.A. König
Pharmaceutical Biology / Organic Chemistry
University of Hamburg

Abstracts

PL-1

Authenticity Assessment of Essential Oils – The Current State and the Future

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Both phenomena, *enantioselectivity* as well as *isotope discrimination* during biosynthesis, may serve as endogenetic parameters in the authenticity control of natural flavour and fragrance compounds, provided that suitable methods and comprehensive data from authentic sources are available. Enantioselective *c*apillary *g*as *c*hromatography (enantio-*cGC*) and comparative *i*sotope *r*atio *m*ass *s*pectrometry (IRMS) have proved to be highly efficient tools in the origin specific analysis.

Nevertheless, analytical authentication of genuine food constituents or essential oils is a permanent challenge, due to the complexity of biological matrices. So far, enantioselective and/or IRMS online coupling techniques are the methods of choice in order to determine the authenticity of flavours, essential oils and extracts of spices. The analytical progress in the origin assignment of flavours is now well accepted for the authenticity assessment in the European food law, but there exist only first steps on obligatory regulations in the field of essential oils.

It seems to be reasonable to discuss the analytical authentication of essential oils in the future of the European Pharmacopoeia.

[1] A. Mosandl
Food Reviews Int. 11, 597-664 (1995)
Enantioselective capillary gas chromatography and stable isotope ratio mass spectrometry in the authenticity control of flavours and essential oils.

L-1

BIOTRANSFORMATION OF (+)- AND (–)-LIMONENE BY FUNGI AND THE USE OF SOLID PHASE MICROEXTRACTION FOR SCREENING

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The biotransformation of the hydrocarbon terpenes (+)- and (–)-limonene was carried out by fungal spores. More than 60 fungal cultures were screened for their ability to bioconvert the substrate, using Solid Phase Microextraction as the monitoring technique. Therefore, a method was developed to cultivate sporulated surface cultures of the fungi in small vials, and the SPME-parameters were optimised. Different SPME-fibers, extraction times and temperatures were compared. After screening, the best fungal strains were selected for further study and were grown as sporulated surface cultures in conical flasks and as submerged liquid cultures. The biotransformation was monitored by dynamic headspace, steam distillation solvent extraction and liquid/liquid extraction. The effect of the culture conditions on the biotransformation of limonene was studied. It was found that (+)- and (–)-limonene were converted by *Penicillium digitatum* to a-terpineol (main metabolite), *cis*- and *trans-p*-menth-2-een-1-ol, neodihydrocarveol and limonene oxide (minor metabolites) using liquid cultures. The bioconversion of (+)- and (–)-limonene by *Corynespora cassiicola* yielded (1*S*,2*S*,4*R*)- and (1*R*,2*R*,4*S*)-limonene-1,2-diol respectively. The bioconversions by liquid cultures were also monitored by Solid Phase Microextraction as a function of time. The optimum conversion of limonene to a-terpineol by *Penicillium digitatum* was obtained after 8 hours (up to 100%). Since an important pH-decrease was noticed in some liquid broths, the stability of limonene under acid conditions was investigated. No acid catalysed conversion products were recovered after 8 days from control flasks at pH 3.5 containing limonene.

L-2

HEADSPACE SORPTIVE EXTRACTION (HSSE) IN THE HEADSPACE ANALYSIS OF AROMATIC AND MEDICINAL PLANTS

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Headspace Sorptive Extraction (HSSE) is a new solventless sampling technique based on the principle of Stir Bar Sorptive Extraction (SBSE). SBSE was recently developed by Sandra's group [1] to extract organic analytes from aqueous samples and is based on the sorption of an analyte (or analytes) onto a thick film of polydimethylsiloxane (PDMS) coating an iron stir bar. The stir bar is inserted into the aqueous sample and extraction takes place during stirring. After a fixed time it is removed and either introduced into the GC injector liner or placed in a glass tube, which is transferred to a thermal desorption system where the analytes are thermally recovered and analysed by GC or GC/MS.

HSSE-PDMS coated stir bars are potentially successful for sampling volatiles in a gaseous or vapour phase because HSSE lies between S-HS and D-HS, as it is for HS-SPME, but involves higher amounts of trapping material than SPME fibres, potentially giving it better concentration efficiency [2,3].

This communication reports the results of HSSE sampling of medicinal and aromatic plants. Analyte partition coefficient between HSSE-PDMS stir bar and sample headspace (K_l), concentration factor (CF), reproducibility and minimum recoverable amount were determined by analysing standard solution of high volatility compounds with different polarities and structures (cyclohexane, propyl acetate, hexanal, 1-hexen-3-ol, isoamyl acetate and 2-heptanol). Four aromatic and medicinal plants: rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.) and valerian (*Valeriana officinalis* L.) were analysed by HSSE-GC with PDMS stir bars, and their concentration capacity was compared with those of S-HS and HS-SPME with different fibres.

[1] E. Baltussen, P. Sandra, F. David, and C. Cramers, J. Microcol. Sep. 11 (1999) 737
[2] B. Tienpont, P. Sandra, F. David, and C. Bicchi, submitted to J. Microcol. Sep.
[3] C. Bicchi, C. Cordero, C. Iori, P. Rubiolo and P. Sandra, J. of HRC, in press

L-3

PLANT VOLATILES AND ENDOPHYTIC FUNGI: HS-SPME-GC ANALYSIS OF PEPPERMINT CULTURED *IN VITRO*.

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Peppermint leaves harbor an endophytic fungus (PGP-HSF), that after aseptical isolation from tissues can be grown in pure culture and reintroduced in axenic plants.

In vitro inoculation of peppermint cuttings results in epibiotic growth of a mycelial net of hyphae, upward spreading from roots to stem and leaves. Plant fungus association is asymptomatic and leads to enhanced vegetative growth, characterized by greater node number, higher LAI (leaf area index) values and a 96 % increase of the total number of trichomes per plant.

In a previous work we have demonstrated that peppermint essential oil and oil components affect fungal cell respiratory activity, thus controlling PGP-HSF development and reproduction. Being the host sensitivity to essential oils a probable main determinant in the plant-fungus mutualism, we wanted to investigate the chemical nature of plant volatiles emitted by peppermint plants during the culture with the endophyte.

Employing the Solid Phase Microextraction technique, an absorbent coated fused-silica fibre - as a part of a syringe needle - is aseptically inserted into the culture tube, thus allowing time-course sampling of peppermint head-space with the least chemical and mechanical disturbance of the culture system.

The chemical profile of peppermint volatiles in response to fungal inoculation has been studied and HS-SPME-GC pattern compared to conventional solvent distillation of plant material.

PL-2

SOME ASPECTS OF CITRUS OIL CHEMISTRY

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All essential oils of the species of the citrus family have common constituents, mainly limonene together with other monoterpene hydrocarbons, which are accompanied by some specific compounds which are responsible for the characteristic organoleptic properties and the analytical fingerprint of each species. This will be demonstrated for the most frequently used oils. Then, an indepth analysis of the volatile components of a mandarin extract will be presented. In mandarin the characteristic tonality is due to a combination of the monoterpenes with α-sinensal, thymol and methyl N-methylanthranilate, but, as in tangerine, fatty, aldehydic notes are overwhelming, which could be identified as uncommon unsaturated aldehydes (decenals, decadienals, decatrienals, dodecenals and dodecadienals). Finally we will walk through an orange processing plant and discuss the chemistry of the various orange by-products used in the flavour and fragrance industry.

L-4

ANALYTICAL INVESTIGATION OF SWEETIE (OROBLANCO) OIL AND GERMACRENES IN CITRUS PEEL OILS

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The analytical investigation of Israeli Sweetie oils and their distinction to grapefruit are within the scope of the present investigation. In the second part, the distribution of the germacrene sesquiterpene class in citrus essential oils will be dealt with.

The citrus variety Sweetie (Oroblanco) is a cross between pummelo and grapefruit. Sweetie is well established on the fresh fruit market and has lately been gaining importance in industrial processing. In comparison to white and pink grapefruit, the oils showed an enhanced sabinene and a lower β-caryophyllene content. The nearly complete absence of nootkatone was conspicuous; it has, however, also been observed with grapefruit oils from Cuba.

GC analysis of the germacrenes turned out to be a useful distinctive feature between Sweetie and grapefruit varieties. While Sweetie oil possessed the whole set of germacrenes A, B, C, D and bicyclogermacrene, germacrenes B and C were missing in grapefruit oils.

Under extremely mild GC conditions, all germacrenes, including the thermally labile germacrenes A and C could be detected. A screening of commercially available citrus oils permitted an overview of the presence of this sesquiterpene class in the oils of bergamot, grapefruit, orange, lemon, lime, pummelo, yuzu and of various mandarin and tangerine varieties.

Germacrene A could be found in all cold-pressed citrus oils. Germacrenes B and C, present in some of the oils and missing in others, always occured together. Germacrene D and bicyclogermacrene could be found in all citrus varieties with the exception of lemon which is free of germacrene D and lime oils which do not contain bicyclogermacrene.

L-5

SESQUITERPENE HYDROCARBONS FROM PATCHOULI AND PATCHOULOL

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The composition of the non polar fraction of patchouli essential oil (*Pogostemon cablin* Benth.) was reinvestigated, using a classical combination of fractional distillation, $AgNO_3$-impregnated silicagel column chromatography and preparative gas chromatography. The identification of isolated hydrocarbons was carried by conventional GC-MS, and, when necessary, by high field NMR studies and IR spectroscopy. On this basis, a compilation of analytical data on the composition of 25 different commercial lots of patchouli essential oil is presented.

New rearranged hydrocarbons are formed upon acid-catalyzed dehydration of pure patchoulol, using perchloric acid in nitropropane at –25°. The distribution of these hydrocarbons was compared to the previously described transformation product of patchoulol, using a strong cation exchange resin [1]. Extensive high field NMR studies (up to 600 MHz) were used to establish the structures of the hydrocarbons.

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[1] B. D. Mookherjee, R. W. Trenkle and W. O.Ledig, *J. Agric. Food Chem.*, 1974, **22**, 771.

L-6

ON-LINE HPLC DETECTION OF RADICAL SCAVENGERS IN PLANT EXTRACTS AND ESSENTIAL OILS

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Oxygen is not only a molecule absolutely essential for life but also one that can exert negative effects. Through a reaction of oxygen with carbon radicals reactive peroxyl radicals are formed which in turn can generate more radical species eventually leading to the oxidative breakdown of essential molecules like DNA, proteins and fatty acids. In foods and cosmetics this can lead to deterioration. To avoid this, antioxidants are added to prolong the shelflife.

Currently there is some concern about the safety of synthetic antioxidants like 2,6-di(*t*-butyl)-4-methylphenol (BHT) and therefore natural antioxidants are increasing in popularity. Well known natural antioxidants include ascorbic acid (vitamin C), tocopherols (vitamin E), flavonoids and rosemary and sage extracts. Many antioxidants are phenolic in nature. Their mode of action can be twofold: (1) inhibition of the formation of active radical species, and (2) scavenging of already formed radicals.

Several different assays for the determination of antioxidant activity exist and the actual outcome may vary widely depending on the compound and assay. All these assays are "total" assays and do not yield information on individual components. Therefore a significant problem in identifying new antioxidants present in plant extracts, is pinpointing the active peaks in often complex HPLC profiles. This can be done by collecting every peak followed by the determination of its activity in off-line assays. However this is time-consuming, requires considerable amounts of compounds and the potential danger of degradation exists.

Therefore we have investigated the possibilities of an on-line antioxidant assay. During oxidation of luminol by hydrogen peroxide catalysed by microperoxidase, an excited molecule of 3-aminophthalate is formed along with nitrogen and water. Upon relaxation to the ground state, 3-aminophthlate emits light at 425 nm. Active radicals, like the superoxide anion, play a role in the formation of the excited molecule. Radical scavengers can interfere with this reaction and thus cause a quenching of the fluorescent signal. Other frequently used relatively stable model radicals include $DPPH^{\bullet}$ and $ABTS^{\bullet +}$. Similarly to the luminol system, these can be added to the HPLC effluent by a syringe pump via a T-junction. As the radicals absorb light at visible wavelengths while the reduced radicals do not, the disappearance of absorption (quenching) in the form of a negative peak is indicative of the presence of a radical scavengers. As many of the more powerful natural antioxidants are also radical scavengers, this can accelerate the identification of new antioxidants in complex mixtures.

Some stages of the method development of on-line radical scavenging detection will be presented as well as some chromatograms of test substances, extracts of plants with known antioxidants and active extracts of plants not known to contain radical scavengers. Detection limits for the three systems will be discussed. As most radical scavengers are phenolic in nature, essential oils are in general a poor source for finding novel antioxidants. Nevertheless some essential oils were separated by gradient RP-HPLC and detected by UV at 210 nm and by on-line radical scavenging detection. Some active compounds could be identified. Thyme essential oil is well suited to illustrate the significant differences between the DPPH and ABTS radicals. Both have their specific advantages.

L-7

COMPARISON OF PEAK PATTERNS – A CUSTOM VERSUS A COMMERCIAL SOFTWARE SOLUTION

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Pharmaceutical products derived from essential oils can exhibit a very complex composition. When using high resolution capillary gas chromatography more than a hundred peaks may be detected. Therefore, by visual inspection only, it is difficult to locate points in the chromatogram of a sample where peaks are new or absent in relation to a reference.

In order to improve the reliability of quality control by comparison of gas chromatograms the author designed a macro program that evaluates the results tables of sample and reference created by conventional chromatography software (1). First, this macro corrects shifted retention times by linear interpolation relative to selected main compounds. Then, peak by peak, it calculates the relative differences of amount between sample and reference. If a peak is present in one chromatogram only, the missing amount value is approximated by the average of zero and the limit of detection to avoid division by zero. Finally, the results are plotted in a chromatogram-like diagram. It indicates new peaks by an increase of several hundred percent, even if they constitute only about one percent of total amount. The disadvantage of this custom solution is the imperfectness of the primitive mode of peak alignment, provocating wrong matches, especially if chromatograms from different years are compared.

Very recently a commercial pattern matching software was introduced (2). It selects an interval from the sample chromatogram and determines the match quality with the corresponding interval of the reference chromatogram. This is done for different shifts of retention time to determine the actual offset of retention times. Then the next interval, overlapping with the first, is selected from the sample chromatogram and analyzed. This is repeated until the whole chromatogram is scanned. Differences between sample and reference are detected primarily by the named match quality and, after peak detection, by the response ratio, i.e. the ratio of peak heights. Both means together allow to filter out significant differences even from intricate patterns. The problems and the benefits of the software will be discussed.

It is expected that within the next decade pattern matching will become a routine method in quality control and stability testing of complex products.

1) A. Zaenglein 1999: "Automated Numerical Comparison of Chromatograms for the Quality Control of the Complex Essential Oil in a Pharmaceutical Product", Joint Meeting of ASP, AFERP, GA and PSE: "2000 Years of Natural Products Research – Past, Present and Future", July 26 - 30, Amsterdam; Poster #767

2) Anon. 2000: "Millennium^{32} Chromatographic Pattern Matching Software", Millennium^{32} Version 3.20, Waters Corporation, 34 Maple Street, Milford, MA 01757

PL-3

The Commercially Important Mint Oils

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Mint oils including pennyroyal (*Mentha pulegium* L.), bergamot mint (*M. citrata* Ehrh.), cornmint (*Mentha arvensis* L. f. *piperascens* Malinv. ex Holmes), Native spearmint (*Mentha spicata* L.), Scotch spearmint (*Mentha gracilis* Sole), and peppermint (*Mentha piperita* L.) are the most important commercial essential oil produced because they are higher valued than the higher volume citrus oils. To put their production in perspective, the farming and processing practices for North American and Indian mint oils and their world volumes will be compared.

If found in the wild or seed produced, *Mentha* species and hybrids are known to exist in chemotypic forms. Also, clonally reproduced mints are supposed to yield the same oil composition if harvested at the same ontogenic time; however, extrinsic conditions can influence the composition of particularly peppermint. To differentiate between the same types of mint oil produced in different geographic regions the use of component ratios has been examined. Not only can these ratios be used to determine the true origin of the oil, but adulteration with mint oils from other regions can also be determined.

After a brief description of the process used to isolate menthol, the use of non-equilibrated SPME analysis will be shown to determine the geographic origin of menthol. Finally, to address the off-notes detected in mint oils by sensory analysis a reproducible SPME procedure to quantitate the off-notes has been developed.

L-8

South African Medicinal Aromatic Plants – an Overview

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South Africa boasts an unique and diverse botanical heritage of almost 28 000 species of which *ca.* 10% have been studied for their possible therapeutic use. In addition to this unique botanical heritage South Africa has a cultural diversity with traditional healing being integral to each ethnic group. A concerted and organized effort is required to record ethnobotanical information and to explore the chemistry and uses of our indigenous aromatic plants with the aim of finding a scientific rationale for the use of the many "Muthi medicines" used by the people of South Africa.

An overview of the most widely used aromatic plants in South Africa will be presented dealing with their essential oil chemistry, medicinal uses and pharmacological activity. Some of the aromatic plants have been developed commercially as herbal remedies. Drawing on these examples the importance of correct botanical sourcing, chemical variation and quality control of essential oil containing plants will be discussed.

L-9

COMPOSITIONS OF VOLATILS FROM SIBERIAN MEDICAL PLANTS

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We have studied chemical composition of a number of species of the *Asteraceae* family growing wild in Siberia as well as the corresponding cultural forms from regional botanical gardens (*Artemisia dracunculus L., Artemisia obtusiloba Ledeb., Artemisia jacutica Drob., Artemisia abrotanum L., Artemisia scoparia Waldst. et Kit., Artemisia glauca Pall. ex Willd., Artemisia altaiensis Krasch., Artemisia glabella Kar. et Kir., Artemisia filatovae A. Kuprijanov ssp. nova, Artemisia pontica L., Brachanthemum baranovii (Krasch. ex Poljak.) Krasch., Ajania fruticulosa (Ledeb.) Pojak.*), and 8 populations of *Thymus serpyllum L.s.l.* (*Lamiaceae* family) growing wild in Altai Mountains. Our data show that various factors, both endogenous and exogenous, can affect the composition of the essential oils of Siberian species of the above plants. Those species, which are characterised by significant polymorphism and broad natural habitats, usually demonstrate great dependence of the ethereal oil composition on geographical and climatic factors (*Thymus serpyllum, Artemisia dracunculus, Artemisia scoparia, Artemisia abrotanum, and Artemisia glauca*). Contrary, compositions of ethereal oils of endemic species, such as *Artemisia jacutica, Artemisia altaiensis, Artemisia obtusiloba*, do not vary significantly and demonstrate a hereditary predetermined set of secondary metabolites.

Compositions of volatile compounds were studies by GC-MS using quadruple MS (Hewlett-Packard MSD 5971) coupled to a HP 5890/II GC fitted with an HP-5 (30 m × 0.25 mm I.D., film thickness 0.25 μm) fused silica column. Qualitative analysis was based on comparison of the retention indexes and full mass spectra of the components with the data for standards prepared by separation of reference oils and identified by NMR spectroscopy.

Details of separation and identification procedures and chemical composition of the oils are discussed as well as dependence of the content of the principal components on different factors: age of plants and their phase, genetic and ecological factors.

Acknowledgements: *The research was made possible in part by Grant "Study of volatile terpenoids of herbs from Siberia and Russian Far East" from The Competitive Centre on Natural Sciences at the Saint-Petersburg University.*

L-10

SESQUI- AND DITERPENOIDS FROM JAPANESE AND EUROPEAN LIVERWORTS

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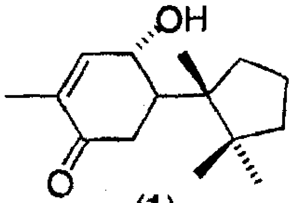
The interest in the liverworts has dramatically increased since their constituents have shown high therapeutic effects such as antitumor, antimicrobial and antifungal and muscle relaxing activities etc.[1-3] In this communication, we report the isolation and structure elucidation of several new sesqui- and diterpenoids from Japanese liverworts *Jungermannia infusca* (**1-3**), *J. trancata* (**4**), *Trocholejeunea sandvicensis* (**5-7**), *Porella subobtusa* and the European *Plagiochila asplenioides* (**8**), *Scapania undulata* (**9**), *S. nemorea* and *Mylia taylorii*, and the microbial transformation of **7** by *Aspergillus niger* and *A. cellulosae*.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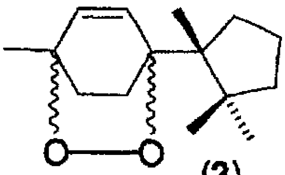
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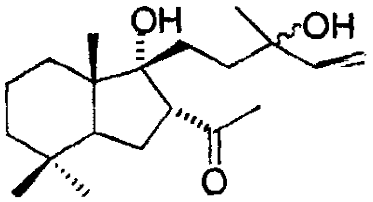
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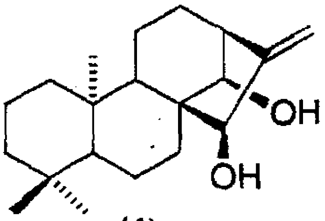
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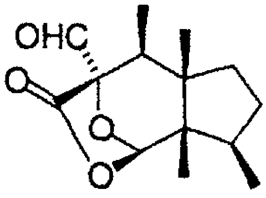
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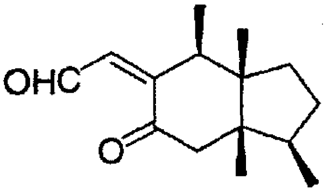
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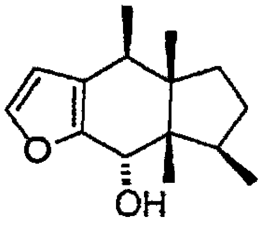
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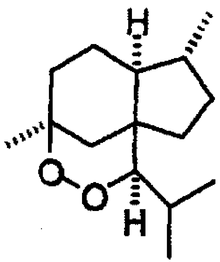
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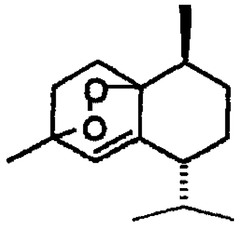
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1)Asakawa, Y. *Progress in the Chemistry of Organic Natural Products*, **42**, 1-285(1982), *ibid.*, **65**, 1-618(1996), Springer, Wien.
2)Asakawa, Y. *Recent Advance in Phytochemistry*, **33**, 319-342 (1999), Kluwer Academic/Plenum Publishers, New York.
3) Asakawa, Y. *Heterocycles* (in press).

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L-11

RESEARCH INTO TURKISH ROSE OIL: RECENT RESULTS

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Rose Oil is produced by hydrodistillation from fresh flowers of *Rosa damascena* Miller Turkey and Bulgaria are the main producers of this precious oil. Results of analysis of Turkish rose oil produced by Gülbirlik Cooperative in the last 15 years will be given and the main odorous components characterising the Turkish rose oil will be indicated. Headspace studies using Solid Phase Micro Extraction (SPME) techniques on living rose plants and freshly picked rose flowers will be presented.

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L-12

Chemotypic Differentiation *versus* Plasticity in indigenous Populations of *Foeniculum vulgare* var. *vulgare* in Israel

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Chemical analysis of the volatile fractions of oleoresines, from fruits (mericarps) of wild populations of bitter fennel (*F. vulgare var. vulgare*) in Israel, exhibited four different chemical groups (1). In each of these groups we aimed to identify the level of heritability, as well as chemical plasticity. To this end representatives of the natural populations were grown under similar conditions. Ripe fruits were collected simultaneously from the natural mother populations, as well as from their cultivated representatives grown together in the Newe' Ya'ar Research Center. Fruits were hexane extracted and the volatile fraction of fruit oleoresins of 7 indigenous populations was analyzed by GC-MS. Data indicated 3 chemically stable populations presenting 2 different chemotypes: (1) a *t*-anethole dominated chemotype of the south in the Negev desert and also in the Mediterranean coastal belt in the North. And (2) an estragole dominated chemotype of Mt. Dov, ca 900m a.s.l., at the North east of the country. Compared with the natural mother populations, cultivation of four populations changed their chemical composition, suggesting plasticity. In the mountainous populations, cultivation reduced the relative content of *trans*-anethole and enhanced that of estargole; And *vis-a-vis* in the lowland populations, here cultivation rather enhanced the relative content of *trans*-anethole and reduced that of estragole. Suggesting, that in *F. vulgare var. vulgare,* chemical plasticity amplifies variability in an inconsistent way.

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L-13

BIODIVERSITY AND RANDOM SAMPLING IN ESSENTIAL OIL PLANTS

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Essential oil plants show in general a high quantitative and qualitative variation in their chemical pattern. This is due to the fact that both terpenoids as well as phenylpropane-derivatives can appear in one and the same species being controlled genetically by several steps, partly depending from each other. In addition, the morpho- and ontogenetic variability as well as the environmental factors influencing the biosynthesis have to be taken into consideration. To get valid information on the chemical profile of a species or population presupposes therefore a correct sampling and exact methodological approach.

As concerns *quantitative* variations of a certain pattern, random samples should consist of at least 15-20 individual plants and their respective parts. Statistically significant results will be obtained with 3-4 replications. Quite different appears the situation with *qualitative* („either/or“) variations, e.g. carvacrol/thymol, 1,8 cineole/camphor/α-/β-thujone. Any random sample can give only a non-specific information on the principal chemical profile of a population provided that the sample is representative. No less than 40-50 plant individuals per replication are needed for that purpose. Much better information will be achieved if the above number of individuals (40-50) will be analysed separately. Very poor, in contrast, are results based on a single sample consisting of a mixture of 10 or less individual plants, as shown in figure 1. Good Laboratory Practice (GLP) should be kept also in this respect, and a simple sniffing test could help the investigator deciding whether random sampling or single plant investigations are more appropriate. The above stated will be discussed by several examples.

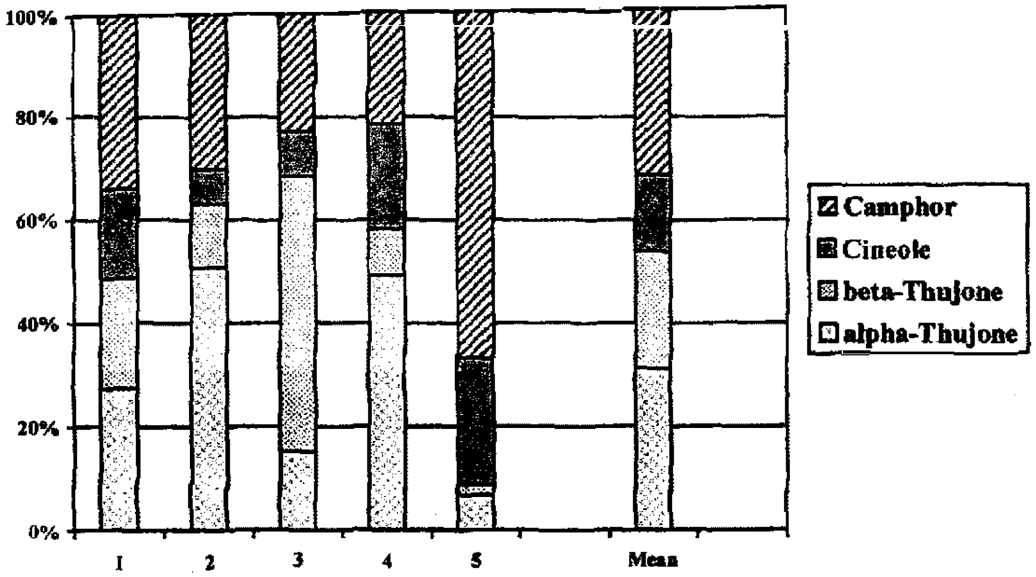

Figure 1: Composition of the essential oil of 5 *Salvia officinalis* individuals in comparison to the mixture

PL-4

REGULATION OF TERPENOID BIOSYNTHESIS

Harro Bouwmeester

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The formation of terpenoids in plants is regulated by both internal as well as external factors. External factors include environmental conditions such as light and temperature as well as biotic stresses such as infection by micro-organisms (formation of phytoalexins) and herbivory (induction of defense compounds). Internal factors include organ specificity and plant development.

Most of this regulation is due to changes in the activity of the enzymes involved in the respective biosynthetic pathways and these enzymes largely determine the accumulation or emission pattern of terpenoids in plants. We are studying the enzymatic and corresponding genetic regulation of a number of biosynthetic pathways. On the one hand these include ecologically important processes such as the induction of nerolidol synthase activity in cucumber and lima bean upon spider mite feeding and the biosynthesis of anti-feedant sesquiterpene lactones in chicory.

On the other hand we are interested in economically important pathways such as artemisinin biosynthesis of which we have elucidated a number of so far unknown steps. Some examples of these pathway studies will be shown in more detail. Finally, for many pathways it would be interesting to study the consequences of changes in the pathway. We have successfully expressed a linalool synthase in petunia which gave rise to linalool production in leaves and flowers where the wildtype does not produce any linalool. Without doubt such transgenic plants will be a valuable tool in the elucidation of the importance of many compounds in plants.

L-14

Induction of Plant Volatile Biosynthesis by Microbial and Insect Elicitors

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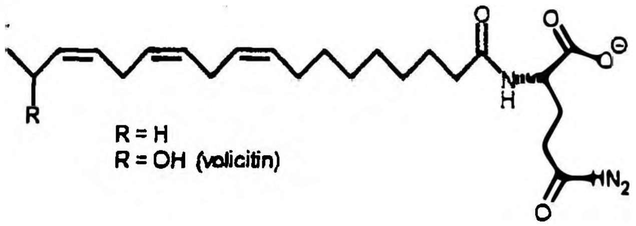
The elicitors of volatile biosynthesis and their mode of action are largely unknown; only a few high- and low molecular weight compounds from micro-organisms and insects have been identified. One of the most potent proteinaceous elicitors is cellulysin, a cocktail of cell wall lytic enzyme from the fungus *Trichoderma viride* [1]. The signal transduction, initiating volatile biosynthesis, proceeds via intermediates of the octadecanoid pathway, especially early (linolenic acid) and late intermediates such as jasmonic acid (JA) both of which induce completely different pattern of volatiles [2]. Ethylene is the first volatile to be released from damaged plants. Its biosynthesis is triggered by herbivory as well as by individual elicitors, but the mode of emission (burst or rhythmic) is different. Alamethicin (ALA), a voltage-gated, ion channel-forming peptide mixture from *T. viride*, is another potent elicitor of volatile biosynthesis. ALA acts via depolarization of membrane potentials and thereby activates the octadecanoid signaling pathway. ALA also up-regulates salicylate biosynthesis, and the time course of the production of endogenous salicylate correlates well with the appearance of the methyl ester in the gas phase. The massive up-regulation of the SA-pathway interferes with late steps of the octadecanoid pathway (downstream of 12-OPDA) and thereby reduces the pattern of emitted volatiles to compounds characteristic for a linolenic acid treatment [4]. Certain amphiphilic elicitors from insects (acyl amino acids; volicitin) apparently also act on membranes by disintegration of their molecular integrity leading to depolarization.



In line with this view is the observation that herbivory not only up-regulates the octadecanoid pathway (wounding response) but also leads to enhanced levels of endogenous salicylate. Evidence will be given that the amphiphilic *N*-acyl amino acids from the insect gut may be synthesized by endosymbiontic micro-organisms, thus, adding a new trophic level to the complex web of interactions between plants and insects [5].

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cellulysin 50µg/ml
[illegible] 3h [illegible]

channel-forming peptides (alamethicin)
40 µg/ml [illegible]

MICROBIOLOGICAL TRANSFORMATION OF α-SANTONIN, 1,2-DIHYDRO-&TETRAHYDRO-α-SANTONIN, NOOTKATONE, OPTICAL ACTIVE α-IONONE AND 2-ADAMANTANONE

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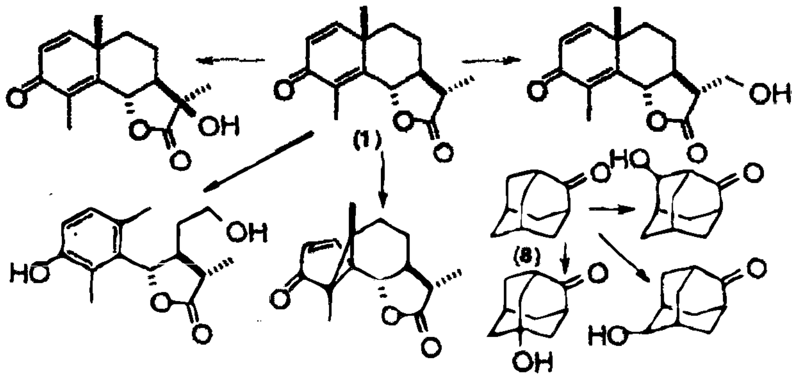
We are continuing to investigate the biotransformation of plant secondary metabolites obtained as a constituents from higher plants and liverworts such as (-)-hinesol [1], α-(-)- and β-(+)-eudesmol[2], (-)-ambrox [2], dehydrocostuslactone[2], α-, β- and γ-cyclocostunolides [2] by various kinds of microorganisms from a pharmacological point of view[3]. Now we report the microbiological transformation of several sesquiterpenoids such as α-santonin (**1**), 1,2-dihydro-1(**2**), 1,2,4α/β, 5α-tetrahydro-1(**3** & **4**), (-)-nootkatone (**5**), optically active (-)- & (+)-α-ionone (**6** & **7**) and 2-adamantanone(**8**) by microorganisms.

Compound **1** was biotransformed to 11β-OH-**1** and phenolic compound as the major metabolites and 13-OH-**1** and lumisantonin as the minor metabolites by *A.niger*. On the other hand, **2** was converted to **1**, 1-OH-**2**, 2-OH-**2**, and 9-OH-**2** by *A.niger* and *A.cellulosae* . Furthermore, **3** was only reduced to 3β-OH-**3**, and **4** was also reduced to 3β-OH-**4** at first, which was further hydroxylated to give 1β, 3β-diOH-**4**, 2α, 3β-diOH-**4**, 1-oxo, 3β-OH-**4**, 2-oxo, 3β-OH-**4** & 2-oxo-3-OH-3,4-dehydro-**4** by *A.niger* . Compound **5** was converted to 11, 12-diOH-**5** as the major product and 12-OH-**5** as the minor product by *A.niger* . Optically pure **6** & **7** were prepared from *dl*-α-ionone. Compounds **6** & **7** were easily hydroxylated nonspecifically to give 3α- & 3β-OH-**6** & 3α- & 3β-OH-**7**, which were further dehydrogenated to give 3-oxo-**6** & 3-oxo-**7**, respectively. Compound **8** was hydroxylated mainly to 4-OH-, 5-OH- & 6-OH-**8**. Furthermore, **8** was reduced to 2-adamantanol, which was further converted via 5-OH-**8** to adamantane-1,4-diol(**9** eq) selectively by *Fusarium culmurorum* and mainly to adamantane-1,4-diol(**9** ax) by *A.niger*. Stereoselective reduction of 5-OH-**8** was observed by microorganisms. In the biotransformation of the above sesquiterpenoids, stereospecific and regiospecific hydroxylation, stereoselective dehydrogenation, hydrogenation, reduction, carboxylation, epoxidation, hydrolysis, cleavage of ether linkage, intramolecular ether linkage formation, formation of sulfur containing compounds were observed. The reaction patterns on the microbiological transformation of nor-/ sesquiterpenoids together with their metabolic pathways were discussed.



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PL-5

CHEMICAL COMMUNICATION: STRUCTURAL CONCEPTS AND EVOLUTIONARY PRINCIPLES

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Molecular recognition is prerequisite to the beginning of life and thus, „chemical communication“ is the oldest means to transmit information.

During evolution, principles of chemical communication may have been developed several times and for different reasons, typical elements being made up by secondary metabolites. Reproductive strategies, dominance, chemical defense, predator-prey-interactions as well as animal-host plant-relations include a broad spectrum of components, the variability of which, however, appears not to be unlimited.

Striking similarities are found between molecular structures of compounds which are used for chemical communication. This conservative scenario may indicate similar biogenetic pathways and common roots including aspects of coevolution. It points to general concepts in the establishment of „chemical languages“ and the principle development of appropriate receptor systems.

Most of the relevant compounds are represented by acetogenins, polyketids and mevalogenins, the biosynthesis of which is not restricted to animals, but is also valid in plants and microorganisms, in terrestrial as well as in aquatic ecosystems. The role of (endo)symbionts in the production and transformation of animal associated chemical signals is yet unknown.

Due to past processes of coevolution, odoriferous compounds from plants, insects and microorganisms can be congruent. The same compound may be used as a chemical messenger by quite different organisms and in entirely different ecological contexts. The information linked to a specific chemical structure is not necessarily constant and may change during evolution. This becomes particularly evident in compounds which cause a high status of alertness in the receiver and which may convert into signals causing attraction, aggression, defense or stampede; compounds evolved in the context of defense, may turn into attractants.

Sequestered food constituents which animals use as chemical signals, provide information about the quality of the environment. The more such compounds are changed through metabolic activities in the animal (finally leading to a totally *de novo* synthesis), the more they reflect the physiological state of the emitter. During biosynthesis of semiochemicals, the action of less specific enzymes will lead to the production of „byproducts“ (stereoisomers, chain length) of the „actual signal“ which may add a fine tuning of the bouquet or facilitate evolution through the introduction of a „disposable variant“.

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

L-16

ESSENTIAL OILS AND SINGLE FRAGRANCE COMPOUNDS: BIOLOGICAL PROPERTIES

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A review is given on scientifically proven activities of essential oils and single fragrance compounds. Essential oils as natural mixtures of a lot of volatiles are used as therapeutic agents against various ailments, disorders and psychosomatic complaints. Contrary to esoteric and holistic believes, the rather small and lipophilic fragrance compounds exhibit distinct physiological activities (besides their sensoric qualities) which can be shown in animal and human experiments. The psychological effects, e.g. the creation of a sort of well feeling, happiness, etc., are not topic of scientific aromatherapy: these should be discussed and studied by aromachologists. Therefore, in this contribution new facts on the way of action as well as new research results concerning the biopharmaceutical behaviour of chiral fragrance compounds are presented.

L-17

BIOSYNTHTETIC ASPECTS OF PIPERITONE IN PEPPERMINT (*MENTHA X PIPERITA* L.)

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Piperitone is a minor component in peppermint oil with an enantiomeric distribution of - 99% in favour of the (4S)-(+) enantiomer. In order to get further insight in the biosynthetic interrelation of this monoterpene in *Mentha x piperita* L. *in vivo*-feeding experiments were designed.

Deuterium labelled monoterpene precursors were dissolved in water and administered t the shoot tip and first leaf pair of young peppermint plants. The essential oil was extract with solid phase microextraction (SPME) or steam distilled and analysed using enantioselective multidimensional gas chromatography mass spectrometry (enantio-MDGC-MS). Thus, genuine and labelled monoterpenes were detected simultaneously.

Deuterium labelled piperitone and piperitenone were used as precursors. The essential c was analysed for menthone and isomenthone enantiomers and/or piperitone enantiomer Labelled (-)-menthone and (+)-isomenthone were biosynthesised after feeding labelled piperitone as well as piperitenone precursors.

The enantiomeric distribution of piperitone in peppermint is determined by the selectivi of the formation, that is the piperitenone-reduction, and the selectivity of further reducti to menthone and isomenthone, indicating, that piperitone is not an inert metabolite in peppermint.

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L-18

COMPARATIVE ANTIMICROBIAL ACTIVITY OF SELECTED PLANT ESSENTIAL OILS AND THEIR RESPECTIVE HYDROSOLS IN A MODEL FOOD SYSTEM.

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Plant essential oils were obtained by distillation using a glass Clavenger-type laboratory still, from fresh leaves of *Pelargonium* 'Attar of Roses', dried leaves of meadowsweet (*Filipendula ulmaria*), dried cinnamon bark (*Cinnamomum verum*), bay leaves (*Laurus nobilis*), clove buds (*Eugenia caryophyllus*). The hydrosol remaining after the distillation in each case, was reduced by a hundred-fold using a rotary evaporator at 50^0C.

A Model Food System, consisting of Porridge oats and vegetables, was inoculated with either *Staphylococcus aureus* or *Escherichia coli* at 10^4 cfu/ml and incubated with or without a plant essential oil or its hydrosol (at 1000ppm) for 24 h at 25^0C. The log cfu g^{-1} reduction in the bacterial numbers was then compared against controls.

The results indicated that meadowsweet oil (at 1000ppm) was totally effective against both *S. aureus* and *E. coli*, but the hydrosol was only slightly effective against *S. aureus* in the porridge system; the oil was totally effective up to 250ppm.The clove, cinnamon and *Pelargonium* oils were very effective against *S. aureus*, with bay oil slightly so; however there was only slight antibacterial effect using cinnamon and bay hydrosols and the rest of the hydrosols were ineffective. Meadowsweet oil had a very strong effect against *E. coli* in porridge, and there was a decreasing effect with cinnamon, clove and *Pelargonium* oils respectively; there was no significant effect against *E. coli* by any plant hydrosol. Antimicrobial effectiveness of all plant essential oils against both the bacteria in MRD was greatly enhanced, suggesting that the porridge-based food system was sequestering the oils or the bacteria or simply inhibiting the effect of essential oils; the effectiveness of hydrosols from meadowsweet, *Pelargonium*, clove and to a lesser extent cinnamon and bay in MRD, again showed the difficulties of the practical application of potential antimicrobial agents in some foods.

L-19

In-vitro Bactericidal Effects of Tea Tree Oils s.l. on *Staphylococcus aureus*

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Staphylococcus aureus ATCC 6538 cells were exposed to increasing concentrations of Australian tea tree oil, terpinen-4-ol type (*Melaleuca alternifolia* Cheel), cajuput oil (*M. cajuputi* ssp. *cajuputi* Powell), niaouli oil (*M. quinquenervia* (Cav.) S.T. Blake), kanuka oil (*Kunzea ericoides* (A. Rich.) J. Thompson) and manuka oil (*Leptospermum scoparium* J.R. et G. Forst.). Two further products were included in this study, a β-triketone complex, which was shown to be the active principle of manuka oil [1,2], and Lema® oil, a blend of Australian tea tree oil and the polar fraction of manuka oil consisting mainly of β-triketones. It was worth examining Lema® oil in detail because of possible synergistic effects. Although the excellent in-vitro bacteriostatic activities of tea tree oils s.l. against Gram-positive bacteria (e.g. *Staphylococcus aureus*) were proved previously [3], their use as antiseptics and disinfectants requires the knowledge of their bactericidal activities [4].

The death kinetics of *S. aureus* were determined by calculation of $\log_{10}$ reduction factors after increasing exposure periods. Niaouli oil turned out to be highly active, followed by Lema® oil, Australian tea tree oil and cajuput oil. Kill rate data indicated that 1.0 % (v/v) were lethal to stationary phase cells in the assay conditions used. At 2.0%, niaouli oil and Lema® oil yielded a complete 6.8 $\log_{10}$ reduction of cell numbers in suspensions within 60 min, whereas cells treated with Australian tea tree oil and cajuput oil were inactivated more slowly within 120 min and 240 min, respectively. Kanuka oil and manuka oil as well as the β-triketone complex, the active principle of manuka oil, lacked any bactericidal properties. Their high effectiveness against Gram-positive bacteria can be explained by bacteriostatic effects. The results obtained with Lema® oil, a blend of Australian tea tree oil and a polar fraction of manuka oil (mainly β-triketones), gave cause to discuss synergistic effects.

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L-20

THE SIGNIFICANCE OF THE OUTER MEMBRANE IN RESISTANCE OF GRAM-NEGATIVE BACTERIA TO MONOTERPENES

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The antimicrobial activity of many essential oils and their monoterpene constituents has been well established. However, the reasons why some monoterpenes are more active than others and why the active monoterpenes differ in their spectrum of activity is less well understood. Structural similarities and the presence of particular functional groups alone do not explain these differences. Our studies have shown that water solubility, H-bonding capacity and log k_{ow} are useful indicators of activity, but do not explain why some terpenes are active against some Gram-negative bacteria, but not others.

This paper will examine the properties of some monoterpenes that demonstrate significant antimicrobial activity against *Escherichia coli*, but not *Pseudomonas aeruginosa*. Both these organisms are Gram-negative bacteria, with many structural similarities. However, there are significant differences in the structure of their outer membranes; differences which have been related to the greater resistance of certain strains of *P. aeruginosa* to antibiotics, disinfectants and some solvents. Monoterpenes that are active against *E. coli*, but not *P. aeruginosa*, are very similar in structure and molecular properties, but discriminant analysis has demonstrated a tendency towards a lower H-bond donor capacity, greater surface area and a higher hydrophilic-lipophilic balance (HLB) in the former group.

Studies with the outer membrane permeabilising agent, polymyxin nonapeptide B (PMBN), suggest that this difference is due to a reduced uptake of the less active monoterpenes by *P. aeruginosa*. The susceptibility of *P. aeruginosa* to several pairs of closely related molecules, including α-terpineol and terpinen-4-ol, was compared with and without treatment of the cells with PMBN. With untreated cells, terpinen-4-ol (0.7%,v/v) caused a $6\log_{10}$ reduction in the number of viable cells over one hour, compared to a $3\log_{10}$ reduction with α-terpineol at a similar concentration. After treatment with PMBN, the activity of both compounds was comparable, suggesting that terpinen-4-ol penetrates the outer membrane of this bacterium more effectively than α-terpineol.

To test whether monoterpenes that are active against *P. aeruginosa*, such as terpinen-4-ol and carvacrol, act by permeabilising the outer membrane, studies were undertaken with the fluorescent stain, 1-phenylnaphthylamine (NPN). Outer membrane damage is indicated by an increased uptake of the stain. Our results demonstrate that monoterpenes differ in their effects on the outer membrane. These differences will be discussed in more detail.

L-21

ALLELOPATHIC EFFECTS OF PEPPERMINT ESSENTIAL OIL IN HIGHER PLANTS: INTRACELLULAR CALCIUM VARIATIONS

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Monoterpenes inhibit seed germination and plant growth in the chemical interaction among plants known as allelopathy. Previous studies have demonstrated that peppermint (*Mentha piperita* L.) essential oil and some of its oil components affect root and mitochondrial respiration as well as membrane permeability and ion uptake of cucumber (*Cucumis sativus* L.). In this communication we report on the effect of the monoterpene (-)-menthol on intracellular calcium concentration $(Ca^{2+})_i$ in cortical root cells of cucumber.

To evaluate $(Ca^{2+})_i$ as a response of menthol concentration two Ca^{2+} indicators, Fura-2 and Fluo-3 AM, were used by using a Nikon Eclipse E-400 Epifluorescence Microscope with a light source and optics transmissive to 340 nm and a Zeiss Axiovert 100M connected to a Zeiss LSM 510 Laser Scanning Confocal Microscope Imaging System with an argon-ion laser emission light at 488 nm, respectively.

When observed under epifluorescence, control tissues treated without Fura-2 and with only menthol showed very low levels of $(Ca^{2+})_i$ and the absence of unspecific autofluorescence, respectively. Menthol-induced increase of $(Ca^{2+})_i$ was evident after 10 min of perfusion with 300 ppm (-)-menthol of cucumber root cells incubated for 2 h in the Ca^{2+} indicator, Fura-2.

In order to verify the kinetics of $(Ca^{2+})_i$ variation, cucumber root cells were incubated for 2 h in the Ca^{2+} indicator Fluo-3 AM and then observed under a Laser Scanning Confocal Microscope. No significant variation was observed in the sole presence of the Ca^{2+} indicator Fluo-3 AM, whereas a few seconds after the addition of 300 ppm (-)-menthol a steep increase in $(Ca^{2+})_i$ was observed, reaching the maximum value after 300 seconds and followed by a consistent decrease in the $(Ca^{2+})_i$ down to the starting values. Figure 1 shows the time-course variation in $(Ca^{2+})_i$ during treatment with (-)-menthol. At the beginning $(Ca^{2+})_i$ increases as the consequence of Ca^{2+} release from storage, then Ca^{2+}-ATPases start pumping out cytosolic Ca^{2+}, by lowering $(Ca^{2+})_i$.

The physiological, biochemical, metabolic and allelopathic effects of (-)-menthol on cucumber root system are discussed along with general considerations on the signal transduction pathway.

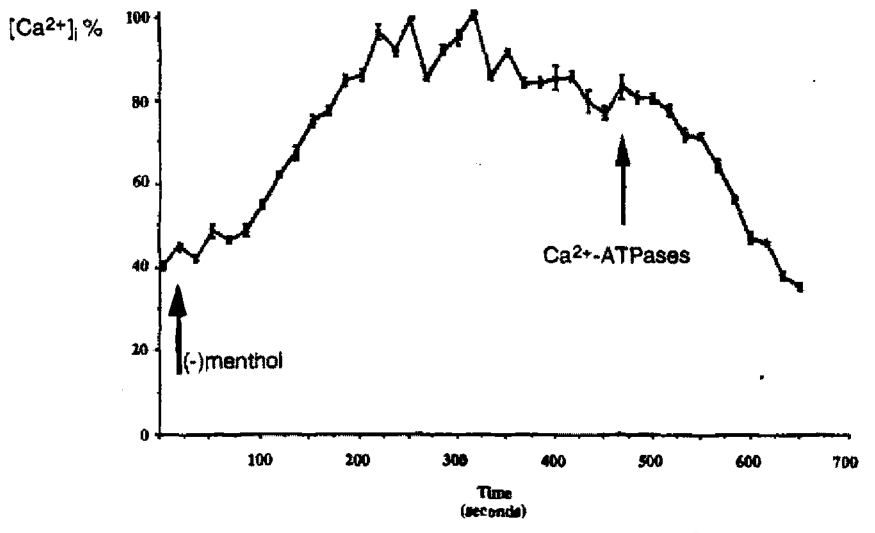

Figure 1. intracellular calcium concentration $(Ca^{2+})_i$ variations in cortical root cells of cucumber (*Cucumis sativus*) after perfusion with 300 ppm (-)-menthol, as measured with Laser Scanning Confocal Microscopy.

Poster Session I (A)

Monday, September 11, 2000
16.50 - 18.00

A-01

CHEMICAL COMPOSITION OF THE VOLATLE CONSTITUENTS OF *CYNANCHUM ACUTUM* L. HERB

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The Genus Cynanchum (Asclepiadaceae) includes several species. Some of these species are used in Chinese folk medicine for the treatment of epilepsy and chronic hepatitis [1], as anti-tumor [2], anti-tussive, expectorant and anti-asthmatic agents [3].

Cynanchum acutum L.is wildly growing perennial herb commonly distributed in Egypt [4].

The volatile constituents of this herb were prepared by hydro-distillation in a modified Lickens and Nikerson apparatus, which allowed the simultaneous extraction of the volatile vapours in pentane. The yield was 0.05%. GC/MS analysis of the volatile constituents using DB-5 column revealed the identification of 69 components representing 87.2% of the total volatile constituents. The major compounds were phytol (15.52%), heptacosane (6.93%), hexadecanoic acid (6.06%), bis(2-ethyl hexyl) phthalate (5.04%) and caryophyllene (5.05%). The n-alkane hydrocarbons (HC) represented 25.67% of the total volatiles, the n-alkene HC (2.11%), the sesquiterpene HC (10.32%). The oxygenated n- alkanes and n- alkenes represented 28.78%, while the oxygenated monoterpens were 3 %, the oxygenated sesquiterpenes represented 5.89 %. The aromatic compounds represented 7.42% and the nitrogenous compounds represented 4.01%. The total oxygenated compounds represented 49.36% of the total constituents while the non-oxygenated compounds were 37.84%. The molluscicidal activity of the volatile constituents was evaluated.

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A-02

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *OCIMUM BASILICUM* GROWING IN KUWAIT

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The family Lamiaceae comprises several genera, the members of which are known for their content of essential oils of economic significance in pharmaceutical, cosmetic and food industries. The genus *Ocimum* includes a number of species which have been the subject of chemical and bioactivity studies. *Ocimum basilicum* L. (Sweet basil) is essentially known as a spice and as a source of essential oil, being cultivated in the Mediterranean area and in the islands of the Indian Ocean[1]. Sweet basil leaf is traditionally used for the symptomatic treatment of gastrointestinal disturbances. Essential oils from *O.basilicum* find outlets in food and perfume industries.

Studies dealing with the chemical composition of essential oils of *O.basilicum* from different geographical sources revealed several chemotypes[2-6]. The main chemotype from Reunion Island, Comoro Islands and Madgashcar contains methyl chavicol, alongside with small amounts of cineole, fenchol, linalool and methyl eugenol. Another chemotype, characteristic of Southern Europe and Egypt, produces an essential oil with high levels of linalool. Chemotypes with methyl cinnamate and linalool as well as chemotypes with methyleugenol are also known. It is the aim of this work to study the chemical composition of the essential oils from the leaves and flowers of *O. basilicum* grown in Kuwait.

The hydrodistilled essential oils prepared from the leaf as well as the flower of *O. basilicum* grown in Kuwait were analyzed by capillary gas chromatography coupled to mass spectrometry. The different oxygenated and non-oxygenated mono- and sesquiterpenoids as well as aromatic compounds were identified by their retention times and mass spectra which were compared with published data. Quantification was based on peak area integration. The composition of the essential oil of *O. basilicum* grown in Kuwait was found different from Basil oils from other geographical sources. While the leaf oil contained 92.8% anethole, the flower oil contained only 16%. On the other hand, the flower oil contained 58% methyl cinnamate, while being 2.4% in the leaf oil. *O. basilicum* growing in Kuwait can be defined as an anethole/methyl cinnamate chemotype.

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A-03

ESSENTIAL OIL OF *OLIVERIA DECUMBENS* VENT. GROWN IN IRAN

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The genus *Oliveria decumbens* Vent, family Umbelliferae, include only 1 species in Iran.It is located on tropical regions of some parts of Iran[1,2]. A literature search reveals that the oil of *O. decumbens* has not been the subject of previous study.The flowers and involucres of the plant , after hydrodistillation in a clevenger apparatus for 3h gave the corresponding colourless oils in a yield of 5.8%(W/W). The oil was analysed using a GC/MS (Varian 3400) and 14 components in amounts greater than 0.1% were identified which constituting 100% of the oil. The major components being carvacrol (29%), thymol (28.1 %), γ-terpinene (20.5 %), p-cymene (15.4%) and myristicin (2.1%).

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A-04

ISOLATION OF GENES INVOLVED IN BIOSYNTHESIS OF GERMACRENE D ENANTIOMERS FROM *SOLIDAGO CANADENSIS*

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Germacrene D is a sesquiterpene common in the essential oils of many higher plants. While in most higher plants germacrene D is present as its (-)-enantiomer [1], *Solidago canadensis* and other *Solidago* species are exceptional by producing both enantiomers in large amounts. As shown in prior investigations *S. canadensis* possesses two individual germacrene D-synthases catalyzing the enantiospecific formation of (+)- or (-)-germacrene D from the common precursor farnesyl diphosphate [2,3].

Two sesquiterpene synthase genes were isolated by polymerase chain reaction (PCR) from *S. canadensis* cDNA using degenerated primers designed against conserved areas of known sesquiterpene synthase genes. To obtain the full-length cDNAs rapid amplification of cDNA ends (RACE-PCR) was performed. Insertion of the genes into the pET11d expression vector and heterologous expression in *E. coli* BL21(DE3) will be performed to show their activity.

The intention of isólating and cloning the gene is to obtain the enzymes in a reasonable amount necessary for the investigation of the 3D-structure of the enzymes and of their catalytic site by X-ray crystallography.

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A-05

THUJONELESS ABSINTHIUM FROM IRAN

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Four samples of *Artemisia absinthium* from two different regions of Damavand mountain (north of Iran) before and after flowering stage were collected and identified. The oils of aerial parts were isolated by hydrodistillation method and the components separated and analyzed under similar conditions by means of GC and GC/MS and identified using both MS spectra and Kovats indices.

Plant sample collected from north of Damavand before flowering had 0.4% essential oil and lacking thujone, whereas the one after flowering from the same region had 0.25% oil with 28% thujone. The samples from south part of Damavand before and after flowering had 1% and 0.45% oil respectively and lacking thujone.

This shows that the amount of thujone in absinthium which is responsible for the unwanted effects of absinthinism might change with the weather and flowering condition of the plant growth.

A-06

Some Hydrocarbon Sesquiterpenes of the Essential Oil of *Laggera tomentosa* from Ethiopia

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As part of our continuing investigation on the essential oil plants of Ethiopia [1-3], we report now the identification of some sesquiterpene hydrocarbons from the essential oil of *Laggera tomentosa* (Asteraceae) which is endemic to Ethiopia. Germacrene D, bicyclogermacrene, γ-curcumene, ar-curcumene, β-bourbenene, and α-copaene have been isolated and characterized from the oil. The compounds were isolated by high vacuum micro-distillation and flash chromatography. The structure elucidation was carried out using MS, Kovat's retention indices, 1D and 2D NMR data. In addition, β-caryophyllene, longifolene, α-cedrene, aromadendrene, and caryophyllene oxide have been identified by peak enhancement and/or GC-MS. Germacrene D (5 %) is the major sesquiterpene of the oil. A literature survey indicated that the essential oil of this plant has been investigated for the first time [1].

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A-07

The Essential Oil and Polyacetylenes From *Sciadophyllum pulchrum* Hort. (Araliaceae)

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Sciadophyllum pulchrum Hort. is a green shrub belonging to the Araliaceae family with digitately composed leaves. The genus *Sciadophyllum* comprises about 25 species, which are native of tropical America[1].

We have analysed the essential oils obtained from different parts of *Sciadophyllum pulchrum* collected from Orman botanical garden in Cairo by GC, GC/MS, TLC, HPLC and NMR spectroscopic techniques.

In the herb oil of the plant mono- and sesquiterpene hydrocarbons were the most abundant constituents with limonene (22%), myrcene (20.4%) and β-sesquiphellandrene (11.3%) being the most prominent components. Differentiation of the plant material into fruits, stalks and roots yielded quantatively divergent results. In the fruit oil relatively high amounts of β-sesquiphellandrene (18.8%) in addition to the before mentioned components were detected. The monoterpene hydrocarbons α-pinene and β-pinene exhibited the highest proportion in the essential oil from the roots (17.4 and 31.4%, respectively) which are present in the fruit oil only in small amounts (1.7 and 1.3%, respectively). The highest concentration of the monoterpene hydrocarbons limonene (29.8%) and myrcene (23.6%) were detected in the stalk oil.

In addition, the root oil from *Sciadophyllum pulchrum* proved to contain a small proportion of the polyacetylene derivative falcarinol, which is a common constituent of many species of the Araliaceae plant family [2] . We, therefore, decided to investigate this type of constituents in more detail with TLC, HPLC and spectroscopic techniques. From a root extract four polyacetylenic compounds were isolated and identified by MS, 1D- and 2D-NMR spectroscopy as falcarinone, falcarinol, falcarindiol and heptadeca-1,8-diene-4,6-diyne-3,10-diol, a constituent which was recently isolated from *Eryngium campestre* [3].

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A-08

QUALITATIVE AND QUANTITATIVE DETERMINATION OF ESSENTIAL OILS FROM SEED VESSELS OF *DICTAMNUS ALBUS* L.

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Dittany (*Dictamnus albus* L.) is an aromatic plant occuring in many regions of the world. Active constituents (show) several complex pharmacological action. Systematical investigations on phytochemical composition, biology of growth of dittany have been conducted by us for three years [1, 2, 3]. In this paper we have been dealing with the composition of the aerial parts of dittany.

The content of the essential oil from seed vessels of *Dictamnus albus* L. cv. *Rosa Purple* and cv. *Albiflorus* was estimated using the methods described in Polish Pharmacopoeia IV [4]. The essential oils were obtained by: I - steam-water distillation, II - steam-water distillation with addition of m-xylene, III - steam-water distillation with addition of m-xylene and 20% H_2SO_4. The percentage content of essential oils determined by methods mentioned above was: for cv. *Rosa Purple* I - 0.08%; II - 0.09%; III - 0.12%; and for cv. *Albiflorus*: I - 0.12%; II - 0.12%; III - 0.16%.

Qualitative and quantitative determination of the compounds contained in essential oils was made using GC/MS method. The main components in both varieties were: limonene (from 33.8% to 50.8%), terpinolene (from 22.0% to 27.6%) and myrcene (from 18.1% to 21.0%). After acidic hydrolysis (method III) additional compounds were ascertained: 2-ethyl tetrahydro-2,6,6-trimethyl-2H-pyrane, 1,4-cineole+α-phelandrene, p-cymene, 1,8-cineole, 2-methyl-6-methylene-7-octen-2-ol, 1-terpineol, cis-terpineol and α-terpineol.

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A-09

CHEMICAL POLYMORPHISM OF THE ESSENTIAL OILS FROM *THYMUS CAESPITITIUS* GROWN ON THE ISLANDS PICO, FAIAL AND GRACIOSA (AZORES)

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Thymus caespititius is a Lamiaceae species characteristic of Atlantic wet zones; it is endemic of the NW Iberian Peninsula, and of the Madeiran and Azorean archipelagos. Samples of the aerial parts of the plant, collected during its flowering period on the islands Pico, Faial and Graciosa (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 (>5% 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 Pico, Faial and Graciosa.

Island		**Pico**				**Faial**			**Graciosa**			
Population		**P1**	**P2**	**P3**	**P4**	**F1**	**F2**	**F3**	**G1**	**G2**	**G3**	**G4**
Altitude (m)		500	1200	650	700	230	300	700	250	350	125	300
Components (>5%)	*p*-Cymene	5.5	4.0	4.2	4.5	5.0	9.6	5.1	9.5	4.4	6.6	2.6
	γ-Terpinene	2.8	2.7	2.1	2.7	2.9	3.1	3.0	8.3	6.8	4.2	4.4
	α-Terpineol	4.0	2.7	0.4	4.6	11.8	6.3	5.3	15.3	27.2	32.5	36.7
	Thymol	9.7	2.4	1.4	12.1	0.7	4.6	0.1	0.1	0.6	1.1	1.4
	Carvacrol	48.1	56.8	48.5	44.9	51.1	51.3	54.0	35.0	32.0	24.3	3.3
	Carvacryl acetate	9.1	15.4	17.0	1.6	3.2	3.2	4.9	4.2	1.6	2.5	0.4
	T-Cadinol	2.3	2.2	3.3	2.5	4.1	3.0	4.4	4.3	6.4	7.0	11.2
Total % identified		95.4	95.8	88.3	86.5	91.9	95.2	90.0	93.2	94.8	92.4	82.4
Oil yield (%)		0.5	0.7	0.2	0.5	0.8	0.5	0.3	0.4	0.4	0.6	nd

nd = not determined

The essential oils from the eleven populations, analysed by GC and GC-MS, were dominated by their monoterpene fraction (55-90%) that consisted mainly of oxygen-containing compounds (44-79%). The sesquiterpenes represented an important fraction of the oils from the populations grown on Graciosa (14-28%). In contrast, this fraction was rather small in the oils from the populations grown on Pico and Faial (6-11%). Despite this difference, oxygen-containing compounds (4-18%) dominated the sesquiterpene fraction of all oils. Cluster analysis of all identified oil components grouped the oils into three main clusters that corresponded with their main components: carvacrol, carvacrol/α-terpineol and α-terpineol/T-cadinol. The essential oils from the populations studied showed a clear chemical polymorphism that, in some cases, was more evident among the populations growing on the same island than among those from different islands.

Acknowledgments: This study was partially funded by the Fundação para a Ciência e Tecnologia (FCT) under research contract PRAXIS/P/BIA/11054/1998.

A-10

ELECTRONIC NOSE AS A FAST SCREENING TOOL POSSIBILITIES AND LIMITS IN THE ANALYSIS OF ESSENTIAL OILS AT THE EXAMPLE OF OREGANO OIL

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The quality control of food and cosmetic products, of products of the packing industry as well as fire and contaminants' control are possible industrial applications of the novel sensor system, also called electronic nose. Different sensor technologies and sampling techniques are used in various commercially available sensor systems.

Non-selective, yet different sensitive sensor elements detect the composition of volatile compounds in the headspace and provide a signal pattern (fingerprint). The signal pattern is evaluated using methods of pattern recognition (PCA, ANN).

The headspace phase of the sample is injected into the sensor cell without gas chromatographic separation thus facilitating a high sample throughput.

Due to the high antimicrobial potential the very popular oregano oil which is used for food flavouring is gaining in importance. Oregano oils with a high carvacrol content (>65 %) and a very low thymol content (1-5 %) have a stronger effect as biologically active components than oils with a balanced thymol/carvacrol ratio.

With the sensor system SAM Select of the company RST Rostock (6 QMB's) tests were performed

- on the classification of preparations of identical matrix and different concentrations of oregano oil, and
- on the classification of oregano oils of different composition.

A-11

(+)-MENTHOFURAN BIOSYNTHESIS IN THE GENUS *MENTHA*: CLONING AND HETEROLOGOUS EXPRESSION OF (+)-MENTHOFURAN SYNTHASE FROM PEPPERMINT

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(+)-Menthofuran is a commercially undesirable monoterpene oxide constituent of mint species essential oil. In peppermint (*Mentha x piperita* L.), high amounts of this monoterpene cause a decrease of the commercial value of the oil, with great economical loss for farmers. From a biochemical point of view, we have demonstrated that the oxidation of (+)-pulegone in mint involved a cytochrome P450 hydroxylase. On the basis of these considerations a search for (+)-menthofuran synthase gene in a peppermint oil gland cDNA library was initiated. A putative full-length clone was isolated and successfully expressed in *Saccharomyces cerevisiae* and *Escherichia coli..* Functional protein overexpression in yeast was lower than in *E. coli:* 20 pmol mg prot^{-1} and 220 pmol mg prot^{-1}, respectively. These results were confirmed by the appearance of an intensely stained band at about 55 kDa in *E.coli* membranes analyzed by SDS-PAGE.

In samples derived from both expression systems, incubated according to the standard cytochrome P450 assay, analyzed by capillary GC and GC-MS, (+)-menthofuran was detected as the sole reaction product with the same retention time and mass spectrum of the standard. Full-length sequencing reactions were run across original plasmids and expression vectors and complete clone sequecing resulted in a 1479 bp open reading frame encoding a deduced protein of 493 aa and showing all the elements related to cytochrome P450 superfamily.

One of the agronomic applications of this research could be the repression of the gene responsible for the conversion of (+)-pulegone to (+)-menthofuran, with a resulting improvement of mint essential oil quality for flavouring purposes. Besides, (+)-menthofuran, like (+)-pulegone, is also known owing to its hepatotoxic properties.

A-12

THE HEADSPACE AND IMMERSION TYPE SPME TRAPPING OF VOLATILES IN THE AROMATIC WATER OF *ORIGANUM ONITES* L.

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Origanum (Kekik) species are used in food industry as spice and flavouring agent, and *Origanum* oils and their aroma chemicals are used in perfumery and food industry as raw material.

The distilled water of *Origanum* oil production left out of the removal of oil is used as aromatic water (or hydrosol). It is called "Kekik Suyu" in Turkish meaning *Oregano* water. It is widely traded and used orally in Turkey for prophylaxy or as a remedy for several diseases, mainly gastrointestinal disorders. It is claimed to reduce blood sugar and cholesterol levels.

In order to analyze the volatiles dissolved in Kekik Suyu, immersion and headspace SPME techniques were employed in combination with a GC/MS system. The results will be presented in a comparative manner.

A-13

SCREENING AND DEVELOPMENT OF NEW AROMA CHEMICALS FROM GENETIC RESOURCES OF GENUS OCIMUM

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Among the aromatic genera in the family Lamiaceae, the genus Ocimum, which comprises 176 species, a number of them are aromatic in nature, although overwhelming number from tropical and sub-tropical Asia and Africa have so far remained unexplored. A limited number of species which have been screened and evaluated for essential oil constituents, revealed heterogenous nature of aromatic compounds. They mainly comprise the terpenes, sesquiterpenes, alcohol, esters, aldehydes, ketones, phenols, ethers etc.. Among non-terpenic constituents, eugenol and thymol that occur either singly or both in *O. gratissimum* L. and *O. viride* Willd. have pharmaceutical and flavouring value. These phenols are not species specific and have been reported from other Ocimum species. It is logical to classify different types of essential oils by their chemical composition than their botanical origin. Screening and evaluation of basil essential oil has led to evolve useful strains which has worldwide acceptability and utilisation. *O. sanctum* is a spectacular example of having age old sanctity as a panacea for different ailments which now includes anti-stress activity and anti-viral hepatitis besides haven proven efficiacy in Ayurvedic System of medicine having based their effect on essential oil constituents. Through genetic upgradation, new sources of aroma chemicals have been developed in the evolved plant types rich in elemicin, methyl cinnamate, linalool and citral. Farnesol which formed the starting material in vitamin E synthesis got located as a minor constituent in the essential oil of *O. adscendens* Willd.

A-14

NECESSITY OF CHIRAL CAPILLARY COLUMNS FOR GC ANALYSIS OF PHARMACOPOEIA ESSENTIAL OILS

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Adulterations in essential oils have become more and more skilled during the last decades. As they are used as pharmaceuticals, it cannot be accepted that synthetic compounds are added. Therefore for the essential oil monographs of the German- (DAB 1999), as well as the European Pharmacopoeia (Ph. Eur.) analytical methods for authenticity and quality control need to be developed, since the existing ones, including gas chromatography on achiral phases, often fail in detecting these adulterations. For this purpose gas chromatography on chiral capillary columns can be applied for a large number of essential oils.

A good example is the essential oil of *Carum carvi* L. In genuine caraway oils not more than 1.0 % of the (-)-enantiomer, but about 50 to 65 % of the (+)-enantiomer are contained. To some commercial samples (-)-carvone, the main component of spearmint oil, had been added to increase the total amount of carvone.

In lavender oil, the essential oil of *Lavandula angustifolia* MILLER, linalylacetate and linalool, the main constituents, both appear in high enantiomeric purity. The analysis of several lavender oils on a modified β-cyclodextrin phase showed that 30 % of the examined samples had been adulterated by adding racemates of these substances.

A further example to demonstrate the necessity of chiral analysis, is bitter-orange-flower oil of *Citrus aurantium* L. subsp. *aurantium*. The enantiomers of the main components limonene and linalool are contained in defined ratios. 25 % of the analysed commercial oils showed deviations and can therefore be no genuine oils.

The analysis on a polyethylenglycol phase, as prescribed in the Ph. Eur. Supp. 1998 for lavender and bitter-orange-flower oil, could not lead to appropriate results and did not result in this conclusions.

δ-TERPINYL ACETATE – ISOLATION AND SYNTHESIS OF A NEW NATURAL COMPONENT

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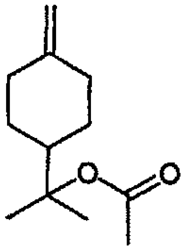
Laurus nobilis (laurel leaf oil) is beside *Persea americana* (avocado oil) and *Cinnamomum zeylanicum* (cinnamon oil) a commercially important representative of the Lauraceae family. Dried or fresh leaves of *Laurus nobilis* L. are commonly known as household culinary herb while the essential leaf oil is mostly used for flavors and fragrances. Laurel leaf oil has a fresh-green, strong, sweet-spicy, aromatic-eucalyptaceous odor and a fresh, delicate spicy, camphoraceous-eucalyptaceous taste.

We report in our poster about the isolation of a new natural component from the essential leaf oil of *Laurus nobilis* - δ-terpinyl acetate [1].



This monoterpene acetate has not yet been, to the best of our knowledge, isolated nor identified as a natural product. In addition we present a detailed analysis of laurel leaf oil.

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A-16

ESSENTIAL OILS OF BRYOPHYTES OF THE *MNIUM* AND *PLAGIOMNIUM* SPECIES: IDENTIFICATION OF BUTENOLIDES AND SESQUITERPENE HYDROCARBONS

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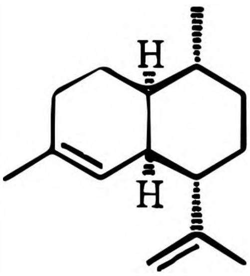
Our current interest concentrates on volatiles of mosses of the genus *Mnium* and *Plagiomnium*. We investigated the species *Mnium hornum, M. marginatum, M. spinulosum* and *M. stellare*, collected at several sites in Europe. The genus *Plagiomnium* includes 20 species spread mostly on the northern hemisphere[1]. Among others, we investigated the species *P. undulatum* collected in various places in northern and southern Germany and in Sicily. In contrast to previous assumptions not only liverworts (Hepaticae) but also mosses (Musci) contain a large variety of terpenoid metabolites. In *M. hornum* the sesquiterpene hydrocarbon (+)-muurola-4,11-diene (**1**) is the most abundant constituent of the essential oil. The (-)-enantiomer of this compound has before been identified as a minor constituent of the essential oil of *Amyris balsamifera* [2]. In addition β-caryophyllene, β-barbatene, *ar*-, β- and γ-curcumene, α- and β-selinene were identified in the sesquiterpene fraction. Also *M. marginatum, M. spinulosum* and *M. stellare* are rich in sesquiterpene hydrocarbons. The volatile fraction of *Plagiomnium undulatum* is dominated by the butenolide **2a** and by a sesquiterpene hydrocarbon of unknown structure. **2b** is present as a minor component.

The isolation of the compounds of interest was achieved by preparative gas chromatography. Structural investigations were performed by 1- and 2-dimensional NMR investigations in conjunction with high resolution mass spectrometry, GC/MS and enantioselective gas chromatography.



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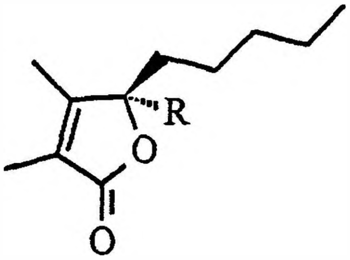

2a, R = CH_3
2b, R = H

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A-17

ESTRAGOLE CONTENT IN BASIL CULTIVARS GROWN IN STYRIA

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Among the genus *Ocimum* (Lamiaceae), which is variing largely both in morphology and chemotypes, *Ocimum basilicum* L. (basil, sweet basil), is the economically most important species which is used not only in food industry and perfumery but also in folk medicine for its carminative and stomachic properties.[1, 2] However, high estragole content in sweet basil oil gave reasons for concern because of its possible cancerogenic potential.[3] Its therapeutic use was not recommended.[4] Yet, depending on type of cultivar and growing conditions essential oil composition of basil is known to show great differences.

Six basil cultivars were grown under uniform conditions in Styria (Landwirtschaftliches Versuchszentrum Steiermark, Wies, Austria). The aerial parts (leaves and flowering tops) were harvested at two times (first cutting at early blooming around begin of July, second cutting around mid August). The hydrodistilled essential oils obtained from fresh and air dried plant material were analysed by GC-MS.

Oils obtained from fresh plant material could be characterised as follows:

3 oils (*O. basilicum* <Genoveser>, <Dark Opal>, <Latino>) showed only very low estragole levels (<0.1 %) but higher amounts of eugenol (23.7 - 35.5 %). Linalool content was similar to or higher than eugenol levels. 1,8-Cineole content varied between 0.4 - 6.0 %. Oils obtained from 2 cultivars (*O. basilicum* <Mittelgrossblättriges>, <A Folia di Lattuga>) represented typical European type sweet basil oils and contained 31.7 - 43.2 % estragole and 30.8 - 40.5 % linalool. 1.8-cineole ranged from 2.7 - 4.9 %. *O. basilicum* <Metalica> contained 41.7 - 49.5 % linalool, a relatively low estragole content of 7.7 - 15.7 % but relatively high amounts of eugenol (9.6 - 14.7 %).

Estragole and eugenol content decreased during air drying of the plant material while 1,8-cineole and linalool content tended to increase.

The finding of basil cultivars with very low estragole levels is commensurate with data obtained by Marotti *et al.* [5] and data summarized in [2]. Restrictions of therapeutic use as suggested in [4] do not seem to be applicable to oils obtained from these cultivars.

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A-18

Comparative Study of Different Types of Poppy Seeds Headspace by Charcoal Trapping and SPME-, GC-FID and GC-NDP Detection

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The poppy is said to be the oldest medicinal plant, and still today, in Europe alone, many thousands of ha are cultivated for the production of its edible and oil producing seeds. Although numerous investigations have been carried out with respect to the constituents of poppy capsules and seeds, very little is yet known about the volatile components of poppy seeds, which contribute to their characteristic odour.

In this study, the distribution of components in the headspace of different types of poppy seeds – blue, white and grey – was compared using charcoal trapping/solvent extraction or SPME, and gas chromatographic detection by FID and NDP, respectively. The detected peak distribution („profiling“) was evaluated by means of Principal Components Analysis (PCA) and by supervised neural network (rel. inf. of 20 peaks were used) enabling, in all cases, the separation of poppy seeds types used.

A-19

New Strategies on Fragrance and Aroma Research

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Although the aroma impression plays an important role in nutrition science and food chemistry, the quantitative relationships between the molecular structure of flavor compounds and the intensity and quality of the aroma perception was not investigated in detail for many years. Moreover, the structure of the receptor proteins is not known up to now, which prevents an understanding of the physico-chemical mechanism of the aroma or the odor recognition. Quantitative Structure Activity Relationships (QSAR) techniques are widespread and rather successful methods in modern drug design and, therefore, these methods should also lead to more extended information in aroma and fragrance chemistry. In the presented studies, structure-flavor relationships on pyrazine-based flavor molecules are analysed by a set of different methods: QSAR investigation with multiple linear regression (MLR) and with partial least square analysis (PLS); Comparative Molecular Field Analysis (CoMFA) as well as Holographic QSAR (HQSAR) methods and Artificial Neural Networks (ANN) method.

It can be shown, that the obtained models are both satisfactory in statistical significance and predictive ability. Moreover, a better knowledge of the interaction of the aroma molecules with the corresponding receptor site can be obtained from the parameters of the models.

A-20

INSECTICIDAL ACTIVITY AND CHEMICAL COMPOSITION OF ESSENTIAL OILS OF *OCIMUM GRATISSIMUM* AND *XYLOPIA AETHIOPICA* FROM CAMEROON

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Insects attack are the major causes of losses in stored products. To prevent these post harvest losses synthetic products have been developed for crop protection. However, their insecticidal activity has an extension to the consumer on the other side, many plant extracts have the potential to protect crops against the insects' damages. Essential oils are natural resources for fighting against pests in general [1] and particulary against insects attacking stored grains.

Ocimum gratissimum (Lamiaceaea) leaves and *Xylopia aethiopica* (Annonaceae) fruits are potential sources of essential oils in Cameroon [1-3]. These essential oils from the 2 species, obtained by hydrodistillation were analyzed by GC/FID and GC/MS, and also tested on *Sitophilus zeamais* for their insecticidal activities.

The *S. zeamais* is a weevil major pest for the stored maize [4]. Insects used for the tests were collected on infected stocks of maize. Insecticidal activity has been tested by putting 20 adult insects of *S. zeamais* with 20g of maize grains powdered with mixtures of essential oil and kaolin (5% and 10%).

The oil of *X. aethiopica* contains more than 100 identified compounds, and the main components are β-pinene (18%), terpinen-4-ol (8.9%), sabinene (7.2%), α-terpineol (4.1%), 1,8-cineole (2.5%) myrtenol (2.4%) and kauran derivatives (4.2%). The essential oil of *O. gratissimum* contains about 40 compounds, dominated by thymol (47.7%), γ-terpinene (14.3%), p-cymene (8.5%), limonene (2.6%), terpinolene (2.3%) and 1,8-cineole (1.6%).

Essential oils of *O. gratissimum* and *X. aethiopica* ensure a control of 66% and 73% of the *Sitophilus zeamais* population respectively after 4 days. A direct application of the essential oil on the pest killed by knock down effect 80% of the weevil population in the case of *O. gratissimum* and 90% in the case of *X. aethiopica* after 48 hours. These studies underline the potential of these essential oils for the control of the *Sitophilus* pest in stored maize.

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A-21

ANALYSIS OF THE ESSENTIAL OIL OF THE ROOTS OF THE MEDICINAL PLANT *KAEMPFERIA GALANGA* L. (ZINGIBERACEAE) FROM SOUTH-INDIA

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The essential oil of the roots of *Kaempferia galanga* L. (Zingiberaceae) from South-India was analyzed by gas chromatographic-spectroscopic methods (GC/FID and GC/MS) to identify those compounds responsible for the reported biological activities as well as for the interesting odor of this medicinally used oil. More than fourty constituents could be identified. Ethyl-trans-p-methoxycinnamate (52.5%), ethyl-trans-cinnamate (26.3%), pentadecane (4.9%) and 1,8-cineole (2.4%) are the main compounds of this essential root oil sample. Further mono- and sesquiterpenes (e.g. borneol, p-cymene, δ-3-carene and α-terpineol as well as α-gurjunene, germacrenes, cadinenes and caryophyllenes) resp. aliphatic hydrocarbons and alcohols are dominating in this essential oil of roots of *K. galanga*. The effects of these constituents for the use in medicinal applications as well as their olfactoric properties are discussed.

A-22

STUDIES ON THE RADICAL SCAVENGING ACTIVITY OF THE ESSENTIAL OIL OF *PEUCEDANUM OSTRUTHIUM* L. KOCH

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The essential oil of the root of *Peucedanum ostruthium*, masterwort (Apiaceae), was tested for its possible radical scavenging activity. Tests started with a TLC screening using two spraying reagents for antioxidants. In the so called diphenylpicrylhydrazyl-assay[1] the essential oil could act as a non-specific donator for hydrogen atoms or electrons (IC_{50} value was 4.85 µl/ml). Furthermore the essential oil could inhibit the non-enzymatic lipid peroxidation in bovine liposomes[2] with an IC_{50} of 0.64 µl/ml. It possessed also $^{\cdot}OH$ radical scavenging abilities when tested in the deoxyribose-degradation-assay[3]. Concentration for 50% inhibition was 0.82 µl/ml. This method was adopted in different ways to assess site-specific actions of the essential oil, like possible prooxidative effects.

To measure the radical scavenging properties of the oil against O_2^- the ability to inhibit pyrogallol-autoxidation[4] was checked.

The compound carvacrol, which is likely to be responsible for the observed antioxidant effect of the volatile oil, was tested in the different assays too.

Additionally the qualitative and quantitative composition of the essential oil was analysed by means of GC-MS.

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A-23

COMPOSITION OF THE ESSENTIAL OIL AND MICROMORPHOLOGY OF *TEUCRIUM POLIUM* SUBSP. *CAPITATUM*, FROM PORTUGAL

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Continuing our research on composition of essential the oils of spontaneous plants from Portugal we undertook the study of *Teucrium polium* L. subsp. *capitatum* (L.) Arcangeli, spontaneous from the centre of Portugal. Now, we report on both, the chemical composition of the essential oil and the micromorphology of the secretory structures.

The essential oils of four samples from different locations, Fonte Coberta - Serra de Sicó, Rabaçal - Serra de Sicó and Portunhos - Cantanhede (two samples) were isolated by hydrodistillation in a Clevenger type apparatus and analyzed by GC and GC-MS.

Identification of components was made by comparison of GC retention indexes and mass spectra with corresponding data of authentic compounds or components from reference oils.

In total, ninety-eight compounds were identified accounting from 83.8% to 90.7% of the essential oil. Relevant qualitative and quantitative differences among the composition of the four samples were observed. One of the samples from Serra de Sicó (Rabaçal) is characterised by a high content of oxygen containing monoterpenes (33.3%), being isomenthone (7.7%) the major constituent. This compound, as well as menthol (2.1%) and pulegone (1.4%), was not detected in all other samples. Nevertheless in the oil of the other sample from Serra do Sicó (Fonte Coberta), monoterpene and sesquiterpene hydrocarbons (43.9% and 23.2%, respectively) are dominant, being α-pinene (7.7%), sabinene (11.2%) and β-pinene (10.3%) the major constituents. Oils of the two samples from Portunhos (Cantanhede) are characterised by high content of sesquiterpene hydrocarbons (23.0% and 32.0%) and oxygen containing sesquiterpenes (39.7% and 23.4%). τ-Cadinol (24.1%) and α-cadinol (9.8%) are the major constituents in one sample while δ-cadinene (7.5%) and *trans*-caryophyllene (5.4%) are the major ones in the other sample. Once ecological and edaphic features in these areas are similar this chemical polymorphism is perhaps due to genetic factors.

The micromorphology and distribution of the trichomes on the vegetative and reproductive organs were studied by SEM. The non-glandular trichomes are multicelullar and arborescent and the glandular ones are of two types (capitates and peltates) differently distributed on the abaxial and adaxial surfaces.

A-24

AUTHENTICITY ASSESSMENT OF LAVENDER ESSENTIAL OIL

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Lavender (*Lavandula angustifolia* Mill.) oil is highly prized and today it continues to grow in popularity; hence it is subject to adulteration. Forty commercial samples of lavender oil and twelve pure organically grown and distilled lavender oils were analyzed for purity. The tests employed were, refractive index, optical rotation, specific gravity, GC/MS using DB-1 column, GC/FID using DB-5, DB-WAX and RT-βDEXse columns. Based on the testing, by the above methods, two of the oils, out of the forty commercial oil samples were found to be heavily adulterated, and were further characterized by Radiocarbon (^{14}C) and Stable Isotope Ratio ($\delta^{13}C$ and δD) analyses. GC-Chiral analysis was found to be a very reliable method to detect adulteration with linalool and linalyl acetate. The Radiocarbon analysis confirmed the adulteration of lavender oil with synthetic linalool and linalyl acetate.

MACROSPORONGENESIS AND DEVELOPMENT OF FEMALE GAMENOPHYTE IN SALVIA NEMORORSA L. (LAMIACEAE)

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S.nemorosa is a well known plant in the Bulgarian folk medicine with wide application against blood expectoration, abdomen ache, wood and furuncles. That is why the study of this plant was focused mainly on the essential oil quality and quantity. To the best of our knowledge, based on the available literature, the female generative sphere of *S.nemorosa* was not investigated. *S.nemorosa* together with *S.officinalis* are known as two representatives of the genus *Salvia* in which male sterility of unestablished nature is known. Male sterility was found also in the Bulgarian representatives of *S.nemorosa*. The aim of this study is to find out if there exist accordance between the sterility of male and female generative organs in male sterile Angiosperms.

The well developed ovule is anatropous, tenuinucellate and unitegmig. The archesporium being hypodermal consists mainly of one cell, but one or two cells also exist in some ovules. The sporogenous tissue in the ovule is formed considerably later than the archesporium in the anthers. The archesporial cell functions directly as a megaspore mother cell (MMC) without building of covering cells. The MMC undergoes meiosis giving rise to a linear tetrad of megaspores. The chalazal megaspore functions as an embryosac mother cell and develops successively into a 2-4 and 8-nucleate embryo sac (ES). Thus ES-formation is of the *Polygonum*-type like all other, studied representatives in *Lamiaceae*. The innermost cell layer of the single integument differentiates in to the endothelium (integumentary tapetum) after the uninucleate stage of ES, or even somewhat later.

The mature ES consists of an egg apparatus (an egg cell and two synergids, two polar nuclei and 3 antipodial cells). First of all the egg apparatus elements differentiated in the 8-cellular ES. The egg-cell is pear-shaped or paddleformis, with big nucleus and clearly seen vacuole. Most often it is in direct contact with the two polar nuclei and after their fusion also with the central cell of the ES.

The synergids are smaller than the egg-cell, their vacuolization is not always distinct and they degenerate before fertilization. The 3 antipodial cells are small, uninuclear and degenerate in the course of porogamous fertzation.

Fertilization of the studied taxon is porogamous. Syngamy and triple fussion take place more or less simultaneously.

During the development of the female gametophyte markedly expressed degeneration processes were found, typical also for other studied male sterile plants.

A-26

SCREENING OF BIOTRANSFORMATION PRODUCTS OF CARVONE ENANTIOMERS BY HEADSPACE-SPME/GC-MS

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In the course of our continuing work on the microbial transformation of aromatic monoterpenes by microorganisms, the biotransformation of *d*-(+)- and *l*-(-)-carvone was carried out. The metabolites formed during microbial transformations were screened using a simple, rapid and efficient technique: Headspace-SPME/GC-MS. The results as well as the application of this technique will be described.

A-27

Micro-distilled Volatile Compounds from *Ferulago* Species Growing in Western Turkey

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The genus *Ferulago* (Umbelliferae) is represented in the Flora of Turkey by 30 species, 16 of which being endemic in Turkey. The essential oils obtained by micro-distillation from fruits of 13 species of *Ferulago* using an Eppendorf MicroDistiller were analysed by GC/MS. A summary of the results is as follows:

***Ferulago* species**	**Collection Place**	**Main Component**
F. asparagifolia	Aydin: Ephesus	2,3,6-Trimethylbenzaldehyde (42.03 %)
*F. aucheri**	Mugla: Sandras Mountain	α-Pinene (35.95 %)
*F. aucheri**	Manisa: Sipyl Mountain	α-Pinene (21.02 %)
F. confusa	Tekirdað: Hayrabolu	2,5-Dimethoxy-*p*-cymene (63.41 %)
F. galbanifera	Bilecik	*trans*-Chrysanthenyl acetate (17.20 %)
*F. humilis**	Mugla: Sandras Mountain	(*Z*)-β-Ocimene (31.86 %)
*F. idaea**	Balikesir: Kazdagý	*p*-Cymene (18.37 %)
*F. macrosciadia**	Balikesir: Kazdagý	Carvacrol methyl ether (52.24-78.11 %)
*F. mughlae**	Mugla: Köycegiz	α-Pinene (25.36 %)
*F. sandrasica**	Mugla: Sandras Mountain	α-Pinene (40.82 %)
*F. silaifolia**	Bursa: Mezitler	*trans*-Chrysanthenyl acetate (83.54 %)
F. sylvatica	Çanakkale: Can, Kazdagý	*p*-Cymene (45.82 %)
F. trachycarpa	Balikesir: Edremit	γ-Terpinene (27.75 %)

* Endemic Species

MD: Micro-distillation

A-28

ESSENTIAL OILS FROM BUDS OF *BETULA* SPECIES GROWING IN TURKEY

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The genus *Betula* (Betulaceae) is represented in Turkey by five species, one being endemic [1,2]. *B. browicziana* A. Güner, *B. litwinowii* Doluch., *B. medwediewii* Regel, *B. pendula* Roth and *B. recurvata* V. Vassil. were collected from North-Eastern provinces of Turkey. The buds were either hydrodistilled using a Clevenger type apparatus for 4 h or were subjected to Likens-Nickerson simultaneous distillation-extraction (SDE) method. The oils were analysed by GC/MS. Main components were detected as 14-hydroxy-β-caryophyllene and 14-hydroxy-4,5-dihydro-β-caryophyllene in all the oil samples. Oil yields and information on collection sites are given in the following Table.

Table Information of *Betula* Bud Essential Oils

Betula species	Collection Site	Altitude	Collection Period	Oil Yield* (%)
B. browicziana (E)	Rize:Camlıhemsin	1765 m	May, 1998	#
B. litwinowii	Artvin	2050 m	May, 1998	6.34
B. medwediewii	Rize:Camlıhemsin	1700 m	May, 1998	1.25
B. pendula	Erzurum	1800 m	May, 1998	3.82
B. recurvata	Rize:Camlıhemsin	1700 m	September,1998	#

***Yields are given on moisture free basis**
(E) Endemic
\# Likens-Nickerson SDE

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A-29

INFLUENCE OF WATER STRESS ON ESSENTIAL OIL CONTENTS AND COMPOSITIONS OF *SATUREJA LAXIFLORA*

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Essential oils, steam distilled from aerial parts of cultivated *Satureja laxiflora* in different drought stresses were analysed by GC/ MS. Results showed that the quality and quantities of oil produced was related to the different amounts of irrigation. Four water stress treatments, field capacity (FC), two moderat drought (66% FC) at two different stages (during the all of vegetative growth and at the flowering stages) and severe drought (33% FC) were imposed on potted samples. As the mean leaf water potential declined from –0.4 to –1.66 Mpa, the essental oil contents increased. Essential oil biosynthesis after moderate stress during the vegetative growth (LS1) had increased 6 fold more than the others treatments. Also this treatment had the highest effect on the quantity of some important compositions of essential oil such as carvacrol which is one of oil constitutes exhibiting the highest level of antibacterial activity. It was found in relatively high quantities (31%) as a percentage of total oil in this treatment but the α-thujene, α-pinene, β-pinene, myrcen, α-terpinen as a percentage of total oil increased as water stress increased (at severe drought). The amounts of irrigation effected on the rate of vegetative growth, fresh and dry matter production too.

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A-30

COMPOSITION OF THE ESSENTIAL OIL FROM *PLECTRANTHUS LAXIFLORUS* COLLECTED DURING THE FLOWERING PHASE

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The genus *Plectranthus* includes both aromatic shrubs and creepers that are currently used as ornamentals. *Plectranthus laxiflorus* Benth. is an erect to spreading aromatic herb, 70-150cm tall. This is one of the only two South African *Plectranthus* species which have the characteristic "Dutchman's pipe" corolla shape, but it can be easily distinguished from the other species, *P. petiolaris*, and from the other species of the genus, namely by the sharp citronellal-like scent, unlike any other South African species (1).
The aerial parts of *P. laxiflorus* were collected during the flowering phase at the Botanical Garden of Lisbon. The essential oils were isolated separately from flowers, leaves and stems, either by hydrodistillation for yield determination, or by distillation-extraction, for qualitative and quantitative analysis by GC and GC-MS.
All the oils showed a yellowish colour and were obtained in a yield <0.05%, Table 1. The oils isolated from both the flowers, leaves and stems were dominated by monoterpenes (64%, 60% and 59%, respectively), the monoterpene hydrocarbons amounting to 43%, 52% and 38% of the total oil, respectively. The identified sesquiterpenes attained 21% and 27% of the flower and leaf oils, respectively, and only 9% of the stem oil.
α-Phellandrene (13%), *trans*-β-ocimene (8%) and α-selinene (7%) were the main components of the flower oil, Table 1. α-Phellandrene (19%) was also the main component of the leaf oil, the second and third main components being germacrene-D (14%) and *trans*-β-ocimene (12%). The oil isolated from the stems was dominated by α-phellandrene, citronellol and *p*-cymene in similar relative amounts (9% each).

Table 1- Main components (>5%) identified in the essential oil of *P. laxiflorus* collected during the flowering phase.

Components	*Plectranthus laxiflorus*		
	Flowers	Leaves	Stems
α-Phellandrene	**13.1**	**19.4**	**9.4**
p-Cymene	2.3	2.9	8.8
β-Phellandrene	5.9	8.0	4.7
trans-β-Ocimene	**8.1**	**12.1**	1.5
γ-Terpinene	4.2	2.1	6.1
Citronellal	6.8	2.7	6.6
Citronellol	6.0	2.1	**9.0**
Germacrene-D	6.3	**13.5**	2.9
α-Selinene	**7.3**	5.4	1.2
Total % identified	85.2	87.0	70.4
Grouped Components			
Monoterpene hydrocarbons	42.7	52.0	38.2
Oxygen-containing monoterpenes	21.3	7.7	20.9
Sesquiterpene hydrocarbons	19.7	25.2	8.3
Oxygen-containing sesquiterpenes	1.3	1.6	0.9
Phenylpropanoids	0.1	0.4	0.0
Others	0.1	0.1	2.1

The composition of the essential oil of *P. laxiflorus* showed marked differences when compared with that of the *Plectranthus* species studied so far (2-6).

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A-31

BIOSYNTHESIS OF MONOTERPENOIDS IN BUCHU – *AGATHOSMA CRENULATA* (L.) PILLANS

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The essential oil of *Agathosma crenulata* (L.) pillans (synonym: *Barosma*) mainly consists of limonene, menthone, isomenthone, pulegone and isopulegone. The constituents responsible for the blackcurrant odour are p-menthane-3-one-8-thiol and its S-acetate.

In vivo feeding experiments with stable isotope labelled monoterpene precursors, headspace extraction and subsequent analysis of the essential oil by enantioselective multidimensional gas chromatography / mass spectrometry were investigated.

On the basis of these results, enantioselectivity and mechanistic aspects of the observed biosynthetic pathways were studied for the first time [1, 2].

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Volatile Constituents of *Phlomis olivieri* Benth.

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Phlomis olivieri Benth. (Lamiaceae) is herbaceous wild plant native to Iran[1]. However, no phytochemical study about this plant have yet been done. In the course of systematic investigation on the essential oils of *Phlomis* species, the constituents of the essential oil obtained by hydrodistillation of *Phlomis olivieri* Benth. were investigated by GC and GC-MS analysis. The oil contians over 39 components. The major components were β-caryophyllene (6.9%), germacrene-D (9.7%), hexahydrofarnesyl acetone (13.3%), spathulenol (11.4%) and caryophyllene oxide (5.3%).

The oil of *Phlomis olivieri* Benth. is characterized by a high content of sesquiterpens, wheras monoterpenes represent only trace amount of the oil.

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A-33

The Study of External Factors on Essential Oil in *Matricaria chamomilla L.*

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Plants were considered to have useful effects in pharmaceutical and cosmetical aspects from long time ago. One of the medicinal plants which has been used since historical era is German Chamomile (Matricaria chamomilla L.) that belong to compositae and is widely distributed in the world.

We studied samples of Hungarian, Pakestanian and Kazeroon (Iran) chamomiles. These plants were cultured in 2 climates (warm :Tehran and cold: Hamedan) and the contents of essential oils were extracted by steam distillation. The oil was investigated by capillary GC (9 A -Shimadzu) and GC/MS (Varian 3400), column DB-5, 30 m x 0.22 mm fused silica capillary column, film thickness 0.25 by temperature program from 40-220° C at a rate of 4° C/min (injector temperature: 220° C, carrier gas: helium). The results show that Hungarian chamomile had a high content of oil in warm climate and contains azulene, β-farnesene, camphene, trans-caryophyllene, α-cubene, α-bisaboloxide B, α- sinensal. This plant was selected for other experiments. The effects of soil texture, the content of N/P/K in soil, and IAA treatment were studied on Hungarian chamomile in warm climate. The results show that the best soil texture for chamomile growth was loam. In loamy soils time of flowering decreased to 29 days and flower production increased. By increasing the N/P/K content of soil, flowering was decreased. In treatment of IAA by increasing of auxin content, flowering time was shorter and essential oil content and number of oil conducts were increased. Study of antimicrobial effects of oil show that this material has effects on g+bacteria (such as staphilococus aureus) and hemolytic effects. Flower extracts prepared by propylene glycol were applied in shampoos and creams and gave good results.

A-34

DETERMINATION OF ESSENTIAL OILS BY MICRO-HYDRO-DISTILLATION VERSUS CONVENTIONAL HYDRO-DISTILLATION ACCORDING TO THE EUROPEAN PHARMACOPOEIA

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Using a micro-distillation technique in combination with capillary GLC, the determination of essential oils in drugs can be simplified for suitable medicinal plants as compared to the conventional distillation according to the European Pharmacopoeia. By means of the micro-distillation device shown in Figure 1, the volatile constituents of several medicinal plant drugs have been separated and analyzed by capillary GC [1,2]. In order to demonstrate the potential of the applied technique, the investigation of rosemary leaves (Rosmarini folium) and fennel fruits (Foeniculi amari fructus) are shown. The results are compared to those obtained by the conventional distillation according to the European Pharmacopoeia, exhibiting a good agreement of the proportions of the individual constituents.

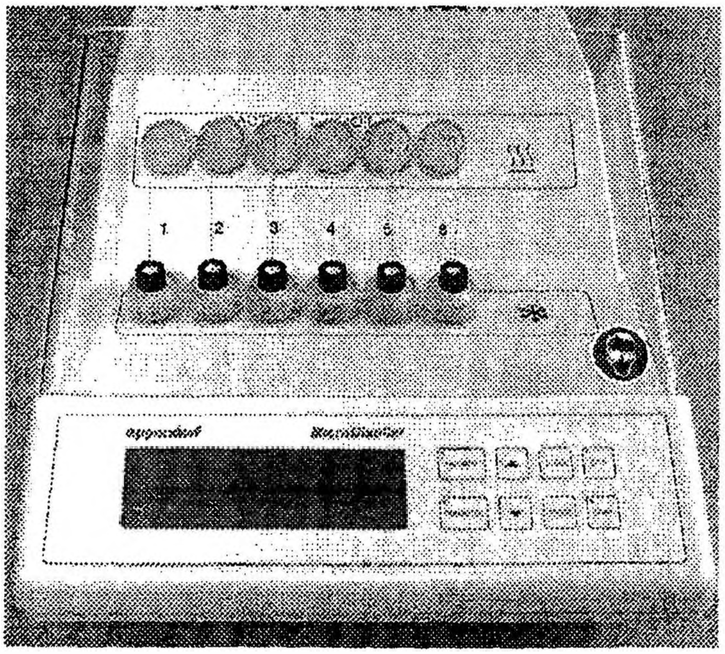

Figure 1: Micro distillation device

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A-36

Influence of salt stress on volatile oil content and major components in thyme *Thymus vulgaris L.*

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Salinity problems occur in nonirrigated croplands and rangelands either as a result of evaporation and transpiration of saline underground water or due to salt input from rainfall. Saline soils are abundant in semiarid and arid regions, where the amount of rainfall is insufficient for substantial leaching. Salinity has been an important historical influence on the life spans of agricultural system. It frequently destroyed ancient agrarian societies.

Thyme plant is antiseptic, anthelmintic, expectorant, carminative, diuretic, and sedative. The herb is used whole or ground for the seasoning of foods. The essential oil is also used extensively in flavoring foods, and in perfumery, and in pharmaceuticals. The main constituent of the oil is thymol, having antiseptic and fungicidal properties. The volatile constituents of oil have been investigated by Russell and Olson (1972).

In a greenhouse experiment, seedling of *Thymus vulgaris* were cultivated under 5 soil salinity concentrations (Control, 500 ppm, 1000 ppm, 2000 ppm and 4000 ppm NaCl + $CaCl_2$) for three seasons.

Salinity concentrations had no significant effect on volatile oil content in the three seasons, whereas there was a significant influence on the components of the distilled volatile oil. However in dichloromethan extract no significant effects on the components of the volatile oil could be observed.

This could be due to pH - dependent artefact formation during distillation.

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A-37

EFFECTS OF SANDALWOOD OIL AND α-SANTALOL ON HUMANS I: INHALATION

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Scientific evaluation of the effects of fragrances on human is rather scarce. The aim of this investigation was to study the effects of essential sandalwood oil and α-santalol on human physiological parameters and self-evaluation. In part I of the investigation the fragrances were administered by inhalation. Physiological parameters recorded were blink rate, blood oxygen saturation, blood pressure, breathing rate, surface-electromyogram, heart rate, skin conductance level, and skin temperature. Self-evaluation was assessed in terms of alertness, attentiveness, calmness, mood, relaxation, and vigor. Additionally, fragrances were rated in terms of pleasantness, intensity, and effect.

The present investigation showed that sandalwood oil caused a significant decrease of skin temperature and significant increases of skin conductance level, subjective attentiveness, nervousness, and alertness which are interpreted in terms of a stimulating effect. α-Santalol caused significant increases of subjective attentiveness and mood but had no effects on physiological parameters, which may be interpreted as a less stimulating effect than sandalwood oil. Correlational analyses revealed that the observed effects are mainly due to subjective odor experience. Moreover, differences between the effects of sandalwood oil and α-santalol seem to be attributable to differences in odor quality.

A-38

ON-LINE GAS CHROMATOGRAPHY PYROLYSIS ISOTOPE RATIO MASS SPECTROMETRY (HRGC-P-IRMS): $^2H/^1H$ RATIO ANALYSIS OF CITRAL FROM VARIOUS ESSENTIAL OILS

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Various commercially available essential oils as well as a number of self-prepared essential oils containing citral either as main component or as a key compound of the aroma were used for the determination of the $^2H/^1H$ ratio of citral (neral/geranial) by on-line gas chromatography pyrolysis isotope ratio mass spectrometry (HRGC-P-IRMS). The data obtained in our HRGC-P-IRMS studies performed with (i) commercially available synthetic and ‚natural' citral samples, as well as with essential oils from (ii) *Cymbopogon flexuosus* and *citratus*, (iii) *Litsea cubeba*, (iv) *Lippia citriadora* and *Melissa officinalis*, (v) *Citrus limon*, (vi) *Citrus sinensis* and *aurantium*, and (vii) *Citrus aurantifolia* and *paradisi* showed reliable differentiation of natural (δ^2H_{SMOW} values from -236 to -314 ‰) from synthetic products (δ^2H_{SMOW} values from +38 to -197 ‰) - even when some samples can also have very negative δ^2H values, e. g. -197 ‰ - but permitted no distinction within the origins from the various plant species.

In spite of the limited number of essential oils under study and of lacking information about the authenticity of some oils the importance of the on-line HRGC-P-IRMS technique was again demonstrated. The limitations of the method will be overcome in future by multielement HRGC-IRMS analysis.

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EFFECTS OF SANDALWOOD OIL AND α-SANTALOL ON HUMANS II: PERCUTANEOUS ADMINISTRATION

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When fragrances are administered by means of inhalation subjective evaluation of the perceived odor seems to have a major influence on their effects on human autonomic nervous system function and behavior. The aim of part II of the investigation was to study the effects of essential sandalwood oil and α-santalol on physiological parameters and self-evaluation excluding olfactory processing of the fragrances. Therefore, substances were administered topically and inhalation of the fragrances was prevented. Physiological parameters recorded were blink rate, blood oxygen saturation, blood pressure, breathing rate, surface-electromyogram, heart rate, skin conductance level, and skin temperature. Self-evaluation was assessed in terms of alertness, attentiveness, calmness, mood, relaxation, and vigor.

The present study revealed that α-santalol caused significant decreases of heart rate, blood pressure, and blink rate, which are interpreted in terms of a relaxing / sedative effect. Sandalwood oil caused significant decreases of blink rate and blood pressure as well as an increase of subjective attentiveness. These findings are likely to represent a harmonizing effect of the substance. Significant changes of physiological parameters were not correlated to self-evaluation.

These findings suggest that the effects of sandalwood oil and α-santalol are due to a direct pharmacological action, e.g. an interaction between odor molecules and anatomical stuctures involved in the control of activation.

A-40

COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *ARTEMISIA SELENGENSIS* TURCZ.

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Artemisia selengensis Turcz. (Asteraceae) is a plant indigenous to East Asia and cultivated in the Medical Plants Garden, Medical Academy in Wrocław, Poland. The aerial parts of the plant were harvested at flowering stage. The essential oil was obtained from dried material by hydrodistillation with the yield of 0.7%. The oil was separated by vacuum distillation and flash chromatography and components were analysed by GC, GC-MS and ^{1}H-NMR.
Antimicrobial studies were carried out by agar dilution method against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.

78 Constituents were identified representing ca 92% of the essential oil. The essential oil was characterised by high percentage of oxygenated monoterpenes, being chrysanthenone (22.9%), 1, 8-cineole (10.9%), α-thujone (8.0%), β-thujone (4.3%) and filifolone (5.4%) the major compounds of this group. α-Pinene (11.6%) and myrcene (7.1%) were the major monoterpene hydrocarbons.

The essential oils showed antibacterial and antifungal activity against all microorganism tested. Gram-negative bacteria were more resistant (MIC 4-5 μl/ml) than Gram-positive bacteria (MIC 2-3 μl/ml), fungi were most sensitive (MIC 1-3 μl/ml).

A-41

STUDY OF THE INFLUENCE OF CERTAIN DISTILLATION PARAMETERS ON THE ESSENTIAL OIL YIELD OF FRUCTUS ANISI.

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The effect of the distillation parameters; a) time, b) quantity of water and c) degree of comminution were studied as regards their influence on the hydrodistilled oil from Pimpinella anisum L. fruits (fructi Anisi).
Different values of distillation time (1, 2, 3 and 4 hours), amount of water (17, 22, 25 and 28 times the amount of the plant material) were introduced in the experimental design, in addition to the factor of the degree of comminution of Anis seeds (integral seeds and comminutted at the degree of 1.40-1 mm, 1-0.75 mm, 0.75-0.50 mm, <0.50mm). The essential oil yield variation resulted between 1.25 and 3.00%. The statistical treatment of the results showed that the duration of the distillation and the comminution affected mostly the Anis oil yield. The latter confirmed as the most significant parameter and the value of particle size < 0.5mm resulted as the most efficient for the essential oil recovery. On the contrary, the parameter of the water amount was not significant for the yield of the obtained oil.

A-42

COMPARATIVE STUDY OF THE INFLUENCE OF DIFFERENT DISTILLATION FACTORS ON THE CONSTITUENTS OF THE ESSENTIAL OIL FROM ANIS SEEDS.

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The quality of the essential oils, among others, is strongly depended on the technological procedures applied for their production. Anis seeds oil is a main source of anethole and has many uses in pharmacy, the flavour and perfumery industry, and in the confectionery as well. Actually, the seeds are distilled in different percentages for various local alcoholic drinks in several countries (*ouzo, raki, sambuca* etc.)
In the present study the influence of certain parameters such as a) the distillation time, b) the amount of the distillation water and c) the comminution of the plant material (of four values each parameter) was investigated on the quality of the obtaining essential oil from Anis seeds. The duration of the distillation and the comminution were determined as the most significant factors, influencing drastically the synthesis of the obtained oil. The identification of the constituents was performed by GC/MS. The GC analysis showed a variation in the percentage of anethole - the predominant constituent of the oil - from 80 to 94%. The highest percentage of anethole was obtained under the combination of conditions A4B3C3 (from comminutted Anis seeds, at particle size less than 0.50 mm, after 3 hours of distillation and with an amount of water 25 times the amount of the plant material). The main constituents percentages of the anis seeds oil a) limonene, b) fenchone and c) estragole varied as follows: a) 0.16-5.65%, b) traces - 0.64% and c) 0.83- 10.06 % under the different applied combinations of conditions.

ANTIBACTERIAL EFFECTS OF PURE PLANT ESSENTIAL OILS AGAINST GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA.

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The antimicrobial properties of plant essential oils and their constituents from a wide variety of plants have been screened, which has shown that higher plants represent a potential source of new anti-infective agents as well as potential lead compounds from plant-derived natural products in drug discovery programs [1,2]. The intention of this study was to examine antibacterial activity of a range of essential oils, some of which have not been investigated (Table 1). The pure oils were tested against 4 Gram-positive (2 strains of *Staphylococcus aureus, S. epidermidis, Bacillus subtilis*) and 4 Gram-negative bacteria (2 strains of *Escherichia coli, Pseudomonas fluorescens, Enterobacter aerogenes*).

Table 1. The studied essential oils and their origins. Examples of one gram-positive and one gram-negative bacteria are included in the table (n= 3, d=diameter as cm)

Essential oil	Origin	Purchase (Crearome Ab)	*S. epidermidis* d	*E. coli* d
Abies sibirica	Siberian	1998	1,600	1,717
~~*Achillea millefolium*~~	France	1998	-	-
Artemisia dracunculus	France	1996	1,642	2,367 (1,800)
Daucus carota	India	1997	1,733	-
Foeniculum vulgare	Spain	1996	1,517	1,867
Hyssopus officinalis	France	1997	2,000	1,800
Illicum verum	China	1998	1,600	1,817
Juniperus communis	Himalaya	1997	-	-
Origanum marjorana	Egypt	1997	2,600 (3,100)	3,967
Origanum vulgare	Spain	1998	6,500	4,800
Pinus cembra	Austria	1998	-	2,008
Pinus nigra	Austria	1999	-	2,550
Thymus serpyllum	former Yugoslavia	1998	3,223	2,650 (3,783)
Valeriana officinalis	East Asia	1998	1,633	-

( ) bacteriostatic effect - no inhibition

Antibacterial activity was tested using an agar diffusion method [3] with the exception that bacteria (245 µl) was spread on the surface of the agar. The oils were used in 50% dilutions with hexane, hexane served also as a negative control. As a positive control were used 4 different antibiotics. After incubation 24 hours in a proper temperature for each bacteria, the inhibition zones (d) were measured.

The most efficient essential oils against bacterial growing were *Origanum vulgare* and *Origanum marjorana*. They exhibited a very strong inhibitorial activity against every tested bacteria, the largest inhibition zone was shown by *O. vulgare* oil against *S. epidermidis* (d = 6.5 cm). On the other hand, some oils showed almost no effects at all, for example those from *Valeriana officinalis* or *Pinus cembra*. The chemical composition of the test essential oils was determined using GC-MS, in order to determine which components may be responsible for the observed activity of the oils.

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A-44

STUDIES ON THE VOLATILE COMPONENTS OF OLIBANUM RESIN

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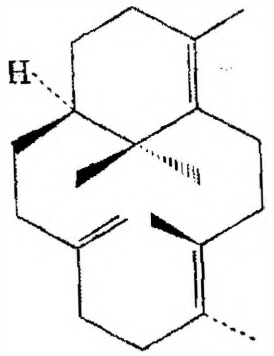
Olibanum belongs to the group of oleogum resins that exudes from incisions in the bark of *Boswellia carterii* (Burseraceae) or other species of the same genus. The trees yielding the resin are native to Ethopia, Somalia, the Arabian peninsula or to India.

Olibanum is known to be used as anti-inflammatory and antiseptic agent in folk medicine which may be attributed to the occurrence of higher terpenoid constituents. Non-medical uses as ingredient for perfumery products and as an insence agent in churches are related to the mono- and sesquiterpenes that dominate the essential oil.

Our studies were carried out with samples of various Olibanum resins of *B. carterii* and *B. serrata* from which the essential oils were prepared by hydrodistillation. The inspection of the GC/MS data and comparison of the mass spectra and retention indices with our own library data showed that both species contain many commonly found mono- and sesquiterpenes. Consequently, the main component of *B. carterii* species is octyl acetate. The presence of the diterpene derivatives incensole, incensole acetate as well as cembrene (cembra-2,4,7,11-tetraene), cembrene A (cembra-3,7,11,15-tetraene) and cembrene C (cembra-1,3,7,11-tetraene) may serve as diagnostic marks for *B. carterii* which is also supported by HPTLC fingerprints. A novel diterpene was isolated from *B. carterii* and identified as verticilla-4(20),7,11-triene (**1**) by 2-dimensional NMR investigations.

Besides the dominating octyl acetate, the oil of *B. carterii* is found to contain α-pinene, limonene, 1,8-cineol, 1-octanol and linalool in considerable amounts, whereas α–pinene is the most abundant component in *B. serrata*. Another compound unique for *B.serrata* is cembrenol (cembra-3,7,11-triene-1-ol), while other diterpenic constituents are lacking.



Verticilla-4(20),7,11-triene (**1**)

Poster Session II (B)

Tuesday, September 12, 2000
16.20 - 18.00

B-01

CHANGE IN ESSENTIAL OIL (*l-MENTHOL*) BY CEMENT COMPONENTS

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Concrete-structures which are social basis as infrastructures have given us a lot of profit conveniences from the beginning of this 20th century. Then, the 21st century is the periods which need to repair these concrete-structures.
On the other hand in recent years, although the opinion about the earth environment is globally spreading, the people who live in urban will be more isolated from the various benefits which the natural environment gives. For example, various smell are one of the elements which are indispensable for our daily inner life. However, natural smell which are given from natural environment intermingle with the artificial smell (unpleasant odor) which are made in urban.
The artificial smell might be stronger than the smell of the natural environment. Thus, estimating the influence of smell from concrete could be also important for us.

In this report, the examination about the relation between the *essential oil* and cement components was carried out. The essential oil was used with "*l-menthol* ($C_{10}H_{20}O$)" as masking-admixture to reduce the unpleasant smell of concrete. Though the main subject was examination of the change in essential oil, the detail of outline and results of this report will be discussed.

B-02

MICROBIAL TRANSFORMATION OF CEDROL

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In the course of microbial transformation of aromatic compounds and sesquiterpenes by microorganisms, the biotransformation of cedrol was carried out. Here we report on the biotransformation and regiospecifically formed metabolites and their antimicrobial activities.

B-03

ELIMINATION AND STORAGE OF TERPENES IN EPIDERMAL GLANDS AND SECRETORY CANALS

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The ultrastructure of two types of secretory structures that produce terpenes – epidermal glands of genera *Agastache*, *Dracocephalum* and *Nepeta* (*Lamiaceae*) and secretory canals of *Delarbrea* and *Schefflera* (*Araliaceae*) has been studied. In the glands of *Lamiaceae* all secretory cells are at the same stage of development, whereas the *Araliaceae* secretory canals consist both of initial cells, and the cells at the stages of active secretion, and senile cells. In both types of the studied structures the secretory cells loose the features that are common for terpenoidogenic cells (such as abundant plastidome and endoplasmic reticulum) after a very short stage of synthetic activity, and get the features of not synthesizing but storage cells. Most time of their life the secretory cells of glands and canals are not secreting.

Vacuoles, extraplasmic space, and intercellular spaces (lumina of secretory canals and subcuticular spaces in the glands) are three main sites of terpene storage. Both the subcuticular space and canal lumina are formed at the first stage of the secretory structure development, simultaneously with the differentiation of initial cells into secretory cells. In glands the secretion is stored in vacuoles of secretory cells, in extraplasmic spaces, and in subcuticular space, whereas in the secretory canals the terpenes are mostly located in lumina. Accumulation of the osmiophilic substances (which we consider to be fixated terpenes) in the extraplasmic space of epithelial cells in secretory canals is not observed. Apparently, the secreted material permeates quickly through the cell wall into the lumina.

The cell wall facing the intercellular space differs from that between neighboring cells in the pattern of cellulose fibril distribution. The apical cell wall is more loosely arranged in gland cells. In secretory canals the fibrils form a loose web with the electron-opaque droplets between its fibrils. The secretory canal cell wall facing the lumen is probably synthesized at the plasmalemma site and destructed at the lumina site during secretion elimination. At the senile stage the cellulose fibriles are distributed regularly throughout the cell walls both in glands of *Lamiaceae* and in *Araliaceae* secretory canals. Such structure of the cell wall is probably related to terpene transport through it, and also shows that terpene secreting cells have some sort of morphological and apparently physiological polarity.

The first author would like to thank Deutsche Akademische Austauschdienst (DAAD) for financial support of investigation of the secretory canals in *Araliaceae* during her research stay in Ordinariat für Holzbiologie, Universität Hamburg. The work has been continued due to Russian Basic Research Foundation (RBRF) grant 00-04-48561.

B-04

COMPOSITION OF ETHEREAL OILS FROM SIBERIAN PLANTS OF THE *ASTERACEAE* FAMILY (SUBTRIB *ASTERNINAE* O.HOFFM.)

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We studied ethereal oils form the following Siberian plants of the *Asteraceae* family (subtrib *Asterinae*): *Galatella biflora (L.) Nees, Galatella dahurica DC, Galatella angustissima (Tausch) Novopokr., Galatella villosa (L.) Reichenb., Galatella hauptii (Ledeb.) Lindl., Heteropappus altaicus (Willd.) Novopokr., Tripolium vulgare Nees, Conyza canadensis (L.) Cronq., Erigeron acris L. s.l., Brachyactis ciliata (Ledeb.) Ledeb.* The oils were prepared by standard steam-distillation procedure from the fresh and dried plants collected in 1998-1999 at different vegetation phases. We prepared ethereal oils from the plants growing wild in the South Siberia (forest-steppe and forest-mountain temperate zones) and compared the compositions with those of the oils from cultivated plants of the same species. The plants were being cultivated at the Central Siberian Botanical Garden (Novosibirsk, Russia) for 20 years without using special agrotechnics and chemicals (fertilizers and pesticides).

Compositions of the oils were studies by GC-MS using quadruple MS (Hewlett-Packard MSD 5971) coupled to a HP 5890/II GC fitted with an HP-5 (30 m × 0.25 mm I.D., film thickness 0.25 µm) fused silica column. The percentage composition of the essential oils was computed from GC peak areas without using a correction factor. Qualitative analysis was based on comparison of the retention indexes and full mass spectra of the components with the data for standards prepared by separation of reference oils and identified by NMR spectroscopy.

The oils contain quite different number of components (from 3 to 104), not more than 10 of them being the main constituents (≥5% of the total). Compositions of the oils depend strongly on the condition of the plant raw material (fresh or dried) and the vegetation phase. The most frequently observed constituents were α-pinene, β-pinene, limonene, α-ocimene (3,7-dimethyl-1,3,7-octatriene), β-myrcene, germacrene D, *trans*-β-ocimene. The highest yields of the oils were determined for the plants of the genera *Conyza* (0.6% from fresh grass and 3.0% from dried plants) and *Galatella* (0.5% and 2.3%, correspondingly), which demonstrated also the highest content of monoterpenes, whereas sesquiterpenoids were predominant in the other species.

B-05

ESSENTIAL OILS FROM SOME SPECIES OF THE GENUS *PINUS*

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The chemical composition of the essential oils from three species of the genus *Pinus* have been determined employing GC-MS method.
The essential oils were obtained by hydrodistillation of dried needles from the following species, growing in Poland: *Pinus strobus, Pinus ponderosa, Pinus resinosa*. From among 61 identified constituents in the above mentioned oils, 15 occured in amounts over 1% in *P.strobus* and only 10 in *P.ponderosa* and *P. resinosa*.
The oil obtained from needles of *P. strobus* differed markedly from that distillated from *P. resinosa* and *P. ponderosa*, by its low concentration of β-pinen-7,9% (in contrary to last two -22,6% and 17,7% respectively). The essential oil has been characterised by the high content of myrcene-15% in *P.resinosa* and germacrene D-12,2% in *P.strobus*. On the other hand, estragole and Δ^3-carene in concentrations about 8% were identified only in *P. ponderosa*. The analysed essential oils, may be classified as rich in hydrocarbons (α–, β-pinen, limonene) but the content of oxygenated monoterpenes is, in contrast to other conifers comparatively low, especially in *P. strobus*.

B-06

STUDIES ON TURKISH ROSE CONCRETE, ABSOLUTE AND HYDROSOL

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Fresh flowers of the cultivated *Rosa damascena* Miller are the source of rose oil, rose concrete and rose water. Rose concrete is produced by hexane-extraction of fresh flowers. Rose absolute is obtained by extracting the concrete with ethanol, followed by cooling, filtering and evaporation. Rose water is the distilled water of rose flowers left after the removal of oil.

Production of Rose absolute was realized using traditional and modern techniques, such as molecular distillation, extraction with liquified gases, etc. Headspace trapping and SPME techniques were employed in the sampling of volatile constituents in the concrete, absolute and hydrosol for analysis by GC/MS.

THE VOLATILE OILS OF SOME SPECIES OF THE FAMILY ASTERACEAE (COMPOSITAE) BELONGING TO THE TRIBES ASTERAE AND INULAE

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Despite the very large number of Australian species of the huge family Asteracae very little has so far been published on the chemical composition of their volatile oils. Maltol has been reported to be the major component of the volatiles of *Helichrysum ramosissimum*[1], (-)-*cis*-chrysanthenyl acetate made up about 45% of the herb oil of *Centipeda cunninghamii*[2] whilst the herb oil of *Kippistia suaedifolia* contained about 65% of (+)-perillyl acetate accompanied by smaller amounts of (+)-limonene, α-pinene and myrcene[3]. Bohlmann and his co-workers have isolated very small amounts of various sesquiterpenoids from *Cassinia longifolia*[4], *C. laevis*[5], *C. subtropica*[5], *C. uncata*[5] and *Actinobole uliginosum*[5] as well as from *Cassinia aculeata*[6].

The present communication reports the results of a GC/MS investigation of the oils of *Ozothamnus diosmifolius* (syn. *Helichrysum diosmifolium*), *Cassinia arcuata, C. aculeata* (leaves and flowering heads), *C. denticulata* and *C. quinquefaria* (tribe Inulae) and *Calotis cuneata, Olearia elliptica* and the introduced *Conyza bonariensis* (syn. *Erigeron bonariensis*) (tribe Asterae). Most of the oils were terpenoid in nature with monoterpenoids predominating over sesquiterpenoids. *Conyza bonariensis* was exceptional in that its herb oil contained very major amounts (up to 82%) of *cis*-lachnophyllum methyl ester, an acetylenic compound. Oil yields were in all cases low, reaching about 0.7% in *Cassinia aculeata.*

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B-08

SYNTHESIS AND ODOR CHARACTERISTICS OF NEW *gem*-DIMETHYLBICYCLO[3.1.0]HEXANE DERIVATIVES

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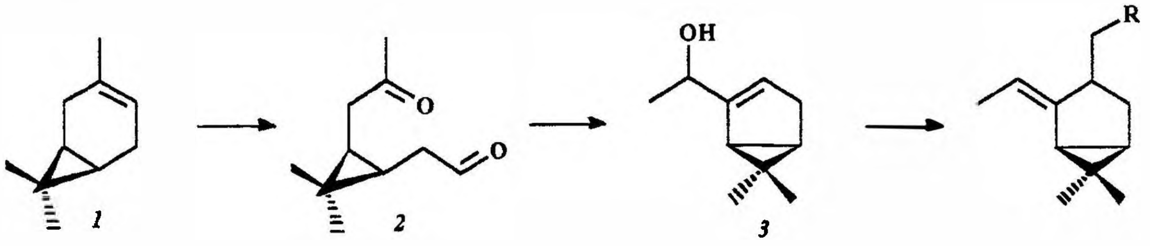
Monoterpenes containing the bicyclo[3.1.0]hexane system for example sabinene, thujone or umbellulone are known as a naturally occurring sensorial components of many essential oils. Synthetic analogs of these compounds and especially their odor properties are relatively little known.

In our previous papers we reported the synthesis of odoriferous monosubstituted 6,6-dimethylbicyclo[3.1.0]hexane derivatives at 2- [1] or 3-position [2]. As a continuation of these works now we present synthesis of 2,3-disubstituted derivatives of this system.

The key compound, allylic alcohol **3** was obtained in four step procedure [3] from terpene hydrocarbon (+)-3-carene **1** *via* intermolecular condensation of ketoaldehyde **2**. Alcohol **3** was subjected to the Claisen rearrangement (orthoacetate modification) giving γ,δ-unsaturated methyl (**4**) or ethyl (**5**) esters. Reduction of both esters with $LiAlH_4$ afforded primary alcohol **6**, which in the reaction esterification with acetyl chloride was transformed to acetate 7. All new compounds obtained possess very interesting olfactory properties.



4 R = COOMe
5 R = COOEt
6 R = CH_2OH
7 R = CH_2OAc

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Supported by State Committee for Scientific Research, Grant No 3 T09B 013 14

B-09

ESSENTIAL OIL OF BRAZILIAN *CROTON MIGRANS* CASARETO

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The genus *Croton* (Euphorbiaceae) comprises about 800 species of which 1/3 is found in America. *Croton* species are wide spread in Brazil and up to now, only a few species collected in the Amazonian and Northeastern areas have already been studied due to their biological activities and the occurrence of essential oils. Cascarila oil from Bahamas (*Croton eluteria* Bennet.), a commercial essential oil, is used as a beverage ingredient. *Croton migrans* Casareto, locally known as "velame", is a shrub found in Rio de Janeiro seashore. The hydrodistillation of aerial parts (leaves and slender stems) using a Clevenger-type apparatus for 4 h yielded an oil (0.6%, fresh weight) which was analysed by gas chromatography and mass spectrometry. GC-MS analysis was carried out on a Hewlett- Packard capillary GC-quadruple MS system (Model 5995C) using a DB-5 column. The substances present in the oil were identified by their mass spectra and retention indices. According to the available data, the major compounds are 1-epi-cubenol (46.3%), α-pinene (9.1%), terpinolene (4.0%), β-pinene (4.0%) and limonene (3.6%).

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B-10

GC-MS ANALYSIS OF ESSENTIAL OIL AND GALLENIC PREPARATIONS RECEIVED FROM LEAVES OF ROSEMARY (*ROSMARINI FOLIUM*).

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Leaves and twigs of rosemary are raw material used for receiving the essential oil and preparations possessing antioxidative properties. This properties are connected with the presence of phenolic compounds and especially diterpenes such as carnosol and carnosic acid [2,4].

Essential oil received from the herb of rosemary is one from more important oils. It is used both in medicine and cosmetic and perfumery industry. Three chemotypes of rosemary oils, depending on the main component: camphor, 1.8-cinneole, verbenon are distinguished. Pharmacological activity of essential oil is strictly connected with its composition. Oils with superiority of camphor act as a stimulant, while oils with superiority of 1.8-cinneole as expectorant, antiphlogistic, antiseptic and mucolytic remedies. Oils with verbenon as the main component are used as a antiphlogistic agent. Rosemary preparations and oils with proved high content of 1.8-cinneole belong to the most safe [1-3].

The aim of lead investigations was the comparison of the composition of essential oil received from leaves of rosemary and commercial preparations. Besides, the composition and the content of oil-components present in gallenic preparations: *Tinctura Roosmarini* and *Extractum Rosmarini 50* was determined.

GC-MS analysis showed, that main component of rosemary oil and all investigated preparations is 1.8-cinneole. Besides in considerable quantities camphor, α-pinene, borneol, bornyl acetate, β-caryophyllene and camphene are present.

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B-11

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *AMBROSIA ARTEMISIIFOLIA* L. ESSENTIAL OIL

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Ambrosia artemisiifolia L., *Asteraceae* is plant species indigenous to North America, but naturalized abroad. In Europe it grows in central and southern parts of the continent, usually at waste places near urban areas, where it constitutes several ruderal plant associations. It is well known as allergenic plant, causing numerous cases of pollen fever, especially in late summer and early autumn. For the purpose of this investigation, aerial parts of *A. artemisiifolia* were collected from *locus typicus* at Kumodraž (southern suburbs of Belgrade) during full-flowering period. Plant material was dried in shaded, well-ventilated place and hydrodistilled. Composition of isolated oil was determined by GC and GC/MS. Fifty-one constituents were identified. The most prominent one was germacrene-D (24.08 %), followed by limonene (16.83 %), α-pinene (8.03 %) and myrcene (7.42 %). Antimicrobial activity of obtained essential oil was determined applying diffusion method on Mueller-Hinton agar and Saburaud agar, as well as dilution method in Mueller-Hinton broth against certain strains of bacteria, yeasts and fungi. Observed effects were expressed through minimum inhibitory concentrations (MICs) or diameters of inhibition zones and compared to the activity of chosen antibiotics. Investigated oil exhibited a considerable, concentration-dependent inhibitory effect against all organisms tested.

B-12

ESSENTIAL OIL OF *ACINOS HUNGARICUS* (Simonkai) Šilić, *LAMIACEAE*

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Acinos hungaricus (Simonkai) Šilić, *Lamiaceae* is perrenial or biennial, herbaceous plant species which range covers lower mountainous regions (up to 1200 m of altitude) of Asia Minor, Eastern and South-Eastern Europe. It is the plant of open habitats, growing mostly at dry, calcareous and rocky soil at mountain meadows and pastures, constituting several, predominantly xerothermous plant associations and possessing very wide ecological amplitude. In Serbia, it is widespread. Plant material for this investigation (herb) was collected from periphery of the Deliblato Sand, near the town of Bela Crkva (North-East of Serbia), during full-flowering period (July 1998). The herb was dried under laboratory conditions and hydrodistilled. Isolated essential oil was submitted to GC and GC/MS analysis. A total of forty-one constituents were identified (88.65 %). The principal component was germacrene-D (46.67 %), followed by β-bourbonene (6.60 %) and 1,8-cineol (5.60 %). A mixture of δ- and γ-cadinene (3.97 %), as well as β-caryophyllene oxide (3.55 %), β-elemene (2.93 %) and β-caryophyllene (2.22 %) were also present in significant quantities.

B-13

ESSENTIAL OIL OF *SENECIO SQUALIDUS* L., *ASTERACEAE*

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Senecio squalidus L., *Asteraceae* is biennial or annual, herbaceous plant species, growing wild in Central and Southern Europe, mainly in the mountains. It could be found in woodland and scrub, and also on disturbed rocky ground, especially in the northern part of its range. This species is abundantly naturalized in Britain and locally elsewhere in Northern and Central Europe. In Serbia, it is widespread. Plant material (herb) was collected near the Vlasina Lake in Southern Serbia, during full-flowering period. Harvested material was dried in a shaded, well-ventilated place, reduced to smaller particles and hydrodistilled. Chemical composition of isolated essential oil was determined by GC and GC/MS. Sixty-nine constituents (98.38 % of total oil) were identified. The most prominent one was p-cymene (29.3 %), along with α-phellandrene (24.9 %), α-pinene (8.0 %) and a mixture of (Z)-β-ocimene and γ-terpinene (7.0 %). Significant presence of β-cubebene (3.2 %), germacrene-D (2.8 %), β-caryophyllene (1.9 %) and *trans*-caryophyllene oxide (1.7 %) was also detected.

B-14

USING ATOMIC FORCE MICROSCOPY TO VIEW THE EFFECTS OF TERPENOIDS ON THE STABILITY AND PACKING OF PHOSPHATIDYLCHOLINE SUPPORTED LIPID BILAYERS

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Most essential oils consist of monoterpenes, sesquiterpenes and other terpenoid compounds and are known to possess antimicrobial activity. Many of the individual components of essential oils have also been tested and shown to have antimicrobial activity. Recent work has indicated that active terpenes damage membrane structure and function.

Phospholipids, the major components of cell membranes, play a crucial role in the functioning of a cell. They provide compartmentalization and a matrix for membrane-associated proteins such as respiratory enzymes, transport channels, receptors and pumps. Changes in the stability and packing of such phospholipids will affect the crucial functions of the membrane. Atomic Force Microscopy (AFM) was used as one method of determining the effect of terpenoids on membrane stability. AFM works by measuring a minute force of contact between a microscopic tip and the sample surface. The amgnitude of the forces required to cause deflection of this tip are in the nano Newton range and, hence, very fine detail can be imaged. This means that AFM can be used to study the surface structure and heterogeneity of supported lipid bilayers in solution, under physiological conditions, without molecular markers or intrusive treatment of the membrane.

In this study, the physical stability and lipid packing of dipalmitoylphosphatidylcholine (DPPC)-supported lipid bilayers was monitored using AFM before and after treatment with oxygenated monoterpenes. The results showed that carvone, perilla alcohol, carveol and carvacrol all caused degradation of the lipid bilayer. Terpinen-4-ol showed no visible destruction of the membrane, however, distinctly different lipid domains did form. Perilla aldehyde was found to have no visible effect on the lipid bilayer at the concentrations tested. Lipid bilayer packing, as monitored by lipid head group arrangement, was also affected by the terpenes.

B-15

Cape Chamomile Oil - the origin of its blue colour

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Commercial Cape chamomile oil is produced from cultivated plants of *Eriocephalus punctulatus* (Asteraceae) in the Cape Province. *E. punctulatus*, endemic in the "Drakensberge" (South Africa), is a small shrub similar to the *E. africanus*, which is widely used in folk medicine. The blue colour of the commercial oil is striking and one associates it automatically with the European chamomile oil. It is due to the content of chamazulene and 1,4-dimethyl azulene [1]. Gas chromatographic analyses of 8 commercial samples of Cape chamomile oil show chamazulene to be present in concentrations of about 0.05 - 0.1%. 1,4-Dimethyl azulene occurs in much lower percetages, only about one fifth of the chamazulene content. When several samples of dried plant material cultivated in the Cape Province were distilled in the laboratory it could be observed that the blue colour developed only one hour after starting the procedure. This observation and the fact that the solvent extract lacks any blue colour indicate that the azulenes are formed during distillation from precursors as it is known from the European chamomile where chamazulene is formed from matricine. The main components of the Cape chamomile oil are 2-methylbutyl-2-methyl propanoate (20,3 %), 2-methylpropyl-2-methyl propanoate (10,0 %), 2-methylbutyl-2-methyl butanoate (4,3 %) and linalyl acetate (5,7 %). Nearly 150 further components were detected which will be described.

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B-16

Components of Essential Oil from *Glehnia littoralis*

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The chemical composition of the essential oils from aerials and roots of different provenances and growing method of *Glehnia littoralis* was determined by GC and GC-MS. As a result, 125 compounds, which amounted to 86.45-94.19% of the total components detected, were identified. The main constituents were found as α-pinene (0.03-13.40%), limonene (0.15-10.71%), β-phellandrene (0.03-22.93%), germacrene B (0.27-8.33%), spatulenol (0.24-6.50%), β-oplopenone (0.06-6.43%), panaxynol (0.38-25.58%), propyl octanoate (3.44-16.89%), hexadecanoic aicd (0.45-15.67%) and linolic acid (0.16-17.56%). Distinct qualitative and quantitative differences were observed in the components of eight oils. Consequently, the constitution of essential oil from *G. littoralis* could be separated North type from South type.

B-17

THE ESSENTAIL FRUIT OILS FROM *SMYRNIUM CRETICUM*, *S. ORPHANIDIS*, AND *S. APIIFOLIUM* (APIACEAE)

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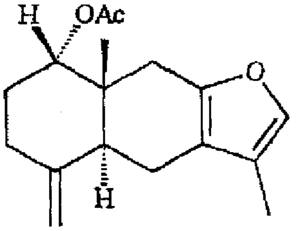
Smyrnium creticum Miller is a biennial plant belonging to the Apiaceae which grows in the South-West of Turkey and on the neighbouring Mediterranean islands. The species *S. orphanidis* Boiss. and *S. apiifolium* Willd., which can be found mainly in Greece are included in *S. creticum* according to the Flora of Turkey (1).

In this study the essential fruit oils of the three mentioned species from different locations were analyzed by GC, GC-MS and NMR exhibiting a high content of oxygenated sesquiterpenoids.

In most of the samples of *S. creticum* and in all the investigated samples of *S. orphanidis* and *S. apiifolium* germacrone, furanodiene (furanogermacrene), as well as isofuranogermacrene (furanoelemene) were the main constituents ranging from 75 to 90%.

In contrast, one sample of *S. creticum* exhibited a considerably different oil composition with a relatively high proportion of sesquiterpene hydrocarbons such as germacrene d (24%) and germacrene b (12%). In addition, 16% of a new furanosesquiterpene derivative were detected. After isolation this component was identified by means of NMR-spectroscopy including 2-dimensional techniques as 1α-acetoxyfurano-4(15)-eudesmene:



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B-18

STUDIES ON INSECTICIDAL COMPOUNDS OF SRI LANKAN ESSENTIAL OIL OF *PIPER BETLE* LEAF

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Piper betle (Family: *Piperaceae)* is widely cultivated in tropical countries such as India, Sri Lanka and Malaysia for chewing purposes. It is reported to have medicinal, antifungal and antiseptic activities [1,2]. The roots are believed to have contraceptive activity in humans[1].

In our studies of Sri Lankan *Piper betle* leaf oil we investigated insecticidal activity against *Musca domestica* (house fly) and *Lobesia botrana* (grapevine moth) and found that this oil is insecticidal to these insects. The LD_{50} values for *Musca domestica* are 0.060 mg/cm^2 and 0.055 mg/cm^2 and for *Lobesia botrana* are 0.054 mg/cm^2 and 0.053 mg/cm^2 for 24 hour and 48 hour exposure periods, respectively.

Published data on Philippine *Piper betle* leaf oil show that it contains phenolic compounds [2,3,4] such as Chavibetol, Chavibetol acetate, Chavicol, Eugenol and Isoeugenol and terpenes [3,4,5], i.e. Camphene, Caryophyllene, 1,8- Cineol, *P*-Cymene, Limonene, Myrcene, (α)-Pinene, (β)-Pinene, (α)-Terpinine, (α)-Terpineol and (α)-Terpineol acetate. Though it is reported that major and secondary components of Philippine *Piper betle* leaf oil are Chavibetol (53.1%) and Chavibetol acetate (15.5%), we report here on GC and GC/MS studies of a *Piper betle* leaf oil from Sri Lanka where Safrole (42.8%) is the major constituent followed by Eugenol (14.3%).

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Acknowledgement
We thank Swiss Federal Scholarship Commission for the financial assistance.

B-19

VARIABILITY OF THE ESSENTIAL OIL CONTENT OF HYSSOP (HYSSOPUS OFFICINALIS L.)

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Hyssop is a medicinal and spice plant, whose drugs are regularly marketed, however, scientific information on its biology is relatively few. During the breeding work since 1993, 15 populations of different origin as well as yearly15-20 selected strains and lines had been investigated. Beside the practical results of breeding, in this presentation we summarise the effect of natural factors on the accumulation level of essential oil, as theoretical results.

The effect of the genetic background can be supposed from the significant differentiation of the 15 investigated populations according to essential oil content. Standard deviation in the populations is varying, the coefficient of variance laid between 7,7 % and 43,5%. The minimum value during seven years was 0,17 %, while the maximum value reached 2,03% d.w. which shows the considerable individual plant differences. The role of inheritance had been demonstrated by the rise of essential oil content by 0,4% during the breeding process. However, heritability is low (narrow sense h^2 0,3-0,4%).

Beside the genetic determination, the effect of the vegetation year might have an outstanding role in the essential oil accumulation. We measured significant differences up to 52% in the essential oil content of the same plant in successive years. On the base of it, we can declare some years (e.g. 1997,1998) being excellent for hyssop, another ones (e.g. 1996, 1999) being unfavourable.

The essential oil content proved to be influenced also by the organic differentiation: in the flowering parts the essential oil content is 3-4 times higher, then in the leaf parts. The stem does not contains it. During the phenological phases, from sprouting till ripening of the seeds significant (18-36%) differences were proved in the essential oil content. In the majority of populations maximal accumulation was proved in bud stadium, minimum level at the time of seed ripening.

The essential oil content of the shoots developing after the first cut, in August-September showed significantly different values. Depending on genotype, they reach only 60% of the level in the main flowering period.

Our results show, that essential oil of hyssop even at the same place is changing considerably according to cultivar, year and method of harvest.

Supported by the Bólyai found of the Hungarian Academy of Sciences

B-20

An α-Pinene Chemotype in *Salvia officinalis*

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The essential oil composition of sage (*Salvia officinalis*) is not only characterised by quantitative, but also by qualitative differences (chemotypes). Such a chemotype has been found e.g. regarding the content of α-thujone. By selfing and crossing experiments a further chemotype ('α-pinene chemotype') was detected changing between a 'low α-pinene group' under 3% and a 'high α-pinene group' of above 8%.
Since it is known, that three different cyclases are responsible for the formation of α-pinene in sage (Coates et al (1987), Croteau et al (1987), Croteau et al. (1988), Croteau et al. (1989) and Gambliel (1984)) it is discussed, which of these enzymes could be responsible for the formation of the 'high α-pinene group'.

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B-21

Composition of Essential Oil Compounds of a historical sample of marjoram (*Origanum majorana*)

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Majoram (*Origanum majorana* L. syn. *Majorana hortensis* MOENCH) was widely used in human, but also in veterinary medicine. Samples of drugs are absolutely necessary to teach the students basic aspects of pharmacognosy. An old sample of marjoram was found in a historical collection of drugs at the university of veterinary medicine, Vienna. The composition of the essential oil compounds is deviating from the composition of cultivated marjoram of today.

B-22

FAST GC OF ESSENTIAL OILS

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Our laboratory has been engaged in the standardisation of essential oils using GC approximately for 20 years. In the first stage we used packed columns, later convenient capillary columns (I.D. 0.20-0.32 mm). For relatively long period we used megabore columns (I.D. 0.53 mm) and in this time we have obtained very interesting results using microbore columns (I.D. 0.10 mm).

Department of Analytical Chemistry of the State Institute for Drug Control has a very good experience with a cooperation with Faculty of Pharmacy in Hradex Králové. Results of this cooperation have been summarised in many diploma works.

Student Davidková compares in this time a chromatographic behaviour of 40 different components of essential oils using DB-5 columns with different inner diameters. This comparison is made by means of a calculation of linear retention indexes, resolutions, efficiencies and symetry of peaks. We present some results, which demonstrate practically the same efficiency of microbore column in comparison with other 2 types of columns. The most positive result is a shortening of the time of analyses from approximately 1 hour to 8 minutes.

B-23

ESSENTIAL OIL COMPOSITION OF SEA FENNEL (*Crithmum maritimum* L.) FROM TURKEY

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Constituents of essential oils of sea fennel (*Crithmum maritimum* L.) collected different localities of Turkey in 1997 asnd 1998 were identified by GC / MS.The samples were classified into groups according to main components: sabinene, ϒ- terpinene, methyl thymol and terpinen-4-ol; ϒ- terpinene, dill apiol and sabinene; ϒ- terpinene , methyl thymol and limonene; *p*-cymene, methyl thymol and ϒ- terpinene. (Z)-β-ocimene and spathulenol were found with high percentage values in some samples. *cis-p*-menth-4-en-1,2-diol was identified in only one oil. Early collected plants yielded oil rich in *p*-cymene. Effect of freezing plant did not have any important effect in oil composition. The effects on collection time and locality on the oil content and composition have been studied.

B-24

DETERMINATION OF THE ENANTIOMERIC DISTRIBUTION OF (1*R*)(+)-AND (1*S*)(-)- CAMPHOR IN VARIOUS ESSENTIAL OILS

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The enantiomeric distribution of camphor was determined using a fused-silica Lipodex E Capillary column in various natural and commercial essential oils. Enantiomerically pure (1*S*) (-)-camphor was detected in *Osmitopsis asteriscoides*, *Tanacetum parthenium*, *T. haradjani*, *Achillea grandifolia*, *A. biebersteinii*, *A. phrygia*, *A. sieheana*, *Arischrada korolkowii* and *Thymus sipyleus* while (1*R*) (+)-camphor was detected only in *Plectranthus grandidentatus*. All other essential oils contained the enantiomers of camphor in different ratios. Both enantiomers of camphor are found in nature but (-)-form is less common than the (+)-form. The paper gives enantiomeric distribution of camphor in 40 essential oils.

B-25

GC-MS INVESTIGATION OF THE ESSENTIAL OIL OF THE LIVERWORT *SPRUCEANTHUS THEOBROMAE* (SPRUCE) GRADST.

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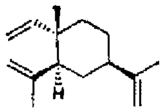
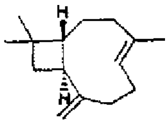
The sesquiterpene constituents of the liverwort *Spruceanthus theobromae* (Spruce) Gradst., which is endemic to western Ecuador, were investigated. The investigation was carried out by GC-MS and comparison with authentic samples.

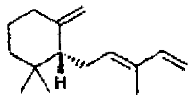
We were able to detect the following sesquiterpene hydrocarbons and a new hydrocarbon which is still to be identified. β-Elemene, β-caryophyllene, cyclofarnesa-5(14),8,10-triene, germacrene D, bicyclogermacrene, germacrene A and δ-cadinene.



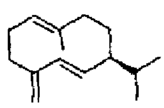
β-Elemene



β-Caryophyllene

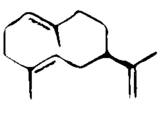


Cyclofarnesa-5(14),8,10-triene

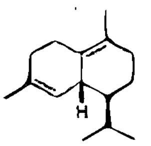


Germacrene D

Bicyclogermacrene



Germacrene A



δ-Cadinene

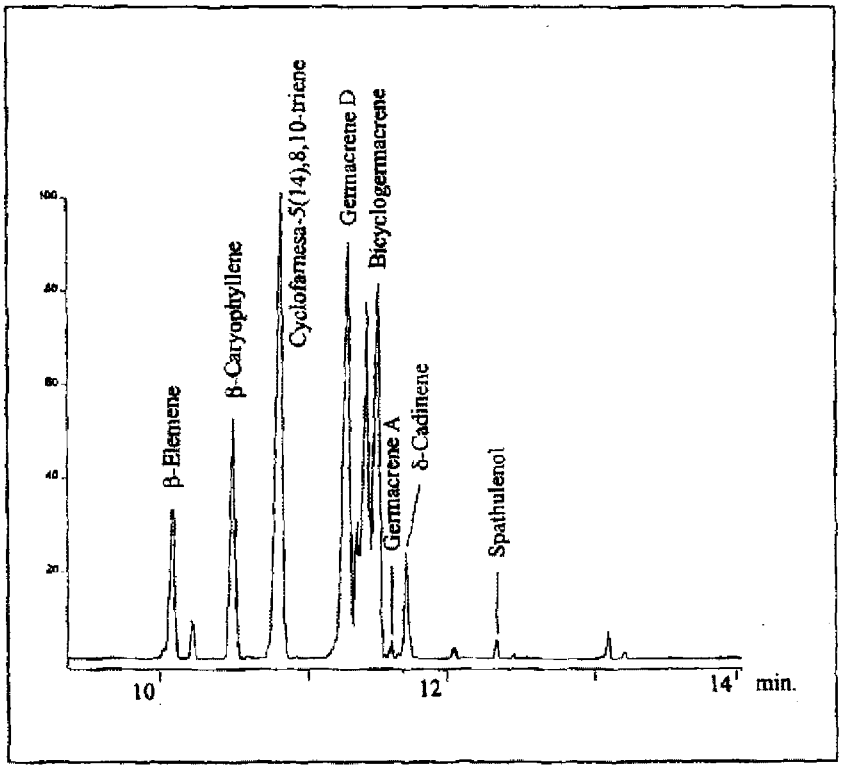


Fig.: Gaschromatographic separation of the essential oil of *Spruceanthus theobromae* (CpSil-5CB, 25 m, 80 °C / 10 °C/ min / 280 °C).

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B-26

ESSENTIAL OILS – SOURCE OF USEFUL PRESERVATIVES ?

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Essential oils or components thereof are allowed to be added to products destined for human use, e.g. food, cosmetics, pharmaceuticals. The stability of such products is endangered by microbial decay, and therefore, the examination of antimicrobial effects of essential oil components is a reasonable assumption.

A valuation of published data about antimicrobial properties of essential oil components is large-scale for several reasons, e.g. use of different testing methods, calculation with different concentration units, different nomenclature for compounds and microorganisms. Transformation of the data material into a standardized form was therefore inevitable as well as computing of such data in a database (1).

To get information about the efficiency of essential oil compounds relative to preservatives, the inhibitory data is scheduled according to compounds groups, which are selected from mono-oxygenated mono- (MT-O) and mono-oxygenated sesquiterpenoids (ST-O), phenols, thymol plus isomers, fatty acids and preservatives (butylated hydroxy anisole, butylated hydroxy toluene, propylparaben). Microorganisms are classified in gram-negative (Bac-), gram-positive bacteria (Bac+), fungi and yeast. Among different types of inhibitory data only MIC- (minimal inhibitory concentration) and MMC-data (minimal microbicidal concentration) is selected (total dh), which are results of agar dilution or serial dilution tests. A gross overview on the inhibitory properties of selected compound groups up to a concentration limit of 200 ppm (dh) is shown in the table:

	Bac-	Bac+	Fungi	Yeast	dh	total dh
1. MT-O	+	+	++	++	377	3102
2. ST-O	++	++++	+++	+++	214	872
3. Fatty acids	++	++++	+++	++	1136	3227
4. Phenols others than 5-8	++	+++	+++	+++	866	2691
5. Thymol plus isomers	+++	+++	++++	+++	506	1072
6. Propylparaben	+	+++	++++	++++	117	242
7. butylated hydroxy anisole	++	+++	++++	+++	79	149
8. butylated hydroxy toluene	++	++++	—	++	20	59

— = no, + = very weak, ++= weak, +++ moderate, ++++ strong inhibitory activity; dh = database hits

The results indicate that compounds of the groups mono-oxygenated sesquiterpenoids, phenols, thymol plus isomers and fatty acids are capable to posses similar antimicrobial activity as selected preservatives. A greater number of resistant species exists towards all selected groups of compounds among gram-negative bacteria.

Reference

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B-27

SUBCRITICAL CO_2 EXTRACTION AND DISTILLATION-EXTRACTION OF THE VOLATILES FROM THREE AZOREAN SPECIES

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The isolation procedure has a major effect on the composition of essential oils. Liquid CO_2 is a non-polar, non-reactive, odourless, tasteless and stable solvent. When compared to the essential oils obtained by distillation and/or solvent extraction, CO_2 extracted oils have more pronounced top notes and show a better recovery of compounds that may be lost, in distillation and/or solvent extraction, through chemical transformation, solubility in the distillation water or by evaporation (1).

As part of a screening programme of the aromatic flora of Macaronesia, we analysed, by GC and GC-MS, the volatiles isolated by distillation-extraction or by subcritical CO_2 extraction from three Azorean species: *Chaerophyllum azoricum*, *Juniperus brevifolia* and *Laurus azorica* (Table 1). Considering the grouped components, up to a 3-fold decrease of the relative amount of the monoterpene fraction was observed in the CO_2 extracted oils. In contrast, the other fractions (sesquiterpenes, diterpenes, phenylpropanoids and other compounds) were present in larger amounts in the CO_2 extracted oils. One of the most remarkable differences was observed in the diterpene fraction of the oils of *J. brevifolia*, with three components, detected only in the CO_2 extracts, representing 3-36% of the total oil. Also noteworthy was the occurrence of six unidentified compounds, present only in the CO_2 extracts of *L. azorica* volatiles, representing 3-27% of the total oil.

Table 1. Percentage composition of the grouped components of the volatiles isolated by distillation-extraction or by subcritical CO_2 extraction from three Azorean species.

	C. azoricum				*J. brevifolia*				*L. azorica*			
Grouped components	Leaves		Flowers		Twigs		Berries		Leaves		Berries	
	Dis-Ext	CO_2	Dis-Ext	CO_2	Dis-Ext	CO_2	Dis-Ext	CO_2	Dis-Ext	CO_2	Dis-Ext	CO_2
Monoterpenes	82	64	91	35	88-94	25-74	84-92	56-73	60-86	17-58	84-94	54-69
Sesquiterpenes	8	14	3	21	1-2	2-7	6-10	7-18	3-17	8-23	3-8	4-28
Diterpenes	-	-	-	-	1-6	20-57	0.2-5	12-33	-	-	-	-
Phenylpropanoids	9	16	5	31	-	-	-	-	3-17	5-19	tr.-2	tr.-3
Others	0.1	0.5	0.2	0.4	1-2	-	tr.-1	-	tr.-0.2	3-27	tr.-0.2	8-20

Acknowledgements - This study was funded by the Fundação para a Ciência e a Tecnologia (FCT), Lisbon, under research contract PBIC/C/BIA/2070/95. The authors are grateful to the FCT for scholarships to P.A.G. Santos and J.A. da Silva.

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B-28

Fractional Distillation of Essential Oils by Vacuum Distillation with the Capillary Distiller

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In addition to chromatographic methods vacuum distillation represents an alternative for fractionating essential oils. The separation of essential oils by vacuum distillation is mainly influenced by the differences of the boiling points of the oil components, which can be divided into the following 5 groups relating to their boiling ranges.

	b.p.
➢ monoterpene hydrocarbons	150-190°C
➢ oxygenated monoterpenes	190-245°C
➢ sesquiterpene hydrocarbons	240-280°C
➢ oxygenated sesquiterpenes	280-300°C
➢ phenylpropanoids	230-270°C

By little modifications of the commercially available capillary distiller it was possible without any difficulties to fractionate 1-10 g volatile oils by distillation under reduced pressure of 55-60 mbar. However, the relative low plate number between 1 and 2 of the distillation device did not allow a significant separation of individual components within one group of components. In the following exemplary the results of separations of eucalyptus oil and chamomile oil are exhibited.

a) *eucalyptus oil*

Distillation of eucalyptus oil at 100°C yielded a distillate of 100% monoterpene hydrocarbons including 1,8-cineole. Increasing the temperature up to 140°C a second fraction containing mainly the oxygenated monoterpenes was obtained, whereas the sesquiterpenoids remained enriched in the residue. As a result a separation into 3 fractions could be achieved.

b) *chamomile oil*

Vacuum distillations of chamomile oil enabled an enrichment of the monoterpene hydrocarbons from 2 to 34%; moreover a separation between the sesquiterpene hydrocarbons and the higher boiling oxygenated sesquiterpenes was achieved.

B-29

Essential Oil Separation from Very Small Amounts of Plant Material

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The separation of essential oils from less than 100 mg of plant material for GC- and GC-MS-investigations can provide valuable information of rarely available samples. As an example the investigation of the essential oils from fruits of *Bunium bulbocastanum*, which could only be obtained in very small amounts, exhibited the fundamental importance of micro hydro-distillation by means of the capillary distiller.

We have investigated fruits from 5 different sources or habitats, respectively, which were declared as *Bunium bulbocastanum*. Four of them exhibited the described morphological characters of *Bunium bulbocastanum* fruits, while the remaining fifth sample could be determined most probably as fruits from *Oenanthe pimpinelloides*.

Even from a fruit sample of approximately 100 mg and an essential oil proportion of 0.1% sufficient oil for GC- and GC-MS-measurements was obtained. According to the gas chromatograms only 3 of the invastigated samples exhibited a high conformity of the composition and indicated together with the morphological characters of the fruits, that the declared species *B. bulbocastanum* most probably had been delivered. GC-MS-analysis of the essential oils from these samples revealed caryophyllene epoxide as main constituent. In addition trans-β-farnesene, humulene epoxide II, β-caryophyllene, salvial-4(14)-ene-1-one and the so far rarely found furanosesquiterpene dendrolasin were identified.

The distillate of the fourth sample, which resembled morphologically the first three samples, exhibited a total different composition with 87% apiole as main constituent. Because of its morphological resemblance it is suggested that in case of the fourth sample fruits of another *Bunium* species had been delivered.

B-30

ESSENTIAL OIL FROM A CRITICALLY ENDANGERED SPECIES - *SYZYGIUM TRAVANCORICUM*

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Syzygium travancoricum, (Myrtaceae) is a critically endangered medicinal plant. Family Myrtaceae is known for its commercial importance owing to rich essential oil contents. When studied, the leaves of *S. travancoricum* were found to possess a characteristic aroma and on analysis of the essential oils of the leaves, trans ocimene, caryophyllene and copaene were identified as the major constituents. The hexane extracts of the leaves exhibited antifungal activity. Also the antimicrobial activity of the oil is discussed since value addition to the plant will help in the conservation measures.

B-31

THE ESSENTIAL OIL OF *TEUCRIUM FLAVUM* L.

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Teucrium flavum L. is a common plant of the Mediterranean region. It is one of more than 400 species of *Teucrium* genera. Unlike some other *Teucrium* species, *T. flavum* L. is not used in folks medicine, and therefore has not been extensively researched. The samples of *T. flavum* L. were collected in Rovinj (Istria, Croatia) approximately at sea level, during flowering period (end of June – beginning of July) 1999. The identity of the plant material was confirmed at the Institute of Pharmaceutical Botany of the Pharmaceutical Faculty in Zagreb. After air drying, the samples were distilled in a Clevenger type distiller. The yield of essential oil was low (less then 0,1%), and it was separated from aqueous phase by organic solvents (pentane - diethylether 1:1). The oil was analyzed by GC and GC/MS using the following instruments:
Perkin Elmer AutoSystem XL / FID
Perkin Elmer AutoSystem XL / Qmass
Varian 3400 / Saturn ITMS
Identification of the components was made based on retention data, co-injection of available standards and mass spectrometric data. A chromatogram of , *T. flavum* L. oil shows more than 80 compounds of which none is present in a concentration of more than 10%.

B-32

ESSENTIAL OIL OF PEPPERMINT AND SACHALINMINT FROM NORWAY WITH REGARD TO LEAF POSITION USING SOLID-PHASE MICROEXTRACTION (SPME) AND GAS CHROMATOGRAPHY/ MASS SPECTROMETRY ANALYSIS (GC-MS)

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The essential oil of leaves and flowers of peppermint (*Mentha × piperita* L.) and sachalinmint (*Mentha arvensis* L. *ssp. sachalinensis* (BRIO) KUDO) has been studied by direct headspace sampling using SPME coupled with GC-MS analysis.
The content of peppermint-characteristic compounds like menthol, menthyl acetate and neomenthol increased in basipetal direction (older plant parts) while menthone and isomenthone showed higher levels in acropetal direction (younger plant parts). Higher levels of menthofuran were found in peppermint flowers in contrast to the leaves.
Sachalinmint was studied in detail with focus on essential oil content and composition by comparing SPME technique with common hydrodistilled solvent-based samples (ethanol). The essential oil content increased linearly in acropetal direction from 1.08 % (0-20 cm plant height) to 1.75 % (60-80 cm; young leaves and flowers). In contrast to peppermint, solvent-based samples of sachalinmint showed a minor complex matrix with a very high menthol and a much less menthone content (87.89 % and 4.05 %, respectively). Having tested SPME exposure times in a range from 10 s to 5 min, an extraction time of 30 s was found sufficient enough to detect both low and high-eluting compounds. Comparing SPME and solvent-based samples, the same tendencies of increasing menthol/menthone content in basipetal/acropetal direction could be detected by both analysis methods. Regarding the detection limit, SPME gave additional detailed information about less important monoterpenic and sesqiterpenic compounds in both peppermint and sachalinmint samples.

B-33

COMPOSITION OF THE ESSENTIAL OIL OF THE ROOTS OF *Vaieriana ficariifolia* Boiss. FROM IRAN

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Valeriana ficariifolia, one of the seven species of Valeriana in Iran was collected from Arak(central region of Iran). The essential oil of the roots isolated by hydrodistillation was analyzed by GC and GC/MS with DB-5 capillary column. Thirty components were identified using MS spectra and Kovats indices constituting 76.9% of the oil, the major components being endo-borneol (17.7%), camphene (10.1%), bornyl isovalerate (7.4%), bornyl acetate (7.2%), α-bisabolen(z) (4.8%), isovaleric acid (4.7%) and drimenol (3.8%). Most of the components were similar to *V. officinalis*. However, some of the components are just new to this species.

B-34

ESSENTIAL OIL CONSTITUENTS OF *OLIVERIA DECUMBENS* VENT.

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Oliveria (Umbelliferae) is represented by only one species in Iran. *Oliveria decumbens* Vent. Is a wild herb having violet flowers with a pleasant fragrance. The plant can be found distributed in some western provinces of country. According to the literature, *O. decumbens* was not the object of any research up to now and this abstract is the first phytochemical investigation on this plant.

The essential oil of aerial parts of *O. decumbens* at full flowering stage was isolated by hydrodistillation method in all-glass apparatus and the oil was dried over anhydrous sodium sulfate. The constituents of the oil were investigated by GC and GC-MS analysis. The major components were, thymol (37.2%), carvacrol (22.1%), γ-terpinene (12.8%), ρ-cymene (11.8%), myristicine (9.6%) and limonene (2.5%). The yield of the oil was 3.5% (w/w). Identification of the compounds was based on retention indices relative to *n*-alkanes, computer matching with the Wiley275.L library, MS data and , whenever possible, by co-injection with authentic samples.

B-35

COMPARATIVE STUDIES ON VOLATILE COMPONENTS OF JAPANESE LIVERWORTS

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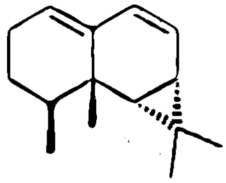
We are continuing our studies on chemical constituents of liverworts from the viewpoint of chemosystematics and biological activity. [1,2] At present, gametophyte constituents of many liverworts have been fully studied but little attention has been paid to the sporophyte, which can be separated into stalk, receptacle and spores in the order Marchantiales. 1) *Marchantia paleacea* subsp. *diptera*, *Marchantia polymorpha*, *Reboulia hemisphaerica*, *Dumortiera hirsuta* were separated into thalli, stalks, receptacles, and spores and each part was extracted in diethylether, n-hexane and/or methanol. 2) Several other liverworts were extracted in diethylether only. The crude extracts were analysed by GC/MS. The identification of each component was carried out by comparison of retention times and mass spectra with those of authentic specimen.

Results: 1) Spores of *Reboulia hemisphaerica* contained three sesquiterpenoids (**1-3**) and one unidentified diterpene-hydrocarbon as major component. 2) 1-Octen-3-yl acetate, which is partly responsible for the strong mushroomy odor, was detected in thalli of *Wiesnerella denudata*, *Conocephalum conicum* and *C. japonicum*, in receptacles of *Reboulia hemisphaerica* and in one sample of female stalks and receptacles of *Marchantia polymorpha.* 3) Thalli, stalks and receptacles of *Marchantia paleacea* subsp. *diptera* elaborated all the same compounds of which β-caryophyllene (**4**) was major.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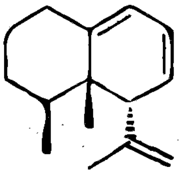
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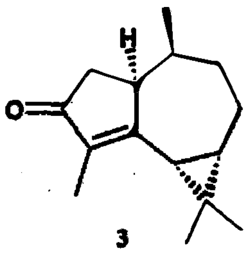

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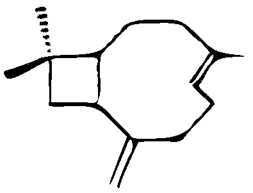
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B-36

THE ESSENTIAL OILS OF THE GENUS *BRACHANTHEMUM* D.C. FROM MONGOLIAN PALCO-ENDEMIC PLANTS

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The genus Brachantemum D.C.(Asteraceae) is represented in the Flora of Mongolia by three species,of which two are paleoendemic and relict plants [1]. Hydrodistilled essential oil of three species of Brachantemum were analysed by GC and GC/MS [2].
Essential oil compositions of above species are described as the follows:
The *Brachantemum mongolorum* Grub. growing only in Eastern Mongolia, are characterized by essential oil components of leaves: A high percentage of chamazulene-cineol type terpenoids were detected. Chamazulene (17,20%) 1,8-cineol (14,67%), camphor (8,52%), germacrene-D (6,28%), β-caryophyllene (6,16%), borneol (4,25%), caryophyllene oxide (4,10%) and myrcene (3,85%).

The *Brachantemum mongolicum* Krasch. which is growing only in the Dzungarian Gobi, is containing a high precentage of cineol, camphor type terpenoids: 1,8-cineol (32%), camphor (16%), borneol (3,12%) and bornylacetate (2,14%).

The *Brachantemum gobicum* Krasch. growing in the Eastern Gobi and Alashan Gobi. The steam oil of *B. gobicum* was characterized by a high guaiol and germacrene-D content, for example, guaiol (8,26%), palamitic acid (6,72%) and camphor (5,62%). The oil of leaves contained 1,8-cineol (17,3-20,5%), camphor (15,9-46,5%).

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B-37

ESSENTIAL OIL COMPOSITION OF *MELISSA OFFICINALIS* L. DEPENDING ON MONGOLIAN CLIMATE

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In the family Lamiaceae the *Melissa officinalis* L has been reportedly been used in food as well as in folk traditional medicine[1].
The chromatographic and mass spectrometric study of the essential oil of *M. officinalis* L from Mongolia (Table 1) is discussed.

Basic components of the essential oil of *M. officinalis* L in Mongolian climate.

N o	Compound	Relative area %	Comparative dates (%)			
			J.Tekel (1997)	M.Usai (1995)	A.Shalady (1995)	A.Clery (1992)
1	citronellal	8,72	10,08	6,48	13,32	2,7-30,5
2	neral	41,42	22,96	28,76	19,75	10,4-47,0
3	geranial		34,20	40,62	26,80	12,9-35,0
4	geranylacetate	37,51	4,71	0,9	1,45	-
5	geraniol	1.20	-	1,99	4,23	2,2-12,5
6		1,67	-	1,85	0,64	0,9-4,0
7	nerol	0,80				
8	β-caryophyllene	2,12	4,12	5,87	4,95	0,6-2,0
	caryoph oxide	0,6	4,66	-	9,99	-

Among these compounds 8 monoterpene hydrocarbons, with a total amount of about 4,19%, 6 oxygenated monoterpenes with 89,31% for whole essential oil components, 11 sesquiterpene hydrocarbons and an oxygenated sesquiterpene were detected in the amount of 6,51%. The main percentage occurs in the oxygenated compounds neral (41,42%), geranial (37,51%), citronellal (8,72%), geraniol (1,67%), geranylacetate (1,20%), and nerol (0,8%).

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B-38

SESQUITERPENE HYDROCARBONS WITH TRIFARANE BACKBONE IN THE LIVERWORT *TROCHOLEJEUNEA SANDVICENSIS*

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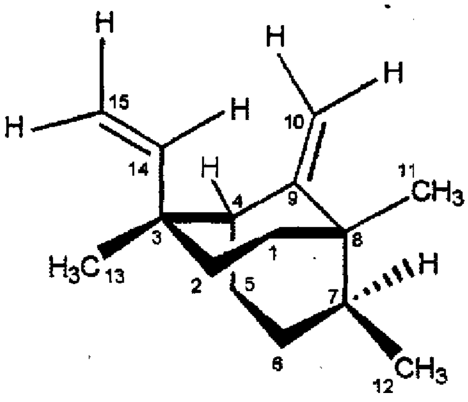
Sesquiterpenes with the unusual bicyclo[3.3.1]nonane (trifarane) skeleton were reported for the first time in a marine sponge *Dysidea fragilis* [1] and later in the Malaysian liverwort *Cheilolejeunea trifaria* [2]. In both cases the trifarane system was highly functionalized. In a recent investigation of the hydrocarbon fraction of the Japanese liverwort *Trocholejeunea sandvicensis*, collected near Tokushima [3], we detected some constituents with unknown structures in significant amounts, which had been observed before as minor components in many other liverworts, e.g *Porella arboris-vitae, P. baueri, P. obtusata, P. platyphylla, Ptilidium ciliare, P. pulcherrimum, Frullania muscicola* and *Cololejeunea cacarea*. The isolation of the new sesquiterpene hydrocarbons from *T. sandvicensis* was carried out by repeated preparative gas chromatography using cyclodextrin stationary phases. 1- and 2-dimensional NMR investigations resulted in the structures of trifara-9,14-diene (**1**) and 3,7-di-*epi*-trifara-9,14-diene (**2**). Both trifaradienes and pinguisanes, another group of sesquiterpenes common to *Porella* and *Ptilidium* species and to the *Lejeuneaceae* family, possess a vinyl side chain. A biogenetic relationship between the pinguisane and trifarane skeletons appears highly probable. **1** and **2** can be considered as precursors of the functionalized trifarane derivatives from *Dysidea fragilis* and *Cheilolejeunea trifaria*.



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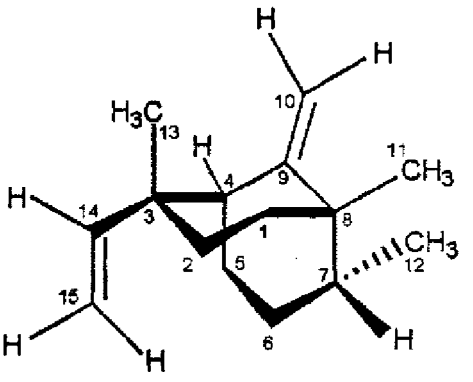

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B-39

COMPARATIVE ANALYSIS OF THE ESSENTIAL OILS OF *SATUREJA WIEDEMANNIANA* (LALLEM.) VELEN (LABIATAE)

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Satureja wiedemanniana (Lallem.) Velen (Labiatae) is an endemic species of Turkey. Aerial parts of the plant collected from 10 different localities in Turkey were subjected to hydrodistillation to produce oils which were analysed by GC/MS. Carvacrol, thymol, p-cymene and γ-terpinene were found as main constituents. The results are presented in a comparative manner.

B-40

The essential oil composition, medicinal uses and antimicrobial activity of the resurrection bush, *Myrothamnus flabellifolius*

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In many parts of Africa a tea is made from the dried leaves of *Myrothamnus flabellifolius* to treat respiratory ailments and 'diseases of the breast'. The plant is often mixed with fat / butter and applied topically to treat abrasions. The rich aromatic smoke is inhaled to treat chests pains and asthma. Eighty-five compounds were identified in the hydro-distilled essential oil of the resurrection bush, *Myrothamnus flabellifolius*. The major compounds in this aromatic medicinal plant from Southern Africa are pinocarvone and trans-pinocarveol. The essential oil were found to have moderate antimicrobial activity against the pathogens tested.

B-41

Osmitopsis asteriscoides – the essential oil composition and bioactivity of a Cape Dutch remedy

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Osmitopsis asteriscoides, known locally as >bels= or 'belskruie'is restricted to the southwestern Cape region in South Africa. This traditional Cape Dutch remedy has been used to treat various ailments and may be taken orally in the form of a brandy tincture for chest complaints. The essential oil composition of *Osmitopsis asteriscoides* is reported together with results on the anti-microbial activity of this South African endemic which has been used in traditional medicinal preparations. Camphor and 1,8-cineole are the main constituents in the hydro-distilled oils. The essential oils were found to be moderately antimicrobial against the eight pathogens tested.

B-42

THE ANALYSIS OF THE CONTENT AND CHEMICAL COMPOSITION OF THE ESSENTIAL OIL IN THE LEAVES OF *SALVIA OFFICINALIS* L. cv. 'BONA' IN THE SECOND YEAR OF ITS VEGETATION

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Sage (*Salvia officinalis* L.) is an oliferous plant, grown on the territory of Poland (3). The raw material of Sage is leaves and leaved tops of shoots (2). It is used sa anti-inflammatory, anti-microbial and astringent agent (4).

The research was conducted in the years 1997-1999. The aim of the study was to determine the content as well as the chemical composition of the essential oil in the leaves of Sage cv. 'Bona', in the second year of utilizing the plantation. The raw material was collected in two phases (in May - first harvesting and in the middle of September - second harvesting).

The three-year study showed that the percentage quantity of the essential oil in the raw material of Sage is linked with the term of collecting the herb. The oil amount was higher in *Folium Salviae* coming from the the second phase of its collection and ranged from 1.6 to 1.8%. The percentage quantity of the essential oil in the leaves of Sage, which was collected in May (first harvesting) fluctuated from 0.8 up to 1.4%.

The GC/MS analysis showed the presence of thirty-two chemical compounds in the essential oil, one of which remained unidentified. We also discovered a changing percentage share of sage essential oil, in particular years of research as well as depending on the term of collecting the raw material.

The main component appearing in sage oil is α- and β- thujone, which cause the characteristic smell and fragrance (1). Studies prove that the essential oil obtained from the raw material collected in the second term contained a higher amount of the substance than in the first one. Moreover, the percentage quantity of camphor in the essential oil extracted from Sage, collected in the middle of September (second crop), was higher.

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B-43

Essential oil Composition from *Thymus albicans* Hoffmanns. & Link collected at different regions of Ria Formosa (Algarve)

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Thymus albicans is an aromatic plant that is only detected in a restrict area of Algarve (Portugal). This species was well studied by Salgueiro (1994) that established several chemotypes according to the major components present in the essential oils. The oil composition was determined from all the aerial parts of the plant collected during the flowering phase.

In the present work, the leaf and flower essential oil composition was established by GC and GC-MS, from *Th. albicans* collected at three areas of Algarve, all of them around a protected region known as Ria Formosa. The oils isolated from the flowers collected at Gambelas were obtained in higher yields (9.2 %, v/w) than those isolated from the leaves (5.7 %, v/w). The remaining oils from the flowers and the leaves were obtained in similar yields. As so, the oil yields from the flowers and the leaves collected at Ancão were 3.5 % (v/w) and 3.6 % (v/w) , respectively, while those obtained from the flowers and the leaves collected at Quinta do Lago were 4.2 % (v/w) and 4.3 % (v/w), respectively. All samples showed 1,8-cineole as the main component of the essential oils. The leaf essential oils had the highest amounts of this compound. The essential oils from the plants collected at Gambelas had the greatest percentages of 1,8-cineole in contrast to the samples harvested at Quinta do Lago that showed the lowest concentrations of this oxygen-containing monoterpene. The second most important component of the essential oils changed depending on the harvesting region. Thus, linalool was the most second one for the plants collected at Quinta do Lago and Ancão, whose concentrations were 30.3 %-36.9 % and 9.8 %-13.5 %, respectively, while those collected at Gambelas such concentrations only reached 0.4 %-1.6 %. In contrast to the 1,8-cineole, linalool was always present in higher amounts in the flower essential oils. The oils from the samples harvested at Gambelas had the highest concentrations of δ-terpineol+borneol, 5.8 % for the leaf oils and 6.9 % for the flower oils. The amounts of those compounds in the leaf and the flower oils from the plants collected at Quinta do Lago only reached 0.2 % and 1.5 %, respectively. Three other significant differences were detected in the essential oils from the plants collected at Quinta do Lago. These samples showed the lowest percentages of α-pinene (1.7 %-1.8 %), camphene (0.2 %) and β-pinene (2.6 %-2.9 %).

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B-44

The study of nitrogen fertilizer effects in essential oil content and different stages of growth of *Dracocephalum moldavica L.*

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Dragonhead (*Dracocephalum moldavica,* L.) is an annual, essential oil containing plant belonging to the Lamiaceae family, which originates from south Siberia and the Himalayas area. However dragonhead is suitable for cultivation under cold climatic conditions.

The aim of this present work was to study the effect of nitrogen fertilizer on the quality and quantity of essential oil and the variation of the components during the growth in *D. moldovica* L. This plant was grown in loamy soil with pH = 7.8, available N 0.07%, available P 8.6 ppm, available K 260 ppm, under four nitrogen (N) levels 0, 20, 40, 60 kg N/ha, supplied as urea [$CO(NH_2)_2$]. Plants were harvested at the flowering and seeding stages, the flowering shoots were separated from stems and essential oil was distilled from them by steam distillation. Extracted oils were analyzed for their components by gas chromatography-mass spectrometry.

Application of N had significant effects on plant height, leaf surface, dry weight, wet weight, length of leaves, number of shootings, total biomass, length of flowering shoots, number of flowers, weight of 1000 seeds and germination, however all of them increased. There were no significant differences ($p<0.05$) in length of root between all plants on the ground.The 60 kg N/ha had a significantly higher effect on these parameters than other treatment.

The oil yields and the main oil components increased significantly up to an application of 60 kg N/ha as against controlled treatments, but generally oil contents of plants in greenhouse were lower than those grown in field conditions. The volatile oil mainly consists of neral, geranial and geranylacetate and these compounds have an antiviral and antimicrobial effect. Nitrogen fertilization also indicated a significant effect on the proportion of other components, like myrcene, α-thujene, camphene, caryophyllene, farnesene, cyclohexanol, and γ-terpinene so that these compounds increased from trace levels (<0.1 %) to detectable rates.

Present measurements clearly indicated that there are considerable changes in the essential oil content in the course of ontogeny. The volatile oil has the highest value after flowering when the green seeds appear and decreases rapidly when the flowering in over and at ripening seed. So harvesting should be carried out at the end of full flowering if dry drug is to be extracted or when the green seeds appear if the aim is essential oil production.

B-45

ESSENTIAL OILS CONTENTS INVESTIGATION IN *MELISSA OFFICINALIS* L. CALLUS CULTURES AND INTACT PLANTS

N.M. Strashnyuk

Laboratory of Ecology and Biotechnology, 2 M.Kryvonis St. Ternopil State Pedagogical University, Ternopil, 46027 UKRAINE

Melissa officinalis L (Laminaceae) is widely used in medicine and is considered to be an official plant in many countries of the world [1]. In shoots and leaves of *M. officinalis* significant amounts of essential oils, ascorbic acid, carotene, resins, tanning and bitter substances, organic acids (caffeic, oleic, ursolic, succinic, chlorogenic, oleanolic), resins and saponins are synthesised [2,3].

There were analysed contents of essential oils obtained from *M. officinalis* 10 samples cultivated under different conditions. Various parts of the plant (stems and leaves), 4-year old and 5-month callus of stem and leaf origin were examined. Callus cultures were grown on nutrient mediums B5 [4] with half content of macro- and microsalts (B5/2) supplemented with different phytohormones combinations of Kin and 2.4-D. The contents of essential oils in the analysed samples of plant material were determined by water distillation method and the components composition was investigated by GLC usage.

All samples demonstrated presence of essential oils but their quantitative and qualitative contents were different. The biggest essential oils amount (0,32%) was found in the leaves taken from the middle stem part of intact plants. Somewhat lower one (0,27%), but close to maximum contents of essential oils, was observed in little top leaves. Intact plants stems comprised 0,18-0,22% of essential oils. Long-term (during 4 years period) cultivation resulted in decrease of essential oil amount in callus cultures of stem (0,04%) and leave (0,06%) origin and change in correlation among its components. There could be observed negative correlation between essential oil contents and relative increase of callus fresh weight. Essential oils amount in 5-month callus cultures constituted correspondingly 0,16 and 0,18%.

According to GLC data citronellal, citral, linalool, neral, caryophyllene were identified as the main components of essential oil. Citral contents in all callus cultures were bigger than in intact plants. The greatest linalool concentration was characteristic for 5-month callus cultures of *M. offisinalis*.

References

[1] V.A.Kurkin, T.V.Kurkina, G.G.Zapesochnaya et al. Chem. of Natur. Comp. (2) (1995) 318-320.

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[4] O. L.Gamborg, J. P.Shyluk, L. C.Fowke et al. Z. Pfllanzenphysiol. **95** (3) (1979) 255-264.

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Bundesstraße 8-10 • 20146 Hamburg • Tel. 040 – 44 82 19 • Fax 45 31 61

ISEO 2000
31st INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

Hamburg / Germany
September 10 - 13, 2000

Chairmen:
Prof. Dr. K.-H. Kubeczka / Prof. Dr. W.A. König
Pharmaceutical Biology / Organic Chemistry
University of Hamburg

Program

List of Participants

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General Information

Chairmen of the Symposium: **K.-H. Kubeczka, W.A. König, Hamburg/Germany**
Honorary Guest: **Prof. Dr. A. Baerheim-Svendsen, Oslo/Norway**

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and the staffs of the Department of Pharmaceutical Biology and the Institute of Organic Chemistry of the University of Hamburg

Location and time

The conference and poster session will be held at the Chemical Institutes, Martin-Luther-King-Platz 6, in the center of the city. The symposium will commence on Monday, September 11th at 9.30 and close at 12.40, Wednesday, September 13th.

Registration

The registration desk will be located in the lounge of the lecture halls of the Chemistry Department, Martin-Luther-King-Platz 6. It is open on Sunday, September 10th from 17.00-19.00. From Monday to Wednesday it will be open from 8.30 onwards; it closes on Wednesday, September 13th at 12.00.
Phone: (040) 42838 4123

Slides

Slide size should be 5x5 cm (2"x2"). The speakers are requested to label the slides adequately and hand them over to the operator before the beginning of each session. An overhead projector and a video-beamer will also be available.

Poster session

The poster boards are located in the passage way on the left of the porter's office. They will be labelled; the number corresponds to the number listed in the program. Please prepare your poster on Sunday, September 10th or in the morning September 11th. You are required to be present at your poster during the indicated poster session for discussion of your results.

Exhibition

An exhibition will be held during the meeting from Monday to Wednesday, September 11-13, in the lounge of the lecture halls. Manufacturers and distributors of analytical equipment will show their products. In addition, informations regarding literature etc. are given.

Lunch

The registration does not include meals. In the vicinity of the conference building are several restaurants. Furthermore, lunches will be offered during the conference in the near student's dining hall at Van-Melle-Park and Bundesstrasse 55 (GEOMATICUM).

Social events

Sunday, September 10, 2000

19.00 Get-Together-Party
An informal welcome with a small buffet will be arranged in the conference building, Martin-Luther-King-Platz 6, opposite to the library.

Tuesday, September 12, 2000

19.00 Symposium-Dinner
Hotel ELYSEE, Spiegelsaal 1
Rothenbaumchaussee 10

Wednesday, September 13, 2000

13.45 Half-day-excursion to "Lüneburger Heide"
There will be the possibility to have supper in a typical restaurant
(meals and drinks are not included in the fee).
Return in Hamburg at approx. 22.00.

SCIENTIFIC PROGRAM

Sunday September 10, 2000

17.00 - 19.00 Registration at the Symposium Office
19.00 - 21.00 Get-Together-Party

Monday September 11, 2000

8.30 Registration at the Symposium Office

9.30 **Opening Session**
Welcome Addresses

10.00 Plenary Lecture 1 Chair: P. Weyerstahl

PL-1 **A. Mosandl**; Frankfurt, Germany
AUTHENTICITY ASSESSMENT OF ESSENTIAL OILS - THE CURRENT STATE AND THE FUTURE

10.50 Coffee break

11.10 L-01 **J.C.R. Demyttenaere**, K. van Belleghem, R. de Kimpe; Gent, Belgium
BIOTRANSFORMATION OF (+)- AND (-)-LIMONENE BY FUNGI AND THE USE OF SOLID PHASE MICROEXTRACTION FOR SCREENING

11.30 L-02 **C. Bicchi**, C. Cordero, C. Iori, P. Rubiolo; Torino, Italy
HEADSPACE SORPTIVE EXTRACTION (HSSE) IN THE HEADSPACE ANALYSIS OF AROMATIC AND MEDICINAL PLANTS

12.00 L-03 **M. Mucciarelli**, C. Iori, M. Maffei, C. Bicchi; Torino, Italy
PLANT VOLATILES AND ENDOPHYTIC FUNGI: HC-SPME-GC ANALYSIS OF PEPPERMINT CULTURED IN VITRO

12.20 Lunch break

14.00 Plenary Lecture 2 Chair: B. Lawrence

PL-2 **R. Naef**; Geneva, Switzerland
SOME ASPECTS OF CITRUS OIL CHEMISTRY

14.50 L-04 **W. Feger**, H. Brandauer, H. Ziegler; Aufsess, Germany
ANALYTICAL INVESTIGATION OF SWEETIE (OROBLANCO) OIL AND GERMANCRENES IN CITRUS PEEL OILS

15.10 L-05 **D. Joulain**, R. Tabacchi; Grasse, France; Neuchatel, Switzerland
SESQUITERPENE HYDROCARBONS FROM PATCHOULI AND PATCHOULOL

15.30 Coffee break

Oral Communications Chair: K.H.C. Baser

16.00 L-06 **T.A. van Beek**, C. van de Haar, A. Dapkevicius, I. Koleva, A. Pukalskas, H.A.G. Niederländer, Wageningen, The Netherlands
ON-LINE HPLC DETECTION OF RADICAL SCAVENGERS IN PLANT EXTRACTS AND ESSENTIAL OILS

16.30 L-07 **A. Zänglein**; Cologne, Germany
COMPARISON OF PEAK PATTERNS - A CUSTOM VERSUS A COMMERCIAL SOFTWARE SOLUTION

16.50 - 18.00 **Poster Session I (A)**

Tuesday September 12, 2000

9.00 Plenary Lecture 3 Chair: E. Stahl-Biskup

PL-3 **B. Lawrence**, Winston-Salem, USA
THE COMMERCIALLY IMPORTANT MINT OILS

9.50 L-08 **A. M. Viljoen,** B.-E. van Wyk, K.H.C. Baser, B. Demirci; Parktown, South Africa; Eskisehir, Turkey
SOUTH AFRICAN MEDICINAL AROMATIC PLANTS - AN OVERVIEW

10.10 L-09 **A. V. Tkachev**, Novosibirsk, Russia
COMPOSITIONS OF VOLATILES FROM SIBERIAN MEDICAL PLANTS

10.30 Coffee break

Oral Communications Chair: C. Bicchi

10.50 L-10 F. Nagashima, E.H. Lahlou, Y. Noma, **Y. Asakawa**; Tokushima, Japan
SESQUI- AND DITERPENOIDS FROM JAPANESE AND EUROPEAN LIVERWORTS

11.10 L-11 **K.H.C. Baser**, M. Kürkcüoglu; Eskisehir, Turkey
RESEARCH INTO TURKISH ROSE OIL: RECENT RESULTS

11.30 L-12 O. Barazani, A. Fait, Y. Cohen, S. Siminshtein, N. Dudai, U. Ravid, E. Putievsky, **J. Friedman; Tel** Aviv, Israel
CHEMOTYPIC DIFFERENTIATION VERSUS PLASTICITY IN INDIGENOUS POPULATIONS OF FOENICULUM VULGARE VAR. VULGARE IN ISREAL

11.50 L-13 **Ch. Franz**, Vienna, Austria
BIODIVERSITY AND RANDOM SAMPLING IN ESSENTIAL OIL PLANTS

12.10 Lunch break

14.00 Plenary Lecture 4 Chair: G. Buchbauer

PL-4 **H.J. Bouwmeester**; Wageningen, The Netherlands
REGULATION OF TERPENOID BIOSYNTHESIS

14.50 L-14 **W. Boland**; Jena, Germany
INDUCTION OF PLANT VOLATILE BIOSYNTHESIS BY MICROBIAL AND INSECT ELICITORS

15.10 L-15 **Y. Noma**, T. Hashimoto, Y. Asakawa; Tokushima, Japan
MICROBIOLOGICAL TRANSFORMATION OF A-SANTONIN, 1,2-DIHYDRO- AND TETRAHYDRO-A-SANTONIN, NOOTKATONE, OPTICAL ACTIVE A-IONONE AND 2-ADAMANTANONE

Software demonstration

15.30 **D.H. Hochmuth**; Hamburg, Germany
A PROGRAMME FOR THE RAPID AND CONVENIENT IDENTIFICATION OF GC-MS MASS SPECTRA OF TERPENOID COMPOUNDS

15.45 **A. Pauli**; Zirndorf, Germany
DATABASE OF VOLATILE COMPOUNDS WITH ANTIMICROBIAL PROPERTIES

16.00 Coffee break

16.20 - 18.00 **Poster Session II (B)**

19.00 **Symposium dinner**
Hotel Elysee, Rothenbaumchaussee 10

Wednesday September 13, 2000

9.00 Plenary Lecture 5 Chair: Ch. Franz

PL-5 **W. Francke**; Hamburg, Germany
CHEMICAL COMMUNICATION: STRUCTURAL CONCEPTS AND EVOLUTIONARY PRINCIPLES

9.50 L-16 **G. Buchbauer**; Vienna, Austria
ESSENTIAL OILS AND SINGLE FRAGRANCE COMPOUNDS: BIOLOGICAL PROPERTIES

10.10 L-17 **S. Fuchs**, A. Mosandl, Frankfurt, Germany
BIOSYNTHETIC ASPECTS OF PIPERITONE IN PEPPERMINT (MENTHA x PIPERITA L.)

10.30 Coffee break

Oral Communications Chair: H. Schilcher

10.50 L-18 **M. Lis-Balchin**, A. Brandstetter, A. Bauer; London, UK; Vienna, Austria
COMPARATIVE ANTIMICROBIAL ACTIVITY OF SELECTED PLANT ESSENTIAL OILS AND THEIR RESPECTIVE HYDROSOLS IN A MODEL FOOD SYSTEM

11.10 L-19 **F. Christoph**, P.M. Kaulfers, E. Stahl-Biskup; Hamburg, Germany
IN-VITRO BACTERICIDAL EFFECTS OF TEA TREE OILS S.L. ON STAPHYLOCOCCUS AUREUS

11.30 L-20 **J.L. Markham,** S.G. Griffin, S.D. Cox, S.G. Wyllie; Hawkesbury R., Australia
THE SIGNIFICANCE OF THE OUTER MEMBRANE IN RESISTANCE OF GRAM-NEGATIVE BACTERIA TO MONOTERPENES

11.50 L-21 **M. Maffei,** W. Camusso; Torino, Italy
ALLELOPATHIC EFFECTS OF PEPPERMINT ESSENTIAL OIL IN HIGHER PLANTS: INTRACELLULAR CALCIUM VARIATIONS

12.10 **Closing session**

12.30 Lunch break

13.45 - 21.00 **Excursion to "Lüneburger Heide"**

Poster Presentation

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Abou Zeid, A.; Ibrahim, N.

A-02
CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF OCIMUM BASILICUM GROWING IN KUWAIT
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A-03
ESSENTIAL OIL OF OLIVERIA DECUMBENS VENT. GROWN IN IRAN
Ahmadi, L.; Mirza, M., Jalilian, N., Nemati, M.

A-04
ISOLATION OF GENES INVOLVED IN BIOSYNTHESIS OF GERMACRENE D ENANTIOMERS FROM SOLIDAGO CANADENSIS
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A-05
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Amanzadeh, Y., Pirali-Hamedani, M.; Ahadi-Barzaki, M.

A-06
SOME HYDROCARBON SESQUITERPENES OF THE ESSENTIAL OIL OF LAGGERA TOMENTOSA FROM ETHIOPIA
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A-07
THE ESSENTIAL OIL AND POLYACETYLENES FROM SCIADOPHYLLUM PULCHRUM HORT. (ARALIACEAE)
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A-08
QUALITATIVE AND QUANTITATIVE DETERMINATION OF ESSENTIAL OILS FROM SEED VESSELS OF DICTAMNUS ALBUS L.
Wolski, T.; Baj, T.

A-09
CHEMICAL POLYMORPHISM OF THE ESSENTIAL OILS FROM THYMUS CAESPITITIUS GROWN ON TH ISLANDS PICO, FAIAL AND GRACIOSA (AZORES)
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A-10
ELECTRONIC NOSE AS A FAST SCREENING TOOL
POSSIBILITIES AND LIMITS IN THE ANALYSIS OF ESSENTIAL OILS AT THE EXAMPLE OF OREGANO OIL
Bauermann, U.

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THE HEADSPACE AND IMMERSION TYPE SPME TRAPPING OF VOLATILES IN THE AROMATIC WATER OF ORIGANUM ONITES L.
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A-13
SCREENING AND DEVELOPMENT OF NEW AROMA CHEMICALS FROM GENETIC RESOURCES OF GENUS OCIMUM
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A-14
NECESSITY OF CHIRAL CAPILLARY COLUMNS FOR GC ANALYSIS OF PHARMACOPOEIA ESSENTIAL OILS
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A-15
Δ-TERPINYL ACETATE - ISOLATION AND SYNTHESIS OF A NEW NATURAL COMPONENT
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A-16
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ESTRAGOLE CONTENT IN BASIL CULTIVARS GROWN IN SYRIA
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Kürkcüoglu, M.; Baser, K.H.C.

B-07
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Lochynski, St.; Frackowiak, B.; Patrzalek, A.; Wawrzenczyk, C.

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Lopes, D.; Bizzo, H.R.; Albuquerque, J.C.; Maia, V.C.R.; Valle, L.C.

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31st INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

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