36th International Symposium on Essential Oils

Programme and Book of Abstracts

4–7 September, 2005
Budapest, Hungary

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Foreword

The importance of medicinal and aromatic plants, including species accumulating volatiles is increasing continuously. Based on the international trend, projected to the 21st century, the utilisation of natural products having medical-, health protection-, special nutritional-, even improving effect on life-standard, or owing other special advantages will increase in both absolute and relative sense.

Scientists, practitioners, experts of firms and wholesale companies and many others interested in the search and production of volatiles join the 36th International Symposium on Essential Oils (ISEO'05) organized at Budapest. According to the pre-registrations at least 245 participants from about 45 countries arrived. The topics of the symposium include all aspects of essential oils and related natural products ranging from analysis, chemistry, biogenesis, taxonomy to biological activity and utilisation.

The Hungarian organizers coming either from the Institutes of Medicinal and Aromatic Plants of Corvinus University, or from Departments of Pharmacognosy of Semmelweis and Szeged Universities are pleased to welcome the participants arrived from different part of the World. We are sure that the opportunity of organizing this Symposium is a great compliment to all of us, Hungarians.

The idea of the Symposium rose in 2003 in Würzburg (Germany) and was motivated by the Hungarian traditions of this scientific field. Our traditions are justified by number of facts:

Medicinal and aromatic plants, especially for self consumption were produced in the territory of Hungary many centuries back. The first written reports about the small scale cultivation are present in the books written by monks settled here in the middle ages arriving from the Mediterranean regions (Italy, France). Further more many medicinal and aromatic plants, especially many of the members of Lamiaceae family had been introduced into the Carpathian Basin at that early time period. However, till the end of 19th century the cultivation of medicinal and aromatic plants was carried out on the "garden" scale and on limited area. The intensification of the production started in the first years of 20th century only, when the processing of the plant raw-material including oil distillation had been started as well. The first Research Station in the world specialised for medicinal and aromatic plants had been established in Hungary by the help of Ministry of Agriculture in 1915. The main activity of the Station was oriented to the control of medicinal and aromatic plant production coming mainly from wild and from limited cultivation. The activity of the Station was completed by R+D activity afterward supported by government.

After the 1st World War - due to the activity of the well organised Medicinal Plant Station as well - Hungary became the "leading" medicinal plant production country of Europe. It was reflected in both the development of drug and essential oil production and the increase of export activity based on it. As example, essential oil in value of 214 000 Hungarian "pengő" had been exported in 1938. The development of the medicinal and aromatic plant sector was completed at that time by overall technical development of processing.

The early period of the development of Hungarian medicinal and aromatic plant sector - after 20es up to the beginning of the 2nd World War - can be characterized as a time period of quick development of Hungarian essential oil industry. The peppermint (*Mentha piperita*), the English and French lavender (*Lavandula intermedia* and *L. angustifolia*) were introduced into the large scale cultivation using propagation material of foreign origin. The "relict" of the first plantation of lavender in the Tihany peninsula (which shows Mediterranean climatic characteristics being part of Balaton region) could be found even nowadays. The quick development of this time can be characterised by some data: till 1941 the amount of peppermint, lavender and dill oil increased up to 15-20 tonnes. That was a time as well, when the Hungarian camomile flower (*Matricaria recutita*) collected from the Hungarian Geat plain became world wide known.

After the 2nd World War the former structure of the medicinal and aromatic plant section - which could have been characterized by increasing effectiveness changed in a great deal. In spite of the administrative, political and economical contradictions existing in the former "socialist" system the medicinal and aromatic plant sector became a successful part of Hungarian Agriculture.

Some of the national products had been accepted as a special Hungarian product ("Hungaricum") evaluated by most respectfully in the world market (*Chamomillae flos*, *Basilici herba* and *folium*). By the estimates the cultivation area of medicinal and aromatic plants increased up to 37,000 - 42,000 ha in the last period of 80ies. The total drug mass reached the 35,000- 40,000 t yearly, from which 25,000-30,000 tonnes came from the cultivation. The production of essential oils was estimated to be in the range of 80-100 tonnes.

The outstanding Hungarian results achieved in the introduction and cultivation of medicinal and essential oil plants, as well as in chemistry, biology and industrial processing became know world-wide. Till the beginning of the changes of Hungarian political system in early 90th, our scientific position was appreciated by regular scientific meetings held in Budapest. As an example GA, ISHS, FIP organized Conferences, Congresses, Workshops, regularly. In 2001 more than 400 participants, scientists, research workers and practitioners from about 90 countries came to Budapest to participate the World Conference on Medicinal and Aromatic plants, which were organized by the experts of Department of Medicinal and Aromatic Plants as well.

However, by organizing ISEO'05 we will celebrate much more than the long history of Hungarian medicinal plant and essential oil sector, only. 2005 will be first whole-year period in which Hungary takes a share in life of new integrated Europe. We intend to celebrate both occasions together.

In this Abstract Book 30 lectures and 161 posters are presented giving a complex overview on scientific and practical result achieved in the filed of aromatic plants, recently.

As an additional program, the participants of the Symposium have an opportunity to visit one of the protected regions of Hungary specialized for collection of aromatic plants, especially that of *Juniperus communis*.

The symposium is held in Budapest, which provide special additional cultural merits. Budapest, the capital of Hungary, is an economic, financial and cultural centre with two million inhabitants. The city which is beautifully situated on both sides of the Danube river has a history dating back over 2000 years. There are ruins from the time of the Roman Empire as well as from the Middle Ages. Its main characteristics reflect the atmosphere of the end of the 19th century when the millennium of the Hungarian State was celebrated. It boasts a number of museums (picture gallery of the museums), theatres, concert halls, a lot of restaurants and other amenities. Several bath and thermal waters of various medicinal springs are also at the disposal of visitors. It provide an exceptional cultural experiences to the participants of the Symposium.

We are convinced, that the production and utilization of medicinal and aromatic plants, including volatiles will be one of the successful branches of the horticulture in the future, producing raw materials and their processed forms. However, to fulfill these requirements all steps of the production, isolation, processing and quality assurance system has to be modernized. The results of the Symposium predict, that the production of high quality volatiles, based on the globalized international co-operations will develop and play an important role in the future, too, on both national and international levels.

Prof. Dr. Jenő Bernáth
Head of the local organizers

General Informations

GENERAL INFORMATION

CONFERENCE VENUE

The conference will be organized in Hotel Bara***
H-1118 Budapest, Hegyalja u. 34-36.
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REGISTRATION

The Registration Desk is open at 08:00-18:30 on Monday and Tuesday, and 08:00-12:30 on Wednesday. On Sunday, 4 September, the registration starts at 14:00 and ends at 20:00.
Conference delegates and their companions will receive their badges, conference materials at the desk. Subscription for optional tours also takes place in this area.
Participants are kindly requested to wear their name badges during all events of the meeting.

CONFERENCE SECRETARIAT & ASSISTANTS

If you need any help during the conference you can find the staff of Diamond Congress at the registration desk. In case of emergency please quote this mobile phone number: +36-20-9362969
Conference assistants will be recognisable by their silver badge and by their T-shirts, signed: „HELP DESK“ They will help you in all practical aspects of conference participation. They also help the speakers and the chairpersons in the lecture rooms and will be at your service at the Registration Desk.

INCOMING MESSAGES AND MESSAGE BOARD

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

LUNCH AND REFRESHMENT

The organized lunch will be served at the Restaurant and the Gallery of Hotel Bara on Monday, Tuesday and Wednesday. Refreshments are included in the registration fee and will be served at the Gallery of Hotel Bara.
Lunches and refreshments are not included in the accompanying registration fee.

CONFERENCE WEBSITE

The Internet homepage of the Conference is kept up-to-date all the time:
http://www.diamond-congress.hu/iseo2005

SMOKE

The Hungarian law strictly regulates the possibility of smoking in all public buildings. Though there are devoted places for smoking inside the building, they cannot be separated in a way to avoid the dissemination of smoke in other areas, so you are kindly requested to smoke preferably outside the building.

INSTRUCTIONS FOR SPEAKERS

The lecture room will be equipped with an overhead projector and a LCD projector (beamer) linked to a PC. Power Point software will be provided. Please, do not use MAC file format and make sure to bring your presentation file written on a CD ROM or floppy disk, please do not bring a zip disk. It is recommended that you ensure a backup file as well. File format is preferred in ppt for Office 2000 or Office XP. Pdf files can also be handled. In order to avoid problems and delays while switching laptops between lectures and to ensure a smooth operation of the presentations, one PC will serve all speakers. There will be a technician in the lecture room to assist you with your presentation. Please bring your pen drive or CD properly closed to the technician during one of the breaks well in advance before your presentation, for checking it and loading it to the PC in the lecture room.

SOCIAL EVENTS

The Welcome Reception will be held at the conference venue, at Hotel Bara on 4 September, from 19:00. This programme is included in all registration fees.

The Banquet will be held in Lajosmizse, on 7 September, at Restaurant "Új Tanyacsárda".
The buses will start at 14:00 in front of Hotel Bara.
This programme is not included in the student registration fee.
Tickets are available at Registration desk. The price is: 40 EUR/person

ACCOMPANYING PERSONS' PROGRAMMES

Participants are encouraged to bring their spouses to Budapest. Companions, paying the accompanying persons' registration fee, are invited to join the following tours:

- Sightseeing tour with visiting the Synagogue on Monday, 5 September (half day)
- Gödöllő tour on Tuesday, 6 September (half day)

Monday, 5 September

The guided sightseeing tours starts at 09:00. This will be a four-hours tour in Budapest by bus.
Among several famous sights the following places will be visited: Heroes' Square, Basilica, Opera House, Buda Castle, Matthias Church, Fisherman's Bastion, Gellért Hill, Citadel.
The tour in the Synagogue, an hour guided tour, which offers the opportunity for a visit of the impressive Synagogue. After a walk around Central Europe's largest synagogue, interior visit of the building. Planned arrival at the conference venue at 14:00.
Extra tickets are available at the Registration Desk for a fee of 30 EUR/person.

Tuesday, 6 September

Excursion to Gödöllő is a half day guided tour (09:00:14:00) by bus to the former summer residence of Queen Elisabeth organised on Tuesday, 6 September. It can be found very near to Budapest. The town's greatest treasure and draw for tourists is its 250 years old Royal Palace. Visitors can see the living quarters of Emperor Franz Joseph and Empress Elizabeth (Sissy).
Transportation from and to the conference venue, entry tickets and English language guidance will be provided. These programmes are included in the accompanying persons' registration fee. Departure is from the registration desk of the congress at 09:00. Planned arrival at the hotel is at 14:00.
Extra tickets are available at the Registration Desk for a fee of 30 EUR/person.

Oral presentation:
Chemotaxonomic aspect of volatiles

PL-1

15 Years research on volatile secondary metabolites tribute to Wilfried A. König

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On November 19th last year, Professor Wilfried König, an outstanding chemist and pioneer in enantioselective gas chromatography passed away, just 3 weeks after his 65th birthday. The aim of this lecture is to commemorate his prolific scientific work.

Wilfried König´s research activities cover a broad spectrum of topics, however, gas chromatographic separation of enantiomers represents a continuous thread throughout his scientific work. He improved permanently chiral stationary phases and finally achieved a breakthrough with the introduction of modified cyclodextrins. Since then, enantioselective gas chromatography has become a standard analytical method worldwide.

The chemistry of natural products was his second field of research. He concentrated on the isolation and structural elucidation of volatile secondary metabolites from higher plants, liverworts, mosses and mushrooms. Especially, the structure elucidation of sesquiterpenes (including their biosynthesis) are strongly represented in his papers and scientific communications. His “Atlas of Spectral Data of Sesquiterpene Hydrocarbons” (together with D. Joulain) and the corresponding electronic version are indispensable for everybody, working in the field of volatile secondary metabolites and their analysis.

For his pioneering investigations and the outstanding achievements in chromatography and the structure elucidation of natural compounds he received in 2004 at the 27th International Symposium on Capillary Chromatography in Riva del Garda, Italy, the prestigious M.J.E. Golay Award.

L-1-01

HS-SPME-GC-PCA as a tool to classify different chemotypes of chamomile flowerheads (*Matricaria recutita* L.)

C. Bicchi, C. Cordero, E. Liberto, P. Rubiolo and B. Sgorbini

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Headspace-solid phase microextraction-gas chromatography-principal component analysis (HS-SPME-GC-PCA) is here proposed as a complement or an alternative to essential oil (E.O.)-GC-PCA to discriminate between chamomile flowerheads belonging to different chemotypes. Ninety-two E.O.s and the headspaces sampled by HS-SPME of the corresponding chamomile flowerheads were analysed by conventional (C-GC) and fast GC (F-GC) and the results submitted to statistical elaboration by principal component analysis (PCA).

HS-SPME-F-GC-PCA showed to be a rapid approach to distinguish the chamomile flowerheads chemotypes in agreement with Schilcher's classification on E.O. reducing the analysis time from at least four hours and a half with E.O.-C-GC to less than one hour with HS-SPME F-GC. HS-SPME F-GC can therefore successfully be used as an analytical decision maker (ADM) to reduce the number of the time-consuming E.O.-C-GC analyses and limiting them to those samples that cannot unequivocally be classified.

PCA results are highly homogeneous, but they do not afford direct and quantitative correlations between the components within the two methods. A more sophisticated statistics and a higher number of samples are necessary to correlate quantitatively through a function components in the headspace sampled by SPME and in the corresponding essential oil.

L-1-02

Determination of suspected allergens in non-volatile matrices

F. David[1], D. Joulain[2], A. Chaintreau[3] and P. Sandra[1]

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The 7th Amendment to the European Cosmetics Directive (Official Journal N° L 66 of the European Union – March 11, 2003) requires the determination of 24 volatile chemicals suspected to elicit dermal reaction under patch testing and therefore classified as "suspected allergens", in cosmetic and related products.

According to their complexity, samples are subdivided into four classes namely class I: simple mixtures of volatiles with small concentration differences, class II: complex volatile mixtures with large concentration differences, class III: natural matrices containing non-volatiles and class IV: finished products with low concentrations and containing non-volatiles. The two last classes are the most challenging ones, as they require a pre-fractionation of volatile from non-volatile components.

To scope with a large number of class-II and –III samples to be analysed, the present work investigate the on-line pre-fractionation, hyphenated with the capillary GC/MS system. Fractionation is performed in the GC inlet liner that is packed with a bed of polydimethylsiloxane (PDMS) foam retaining the high boiling and non-volatile solutes. After each injection the liner is replaced by means of an automatic liner exchanger (ALEX). The features of this system will be highlighted in the presentation. The potential will be illustrated with several real world samples and the figures of merit in terms of precision, accuracy, repeatability and reproducibility will be presented.

L-1-03

New chromatographic methods for the analysis of essential oils

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Essential oils may be considered medium to highly complex samples, which are formed of a volatile and non-volatile fraction. Gas chromatography (GC) is the analytical technique that has given the major contribution towards the determination of volatile profiles in essential oils. The study of the non-volatile fraction is, on the other hand, is generally achieved through high performance liquid chromatography (HPLC).

The development of a series of methods has allowed not only the characterization of several essential oils but also, as a consequence, accurate judgements on genuineness, geographic origin, possible contamination and adulteration. A series of limitations, though, must be considered:

- single column chromatography often lacks the necessary resolving power to isolate all matrix components in an acceptable analytical time. Furthermore, extensive peak overlapping is a hinderance towards reliable MS structural elucidation.
- conventional chromatographic methods, while sometimes ensuring satisfactory separations on essential oils samples, are also characterized by a substantial disadvantage: the cost in analytical time. This becomes a limitating factor especially for laboratories with a high sample throughput and/or where there is a need for quick and correct results.

These aspects regard not only essential oil analysis but also a vast amount of matrices in different fields. As such, in the past years, there has been an increasing interest, within the chromatographic community, towards the development of more effective separation methods.

The present contribution is focussed on the most advanced monodimensional (micro-bore column GC, micro HPLC) and multidimensional chromatographic techniques (comprehensive GC, comprehensive LC, heart-cutting multidimensional GC) today employed in essential oil analysis. A series of applications on different essential oil samples will be described in order to demonstrate the effectiveness of these approaches.

L-1-04

Influence of technological and biological conditions on essential oil composition

É. Lemberkovics[1], A. Kakasy[1], A. Böszörményi[1], A. Balázs[1], É. Héthelyi[1], B. Simándi[2], Á. Kéry[1] and É. Szőke[1]

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In our institute the research of essential oils in a permanent project for some decades. The research includes the gas chromatographic evaluation and studying the influence of some technological and biological factors on composition of essential oils.

The plant materials for the various investigations were selected first from *Lamiaceae* and *Asteraceae* families. The essential oils were usual obtained by steam distillation using the special apparatus of Seventh Hungarian Pharmacopoea. For supercritical fluid extraction the apparatus was developed by Technical University of Budapest. Carbon dioxide was used as fluid solvent, the extracts were collected by stage wise precipitation in two separators. The second separator contained the extracts rich in volatile compounds.

The gas chromatographic analysis was carried out on capillary silica fused columns coated with DB-1701 and the specific chiral columns with Rt-βDEXm or Rt-βDEXsm. The GC results were confirmed by GC-MS measuring.

Comparing the composition of the traditional *steam distilled oils* with that of *volatile SFE fractions* the main characteristics were the following: The SFE fractions were richer in monoterpene-esters and poorer in alcohols than the traditional essential oils (clary sage, lavander, moldavian dragonhead). The azulenogene sesquiterpene lactones did not transform to azulenes (chamomile, yarrow), but SFE fractions of some *Asteraceae* plants contained sesquiterpene-γ-lactones of unchanged structure and coumarins were also detectable.

Certain essential oils and volatile compounds respectively are specially sensitive for *storage* conditions: for example the citral isomers in essential oil of Melissae herba and Zingiberis rhizoma or β-pinene in yarrow oil. After storing during several monthes the percentage value of above mentioned components decreases and the ratio of sesquiterpenes increases.

Following the essential oil composition of *Ocimum basilicum* during the *vegetation period* in six developing stages and the distribution of the oil components in the *plant organs* we established that the leaf oil is rich in monoterpenes (e.g. linalool, camphor, borneol) in the budding and early-flowering stadium and the quantity of sesquiterpenes (e.g. β-, γ-cadinene, δ-cadinol) and aromatic compounds (methylchavicol) increases only in the later stages in the flower-oil.

Studying the formation of the essential oil composition inside three plant species: *Artemisia, Dracocephalum* and *Thymus,* it was established that the toxical β-thujone was present in the oil of *Artemisia absinthium* in 8% and the oil of *A. vulgaris* contained it only in traces. Comparing the oil composition of the four *Dracocephalum* species we didn't found common characteristic compounds. The characteristic oil-constituents of *D.moldavica* are acyclic, oxigenated monoterpenes. In the essential oil of *D.ruyschiana* the dominant compounds are the oxygenated, bicyclic monoterpenes, as camphor and isopinocamphone. Sesquiterpene hydrocarbons characterize the oil of *D.grandifolium;* a monocyclic monoterpene as the limonene is the main component of *D.renati* oil. The main components of *Thymus vulgaris* and *T.serpyllum* are the same: p-cymene, thymol and carvacrol but the ratio of the latter two components are different. At the same time the oil of *T.citriodorus* Archers Gold contains thymol only in traces; its main component is carvacrol (~40%).

Genetic and biosynthetic background

PL-2

Essential oil compounds as chemotaxonomic markers

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Essential oils are often used as markers in chemotaxonomy. However, as they are mixtures of compounds originating from diverse biosynthetic routes, the evaluation should take place at compositional level to obtain proper consequences.

Occurrence of individual compounds is variable. Based on a wide range of References, the plant essential oils consists of mainly terpenes (in more than 90%), phenylpropanes represent 2-6% and others e.g. alyphatic compounds 4-6%. Monoterpenes seem to be more abundant, than sesquiterpenes, their proportion to each other is about 3:1.

The appearance of some compounds may strictly be connected to certain plant taxa, while others are more common. Pinenes, limonene, myrcene, furthermore caryophyllene and germacrenes are without doubt the most universal compounds, practically present in each accumulated oil. On the other side, e.g. mentane or carane sceleton compounds are rather specific for genera or species (*Mentha spp., Pinus spp, etc.).*

A highly specified compound seems to have the most significant taxonomical value. As examples, for species of the *Achillea* genus 1,8-cineole, camphor and borneol are universal constituents. However, chamazulene has real taxonomical value, especially in the group *Millefolium*, where morphological and even cytological features are very variable and proper taxonomical identification needs additional chemical markers. In this genus, several species are characterised by intraspecific chemical taxa of different essential oil quality. Geographically separated chemodemes illustrate the process of continuous chemical diversification. Chemical profile of the plant changes during ontogenesis and vary according to plant organs, which will be illustrated by examples from the genus *Achillea*. However, the ontogenetical- filogenetical connection (Tétényi, 1986) in essential oil composition can not be proved in this genus. It is a big problem to decide, what is the minimal proportion for a compound to possess chemotaxonomical significance. Recently, the relative amounts of defined marker compounds are suggested to be used in identification and differentiation of *Achillea* species (Rauchensteiner et al., 2002). As it is accepted, that never the substance but its formation has the chemotaxonomic value (Tétényi, 1986), studies of the last years are focused on biosynthetic aspects, enzyme systems, genomic sequences (Dudareva et al., 2004).

Essential oil compounds are used today in identification of plant material and clearing up taxonomical relationships, besides, they may help in detecting new sources for biologically active compounds and in breeding of suitable varieties.

References:

Dudareva, N., Pichersky, E., Gershenzon, J. 2004. Biochemistry of plant volatiles. Plant Physiology, vol. 135. 1893-1902.

Rauchensteiner, F., Nejati, S., Werner, I., Glasl, S., Saukel, J., Jurenits, J., Kubelka, W. 2002. Determination of taxa of the Achillea millefolium group and A. crithmifolia by morphological and phytochemical methods, Sci. Pharm. 70. 199-230.

Tétényi, P.1975. Polychemismus bei Ätherischölhaltigen Pflanzenarten. Planta Medica, vol. 28. 244-254.

L-2-01

Essential oils of the genus *Calamintha* in Turkey

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[2]*Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey*

The genus *Calamintha* Miller (Lamiaceae) is represented in Turkey by 9 species and altogether 12 taxa including 5 endemics. The endemism ratio is over 45%.

Water distilled and microdistilled essential oils from aerial parts of the species growing in Turkey were subjected to gas chromatographic/mass spectrometric (GC/MS) analysis.

According to chemical compositions of the oils, the taxa were clustered mainly into two groups of chemotypes according to the degree of oxidation of the C-3-oxo p-menthane skeleton. Some species showed discrepancies.

The species covered in this paper are as follows (*E denotes endemic*): *Calamintha betulifolia, C. caroli-henricana* (E), *C. grandiflora, C. incana, C. nepeta subsp. glandulosa, C. nepeta subsp. nepeta, C. pamphylica subsp, davisii* (E), *C. pamphylica subsp. pamphylica* (E), *C. piperelloides* (E), *C. sylvatica, C. tauricola* (E).

L-2-02

Volatile components of *Polygonum punctatum* var. *punctatum* and two ferns, *Thelypteris hispidula* and *Brechnum fluviatile*: Distribution of polygodial and their related compounds

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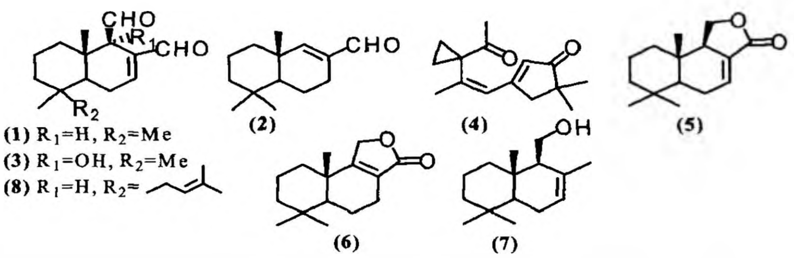
[b]*Facultad de Bioquimica, Quimica y Farmacia, Universidad Nacional de Tucuman, Ayacucho 471-4000-San Miguel de Tucuman, Argentina*

We reported the distribution of drimane sesquiterpenoid (1-3,5,6) in the Japanese *Polygonum hydropiper* (Fukuyama et al., 1982, 1985; Nishiki et al., 2004). The Argentinean *P. punctatum* var. *punctatum* also shows the same taste as that of the Japanese *P. hydropiper*. The Japanese fern, *Hypolepis punctata* elaborates strong pungent sesquiterpenoid (4) (Nishizawa et al., 1977). The Argentinean fern, *Thelypteris hispodula* and the New Zealand one, *Brechnum fluviatile* which indicate strong hot-taste dispaly potent haemolytic, antibacterial, antifungal and antifeedant activity (Asakawa 1982, 1995).

The above three pungent plants were extracted with ether to obtain the crude extracts which were purified by HPLC.

Fractionation of the ether extract of *P. punctatum* var. *punctatum* resulted in the isolation of polygodidal (1), cinnamolide (5) and drimenol (7) together with farnesane-type sesquiterpenoids. *T. hispidula* contained polygodial (1) as a major component along with cinnamolide (5). From *B. fluviatile* a high amount of



polygodial (1) was obtained together with several related compounds like (5). Since polygodial shows strong haemolytic activity, sheep, deer and other cattle avoid polygodial-containing ferns, the higher plants *Polygonum* species and *Pseudowintera colorata* (Winteraceae). On the other hand, many liverworts produce strong pungent substances, for example, the *Porella vernicosa* complex and *Pellia endiviifolia* biosynthesize polygodial (1) and sacculatal (8) which also shows haemolysis. The present paper will discuss the distribution of drimane sesquiterpenoids in higher plants, ferns and liverworts and their biological activity.

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L-2-03

New insight in the knowledge of the qualitative and quantitative composition of oakmoss resinoids

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Oakmoss absolute oil is prepared from *Evernia prunastri* resinoids. Although it has previously been subject to thorough analytical studies, these are mostly qualitative in nature and very little has been published, so far, on the quantitative composition of any oakmoss extract *(1)(2)*. Using GC-MS with chemical derivatization and LC-MS, we report herein some quantitative data obtained from a commercial absolute oil.

We also revisited some aspects of the qualitative analysis of oakmoss and now report that the thujone *(3)* and irone *(4)* isomers previously described in the literature, cannot be detected in a laboratory-made extract from pure *Evernia prunastri* lichen. This suggests that earlier results were either inaccurate or were derived from contaminated products.

Further to our previous measurements of natural abundance of ^{2}H and ^{18}O in lichen substances, we report new data on site-specific natural abundance ^{13}C in oakmoss components, measured by quantitative NMR.

Lastly, using the recent described technique of ALEX/GC-MS, we show that the quantitative determination of atranol and chloroatranol in oakmoss extracts containing reduced levels of these two allergens, can be achieved without significant contamination of the instrumentation.

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L-2-04

Essential oil content of crossing progenies of annual and perennial caraway (*Carum carvi* L.)

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Caraway is used as spice and medicinal plant. Important quality parameters are the essential oil content of the fruits and the carvon content of the essential oil. Beside the biennial caraway, there exists also an annual form which provides economic advantages due to the reduced vegetation period. Currently, there are some efforts to adjust the performance of annual caraway to the level of biennial caraway (Pank et al., 2001).

Ten different biennial caraway cultivars (as female partners only) were crossed with one annual population to generate genetically diverse initial material for breeding inbred lines for a projected synthetic variety. The F_1 generation was cultivated in isolation and the resulting F_2 generation was evaluated and used as initial selection population for inbred line development. First year blossoming of the F_1 generation plants was used as marker of the crossing success. The essential oil and the carvon contents were determined by near infrared spectroscopy (Schulz et al., 2000).

All the F_1 individual plants and 97.7% of the individual plants of the F_2 blossomed in the first year of life. In accordance with Németh et al. (1996) it can be concluded that „first year flowering" is a dominantly inherited trait. The delay of flower initiation and the emergence of only 2.3% biennial genotypes in the F_2 reveal that more than one gene contributes to the control of flower initiation. The essential oil content of the F_2 was positively correlated with the essential oil content of the cultivars used as maternal crossing partners. The generation mean analysis of the essential oil content of P_1, P_2 and F_2 according to Mather and Jinks (1982) revealed that the breeding value of the mother cultivars is limited due to a considerable rate of dominance deviation. Clear transgression of the essential oil content could not be observed. The average essential oil content of the parents and of the F_2 was in the range of 3.51 and 5.22% and met the demand of the European Pharmacopoeia of at least 3%. The variability of the essential oil content of the F_2 was only in some cases remarkably increased in comparison to the variability of the annual pollinator. The average carvon content of the essential oil of parents and F_2 was between 58.0 and 63.8%. According to the European Pharmacopoeia, a carvon content of the essential oil of 50 - 65% is required.

Crossing biennial caraway cultivars with an annual caraway genotype proved to be an appropriate method for the generation of selection populations that serve the development of annual caraway synthetic varieties. These populations suit well for breeding the needed inbred lines because this initial material is characterised by the necessary genetic diversity, adequate per se performance and an appropriate variability.

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L-2-05

New necrodane monoterpenoids from *Lavandula luisieri* essential oil

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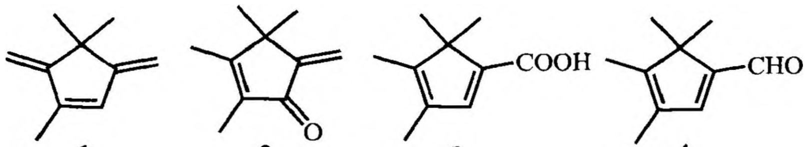
In contrast with the regular monoterpenoids formed by a classical head-to-tail coupling of the isoprenic units, the biosynthesis of the irregular monoterpenoids is supposed to derive from the cleavage of the chrysanthemane skeleton, but few experimental data on this pathway have been reported. Hence, the quest for new irregular monoterpenoids is an interesting research field, as it can set the basis of biosynthetic studies on original isoprenic couplings.

In connection with our precedent works on rare, irregular monoterpenoids (Vellutini *et al.*, 2005), we report here on the identification of four new compounds (1-4) in the essential oil of *Lavandula luisieri* (Rozeira) Riv. Mart. This plant is a Labiatae endemic to the southern iberian peninsula and while it is morphologically nearly identical to *Lavandula stoechas* L. subsp. *stoechas.*, the volatile components of the two plants are significatively different (Garcia-Vallejo *et al.*, 1994). Compounds 1-3 were isolated by fractionment of the essential oil of *L. luisieri* by flash chromatography on silica gel and $AgNO_3$-doped silica gel and identified by MS, mono- and bidimensionnal NMR. On the other hand, the presence of compound 4 in the essential oil was confirmed by hemisynthesis from 3.



1 2 3 4

These compounds are characterised by the 1,2,2,3,4-pentamethylcyclopentane (necrodane) irregular monoterpenic skeleton, which is extremely rare: besides from *Lavandula luisieri*, the only other reported case of isolation of natural necrodane compounds was from the defensive spray of a South American carrion beetle: *Necrodes surinamensis* (Eisner *et al.*, 1986). Finally, we showed that a significant antibacterial activity of *L. luisieri* essential oil was observed against several strains of bacteriae.

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L-2-06

Chemotaxonomically significant 2-ethyl substituted fatty acids from *Stachys milanii* Petrović (*Lamiaceae*)

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Stachys milanii Petrović is endemic to central and eastern regions of the Balkan Peninsula. It is an annual plant with pale-yellow flowers. The studied plant species belongs to section *Olisia*, disjunctly distributed in E Mediterranean, S Africa and S Andes. The aerial parts of the plant were collected in the blooming stage in the region of Lalinačka slatina Salt Marsh (nearby Niš, Southern Serbia).

Ground sample of air-dried aerial parts of *S. milanii* was extracted with diethyl ether. The analysis of the extract was performed by means of analytical GC (FID) and GC/MS.

Nonacosane (17.2 %), palmitic acid (11.5 %), methyl linoleate (11.4 %) and 2-ethylhexadecanoic acid (8.5 %) are the most abundant components. 2-Ethyl substituted fatty acids, 2-ethyloctadecanoic acid (1.1 %) and 2-ethyleicosanoic acid (0.4 %), are two other present in the extract. The rest of the compounds identified in the extract represent common plant constituents.

2-Substituted aliphatic carboxylic acids seem to occur rarely as secondary metabolites in the Plant kingdom. This type of lipophilic constituents were a chemotaxonomical feature previously reported only for the genus *Adesmia* belonging to the Leguminosae family and have been detected as volatile constituens of *Thymus striatus* and *Sambucus*. 2-Ethylhexadecanoic acid has been detected in the essential oil hydrodistilled from *Thymus striatus* (*T. striatus* is also a member of the Lamiaceae family as *S. milanii*). 2-Ethyloctadecanoic acid was found to be a component of the lichen species belonging to the *Parmelia* genus, while 2-ethyleicosanic acid was identified among the volatile constituents of the flowers of *Sambucus nigra*. All three mentioned acids were found to be lipophilic compounds in the *Adesmia* genus.

Thus, not only do the 2-ethyl acids represent a good chemotaxonomical marker for *S. milanii* within the genus *Stachys* and the section *Olisia* but present a remarkable characterictic in the Plant kingdom.

L-2-07

A detailed study of the essential oils of a new chemotype of *Tanacetum vulgare* L. from Serbia

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Common tansy (*Tanacetum vulgare* L., Compositae) is an aromatic perennial plant, widely spread in the northern hemisphere. As a herbal remedy, tansy has traditionally been used in balsams, cosmetics, dyes, insecticides, medicines and preservatives and as anthelmintic, for migraine, neuralgia, rheumatism and loss of appetite, though the effectiveness for these uses have not been documented. Recent studies have also shown that the essential oil or extract of tansy exhibits anti-inflammatory, antibactericidal, antifungicidal and a repellent against insects. The activity against the microorganisms and insects was dependent on the chemical composition of the essential oil. The composition of tansy oils varies markedly and several chemotypes from different geographical origins have already been classified.

The investigation of hydro-distilled essential oils from the aerial parts and roots of *T. vulgare* L. in full bloom growing spontaneously on the river banks of Nišava, Niš, Serbia reveled a new chemotype of tansy. The oils were obtained in the following yields (w/w) 0.27% (aerial parts) and 0.21% (roots). The oil from the aerial parts was subjected to column chromatography on silica gel (gradient elution hexane : ether) and separated into four fractions. All of the oils and fractions were analyzed by GC, GC-MS and ^{13}C-NMR. The essential oil of *T. vulgare* from Serbia has been studied for the first time and the root oil for the first time ever. An apparently simple profile of the total essential oil of aerial parts showing only three major constituents (*trans*-chrysanthenyl acetate 43.9%, β-thujone 28.9% and 1,8-cineole 9.9%) amounting to 82.7% of the oil was an underestimate of the true complexity of the oil composition revealed by the analysis of the fractions. We identified 70 compounds (monoterpene and sesquiterepene hydrocarbons) in the least polar fraction and further 15 from the three remaining fractions containing mostly oxygenated monoterpene derivatives. The following compounds were the major contributors of the first three fractions: α-terpinene 10.3% and γ-terpinene 19.2% (fraction 1), 1,8-cineole 12.3%, β-thujone 26.0%, *trans*-chrysathenyl acetate 54.8% (fraction 2), *cis*-chrysanthenol 41.2% and *trans*-chrysanthenol 54.0% (fraction 3). The fourth (last) fraction was almost entirely constituted of terpinene-4-ol. Constituents belonging to the angular triquinane sesquiterpenes from the first fraction are reported for the first time not only for *T. vulgare* essential oil but are new for the genus *Tanacetum*. The root oil contrary to the oil from aerial parts was rich in triquinane sesquiterpenes (α-isocomene 18.3% and β-isocomene 8.5%). Totally in this study 85 compounds was identified in the volatile oils of common tansy. Further investigation on the antimicrobial activity of oils investigated here is under way.

L-2-09

Variability of the content of chamazulene in essential oil during *Achillea collina* Becker ex. Rchb. 'Alba' ontogenesis

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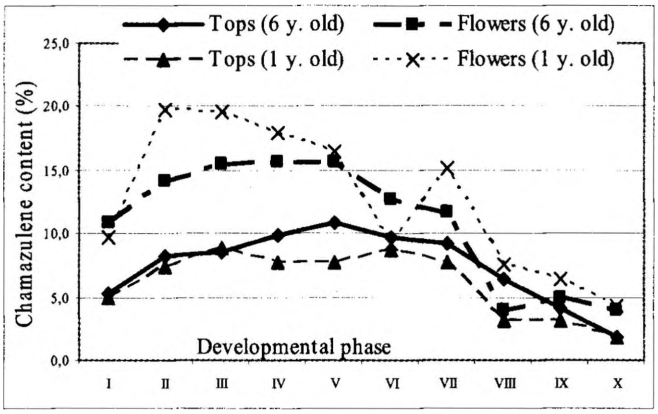
Chamazulene (C_{14} H_{16}) is the main efficient compound of yarrow (*Achillea* L.) essential oil and *Achillea collina* Becker ex. Rchb. var. 'Alba' is one of the pharmaceutically most high-quality taxa of *Achillea* genus. To determine the accumulation of chamazulene in the essential oil during ten developmental stages (from the beginning of bud differentiation until seed ripening) of 'Alba' variety (1 and 6 years old 'Alba' plants) was the aim of the work.

Both the flowering tops with stems (30 cm long) and inflorescences were harvested in ten ontogenetical stages, dried up and ground into fine parts according Czech Pharmacopoeia (ČsL 1997). The essential oil was obtained by steam distillation with addition of xylene (ČsL 1997) and the chamazulene content was measured spectrophotometricaly. Absorbance of essential oil and xylene mixture is measured at 336 nm against blank sample of xylene. The chamazulene content (X) is expressed after recalculation: X [%] = (A * 210) / (W * EO), where A = absorbance (λ = 608 nm), W = weight of plant material for distillation [g], EO = essential oil content [%].

After distillation it seemed the essential oil of all samples has a uniform dark blue colour which is typical for variety 'Alba' and which marks a high chamazulene content. Spectroscopy analysis on the other hand has seriously detect that chamazulene content in essential oil is changed depend on yarrow ontogenesis. Its amount is increased from 5% resp. 10% of essential oil in tops and flowers (beginning of flower differentiation – phase I) to maximal value 10% resp. 18% and later on it is decreased again. The maximal content is reached at the beginning of flowering in yarrow tops (phase V) and – surprising – in green flower buds in yarrow flowers (phase II – III; see. picture). Statistically significant differs (two-factor analysis of variance) have been found between chamazulene content in individual phases and also between tops and flowers. The chamazulene content is not influenced by age of plants. These results are in contrast with Černaj et al. (1983) where is published that chamazulene content in *A. collina* is fluctuated and the highest value is reached in full flowering phase. Figueiredo et al. (1992) even found that chamazulene content in yarrow is continuously decreased during all the developing time.



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Advances in analytics and identification

PL-3

The genus *Juniperus*, evolution, biogeography and essential oils

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Juniperus is the second most diverse genus (67 species, 28 varieties) of the conifers (Adams, 2004). *Juniperus* species are found from sea level (several, *J. virginiana* var. *silicicola*, *J. lutchuensis*, *J. procumbens*) to above timberline (*J. monticola* f. *compacta*). Although limestone is the preferred substrate for many *Juniperus* species, other taxa grow on sand dunes, granite and sandstone and range from deserts (*J. californica* etc.,) to marshes (*J. communis* var. *saxatilis* on Queen Charlotte Island, B.C.). In North America, *Juniperus* species have become weedy and invaded millions of acres of rangeland and abandoned farms.

Juniperus species have evolved a fleshy female cone in which the cone scales are fused. These are often called “fruits” or “berries”. This reproductive structure is especially consumed by birds and small mammals. In fact, the long distance dispersal by birds has resulted in *Juniperus* being established on Atlantic islands such as the Azores, Bermuda, the Caribbean and Canary Islands.

The junipers grow only in the Northern hemisphere, except *J. procera* Hochst. ex Endl., which grows along the rift mountains in east Africa thence, into the southern hemisphere. Some of the Mediterranean *Juniperus* species such as *J. oxycedrus*, *J. phoenicea* and *J. thurifera* also grow in the mountains of the northernmost part of Africa (Morocco, Algeria).

The genus is separated into three sections: *Caryocedrus* (1 species), *Juniperus* (11 species including *J. communis*, whose berry oil is used for gin flavoring; *J. oxycedrus*, the source of Cade oil) and *Sabina* (55 species including *J. ashei*, *J. virginiana*, the sources of most cedarwood oil).

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L-3-01

Essential oil production in Hungary: Past, present and future

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Hungary has a long history as a producer of essential oils. However, the industry has undergone a number of major changes over last century. My intention is to give a brief overview of the whole history of commercial production and then to focus on the more recent developments.

The following time periods of Hungarian essential oil industry is overviewed:

- Before WW II.
- The Communist era, 1945-90.
- The transition period, 1990-94.
- The years of a market economy, 1994-2005.
- Future challenges

The following areas will be covered in details:

- Major crops, their changes from the birth of commercial essential oil production till present days
- Distillery spots, major players, manufacturers and export companies
- Production and export figures, diagrams
- Background information on how the major political and economical changes influenced our industry
- General challenges encountered by all essential oil producers anywhere in the World, such as instable supply, volatile demand, new EU legislation threatening essential oils
- Specific local challenges, such as too strong local currency (HUF), the lack of experienced farmers with large landholdings and cultivation equipment, continuously increasing labour cost
- Our answers to these challenges, including specie development, reorganization of raw material supply and production systems

L-3-02

Superheated water extraction of essential oil-bearing materials: An experimental study

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In recent years there has been a search for methods to replace the use of organic solvents in the extraction of natural products, especially plant materials. The purpose has been to reduce pollution in the work place and environment generally and to avoid residues of undesirable organic solvents in the products, which often are used in foods and fragrances. One of these methods is the use of superheated water, which is liquid water above its boiling point at atmospheric pressure, 100 °C, under pressure (Clark and Macquarrie, 2002, Smith, 2002). The solubility of organic compounds in superheated water is quite high for two reasons, first because solubility increases with increasing temperature and second because water becomes less polar as the temperature rises (Ozel and Clifford, 2004).

Extraction of essential oils is one of the most interesting fields of superheated water extraction. The common methods used so far are mainly based on solvent extraction and steam distillation. The drawbacks linked to these techniques have led to the searching for new alternative extraction processes (Basile et al., 1998, Luque de Castro et al., 1999). The aim of this research work is to extract high quality essential oil from the Iranian essential oil-bearing materials such as lavender, rosemary and cumin. A bench-scale apparatus consists of water deoxygenation system, temperature-controlled oven, preheating coil, extraction cell, cooling system and extract receiver was designed and fabricated. Qualification and quantification were carried out using GC and GC-MS. The effect of different operating conditions such as temperature, pressure and flow rate was investigated and the optimum conditions were determined.

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L-3-04

Comparative investigations on wild growing chamomile (*Matricaria recutita* L.) populations of different origin

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We compared 8 populations of chamomile originating from different Hungarian habitats, both at the original growing areas and as experimental populations cultivated in the same environment at the research field of the department in Budapest, Soroksár. We examined the height of the plants, the diameter of the flowers, content and main components of the essential oil as well as the content of flavonoids. Aim of the investigations was the evaluation of Hungarian wild growing genotypes in order of the development of a new, special quality cultivar.

The population originating from Transdanubia could be separated from the ones originating from the Great Plain ('Hortobágy') according to both morphological traits and active ingredients (Figures 4-5.). The single population of 'Polgár' can not be integrated in either group, it seems to be some kind of transition.

The populations from Transdanubia showed more intensive growth and bigger flower size than the others, both in the original areas and in cultivation. Their essential oil content was by about 40% higher too, than that of the populations from 'Hortobágy'. The proportion of bisabolol-oxide I. (34-43 %) and of chamazulene (7-15 %) are quite high, however the proportion of α- bisabolol was low (7-9 %). The materials from 'Hortobágy', in the contrary, showed a high proportion of α- bisabolol (45-58 %). Thus, according to the aims of the utilisation, we have two chemotypes for cultivar selection.

The environment and the vegetation year modified several characteristics of each populations (growth intensity, spectrum of essential oil and flavonoids), which calls the attention to the importance of interaction between variety and environment.

We propose the definition and standardisation of the actual quality of chamomile as „Hungaricum" (special Hungarian product), because the collected material in Hungary seems to be rather diverse concerned both morphological features and active ingredients.

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L-3-05

Planning scheme for introducing new aromatic plants in India – Patchouli (*Pogestemon cablin*) a case study

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There are approximately more than 300 natural products used as raw materials in flavours and fragrance industry. These raw materials can be found in the form of essential oils, extracts, oleoresins, concretes, absolutes, resinoids and tinctures t name the major groups. Of these materials about half are produced from cultivated plants while the remaining 50 % are obtained either as by-products of a primary industry or are harvested from natural wild plants.

Due to unethical harvesting from the natural resources and wilds, some of the plant species have become extinct or near the point of extinction. Foreseeing this problem of the future a planning scheme should be evaluated for the commercialization of new aromatic plants in the favourable climatic zones/areas and hence cultivation on a mass scale in the new region. India is a country rich in the natural resources (land, water and manpower)and having diversified flora and agroclimatic zones. Biotic and abiotic factors factors of different agroclimatic zones affect the growth, yield and the chemical constitution of the same species. As a result systematic planning and evaluation is necessary for introducing new plant species in a new area. Apart from the biotic and abiotic factors various other points must be considered such as :

Stage 1. Initial selection of plant species.
Stage 2. Plant growth Evaluation.
Stage 3. Standardization of Agro-technology.
Stage 4. Commercialization of the plant
Stage 5. Value addition

After getting positive and optimum results in each of the stages the new crop can be successfully cultivated in that particular agroclimatic zone whereby the technical and economic feasibility is favourable for the farmer and hence the nation.

Patchouli (*Pogestemon cablin*) is taken as a case study. Introducing Patchouli in India and especially in South Gujarat region, evaluating the plant growth, standardization of agrotechniques, commercialization of the crop and the value addition were successfully worked out. The crop now is successfully taken up by the farmers exhibiting splendid growth in the fields as well as the quality and quantity of oil (oil recovery) is best too. Just like patchouli there are many other aromatic plants which can be taken on a commercial scale in South Gujarat region owing to the various ecological factors. Efforts must be made to introduce high value aromatic plants in this part of the world and maximize the production meeting the world standards.

L-3-06

Effect of drying on the essential oil in tarragon (*Artemisia dracunculus* L.) leaves

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Tarragon is a herbaceous plant belonging to Asteraceae family. Two species of tarragon can be distinguished, namely French tarragon (*A. dracunculus* L.) and Russian tarragon (*A. dracunculoides* L.), which are morphologically different (Yaichibe, Masanori et al. 1997). The essential oil compounds are sensitive components in the process of herbs drying and correct drying helps to keep of aromatic plants with high quality (Deans and Svoboda 1992).

The effect of air-drying on the oil components in French and Russian tarragon leaves was studied. The tarragon leaves were dried at air temperatures ranging from 40 to 90 °C. Mostly the drying stopped when the moisture content of the samples reached to 10% and for some of the treatments at 7, 20 and 30%. The essential oil of the fresh and dried leaves was isolated by hydro distillation and analyzed by capillary gas chromatography and gas chromatography-mass spectrometry.

The drying process decreased the total oil recovery and the major oil components and the effect was similar on both varieties. The decrease was highest at 60 °C drying temperature. For the French tarragon the decrease of oil recovery was significantly lower at 90 °C. It seems that lower temperatures and shorter drying times yield more oil. The effect of relative humidity at each temperature was not significant. For the drying at 60 °C, the main oil losses occur in the initial phase of the drying process, i.e. when the moisture content is higher than 30%. The main compounds were estragole in French tarragon with 69 % and sabinene in Russian tarragon with 40%. The relative amount of the constituents was changed differently, for instant the relative value decreased for estragole and increased for sabinene in French tarragon.

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Biological activity and utilization

PL-4

Genetics of monoterpenes in the genera *Origanum* and *Salvia*

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From the genus *Origanum* two different herbs are offered on the market, namely marjoram from *Origanum majorana* L. and oregano from a variety of different *Origanum* species (and species of other genera). While marjoram is characterised by the bicyclic *cis*-sabinene hydrate and *cis*-sabinene hydrate acetate, oregano's character compounds are the phenolic monoterpenes carvacrol and thymol.

Only a few species from the genus *Salvia* are used as medicinal and aromatic plants in a commercial scale, amongst them are *Salvia officinalis*, *S. fruticosa* and *S. pomifera.* These three are not differing with respect to the composition of monoterpenes, but are characterised by different quantities (ratios) of their main compounds 1,8-cineol, α- and β-thujone and camphor.

In the last years, a fast progress was achieved in the elucidation of the genetic basis of terpenes. Several genes for monoterpene synthases were identified and characterised in many interesting medicinal and aromatic plants.

This presentation intends to give an update on the progress of the genetic base of monoterpene formation in the two genera *Origanum* and *Salvia.* The update includes not only the 'vertical' view from the blueprints (genes) to the end-products (monoterpenes) but presents shortly a first 'horizontal' view about the (bio-)diversity of genes responsible for the same end-product.

L-4-01

New metabolic pathways of β-pinene and related compounds by *Aspergillus niger*

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In the continuing study on the microbial biotransformation of terpenoids (Noma and Asakawa,1995,2003), the biotransformation of (-)- & (+)-β-pinene (1&1'), (+)-*trans*-pinocarveol (2), (+)-pino- carvone (3), (-)- & (+)-3-pinanone (4&4'), (+)- & (-)-2-hydroxy-3-pinanone (5&5'), (-)- & (+)-pinane-2,3-diol (6&6'), (+)-fenchol (7) and (-)-fenchone (8) was carried out by *Aspergillus niger* TBUYN-2.

Compound 1 was mainly biotransformed to (-)-α-terpineol (9) and (-)-oleuropeyl alcohol(10) together with the formation of 2, 3 and 4, whereas 1' was also biotransformed in the same manner to form enantiomers of 2-4, 9 and 10. When compound 2 was used as substrate, it was biotransformed via 3, 4 and 5 to (+)-2,5-dihydroxy-3-pinanone(11) together with (+)-fenchane-2α,6β-diol(12) and (-)-fenchane-2α,6β,10-triol(13) as main products. Synthesized 3 from 2 was easily metabolized to 4, which was further transformed to 5 and 11. Compound 5 was easily metabolized to 11 and (+)-2,8-dihydroxy-3-pinanone (14). Furthermore, compound 6 was hydroxylated to (+)-pinane-2,3,5-triol(15) together with the formation of 5 and 11.

The enantiomers of 4-6 were also biotransformed in the same manner. Compound 7 was easily biotransformed to 8 and 12. However, 12 was not converted to 13 at all.

We will discuss the new metabolic pathways of 1 & 1' and the related compounds together with the biological activity of the metabolites.

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L-4-02

Lavandula officinalis L. – selection of optimal essential oil producing type

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A collection of 51 genotypes which includes 10 *Lavandula* L. species is carried out in the gene bank in Olomouc. *Lavandula angustifolia* L., the species often used as an aromatic plant and plant interesting for essential oil producing, is presented by 34 accessions in Czech collection and there are wild genotypes as well as cultivars presented in the collection. The evaluation and selection of perspective lavender genotypes with high essential oil yield and interesting essential oil composition together with account to Czech climatic conditions was the aim of the work.

Because the high variability of both morphological and chemical characters of genotypes propagated by seeds is typical mainly for wild plants, the individual evaluation of each plant (minimally 15 plants per genotype) was done. All together 650 individuals was tested. The yield and composition of essential oil was analysed by steam distillation and gas chromatography respectively, according Pharmacopoeia Bohemica (ČsL 4 1987) and the results was compared with Czech Pharmacopoeia (ČL 2002) requests and with control cultivar 'Krajová'.

The Czech Pharmacopoeia requests in lavender 13 $ml.kg^{-1}$ of essential oil and it should content < 1.0% of limonen, < 2.5% 1.8 - cineol, < 1.2% camphor, 20.0 – 45.0% linalool, 25.0 – 46.0% linalyl acetat, > 0.2% lavandulol acetat and > 0.1% lavandulol. According to this quality, the plants with significantly higher essential oil content or different essential oil composition - all together 32 plants - was chosen as an interesting ones. The highest essential oil content (average from two years analysing) was established at 44.2% and the results of essential oil quality are expressed in the table.

Compound	Ph. request (%)	Evaluated samples (%)	
limonen	< 1.0	0.2 – 3.1	3 plants above the limit
1,8 - cineol	< 2.5	0.5 – 10.3	9 plants above the limit
camphor	< 1.2	0.0 – 1.9	3 plants above the limit
linalool	20.0 – 45.0	18.9 – 52.8	3 plants under and 2 plants above the range
linalyl acetat	25.0 – 46.0	2.9 – 28.6	29 plants under the range
lavandulol acetat	> 0.2	3.0 – 29.0	
lavandulol	> 0.1	0.1 – 8.7	

From all 32 interesting plants the nursery of stool beds was found by vegetative propagation and the mother plantation of all chosen plants is for potential users available for cuttings harvesting or as an original stock for breeding in gene bank working place in Olomouc.

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L-4-03

Essential oils of *Origanum*, *Thymus*, *Satureja* and *Thymbra* species growing wild in lakes region in Turkey

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Lakes region in Southwest Anatolia is known as valley of the oil rose in Turkey. Since It is located in transition zone of Mediterranenan and Irano-Turanian regions, it is very rich in medicinal and aromatic plants. In the present study, it was aimed to determine the essential oils from *Thymbra, Satureja*, *Origanum* and *Thymus* species growing wild in Lakes region. These species are traditionally consumed as 'kekik' which is the name given to those species with a thymol/carvacrol type odor. 1 *Thymbra* species (*T. spicata*), 2 *Satureja* species (*S. cuneifolia* and *S. thymbra*), 5 *Origanum* species (*O. minutiflorum, O. onites, O. majorana, O. sipyleum* ve *O. vulgare*) and 5 *Thymus* species (*T. leucostomus, T. samius, T. longicaulis, T. zygoides* ve *T. cyclotrichum*) were determined during the survey. Although all these species could be considered as potentially valuable crops, the most economic species were from *Origanum* and the least economic species were from *Thymus*. Essential oil-rich (1.0-7.0%) species were belonging to the genera *Origanum, Satureja* and *Thymbra*. The essential oils of *Thymus* genera were moderate level (0.3-1.5%). While the essential oils from *Origanum, Satureja* ve *Thymbra* genera were rich in carvacrol, the oils from *Thymus* genera were rich in thymol. However, some *Origanum* species were rich in linalool and some *Thymus* species were rich in carvacrol and geraniol. Altitude seemed to be the most important environmental factor influencing oil content and composition. In general, the plants collected from low altitudes contained more essential oil than that of the plants collected from high altitudes. But, high values for carvacrol were recorded at high altitudes.

Keywords: *Lamiaceae,* Turkey, Lakes region, medicinal and aromatic plants, essential oils

L-4-04

Essential oil composition of a new *Eryngium* species from Australia: *Eryngium rosulatum* P.W. Michael ined

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The genus *Eryngium* L. belongs to the Apiaceae family and, with about 250 species, has a cosmopolitan distribution. *Eryngium* includes mostly hemicryptophytes and geophytes, although therophyte species have also been described. Relatively few Eryngium species have been described from Australia (Powell and Wieceh 1992): *E. expansum* F. Muell, *E. plantagineum* F. Muell, *E. rostratum* Cav. and *E. vesiculosum* Labill. are native while *E. maritimum* L. and *E. pandanifolium* Cham. et Schldl. are naturalised. An impending revision of the Australian species is likely to include several new taxa including *Eryngium rosulatum* P.W. Michael ined., a rare species from New South Wales and southern Queensland. Plants of this new species are prostrate and form a loose mat up to 20 cm wide.

The essential oil composition from the aerial parts of this new *Erynigum* species have been analysed by GC and GC/MS. The main constituents of the oil were found to be β-elemene (16.0%) and bicyclogermacrene (12.5%). Other representative compounds were identified as δ-elemene (7.0%) and β-caryophyllene (6.0%). It is worth mentioning that most of *Eryngium* essential oils analysed to date contain sesquiterpenes as main

The essential oil composition from the aerial parts of *Erynigum rosulatum* ined. have been analysed by GC and GC/MS. The main constituents of the oil were found to be β-elemene (16.0%) and bicyclogermacrene (12.5%). Other representative compounds were identified as δ-elemene (7.0%) and β-caryophyllene (6.0%). It is worth mentioning that most of *Eryngium* essential oils analysed to date contain sesquiterpenes as main constituents (Palá-Paúl et al. 2003; Palá-Paúl et al. 2005).

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Regional aspects – production of essential oils and their products

PL-5

Metabolism of terpenoids: impact on flavour and biological activity

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Terpenoids are main constituents of plant derived essential oils. Because of their pleasant odour or flavour they are widely used in the food, fragrance and pharmaceutical industry. Furthermore, in traditional medicine, terpenoids are also well known for their analeptic, antibacterial, antifungal, antitumor and sedative activities. Though large amounts are used in the industry the knowledge about their biotransformation in humans is still scarce. Yet, metabolism of terpenoids can lead to the formation of new biotransformation products with unique structures and often different flavour and biological activities compared to the parent compounds. A concise review and discussion of the nature of the pathway of mammalian terpenoid metabolism, metabolites and physiological and biochemical consequences will be presented. Special emphasis will be given to the biotransformation of mono- and sesquiterpenoids in the skin, nasal mucosa, small intestine, liver and lung. Moreover, this review also discusses the most important families of enzymes involved in terpenoid metabolism resulting in a loss of biological activity or, in many cases, the generation of bioactive or even toxic metabolites responsible for allergic reactions.

L-5-01

Chemical composition, antifungal and anti-aflatoxin properties of essential oils of *Thymus eriocalyx* and *Thymus x-porlock* with special references to ultrastructural alterations of *Aspergillus parasiticus*

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Some Aspergillus species are are responsible for many cases of food and feed contamination. Aflatoxin producing fungi grow rapidly on a variety of natural substrates and consumption of contaminated food feedstuffs can pose serious health hazards to human and animals. The use of natural antimicrobial compounds is important not only in the preservation of food but also in the control of human and plant diseases of microbial origin. The antifungal effects of essential oils from *Thymus eriocalyx* and *Thymus x-porlock* were studied with special References to the inhibition of *Aspergillus parasiticus* growth and aflatoxin production. Minimal inhibitory (MIC) and minimal fungicidal (MFC) concentrations of the oils were determined. The oils from the above plants were found to be strongly antimicrobial and inhibitory to aflatoxin production. Static and lethal effects of the above oils against *A.parasiticus* were at 250 and 500-1000ppm of the oils respectively. Aflatoxin production was inhibited at 250ppm of both oils with that of *T.eriocalyx* being stronger inhibitor. The oils analyzed by GC and GC/MS lead to identification of 18 and 19 components in *Thymus eriocalyx* and *Thymus x-porlock* oils respectively. The profile of the oil components from *Thymus eriocalyx* was similar to that of *Thymus x-porlock* in almost all the compounds but at different concentrations. The major components of *Thymus eriocalyx* and *Thymus x-porlock* oils were Thymol (64.3, 30.7%), β-phellandrene (13.2, 39.4%) and Cis Sabinene hydroxide (8.4, 9.7%) respectively. Transmission electron microscopy (TEM) of *A. parasiticus* exposed to MIC level (250 ppm) of the oils showed irreversible damage to cell wall, cell membrane and cellular organelles. Substitution of currently used antifungal and aflatoxin inhibiting chemicals by natural compounds such as thyme is recommended.

Keywords: Essential oils, Thyme, *Aspergillus parasiticus*, Aflatoxin, Ultrastructure

L-5-02

Purity and antimicrobial activities of geraniol and various geranyl derivatives

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In continuation of our research work of combined interpretation of analytical and biological data of various aroma samples (essential oils, extracts and pure compounds) using gas chromatographic-spectroscopic (GC and GC-MS) and olfactoric method as well as antimicrobial tests (e.g. Jirovetz, 2003 and 2004, Schmidt, 2004), geraniol and various natural or synthetic geranyl derivatives were investigated.

Following compounds were used: Geraniol, geranial, geranic acid, geranyl acetate, geranyl acetone, geranyl amine, geranyl bromide, geranyl butyrate, geranyl chloride, geranyl formiate, geranyl isobutyrate, geranyl linalool, geranyl propionate and geranyl tiglate.

The identity and purity of each compound was ascertained by the use of GC and GC (apolar and polar fused silica columns) and in addition, olfactoric evaluations done.

In totally 14 samples showed activity (References component: eugenol) using two different agar methods (diffusion and dilution) against the microorganisms gram-(+)-bacterium *Staphylococcus aureus* ATCC 6538P and *Enterococcus faecalis* (clinical isolated), gram-(-)-bakteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* G 28, *Proteus vulgaris* (clinical isolated), *Klebsiella pneumoniae* (clinical isolated) and *Salmonella sp.* (clinical isolated) as well as against the yeast *Candida albicans* ATCC 10231, but with more significant IZ-data (agar diffusion method) in correlation to the MIC-data (agar dilution method).

The antimicrobial activity of each compound was correlated to its structural properties and the results will be discussed.

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L-5-03

Traditional utilisation of aromatic plants in Peninsular Malaysia

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Malaysia is recognized as one of the 12th mega biodiversity centre in the world with an estimated flora diversity of 15,000 species. With such rich biodiversity there is bounteous knowledge on its ethnic cultural, social and traditional medicinal practices which remain one of Asia's unique treasure and heritage. Among these include the traditional utilization of aromatic plants in particular species from the families Zingiberaceae, Rutaceae, Magnoliaceae, Piperaceae, Annonaceae, Oleaceae etc.

A number of species from the families Zingiberaceae and Rutaceae, for instance, are often utilized as alternative cures for women-related illnesses such as post-partum medicine, menstrual disorders and other gynaecological complications. Zingiberaceous species such as *Alpinia galanga*, *kaempferia galanga*, *Zingiber* spp. Are prepared into poultices for the treatment of aching joints, sprains and inflammation or juice taken orally for the treatment of flatulence, colic, stomache or other digestive problems. Some herbs go into mixtures for preparations of traditional cosmetic powder (Ibrahim *et al* 2000).

Aromatic plants are also used as flavours, spices and condiments in diverse ethnic dishes. While Lemon grass oil is useful in aromatherapy, the leaf bases have an important culinary use in Peninsular Malaysia (PROSEA 1999). Scents are sometimes associated with evil spirits and selected aromatic plants are utilized in rituals for exorcising spirits. Specific examples of aromatic plants and their traditional utilization will be further discussed and presented

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L-5-04

Volatile constituents and antimicrobial activity of Corsican *Achillea ligustica* essential oil

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The Mediterranean herbaceous plant *Achillea ligustica* of the *Asteraceae* family has atracted scientific interest for its use in traditional medicine and for the diversity of its constituents (Maffei et al., 1993, Tzakou et al., 1995, Ahmed et al., 2003). In Corsica, this plant is found under the vernacular name "Erba Santa" (holy herb) and has been used to cure sprains, insect bites and hemorrhages. Here, we report on the molecular identification, the chiral analysis and the antimicrobial activity of the volatile components of *A. ligustica.*

The essential oil of *A. ligustica* was prepared by Clevenger hydrodistillation of a wild sample collected near Ajaccio, Corsica. The essential oil was fractionated by pentane/diethyl ether flash column chromatography on silica gel and the whole oil and its fractions were analyzed by conventional and enantioselective gas chromatography-mass spectrometry (cGC-MS) using a Hydrodex β-6-TBDM stationary phase. A total of 80 compounds representing 93.7 % of the oil were identified and the enantiomeric distribution of seven compounds was characterized. In contrast with the italian and greek chemotypes, the major constituents were determined as santolina alcohol (19.3 %) and camphor (21.3 %).

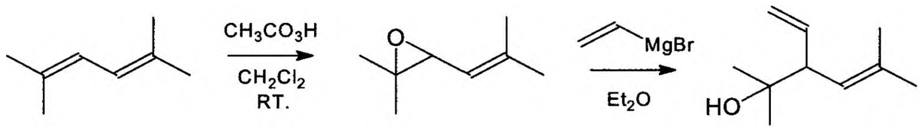

Figure 1. Two-step synthesis of santolina alcohol

In order to prepare a racemic sample of santolina alcohol for the chiral studies, we performed an original two-step synthesis (Fig. 1) from commercial 2,5-dimethylocta-2,4-diene. After optimization of the cGC-MS conditions for the enantiomeric differentiation of this synthetic sample, we observed that the natural sample (purified by $AgNO_3$ doped silica gel column chromatography) was enantiomerically pure. Finally, the biological activity of the essential oil, tested against several bacteriae and fungi, was found to be higher than ampicillin against three *Streptomyces* species.

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POSTER SESSION

Analysis, elucidation and biotransformation of volatiles

P-01

A new chiral stationary phase derivate of β-cyclodextrin for enantioselective separation by Gas Chromatography

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For approximately 38 yiears, gas chromatography has been used to separate enantiomers. GC is an important analytical method for separation and identification of various chiral compounds present in essential oils. Columns coated with cyclodextrins, selectivity alkylated and acylated, are useful for chiral resolution of these compounds. In this work we report the successful preparation and use of the new chiral stationary phase heptakis(6-O-pentafluoropropionyl-2,3-di-O-pentyl)-β-cyclodextrin, for the separation of enantiomeric volatile constituents of essential oils. The pentafluoropropionyl group was never used before as a cyclodextrin substituent and this phase shows to be very efficient. The enantiomers α-pinene, limonene, α-tujene and (+/-)camphor, commonly presents in different essential oils could be resolved. Other chiral analytes like sulfoxides, lactones, acids, amino acids, amines and alcohols, were also analyzed.

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P-02

Automated fast Solid Phase Microextraction-Gas Chromatography with analyte cryo-focussing for the headspace analysis of complex essential oils

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The major aims to be considered in the development of any GC method are both a rapid and efficient sample preparation procedure and the separation of the most critical peaks in the minimum time. This, obviously, becomes of fundamental importance for laboratories with a high sample throughput. As a consequence, there has been considerable attention within the chromatographic community for the introduction of faster analytical methods.

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

The primary aim, relative to any fast GC technique, is to maintain (compared to traditional GC) sufficient resolving power for the separation between the compounds of interest. In respect to this aspect, the micro-bore column approach is a very efficient way of increasing analysis speed. Although the use and effectiveness of these columns was demonstrated more than forty years ago, their routine use in fast GC applications is only quite recent. The reason behind this delay was due to the lack of suitable GC equipment. Modern GC systems are now capable of supplying the drastic experimental conditions that micro-bore columns require: high inlet pressures and split flows, rapid oven temperature heating/cooling and fast electronics for detection.

The present research is based on the rapid automated extraction of HS analytes relative to a bergamot oil sample by using solid phase microextraction (a 7 μm PDMS fibre was used) and the subsequent fast GC separation of the isolated compounds on a 0.1 mm ID micro-bore column. The width of the injected sample band was focussed by using a cryo-trap positioned at the head of the capillary column. With respect to conventional methods (a 30 μm PDMS fibre and 0.25 mm ID column were used) a great reduction of analytical time-costs was observed both in terms of SPME procedure (equilibration time) and GC run time.

P-03

Benefits of a Sol-Gel wax stationary phase in comparison to conventional stationary phases in the Gas Chromatographic analysis of essential oils

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The most common and important method for the determination of volatile components in complex samples is gas chromatography (GC). It is obvious that the continuous advancement of GC analytical performances is a fundamental aim in the field of separation science. In this respect, the improvement of stationary phase technology has led to an increased chromatographic quality of GC separations. The most common stationary phases used are: side chain (methyl, phenyl or cyano) functionalized polisiloxanes; silarylene or carbonate incorporated into the polysiloxane backbone. Polymeric stationaty phases must meet the following criteria to be useful: thermal and physical stability, high degree of crosslinking, partitioning capability, chemical inertness, phase selectivity, polymer synthesis reproducibility. Recently, a new generation of stationary phases, consisting of sol-gel materials, have been developed. These compounds, which may be considered essentially as a synthetic glass, are chemically bonded to the fused silica and are the result of the polycondensation of hydrolyzed monomers of metal alkoxides with crosslinking to form a three dimensional network. The use of sol-gel as a stationary phase is an attractive option due to the good retentive characteristics, excellent inertness, extreme resistance to strong acids and bases, high reproducibility and thermal stability. In particular, the sol-gel wax column has a great versatility in separating volatile mixtures. The aim of this work is to employ sol-gel phases in essential oil analysis and compare there effectiveness with that of widely-used commercially available column stationary phases.

P-04

Biotransformation of sesquiterpenoids isolated from the crude drugs and liverworts by microorganisms

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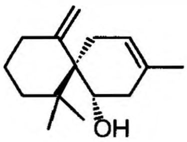
We are continuing to study the biotransformation of terpenoids from the crude drugs and liverworts by microorganisms to obtain functionalized substances such as aroma, insect pheromones and anti-malarial components (Hashimoto, Noma, Asakawa, 2001).

A new chamigrane- (1) and a new cylomyltaylane-type sesquiterpenoids (2), together with cyclomyltaylan-5-ol (3) isolated from the liverwort *Reboulia hemispaerica* and diol (9) prepared from the pungent polygodial (8) were biotransformed by by *Aspergillus niger.* A Zapek-peptone medium [1.5% sucrose, 1.5% glucose, 0.5% polypeptone, 0.1% K_2HPO_4, 0.05% KCl and 0.001% $FeSO_4 \cdot 7H_2O$ in distilled water (pH 7.0)] was used for biotransformation of the substrates by *A. niger.* The cultures were filtered *in vacuo* and each broth was extracted with EtOAc to give the metabolites which were chromatographed on silica gel to give oxygenated products. Their structures were characterized by GC/MS and NMR spectroscopy.

From compound 3 five metabolites 4- 7 were isoated. Their absolute structures were determined by 2D-NMR and modified Mosher's method.



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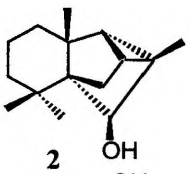

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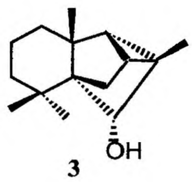

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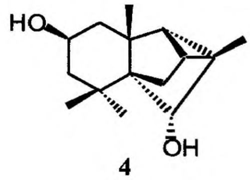

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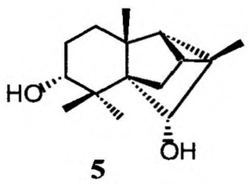

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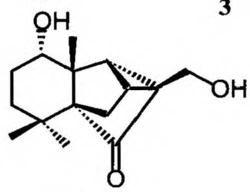

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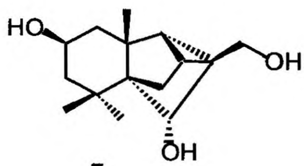

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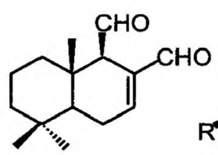

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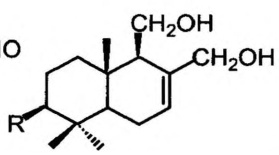
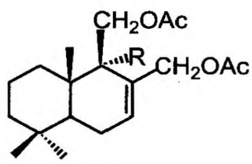
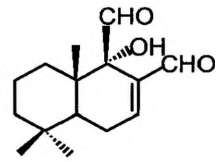


9: R=H
10:R=OH



11:R=H
12:R=OH



13

Compound 9 was converted to 3-hydroxylated compound (10) in good yield. The diacetate (11) of 9 was oxidized with SeO_2 to afford 9-hydroxylated compound (12). The derivatives of warburganal (13) which shows insect antifeedant activity will be synthesized by biotransformation of 12.

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P-05

Bound volatile compounds from osage orange fruit (*Maclura pomifera*) in comparison with its essential oil

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The type of compounds isolated from the various parts of osage orange, *Maclura pomifera* (Raf.) Schneid (Moraceae), belong to different classes such as flavonoids, xanthones, triterpenes and stilbenes (Lee et al., 1998). The fruit of osage orange has been utilized as an insect repellent (Peterson et al., 2002). Among others, volatility is viewed as being important to repellent activity. Phytochemical studies of this fruit made no attempt to compare its free and bound volatiles. If the bound volatile aglycones are compared with the corresponding free volatiles of the same plant, only limited similarity turns out (Stahl-Biskup *et al.*, 1993). In the literature, only one References about *M. pomifera* fruit oil of American origin was found (Peterson *et al.*, 2002). Mostly sesquiterpenoids were determined to be present in the fruit volatile isolates and many of them were repellent to German cockroaches.

As a part of our research project (Croatian grant No. 0011010), we have studied the free and bound volatile composition of *M. pomifera* fruit by GC and GC-MS. The free volatiles (230 mg kg^{-1}) were obtained by hydrodistillation (Clevenger apparatus) and bound volatiles (10 mg kg^{-1}) were liberated after the hydrolysis of the glycosidic fraction (obtained by water extraction and purified by "flash" CC-chromatography) using β-glucosidase. The oil (50 μL) was separated in two fractions – with hydrocarbons and oxygen-containing compounds, respectively – by eluting the oil on a silica gel microcolumn with subsequently *n*-pentane and diethyl ether. Identified compounds consist of aliphatic constituents (free 28.3%; bound 29.1%), phenylpropane derivatives along with related compounds (free 5.1%; bound 36.0%), monoterpenes along with sesquiterpenes (free 55.2%; bound 19.3%) and other compounds (bound 3.5%). In total, 43 bound volatiles were identified, with eugenol (9.9%) and *p*-cresol (9.6%) being the major components. As far as we know, this is the first report on bound volatiles of osage orange fruit and 12 compounds were established to be identical by comparison with the free volatiles. The principal constituents of the essential oil were elemol (19.2%), 1-dodecanol (9.7%), *trans*-caryophyllene (5.6%) and hexyl hexanoate (5.4%).

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P-06

(+)-3-Carene as a natural source in synthesis of fragrant compounds with *gem*-dimethylbicyclo[4.1.0]heptane system

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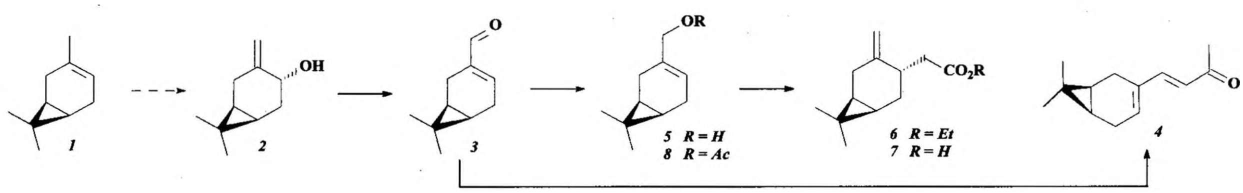
Our interest in synthesis of terpenoid derivatives from natural source is connected with a possibility of their application as fragrant and flavor components for cosmetic and food industry. One of such natural substances is (+)-3-carene, monoterpene, bicyclic hydrocarbon, a major constituent of turpentine from Scotch Pine (*Pinus sylvestris* L.). It is very convenient starting material for synthesis of oxoderivatives with preserved carane (*gem*-dimethyl-bicyclo[4.1.0]heptane) skeleton as well as *gem*-dimethylbicyclo[3.1.0]hexane moiety.

In previous paper we reported syntheses of odorants with di- or trimethyl-bicyclo[3.1.0]hexane system, substituted at C-3 (Lochyński, 1997) or C-2 and C-3 positions (Lochyński, 2001), as well as those with preserved carane system with elongated side chain at C-10 position *via* allylic alcohol **2** (Lochyński, 2002).

As a continuation of our investigations, now we present further syntheses of *gem*-dimethylbicyclo[4.1.0]heptane derivatives substituted at C-3 and C-4 positions.

Oxidation of 2 afforded known α,β-unsaturated aldehyde 3, which was transformed in Horner-Wadsworth-Emmons reaction to the ketone 4. Reduction of 3 led to the allylic alcohol 5, which was subjected to the Claisen rearrangement (orthoacetate modification) giving γ,δ-unsaturated esters 6 and after hydrolysis acid 7. Esterification of acetyl chloride with alcohol 5 afforded appropriate acetate 8.



All newly obtained terpenoid compounds possess interesting olfactory properties. Odour characteristics and synthetic details will be presented with special emphasis on stereochemical aspects.

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Supported by State Committee for Scientific Research, Grant No 3 T09B 092 28

P-07

Comparative studies for the analysis of volatile sulphur compounds in onion and garlic by SPME-GC and SBSE-GC

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The characteristic flavour of onion and garlic has been the subject of chemical investigations for over 100 years. First steam-distillations of fresh onion bulbs have already been performed in 1892 and first empirical identifications have been obtained based on these studies [1]. Later on the formation of sulphur volatiles, which contribute mainly to the specific aroma of the *Allium* species, have been extensively studied applying predominantly various GC headspace sampling procedures [2, 3]. Some years ago solid-phase micro extraction (SPME) has been successfully introduced for the extraction of aroma substances released from onions after rupture of the plant cells [4, 5]. Best results were obtained when the freshly minced plant material was suspended in water and the volatiles in the headspace were adsorbed on a 100 µm polydimethylsiloxane (PDMS) fibre. However, the disadvantage of this approach is that low volatile plant components are more or less discriminated. Therefore a new extraction technique, known as "stir bar-sorptive extraction (SBSE) method", which is based on the partition coefficient between PDMS and water, has been applied in order to get more representative data regarding the aroma profile of both *Allium* species. In this context a magnetic stir bar coated with 50 – 300 µl of PDMS was used. The aim of this study was to compare the applicability of both methods with special regard to sensitivity and to examine their association with sensory characteristics.

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P-08

Fast GCMS-analysis of flavours: liquid injection and Solid Phase Microextraction (SPME)

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In the past fast GC and GCMS using narrow bore columns became more and more a powerful tool to increase analysis efficiency in different fields [1-4]. This approach reduces analysis time drastically by mainly maintaining the resolution [3,4]. Columns with reduced inner diameter and phase ratios of 250 or larger have minimum values for the height equivalent of a theoretical plate (HETPmin) which approach the inner diameter of the columns. By using these columns the instrument hardware has to fulfil some needs. To run the columns at optimum separation efficiency for different temperatures the GC part should be able to maintain the mean linear velocity of the carrier gas. Other parameters like pressure range linear temperature ramp and rapid cooling contribute also to the efficiency of the system. Regarding the detector part the system must be able to follow sharp increases of signals as the peak widths at half height (FWHM) in fast GC with narrow bore columns of 0.1 mm inner diameter are expected to be about or even below 0.5 s [5] . For a quadrupole GCMS system this means it should provide a high number of scans per seconds to ensure reliable quantitative work and a high scanning speed in order to have a non distorted spectrum. Here the instrument (Shimadzu GCMS-QP2010) provides up to 50 scans/s and 10000 amu/s, respectively. The column used for the flavours (strawberry, vanilla 10 % diluted in ethanol) was a DB WAX 10 m, 0.1 mm ID, 0.2 µm. More than 60 compounds were separated in less than 7 min analysis time. The peak widths (FWHM) were about 0.5 s which needs about every 0.05s a scan or 20 spectra per second acquisition rate. The mass range selected was 40 to 300 amu. The spectrum quality was very high and there was no difference in similarity indices observed when compared to standard analysis (up to 98% similarity with the wiley library).

For the control of end products like soft drinks volatile and semi volatile flavour compounds can be analyzed by using solid phase micro extraction (SPME). This technique has been proven to be compatible with fast GCMS. The peak width for liquid and SPME injection was identical indicating that the desorption process is as fast as the transfer in liquid injection onto the column. Volatile compounds were extracted by using a PDMS/DVB 30/50 µm phase (Supelco). Extraction was done with the fiber equilibrated for 30 minutes at 50 °C (headspace SPME) and desorption was performed in the injection port at 250 °C for 1 min. The volatiles detected (multivitamin juice) contain also some of the potential allergens found in cosmetic products like limonene, anisyl alcohol, geraniol, cinnamic alcohol, amyl cinnamyl alcohol and farnesol.

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P-09

Fast multidimensional Gas Chromatography – an innovative approach for chiral separations

A. Casilli[1], P. Tranchida[1], M. Lo Presti[1], P. Dugo[2], G. Dugo[1] M. Furukawa[3], K. Komori[3], K. Miseki [3] and L. Mondello[1]

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It is well-known that single column GC is often an insufficient tool when the total separation of a complex matrix is required. Component co-elution is a common event even for low-complexity samples, as the distribution of chromatographic peaks is, generally, random. In particular, major difficulties are encountered when trace-level analytes overlap with more concentrated solutes. As a consequence, the development of multidimensional instrumentation and methods is one of the foremost goals of modern-day gas chromatography.

The present research is focussed on the evaluation of a recently developed high performance multidimensional gas chromatogaphic (MDGC) system employed in the fast analysis of a series of chiral compounds contained in rosemary essential oil.

The heart of the MDGC system consists in a simple transfer device for the rapid sequential re-injection of analyte "heart-cuts" from the first to the second dimension.

The transfer system has no temperature restrictions, presents very low dead volumes and achieves multidimensional analysis through a pressure-balance mechanism. The MDGC set-up is characterized by two GC ovens (enabling indipendent temperature programming) and the possibility of mass spectrometric (MS) and/or flame ionization detection (FID).

P-10

Free and bond volatile compounds from candytuft, *Iberis sempervirens* L.

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Iberis sempervirens L. belongs to Brassicaceae family. The plants of this family contain glucosinolates. The glucosinolates and their aglycones are biological active compounds. Composition of the obtained aglycones depends on the structure of the side chains in glucosinolates and degradation conditions such as pH-values, temperatures, Fe^{2+} ions and specific protein (Ikan, 1999.).

Up to now, the chemical composition of these aglycones and O-aglycones from candytuft has not been reported. The volatiles of *Iberis sempervirens* were isolated by hydrodistillation and analyzed using GC and GC/MS. The volatiles were isolated from fresh plant material prior and after the autolysis (Al-Gendy *et al.*, 2003.) and from dried plant material. Isothiocyanates, nitriles aliphatic alcohols, carbonyls, fatty acids, hydrocarbons, terpene compounds, C_{13}-norisoprenoids and phenylpropane derivatives were identified. The major components in all samples were: 3-butenyl isothiocyanate (17.4-63.3%), 5-methylthiopentanonitrile (2.2-9.5%), 4-methylthiobutanonitrile (4.8-6.9%), allyl isothiocyanate (4.0-6.6%). Other compounds were 3-methylthiopropyl isothiocyanate, phenyl isothiocyanate, benzenepropanonitrile, 1-cyano-4,5-epithiopentane. The volatiles that were isolated after the autolysis are more complex then other two isolates. Some of them were identified in O-aglycones as eugenol, (Z)-3-hexene-1-ol, 1H-indole.

Further, from the fresh plant material O-glycosides of volatile compounds were isolated and purified by selective extraction and column chromatography. After hydrolysis of O-glycosides using β-glucosidase liberated volatile O-aglycones were also analysed by GC and GC/MS. Compounds with sulfur and nitrogen (which are characteristic for glucosinolates) were not identified among these compounds. Nineteen O-aglycones were identified. The main O-aglycones were: eugenol (15.2%), 2-phenylethanol (12.9 %), 2-hydroxy-β-ionone (10.6%), 2-tert-buthyl-5-methylphenol (9.2%) and methyl-2,5-dihidroxybenzoate (4.5%).

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P-11

Investigations of essential oils with enantioselective Gas Chromatography

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in memoriam Prof. Dr. Wilfried A. König

Professor König invented and established the use of modified cyclodextrins as stationary phases in capillary gas chromatography. Today, most volatile chiral compounds can be separated in their enantiomers using one of several available cyclodextrin-based capillary GC columns. Professor König significantly contributed to the knowledge about essential oils and plant volatiles. He discovered several new sesquiterpenes and improved analytical techniques required for the detailed analysis of essential oils, particularly in structure elucidation, mass spectrometry and gas chromatography.

We demonstrate the value of enantioselective gas chromatography in essential oil chemistry on prominent examples like methyl jasmonate in natural jasmine, α-phellandrene in eucalyptus oil and dill oil, or the separation of peppermint oil constituents.

Further, we show modern applications using *fast GC* and GC/MS with 100 μm narrow-bore columns for the quality assessment of lavandin oil and lemon oil as typical examples of state-of-the-art analytical procedures in today's flavour and fragrance industry.

P-12

Investigations of the suitability of Solid Phase Dynamique Extraction (SPDE) to determine the composition of aroma active components in herbs

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In the context of a research project in which the aroma profile of spicy plants is investigated, the suitability of SPDE as an easy to handle preparation technique – that accumulates aroma components on a specific layer in a syringe – should be determined.

Therefore several influencing factors for the SPDE method particularly the numbers of strokes, the sample weight and the incubation temperature needed to be optimized. Verification was performed by the use of gaschromatographic analysis.

After finishing the considerably period of method development it is safe to say that the pre-analysis technique SPDE can be used to reproducible determinate aroma active components in herbs. However attention needs to be payed on the sample weight which might lead to less reproducible results if it undergoes a certain amount.

Raising the stroke number to high degrees often causes degradation or heightening for some components. Thus optimization is important and often there is a middle course that gives most satisfying outcomes.

Additionally it must be mentioned that there is an advantage compared to other extraction techniques, as for example Solid Phase Micro Extraction (SPME), including a higher detection limit for minor components. This matter of fact is especially important for establishing aroma profiles due to the sensory affectivity of secondary components.

P-13

Isolation of the components of the essential oil of the leaves of *Solanum aethiopicum* and its antimicrobal activity

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The essential oil of *Solanum aethiopicum* was obtained by hydrodistillation from the leaves and was comprehensively analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) to reveal thirty-three (33) constituents, which accounted for 63.47% of the total oil composition. Of the thirty-three constituents isolated, twenty-six (26) compounds accounting for 58.16% of the leaf oil were identified.

The major compound was a diterpene, phytol (17.73%), other prominent compounds are alpha-pinene (7.5%), trans-methylisoeugenol (4.76%), elemicin (4.50%), T-muurolol (4013%) and elemol (4.02%). Some sesquiterpenoids has been reported from the exudates of the plant by Toshino et al. (2001).

The leaf essential oil of *S. aethiopicum* was confirmed to possess no antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Klebsiella spp., Nesseria spp., Candida albican* and yeast. Ayato et al (2001) reported the antifungal activities of lubimin and epilubimin, phytolexins from the plant.

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P-14

Molecular modeling of odor receptors

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Humans possess approximately 300 intact odorant receptors and more than 700 non-functional pseudoreceptors. They are integral membrane proteins belonging to family A of the large superfamily of G-protein-coupled receptors (GPCRs). GPCRs consist of 7 transmembrane helices connected by intra- and extracellular loops. With this repertoire, humans can distinguish between numerous chemical diverse odorants.

To explain this enormous discriminating power, knowledge of the three dimensional structure and mapping of ligands to specific receptors are necessary. Due to experimental difficulties in determining the geometry of membrane proteins, up to date only the crystal structure of bovine rhodopsin (Palczewski et al, 2000) from family A has been solved. Therefore, homology-modeling techniques on two human odorant receptors, hOR17-4 and hOR17-40 were applied using rhodopsin as template. These two receptors are excellent targets because hOR17-4 selectively binds canthoxal®, which has a floral anisic smell, while hOR17-40 is solely activated by the structural related helional® with a green floral note. The location of the putative binding site was identified with the site finder module in MOE. It corresponds well to binding pockets found in other GPCRs. To analyse the nature of the active site, docking studies were performed with known odorants and structurally related molecules.

Key residues involved in odorant recognition are located in the space formed by the transmembrane helices 3, 4, 5 and 6 as well as by the extracellular loop 2. By comparing the docking results of helional®, which exclusively activates hOR17-40 (Spehr et al, 2003) and the structurally related canthoxal® which only activates hOR17-4 (Wetzel et al, 1999) we were able to explicitly assign ligand specificity to structural features in the binding site. Comparison of key residues involved in odorant binding of 10 structurally diverse receptors made it possible to define a olfactory receptor unique ligand binding site.

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P-15

Sensory proprties of rosemary flavoured edible oil

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Rosemary is not only a valuable medicinal plant but a well.known flavourity of the food industry. Beside the traditional use in meat industry and canning technologies, its slight camphoreous character makes possible to improve the taste of edible oils.

In our experiment different amount of dried leaves of rosemary (Rosmarinus officinalis L.spec.Harmat) were dipped in sunflower oil over a four weeks period and the changes of the gust characteristics were investigated.

The following concentrations were prepared : conroll, 0.5 %, 1.0 %, 1.5 % and 2.0 %. Colour, smell, taste and the general acceptance were judged at the 1st, 4th, 7th, 14th, 21st and 28th day of the treatment. For evaluation an unstructured scale was applied in which the panel members marked the position of the judged factor.The panel characterized the samples by terms as well.

Results were analysed by one way ANOVA and paired Welch-tests. The least significant differencies were calculated. After ranking the sensory data Kruskal-Wallis and paired Wilcoxon test were performed. Profil analysis was done to evaluate the textual comments of the panel.

We could establish that during the one month course fragrance appeared firstly and the flavour developed rather slowly. At the end of the dipping, secondary aroma components were observable.

Comparing to the controll there were two distinct groups : the two lower and the two higher concentrations. At the end of dipping the aroma losed freshness and modified to the rich way.

The two higher concentrations were preferred by the panel members.

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P-16

Terpenoids and aromatic components from selected New Zealand liverworts

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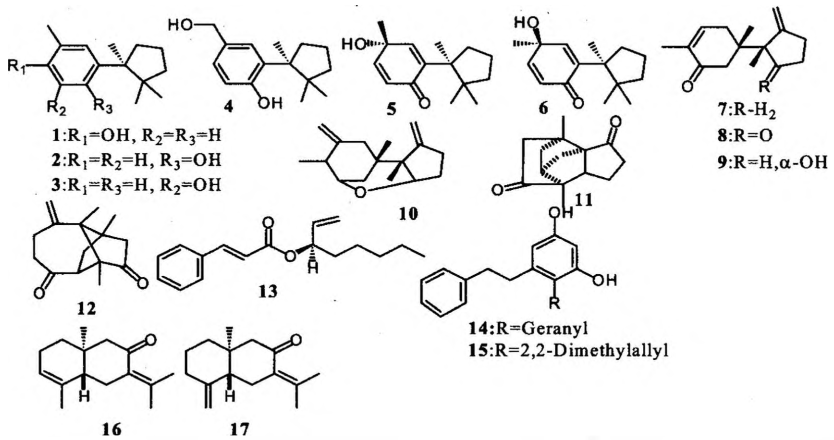
Liverworts produce many peculiar terpenoids and aromatic compounds. We have reported more than 300 new compounds from bryophytes (liverworts, mosses and hornworts) (Asakawa 1982, 1995, 2005). We focus on the chemical constituents of southern hemispheric liverworts since ca 50 endemic genera have been recorded

(Asakawa 2001). Here the structures of several newly isolated compounds from the New Zealand liverworts will be discussed.

Each liverwort was extracted with ether. The crude extracts were purified by a combination of column chromatography and HPLC. Their structures were elucidated by NMR and X-ray crystallographic analysis.



Here the structures of several newly isolated compounds from the ether extracts of New Zealand liverworts will be discussed.

Dendromastigophora flagellifera contained herbertane sesquiterpenoids (1-6). The species is closely related chemically to Japanese *Herbertus aduncus* and *H. sakuraii* since the latter species produce the same herbertanes as those of *D. flagllifera*. Bazzananes (7-10) which are chemical markers of *Bazzania* species were isolated from *Frullania squarrosula. Isotachis lyallii* elaborates 3-octen-3-yl cinnamate (13). *Radula silvosa* and *R. sainsburiana* produced ubiquitous bibenzyls (14, 15). These species are chemically very similar to several Japanese *Radula* species. The following liverworts also produced characteristic secondary metabolites: Unidentified *Chiloscyphus* sp.: *ent*-Germacrones (17, 18), *Bazzania hochstetterii: ent*-Eudesmane, *Plagiochila cricinalis:* two fusicoccanes, two labdanes, three fusiccocane-labdane dimers, *Heteroscyphus billardieri: cis*-Clerodanes. *Jamesoniella colorata*: Barbatanes, Rearranged *cis*-clerodanes.

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P-17

Volatile components of *Polygonum punctatum* var. *punctatum* and two ferns, *Thelypteris hispidula* and *Brechnum fluviatile*: Distribution of polygodial and their related compounds

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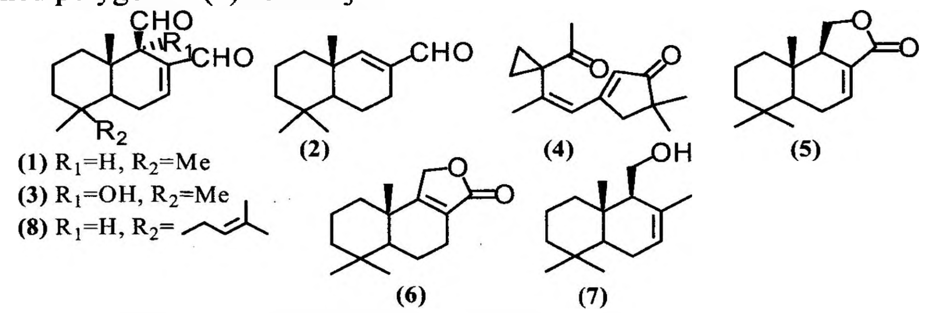
We reported the distribution of drimane sesquiterpenoid (1-3,5,6) in the Japanese *Polygonum. hydropiper* (Fukuyama et al. 1982, 1985; Nishiki et al. 2004). The Argentinean *P. punctatum* var. *punctatum* also shows the same taste as that of the Japanese *P. hydropiper*. The Japanese fern, *Hypolepis punctata* elaborates strong pungent sesquiterpenoid (4) (Nishizawa et al., 1977). The Argentinean fern, *Thelypteris hispodula* and the New Zealand one, *Brechnum fluviatile* indicate strong hot-taste, respectively. We have been interested in such pungent plants since their crude extracts possess potent haemolytic, antibacterial, antifungal and antifeedant activity (Asakawa 1982, 1995). Fractionation of the ether extracts of *P. punctatum* var. *punctatum* resulted in the isolation of polygodidal (1), cinnamolide (5) and drimenol (7) together with farnesane-type sesquiterpenoids. *T. hispidula* contained polygodial (1) as a major



(1) R_1=H, R_2=Me (2) (4) (5)
(3) R_1=OH, R_2=Me
(8) R_1=H, R_2=
(6) (7)

component along with cinnamolide (5). From *B. fluviatile* a high amount of polygodial (1) was obtained together with several related compounds like (5). Since polygodial shows strong haemolytic activity, sheep, deer and other cattle avoid polygodial-containing ferns, the higher plants *Polygonum* species and *Pseudowintera colorata* (Winteraceae). On the other hand, many liverworts produce strong pungent substances, for example, *Porella vernicosa* complex and *Pellia endiviifolia* biosynthesize polygodial (1) and sacculatal (8) which also shows haemolysis. The present paper will discuss the distribution of drimane sesquiterpenoids in higher plants, ferns and liverworts and their biological activity.

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P-18

Volatile components of selected Japanese medicinal plants

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We reported the distribution of drimane sesquiterpenoids and monoterpene hydrocarbons in the ether extracts of Japanese *Polygonum hydropiper, Alpinia japonica* and *Cryptotaenia canadensis* var. *japonica*. (Nishiki et al. 2004). The present paper concerns with the distribution of the volatile components obtained from the above three plants and the following medicinal plants.

Each plant was hydrodistilled and extracted with different solvents. Each extract was analyzed by TLC and GC/MS. The names shown by underline in parenthesis indicate the major components.

Hydrodistillation: *Artemisia vulgaris* var. *indica* (many monoterpene hydrocarbons; 1,8-cineol, camphor, geranyl acetate, linalyl acetate, β-caryophyllene, germacrene-D, β-caryohyllene oxide, T-muurolol); *Cryptotaenia canadensis* var. *japonica* (α-pinene, camphene, β-pinene, myrcene, linalool, β-elemene, β-caryophyllene, γ-maalinene, β-selinene, *trans*-nerolidol, β-caryophyllene oxide); *Polygonum hydropiper* (2- and 3-hexenol (*E/Z*), β-myrcene, β-caryophyllene, β-farnesene, polygodial, isopolygodial, drimenol, drimenin); *Polygonum thumbergii* (3-hexen-1-ol (*E/Z*), 1-octene-3-ol, linalool, *a*-terpineol, decanal, geraniol, 1-decanol, undecanone, bornyl acetate, geranyl acetate); *Erythrina crista-galli* (β-elemene, guaiol, eudesmane-11-ene-4-ol); *Houttuynia cordata* (3-hexen-1-ol (*E/Z*), 4-terpineol, α-terpineol); *Cinnamomum camphora* (camphene, camphor, borneol, 4-terpeineol, α-terpineol, bicycloelemene, β-caryophyllene, bicylogermacrene); *Cinnamomum loureirii* (1,8-cineol, linalool, benzenepropanal, α-terpineol, 3,7-dimethyl-2,6-octadienal (*E/Z*), cinnamaldehyde, geraniol, bornyl acetate, eugenol, geranyl acetate, β-elemene, caryophyllene oxide, *Cryptomeria japonica* (sabinene, 4-terpineol, bornyl acetate, elemol, β-eudesmol, eudesm-3-ene-11-ol, kaurene); *Alpinia japonica* (1,8-cineol, *α*-fenchene, fenchyl alcohol, camphor, β-elemene, aristolene, β-caryophyllene, guaiol, intermedeol, kaurene); *Lantana camara* (linalool, fenchyl acetate, β-elemene, β-caryophyllene, germacrene-D)

n-Hexane extracts: *Pittosporum tobira* (n-nonanone, α-pinene, benzyl alcohol, linalool, undecanone, germacrene-D, secodammarane triterpenoids); *Thujopsis dolabrata* (thujopsene, α-chamigrene, cuparene, δ-cupurenene, α-cedrol); *Hottunia cordata* (7-monoterpene hydrocarbons, bornyl acetate, 2-undecane, fatty acids methyl esters); *Elaeagnus multiflora* var. *hortensis* (fatty acids methyl esters); *Polygonum hydropiper* (β-myrcene, β-caryophyllene, β-farnesene).

Ether extracts: *Ginkgo biloba*, male flowers, stems and leaves: (3-alkyl and 3-alkenylphenols); *Solidago altessima* were collected in seven different places (4 in Kyoto and 3 in Tokushima). GC/MS analysis of the ether extract indicated that one specimen collected in Tokushima differs chemically from the other six specimens which are chemically identical. The fomer sample produced germacrene-D which is the major component of all samples plus cyclocolorenone as the second major component.

MeOH extracts: *Pittosporum tobira* (fatty acids methyl esters)

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Chemical diversity of plants accumulating essential oils

P-19

Aromatic plants from Senegal

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Lower-Casamance, the south west part of Senegal, is an important source of herbs traditionally used by the natives as healing plants. The potentiality of these herbs has not been yet closely examined but they can represent a source of products of economical importance.

In this work, to continue our researches on the biodiversity of aromatic plants and their secondary metabolites having biological properties, the essential oils of four indigenous herbs employed for their "medicinal" properties, *Citrus aurantium* L., *Melaleuca leucadendron* L., *Eucaliptus camaldulensis* L. Dhnh and *Lippia chevalieri* Mold. were characterize by GC-MS. The oils, were supplied by A.S.D.I. (Association Sénégalaise pour le Développement Intégré).

Citrus aurantium L. oil was rich of limonene (27%), linalyl acetate (25%) and linalool (22%), that from *Melaleuca leucadendron* L. showed methyl eugenol as main compound (61%) followed by methyl isoeugenol (29%), the oil from *Eucaliptus camaldulensis* L. Dhnh had 1,8-cineole as main compound (63%) and limonene as second more abundant constituent (22%) whereas that from *Lippia chevalieri* Mold. was characterized by a high content of 1,8-cineole (23%) and by the presence of numerous sesquiterpenes with relevant amounts of germacrene D (12%) and β-caryophyllene (11%).

Most of the oil main components possess recognized antimicrobial activity (Dorman and Deans, 2000) and this aspect can confirm the therapeutic use of the studied plants by local people and can open perspectives in the exploitation of these species.

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P-20

Artemisia essential oils

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Artemisia oils are obtained by steam distillation of the whole over-ground parts of wild growing and cultivated herbs such as *Artemisia herba-alba* in Morocco, *Artemisia alba* in Tunisia, *Artemisia mendozana* in Argentina, *Artemisia annua* in Yugoslavia, Bulgaria, Romania and *Artemisia vulgaris* in the South of France, Morocco, Germany, Hungary, India, China and Japan. They are light yellow to green liquids with a herbaceous, balsamic and fresh-camphoraceous odor. Some *Artemisia* species serve as a source of locally distilled and locally used essential oils for the cosmetic and toilet industry. *Artemisia herba-alba* oil is used in fairly large amounts worldwide in fine fragrances (e.g., for chypre notes) (Bauer *et al,* 1997; Arctander, 1960).

The aim of the present study was to determine the content and the chemical composition of essential oils from *Artemisia* species grown in Western Canada.

A. biennis, A. cana, A. dracunculus, A. frigida, A. longifolia and *A. ludoviciana* were harvested just before the flower-buds open when the essential oil content is at its maximum. Flowers, leaves and thin stalks were dried at ambient temperature, separated from wooden-type stalks and comminuted using a hammer mill. The oils were extracted by hydrodistillation using a Clevenger-type apparatus until total recovery of oil. Analyses were performed by GC-MS using two columns of different polarities, HP-5MS column (5% phenyl 95% polydimethylsiloxane) and DB-Wax column (polyethylene glycol). The identification of single components was performed by comparison of GC retention indices, mass spectra and co-injection with authentic standards.

From the initial laboratory tests it was found that stalks of *Artemisia* species contained insignificant amounts of essential oils. The major components identified were 1,8-cineole and camphor in the oils of *A. cana, A. frigida, A. longifolia and A. ludoviciana.* Methyl chavicol and methyl eugenol were the representative constituents of *A. dracunculus.* (Z)-beta-ocimene and (E)-beta-farnesene were present in *A. biennis. Artemisia cana* provided the highest yield of essential oil. Up to 2.3% of oil was obtained after 8 hours of distillation of the aerial parts including only thin stalks. The oil contents in the flowers, leaves and stalks were 2.8%, 2.4% and 0.1% respectively. The aerial parts from *A. longifolia, A. dracunculus* and *A. biennis* yielded very low amounts of essential oils (0.5%, 0.4% and 0.3% respectively).

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P-21

Characterization of essential oil of *Salvia verticillata* L. from the Pannonian and mountainous parts of Serbia

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In the flora of Serbia, 15 species of *Salvia* genus have been described. *Salvia verticillata* L. (lilac, whorled sage; *Lamiales-Lamiaceae*) is a herbaceous perennial plant, native to Europe and west Asia. In Serbia it grows under semi-arid and continental climate conditions. Since there are very few chemotaxonomical data on *S. verticillata* from Balkan flora, our aim was to investigate the content and composition of volatile constituents of this wild growing sage species.

Plant material was collected during full blossoming at different localities in Serbia: 1. Southern Bačka region (Panonnian part of Serbia, 80 m above sea level, chernozem soil), 2. Fruška Gora mountain (Northern Serbia, 400 m, brown forest soil) and 3. Tara mountain (Southwest Serbia, 1000 m, brown forest soil). Essential oil was analyzed by method of GC/MS from the n-hexane extract. Identification of individual compounds were made by comparison of their retention time with the mass spectra of those of the authentic samples or by computer matching with the library of mass spectra data (Wiley275).

Essential oil content in dry herba of *S. verticillata* specimen ranged from 0.40-0.42%. The yield of the essential oil permit the assignment of this species to oil-poor group of *Lamiaceae* (Malenčić *et al.*, 2004). Number of volatile constituents identified in the oil in amount higher than 1% was the highest in Tara specimen, with the dominant component (E)-caryophyllene (10.2%). This sample also contained long-chained hydrocarbons and other sesquiterpenes. In the Fruska Gora specimen the dominant component was germacrene D (48.0%). The greater amount of some other sesquiterpenes was also established, such as (E)-caryophyllene (13.4%) and α-cadinol (10.4%). In the Backa specimen germacrene D (24.6%) and (E)-caryophyllene (19.0%) were also the dominant constituents. Comparative analysis of the specimen showed differences. In Tara and especially in Backa specimen (32.1%), the presence of monoterpenes has been established while in the Fruska Gora specimen their presence was only in traces. Plants differ in their hydrocarbons content as well. Specimen from the north of Serbia lack these compounds while plants from southwest mountainous regions were rich in them. In order to clarify species relationship of *Salvia* genus considering the effect of various environmental factors, such as geographical and climatic conditions, further studies of wild growing *Salvia* species are necessary.

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P-22

Characterisation of volatile compounds in *Dracocephalum* species

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We have investigated the essential oil composition of 4 *Dracocephalum* (dreagonhead) species: *D. moldavica* L., *D. ruyschiana* L., *D. grandiflorum* L., *D. renati* Emberg.

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

The cultivated plant material was hydro-distilled using the apparatus official in the 7th edition of the Hungarian Pharmacopoeia. GC analysis was performed on a Fisons 8000 gas chromatograph equipped with flame ionisation detector; 30 m x 0.25 I. D. mm capillary column with enantioselective Rt-β-DEXm stationary phase (film thickness 0.25 μm); injector at 210 °C, detector at 240 °C; column temperature: 8 °C min^{-1} from 60 to 230 °C, then 230 °C for 5 min. GC-MS apparatus: Finnigan GC with 30 m x 0.25 mm I. D. capillary column (MDN-5S stationary phase, film thickness 0.25 μm); injector at 200 °C; temperature program: 60 °C for 3 min., 8 °C min^{-1} from 60 to 200 °C, 200 °C for 2 min., 10 °C min^{-1} from 200 to 250 °C, finally 15 min. at 250 °C. Mass spectrometric parameters: electron impact ionisation with 70 eV energy; the mass range scanned was 40 – 650 a.m.u. The identity of constituents was confirmed using data reported in the literature and by comparison with the mass spectra of the reference compounds.

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

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

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

The main constituents of the essential oil of *D. renati* (0.5%) are monoterpenes: a yet non-identified compound and the limononene. Carvone, neral, geranial, linalool, linalyl acetate, β-caryophyllene and bicyclo-vetivenol (a sesquiterpene alcohol) were also identified in the oil.

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P-23

Chemical and morphological variations of Czech and Finnish *Acorus calamus* L. accessions in gene bank collections

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Sweet-flag (*Acorus calamus* L., *Araceae*) is an aromatic perennial herb. Its dried roots have long been used in medicinal preparations and for the flavouring of bitter liqueurs and appetizers. Due to the habitat loss of *Acorus* its populations have become rare in several European countries. The focus now is on the conservation of its genetic resources and to start its field cultivation to meet the raw material demand. The aim of this study was to analyse the chemical and morphological characters of *Acorus calamus* collected from the nature in Finland - before deposit to the Nordic Gene Bank - and to compare them to the *Acorus* accessions collected in the Czech Gene Bank.

The Finnish accessions have been collected during 2002 in South-West Finland at seven natural populations. One accession was obtained from Slovenia (Zalec) and their cuttings were transplanted into the field (moisture sandy till, pH 6.8) of Agrifood Research Finland, Mikkeli. The collection of Czech sweet-flags has been found in 1998 and it consists from 27 accessories originated from all country. The cuttings were replanted into the growing containers to Gene Bank in Olomouc, where a special basin was built to keep them in optimal wet conditions and to protect sprouting of rhizomes and mixing. Leave and rhizome morphological characters, essential oil content and composition of the dried rhizome were determined (Czech accessions during 1997 – 2000, Finnish accessions during 2004).

The average oil content of the Finnish and Slovenian accessions was 1.47% (1.15 - 1.87%) and the oil composition was quite similar. Their main compounds were solavetivon (x = 11.07 %) and beta – asarone (10.08%). The Czech accessions had a similar essential oil content and composition as the Finnish and Slovenian origin ones. The average essential oil content of 1.91% (1.20% – 2.92%) is slightly higher than in Finland, but anyway 14 from 24 analysed samples did not execute the norm defined for quality of Radix calami (minimum 2% of essential oil) by Czech Pharmaceutical Codex. The main components of the essential oils were beta- and gamma- asarones. The average content of gamma-asarone was 18.65% (12.52 – 25.35%) and that of the beta-asarone was 16.11% (11.34 – 21.30%). The content of the beta-asarone in sweet-flag drug did not exceed the maximal recommended value 0.5% of dry matter content.

The Finnish, Slovenian and Czech origin sweet-flags in both Finnish and Czech collections seemed to be phenotypically and chemically very similar to each other. They all represent the European triploid type of *Acorus calamus var. calamus.* The average essential oil content (1.47% in Finland and 1.91% in the Czech Republic) is not sufficient according to the Czech norm for Radix calami quality, but the content of carcinogen beta-asarone did not exceeded secure limit 0.5% of dry drug mass.

P-24

Chemical composition of essential oils of purple perilla (*Perilla frutescens*) from Korea

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This study was carried to investigate the volatile oil components in *P. frutescens* strains collected from different regions in South Korea and identify the chemotype based on the contents of major volatile oil components.

Thirty kinds of volatile components were identified and the major components out of 30 compounds were limonene, perillaldehyde, perillaketone, isoegomaketone, beta-caryophyllene, beta-farnesene, myristicin and dillapiole. *P. frutescens* collections were classified as four chemotype: PA type (limonene 57.7% and perillaldehyde 19.8%), PK type (perillaketone 89.8%), ST type(sesquiterpene 82.4% such as beta-caryophyllene 54.5% and beta-farnesene 27.9%) and PP type (phenylpropenes 40.3% and sesquiterpes 37.8%) based on their differences in major volatile oil components. The majority of *P. frutescens* collections in this study belongs to PA type (41.9%) and PK type(38.8%).

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P-25

Chemical composition of extracts from the *Aesculus hippocastanum* and *Aesculus x carnea*

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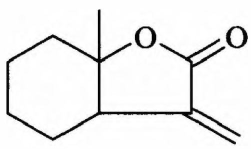
In the last years the horse chestnut leafminer (*Camereria orhidella*), an invasive parasite insect, has rapidly spread his population in Central Europe region. Heavily infested trees have drastically shortened period for photosynthesis. Interestingly, females of the horse chestnut leafminer prefer to oviposit rather on leaves of *Aesculus hippocastanum* than *Aesculus x carnea*.

Here we present the comparative analysis of the composition of volatile oils of leaves of both titled species of the chestnut. The material examined was distilled with water vapour in Deryng apparatus. Chromatographic analysis coupled with mass spectrometer was performed.

Analyses showed no significant difference, except the amount of eugenol, between compositions of volatile oils obtained from both species of chestnut. The content of eugenol in the oil of *A. carnea* was higher than in the oil of *A. hippocastanum*. The GC-MS analysis indicated the presence of simple bicyclic methylenelactone (1) as one of the components of essential oil from leaves of *A. hippocastanum*. The quantitative analysis of saponines detected in water-methanol extracts from showed their higher concentration in *A. x carnea* than *A. hippocastanum*.



1

P-26

Chemical composition of the essential oil of *Salvia microstegia* Boiss. et Bal. growing wild in Lebanon

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Salvia genus comprises herbaceous, suffructicous or shrubby perennials, rarely biennial or annual. About 900 species have been recorded. Members of the genus are of economic importance, as they are used in folk medicine for their biological activities (Ulubelen, 1997, 1998) and as flavouring agents in perfumery and cosmetics (Tzakou, 2001). Previous papers report the presence in the aerial parts of *Salvia microstegia* Boiss. et Bal., a plant used in popular medicine for its diuretic, antiseptic and wound healing properties (Baytop, 1984), of some diterpenoids (Ulubelen, 1991). In the framework of our studies on the oils of *Salvia* spp. (Senatore, 1998, 2004), we report the chemical composition of *S. microstegia* essential oil. To the best of our knowledge, this is the first report on its essential oil. Aerial parts of *S. microstegia* were collected at the full flowering stage from plants wild growing at Ouyoun Ourghouch, Lebanon, at 1800 msl. in June 2003. The oil from air-dried and ground aerial parts was isolated by hydrodistillation and analyzed by GC and GC/MS as previously described (Senatore, 2004). Seventy compounds, representing 93.0% of the oil, were identified. The major components were caryophyllene oxide, pulegone, 4-vinylguaiacole, hexadecanoic acid and menthone. The oil was tested for inhibiting activity on some bacteria and fungi that are phytopathogenic for several vegetables widely cultivated also in Campania region (Southern Italy). *Agrobacterium tumefaciens*, *Erwinia carotovora* subsp. *carotovora*, *Pseudomonas syringae* pv. *syringae* and *Rhocococcus fascians* were the tested bacteria while *Fusarium oxysporum* f. sp. *lycopersici*, *Phytrium ultimum* and *Rhizoctonia solani* were the fungi tested. No inhibiting activity was detected

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P-27

Chemical composition of the essential oil of the selected *Stachys* L. species

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The genus *Stachys* L. comprises more than 270 species and is considered as one of the largest genera of the Labiate family. Even though Serbia is not very rich in *Stachys* representatives eight species are recognized as endemics to the Balkan Peninsula or even narrower regions.

Plants of the *Stachys* genus have long tradition in folk medicine as agents for treating genital tumours, sclerosis of the spleen, inflammatory tumours, cough and ulcers. Phytotherapy suggests whole plant consumption for preparing teas having sedative, antispasmodic, diuretic and emmenagogue effects.

The essential oil chemical composition of three Balkan endemic species: *S. iva*, *S. plumosa* and *S. scardica* as well as *S. germanica*, European widely distributed specia, was determined. The plant materials were collected in blooming stage and the oil was obtained by hydrodistillation. All examined species were characterized by the low essential oil yield: 0.035, 0.037, 0.025 and 0.024 % respectively. The main constituents of the analyzed *Stachys* essential oils are the following: abietatriene (35.8 %, *S. plumosa*), caryophyllene oxide (20.1 %, *S. germanica*), germacrene D (15.7 %, *S. scardica*) 2,6-dimethyl-10-p-tolyl-2,6(*E*)-undecadiene (15.2 %, *S. iva*), spathulenol (12.9 %, *S. iva*) and δ-amorphene (11.3 % and 10.1 % *S. iva* and *S. germanica* respectively) and valeranon (9.7 %, *S. germanica*).

Generally, all taxa have rather low amounts of monoterpenes, while sesquiterpenes and diterpenes (*S. iva*, *S. plumosa*) are predominantly detected compounds.

P-28

Chemical compostions of the essential oils of stems, leaves and roots of Prangos latiloba Korov

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The genus *Prangos* consists of about 30 species [Evans, 1989]. In Iran, fifteen species of Umbelliferae family exist, among which five are endemic [Mozaffarian et al., 1996]. Medicinal applications have been reported for some *Prangos* species as: emollient, carminative [Zargari, 1988], antifungal [Oczan, 1999], antioxidant [Mavi et al., 2004], cytokine release inhibitor [Tada et al., 2002] and anti-HIV [Sikishima et al., 2001].

In this work, air-dried stems, leaves and roots of *Prangos latiloba* Korov (100 g) growing wild in Iran, were subjected to hydrodistillation for 3h using Clevenger-type apparatus to produce oils. Volatile oils of stems, leaves and roots, were analyzed by GC (Shimadzu GC-9A equipped with a HP-5MS fused silica column and a FID detector) and GC/MS (Hewlett-Packard 6890 fitted with a fused silica HP-5MS capillary column). Eight compounds constituting 84.72% of stems oil and twelve compounds constituting 95.39% of leaves oil and nine compounds constituting 88.73% of roots oil have been identified. The main components of stems oil were γ-cadinene (30.39%), α-pinene (25.47%) and sabinene (12.55%). The main components of leaves oil were germacrene D (27.79%), α-pinene (17.81%), β-caryophyllene (12.75%) and β-pinene (11.23%). The main components of roots oil were spathulenol (29.5%), 1,8-cineol (19.42%), p-cymene (17.03%) and α-bisabolol (15.33%).

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P-29

Chemical constituents of the essential oil of *Platychaete aucheri* Boiss. from Iran

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The genus *Platychaete* consists of five species growing wild in Iran. They distributed in south of Iran and three of them are endemic plants. *Platychaete aucheri* Boiss. with synonym name *Pulicaria persica* Jaub. et Spach. is an endemic plant of Iran and grown in mountains areas, rocky and stony slopes at an altitude of 750-1900m (Mozaffarian, 1998).

Aerial parts of the plant were collected in June 2003 during flowering, from Bandarabbas in Hormozgan provenance and the essential oil was obtained by Clevenger-type apparatus. In this study the essential oil composition of the oils were examined by GC and GC-MS. Constituents of the oil were identified by using retention indices and mass spectra (Adams, 2001).

Seventy-three components representing 98.2% of the total oil were characterized. It contains about 83% oxygenated monoterpenes, with myrtenol (60.4%) and borneol (16.8%) as the main constituents.

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P-30

Chemical constituents of the essential oil of *Stachys inflata* Benth wild growing in Iran

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In the Iranian flora genus of *Stachys* is represented by thirty-one species. *Stachys inflata* is a perennial essential oil plant belonging to the Lamiaceae (Labiatae) family. Stem is erect, richly branching from the base and 15-40 cm high. The characteristics spicy odor and taste of this plant result from the essential oil accumulated in the leaves and flowers. The oil has been found to posses anti-inflammatory activity and anti-anoxia action in mice. *S. inflata* herb was collected from Sepidan region located on the west of Iran. The essential oil was extracted by hydrodistillation. The oil was analyzed by a combination to GC and GC/MS. Thirty-two components were identified that approximately constitute 95.3% of the oil. The main constituents of the essential oil were cis-chrysanthenyl acetate (29.2%), cis- pinocarveol (14.7%), carvacrol (7.4%), linalool (7.2%), β-caryophyllene (4.6%), linalyl acetate (3.8%) and limonene + β- phellandrene (3.8%).

Keywords: *Stachy inflata*, Lamiaceae, hydrodistillation, essential oil, GC/MS.

Chemotaxonomy of *Hypericum* L. genus from Portugal: Essential Oils Composition of Sections *Drosocarpium, Oligostema* and *Taeniocarpium*

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Following previous chemotaxonomical studies in the genus *Hypericum* L. (Guttiferae/ Clusiaceae) from Portugal (Nogueira *et al.* 1998, Nogueira 2002), a contribution to the analysis of the essential oils of three sections, out of nine, is presented. *Hypericum perfoliatum* (section *Drosocarpium*) grows wild in the Center and South of Portugal; *H. humifusum* and *H. linarifolium* are both from section *Oligostema*, the first occurring throughout the country, while the second is distributed mainly in the North and Center; *H. pulchrum* (section *Taeniocarpium*) is confined to the littoral North of Portugal.

The essential oils were isolated by distillation-extraction and analysed by GC and GC-MS.

Monoterpene hydrocarbons constituted the main fraction in all oils (44-69%, 56%, 45% and 49% for *H. perfoliatum*, *H. humifusum*, *H. linarifolium* and *H. pulchrum*, respectively). Sesquiterpene hydrocarbons (2-13%, 16%, 20% and 16%, respectively) and a third fraction of non-terpenic compounds (20-29%, 16%, 14% and 11%, respectively) attained also relatively high amounts in all oils. The main components (≥5% in at least one sample) of each oil are given in Table 1.

The essential oil composition of the four species shows some qualitative resemblances which are well correlated with morphological taxonomical characters.

Table 1. Main components identified in the essential oil of four studied *Hypericum* species.

Main components (≥5%)	*Hypericum* sections			
	Drosocarpium	*Oligostema*		*Taeniocarpium*
	H. perfoliatum	*H. humifusum*	*H. linarifolium*	*H. pulchrum*
n-Nonane	11.9 - 23.8	0.4	0.9	4.6
α-Pinene	39.4 - 64.3	44.7	29.4	35.7
β-Pinene	1.9 - 3.2	7.7	9.3	9.0
n-Undecane	2.8 - 6.8	6.8	4.5	3.4
β-Caryophyllene	t - 1.4	4.0	6.6	0.9

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P-32

Comparative study of Iranian *Satureja* species for their essential oils

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Satureja genus (Labiatae) presents 14 aromatic species in Iran, eight of them are endemic. *Satureja* species are present in mountainous areas in Iran, mainly in western and northern parts. The green leaves and herbaceous parts of stems from some of these species are used fresh and dried as flavoring agents in seasonings, stews, meat dishes, poultry, sausages and vegetables. As the medicinal plants, *Satureja* species have been traditionally used as a stimulant, stomachic, carminative, expectorant, antidiarrheic and aphrodisiac. The essential oils of many of these species have demonstrated antimicrobial and antidiarrheic activity because of the phenols in the oil.

Essential oils from aerial parts of *Satureja mutica* Fisch. & C. A. Mey., *Satureja macrantha* C. A. Mey., *Satureja intermedia* C. A. Mey., *Satureja sahandica* Bornm., *Satureja edmondi* Briquet, *Satureja isophylla* Rech., *Satureja khuzistanica* Jamzad, *Satureja bachtiarica* Bunge., *Satureja Boissieri* Hausskn. Ex Boiss., *Satureja rechingeri* and cultivated *Satureja hortensis* L. were obtained by hydro-distillation. The oils were analyzed by capillary gas chromatography, using flame ionization and mass spectrometric detection.

Forty-five components were identified in the oil of *S. mutica* with carvacrol (30.9%), thymol (26.5%), gamma-terpinene (14.9%) and p-cymene (10.3%) as main constituents. Sixty-five compounds were identified in the oil of *S. macrantha* with p-cymene (25.8%), limonene (16.3%) and thymol (8.1%) as main components. Thirty-eight compounds were characterized in the oil of *S. intermedia* with thymol (32.3%), gamma-terpinene (29.3%) and p-cymene (14.7%) as main constituents. Thirty components were identified in the oil of *S. edmondi* with p-cymene (61.1%), gamma-terpinene (9.6%), thymol (5.0%) and alpha-terpineol (4.8%) as main constituents. Fifty-five compounds were identified in the oil of *S. isophylla* with alpha-eudesmol (11.3%), beta-eudesmol (9.6%), camphor (7.1%), beta-caryophyllene (6.1%), gamma-eudesmol (5.8%) and geranial (5.5%) as main components. Thirty-nine components were identified in the oils of eight populations of *S. sahandica*. The main constituents of the essential oils were thymol (19.6%-41.7%), p-cymene (32.5%-54.9%) and gamma-terpinene (1.0%-12.8%). The major components of *S. khuzistanica* were p-cymene (39.6%) and carvacrol (29.6%), while that of *S. bachtiarica* were thymol (44.5%) and gamma-terpinene (23.9%). The main constituents of the essential oil of *S. spicigera* were thymol (35.1%), p-cymene (22.1%) and gamma-terpinene (13.7%). Twenty-three components were identified in the oil of *S. hortensis* with carvacrol (46.0%-48.1%) and gamma-terpinene (37.7%-39.4%) as main components.

P-33

Composition of the essential oils from flowers, fruits and leaves of *Salvia fruticosa* Miller cultivated in Portugal

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Salvia fruticosa Miller (Greek sage) is a well-known medicinal plant endemic of the Eastern Mediterranean region. In some applications, this species can advantageously be used as a substitute of *S. officinalis* L., given its lower levels of thujone, a neurotoxic compound. The *S. futicosa* essential oils have been studied by several authors being normally obtained by hydrodistillation of leaves or entire aerial parts. Several reports suggest it possesses antimicrobial, antiviral and anti-tumor activities (Sivropoulou, 1997; Gali-Muhtasib, 2000).

The objective of this study was to compare the composition of the essential oils from flowers and fruits with the essential oil from leaves of *S. fruticosa* at bloom stage.

Flowers, fruits and leaves were collected in early June from *Salvia fruticosa* plants cultivated in an experimental field from DRAEDM located in Merelim, Braga (Portugal). Small samples (≤ 5g) of fresh material (flowers, fruits, leaves) were hydrodistilled and the resulting essential oils were analyzed by GC and GC/MS. A total of 46 compounds were identified, representing always more than 95% of the total essential oil. All samples possessed high levels of oxygenated monoterpenes (54,1% - 63,3% of the identified compounds) and monoterpene hydrocarbons (22,5% - 28,9% of the identified compounds). The major compound in the essential oils was 1,8-cineole (40,1% - 51,0%). Fruits and flowers contained also high levels of β-pinene, (*E*)-caryophyllene, α-terpineol and α-pinene. Lower percentages of β-pinene and higher percentages of myrcene were observed in the essential oil obtained from leaves. The levels of camphor, *cis*- and *trans*-thujone were very low in all samples.

Keywords: *Salvia fruticosa*, essential oils, monoterpenes, sesquiterpenes

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P-34

Composition of the essential oil from seeds of *Matthiola anchoniifolia* Hub.-Mor. obtained by microdistillation

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Matthiola (Cruciferae) is represented in Turkey by 9 species, three of them being endemic (Cullen, 1965; Davis et al., 1988). The seed essential oil of *Matthiola anchoniifolia* has not previously been studied. Essential oil obtained from crushed seeds of *Matthiola anchoniifolia* Hub.-Mor. by microdistillation was analysed by GC using a Hewlett Packard HP6890 system and GC/MS using a Hewlett Packard G1800A GCD system. The components of essential oil were identified by comparison of their mass spectra with those of Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, Mass Finder 3 Library and confirmed by comparison of their retention indices.

Octyl acetate (21.2%) was identified as the main constituent in the oil. Sulphur compounds responsible for the foul odor comprised 34.7% of the oil. They consisted of isopropyl isothiocyanate (16.9%), dimethyl trisulfide (8.8%), dimethyl disulfide (4.7%) and isobutyl isothiocyanate (4.3%). Non-volatile sulphur compounds have previously been reported from some *Matthiola* species. (Loffelhardt and Kindl, 1975; Kjaer and Gmelin, 1955, Kjaer and Gmelin, 1955; Brinker and Spencer, 1993; Al-Shehbaz and Al-Shammary, 1987, Carreras Matas, 1960).

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P-36

Composition of the essential oil of *Lathyrus rothondifolious*

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The purpose of the investigation was to analyze the chemical composition of the aerial parts of *Lathyrus rothondifolious,* which were collected from Gaduk (in May 2004). Research on secondary metabolites of medicinal and essential oil plants from dry Iran deserts is very important. These species have resistance to environmental pressures such as saltiness, different temperature regime and show ecological flexibility. In addition, there are a lot of studies about medicinal plant of Iran, which have industrial importance.

The essential oil of the plant was taken by hydrodistillation and was investigated by means of GC and GC/MS instruments. Some of the important components have been identified by using of Kovats indices, fragmentation of the components from mass spectra and GC chromatogram.

Retention indices (RI) were calculated by estimation the retention times of the eight peaks with those of C_7-C_{25} alkanes. Thirteen components representing more than 96% of the oil, were identified.

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P-37

Composition of the essential oil of *Lycium barbarum* and *L. ruthenicum* (Solanaceae) fruits

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Lycii fructus has been used as a remedy since ancient times in many countries, especially in China for its emmenagog, diuretic, antipyretic, tonic, aphrodisiac, hypnotic and hepatoprotective effects. The genus *Lycium* (Solanaceae) is represented by 8 species in Turkey (*L. barbarum* L., *L. europaeum* L., *L. ruthenicum* Murray, *L. depressum* Stocks, *L. schweinfurthii* Dammer, *L. anatolicum* A. Baytop et R. Mill, *L. shawii* Roemer et Schultes and *L. chinense* Miller). Two *Lycium* species, *L. barbarum* and *L. ruthenicum*, have been subjected to pharmacognostical investigations. Here, we report on the analysis of the fruit essential oils of *L. barbarum* and *L. ruthenicum*. To the best of our knowledge, this is the first study of their essential oils.

The fruits of *L. barbarum* and *L. ruthenicum* were collected from Eskisehir in October 2001 and from Malatya in August 2001, respectively. The air-dried fruits were hydrodistilled using a Clevenger-type apparatus to obtain essential oils in 0.09% and 0.07% yield on dry weight basis, respectively. Essential oils were analyzed by GC-MS (Hewlett-Packard GCD system with HP- Innowax FSC column). A library search was carried out using the Wiley GC/MS Library and the in-house Baser Library of Essential Constituents.

The main components in the oil of *L. barbarum* were found to be hexadecanoic acid (47.5%), linoleic acid (9.1%), β-elemene (5.4%), myristic acid (4.2%) and ethyl hexadecanoate (4.0%). The essential oil of *L. ruthenicum* has heptacosane (14.3%), ethyl linoleate (10.0%), hexacosane (7.0%), nonacosane (6.2%) and ethyl hexadecanoate (5.8%) as the main compounds.

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P-38

Composition of the essential oil of *Phlomis persica* Boiss

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The purpose of the investigation was to analyse the chemical composition of the aerial parts of *phlomis persica* which was collected from Taleghan (in June 2002). Research on secondary metabolites of medicinal and essential oily plants from dry state to super dry one about Iran s deserts is very important. Because of the resistance on environmental pressures such as saltines, differential temperature and ecological felexibility. [1,2]

In addition, there are a lot of studies about medicinal plant of Iran regions, which have important industrial components.

The essential oil of the plant was taken by hydrodistillation and was investigated by means of G.C and G.C-M.S instruments. Some of the important components have been identified by using of Kovats indices, fragmentation of the components from mass spectra and G.C chromatogram. Retention indices (RI) were calculated by estimation the retention times of the eluting peaks with those of C7-C25 alkanes.

Twenty three components representing 94.7% of the oil, were identified from which 23.7% ws monoterpenes and 71.2% was sesquiterpenes. The main identified components are as follows: germacrene-d (38.2%), bicyclogermacrene (16.3%), alpha pinene (13.3%), germacrene B (8.8%) and limonene (6.2%).

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P-39

Composition of the essential oil of *Salvia anatolica* Hamzaoglu & A. Duran, a new endemic in Turkey

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The genus *Salvia* (Lamiaceae) is represented in Turkey by 89 species and altogether 94 taxa, 45 of which are endemic in Turkey. The ratio of endemism in the genus *Salvia* in Turkey is 50 % (1,2). Some species of *Salvia* are used as medicinal and aromatic plants. *Salvia anatolica* Hamzaoglu & A. Duran has recently been described as a new endemic species in Turkey (3). This is the first report on the chemical composition of the essential oil of this species.

The aerial parts of *S. anatolica* were collected at an altitude 1695 m in Sivas: 22 km from Divriği - Gedikbasi village to Ilic (39° 31.43N, 38° 10.61 E) in June 2004. Voucher specimens are kept at the Herbarium of the Faculty of Education, Department of Biology of Selcuk University in Konya, Turkey (A. Duran 6595).

The plant material was hydrodistilled for 3 h using a Clevenger-type apparatus to yield (0.03 %) essential oil. The yield was calculated on dry weight basis. The oil was analysed by Agilent 6890N Network GC System-5973 Network Mass Selective Detector. An HP-Innowax FSC column (60 m x 0.25 mm *i.d.*, film thickness 0.25 μm) was used for separation of components in the oil.

Ninety-two components were characterized representing 96.2 % of the total components detected. α-Pinene (9.6 %), α-copaene (9.5 %) and β-pinene (5.4 %) were identified as major constituents in the oil.

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P-40

Composition of the essential oil of *Stachys alpina* L. subsp. *dinarica* Murb

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The chemical composition of the essential oil of *Stachys alpina* L. subsp. *dinarica* Murb., an endemic plant of Balkan Peninsula (Croatia, Bosnia and Herzegovina, Montenegro, Serbia and southwestern Bulgaria), was investigated. This plant mostly grows at higher altitudes, on mountain pastures and meadows and according to its morphological features is fairly similar to the Italian-Balkan species *S. tymphaea* Hausskn (1-3).

Essential oil was obtained by hydrodistillation from aerial blooming parts of *S. alpina* subsp. *dinarica*, collected from two localities in Bosnia and Herzegovina: Mt. Jahorina (sample I) and Mt. Maglić (sample II). Essential oil yields were 0.03 and 0.05% (w/w) (sample I and sample II, respectively). Seventy one components were identified by GC and GC-MS, representing 87.2 and 89.3% respectively of the total oil.

Sesquiterpenes were dominant in both samples (60.9 and 50.4%, respectively), with hydrocarbons prevailing (41.4 and 27.5%). Main constituents in both essential oils were: (*E*)-caryophyllene (13.4 and 9.1%), germacrene D (12.3 and 5.2%), caryophyllene oxide (5.7 and 8.0%) and (*E*)-nerolidol (4.8 and 5.3%, respectively).

Monoterpene fraction was significantly higher in sample II than in sample I (19.8 and 4.3%, respectively), with oxygenated monoterpenes dominating (18.1 and 3%). The main monoterpene in both samples was linalool (5.7 and 1.7%).

Considering high sesquiterpene content and distribution of sesquiterpenes, the essential oil of *S. alpina* subsp. *dinarica* is similar to the essential oils of some others *Stachys* species: *S. aleurites* (4), *S. balansae* (5) and *S. swainsonii* group (6).

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P-41

Composition of the essential oils of two *Achillea* species from Iran

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Achillea, is one of the most important genera of the Compositae family. *Achillea millefolium* (Yarrow) was known for many years in the folk medicine. It has been used to reduce sweating and to stop bleeding. It helps regulation of the menstrual cycle and reduces heavy bleeding and pain. The main component of the essential oil of *A. millefolium* is chamazulene which has anti-inflammatory and anti-allergic properties (Der Marderosian, 2001).

The aerial part of two specie, *Achillea eriophora* DC. and *Achillea wilhelmsii* were collected from Banddarabbas in Hormozgan province and Kazeroon in Fars province respectively. Air-dried aerial parts of the plant were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce oil in 0.3% and 0.15% yield respectively.

In this study the essential oil composition of the oils were examined by GC and GC-MS. Constituents of the oil were identified by using retention indices and mass spectra (Adams, 2001). Eighty-three components representing 96.0% of the total oil were characterized in the oil of *A. eriophora*. The main components of the oil were 1,8-Cineole (19.0%), Linalool (17.3%), α-terpineol (9.7%), santolina alcohol (7.6%), β-caryophyllene (5.9%) and β-pinene (5.7%), while Fifty-seven components representing 98.5% of the essential oil were characterized in the oil of *A. wilhelmsii* with the major compounds, carvacrol (25.1%), linalool (11.0%), 1,8-cineol (10.3%), E-nerolidol (9.0%) and borneol (6.4%). As it can be seen linalool is the main component in both oils.

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Composition of the essential oil of Sudanese basil

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Ocimum species (Lamiaceae) are annual or perennial herbaceous shrubs wild or cultivated in many parts of the world. *Ocimum basilicum* "Reihan" is a well known aromatic and medicinal plant in Sudan. In Central Sudan this plant is used to treat jaundice and also used as expectorant.

The chemical composition of the basil oil has been the subject to several studies (Modawi et al., 1984; Lemberkovics et al., 1998; Altantsetseg and Shatar, 2001; Zhongguo, 2003).

Fresh aerial parts of cultivated *O. basilicum* were subjected to hydro-distillation for three hours using Cleavenger type apparatus to produce oil in 0.5 % yield. The oil analyzed using GC/MS, thirteen (13) components have been identified of which the major constituents were found to be l-linalool (14.38%), beta-linalool (9.86%), estragole (9.42%), 1,8-cineol (8.78%), eugenol (8.11%) and iso-eugenol (7.0%). The presence of eugenol and methyleugenol in *O. basilicum* is not surprising as these compounds are present in Mongolian and Egyptian species studied previously (Altantsetseg and Shatar, 2001).

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P-43

Composition of the essential oil within summer savory (*Satureja hortensis* L., Lamiaceae) from Syria based on SPME single gland analysis

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Summer savory (*Satureja hortensis*, Lamiaceae) is a well known medicinal and aromatic plant. It is used in folk medicine especially in the Eastern Mediterranean to treat various ailments based on the plants antispasmodic, anti-diarrhoeal, antioxidant, sedative and antimicrobial activities.

The essential oil of Lamiaceae is produced in oil glands on the leaf epidermis and stored in a subcuticular space of these specialised leaf structures. An interesting approach to better understand the variability of the essential oil composition within a plant is the analysis of single oil glands.

Oil glands from different positions on the plant (petals, calyces, young, medium and old leaves) were analysed by sampling the content of each oil gland with a SPME-fibre. The main compounds identified were γ-terpinene and carvacrol responsible for the major part (approx. 90%) of the total essential oil composition.

The portion of carvacrol was highest in the petals (85%). The calyx showed intermediate values of 75% while carvacrol was equal in all three leaf positions (young, medium and old leaf) with 58%. γ-Terpinen increased from 9% in the calyx to 30% in the leaves.

The lanceolate leaves were divided into four sections starting from the leaf base (no. 1) to the leaf top (no. 4). The content of carvacrol increased from 56% on sector no. 1 to 66% in sector no. 2 and decreased then steadily down to 53% in sector no. 4. γ-Terpinene showed the opposite trend with a decrease from 32% (sector no. 1) to 25% (sector no. 2) and a steady increase to 36% in sector no. 4.

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P-45

Constituents of the volatile oil of *Artemisia sieberi* Besser. from Iran

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Artemisia is a fairly large genus belonging to family of Compositae, with the common Persian name of 'dermane', includes 34 species that are found wild all over Iran (Mozaffarian, 1996). The anticonvulsant potential and sedative effects obtained from the aerial parts of *A. dracunculus L.* assessed to be related to the presence of monoterpenoids in the essential oil (Sayyah, 2004).

In this study the aerial parts of *Artemisia sieberi* Besser. was hydrodistilled by use of Clevenger type apparatus. The yield of the oil was 0.3%. The oil was analyzed by GC and GC/MS (Adams, 2001). Fifty-one components representing 94.3% of the oil were identified. The main components of the oil were1,8-Cineole (23.2%), Comphor (22.2%), borneol (9.0%) and Hotrienol (7.0%).

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P-46

Distribution of volatile compounds in *Tanacetum* species of Turkey

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The family Asteraceae consisting large number of species which have uses as medicinal plants. *Tanacetum* is one of the largest genus in this family. *Tanacetum* grows widespread in the world specially in northern temperate regions (Abad, 1995). *Tanacetum* is represented by 45 species, with subspecies and varieties altogether 60 taxa in Turkey. It shows a high rate of endemism in Turkey (Güner, 2001, Davis, 1975).

Various biological activities have been reported for the extracts and essential oils of *Tanacetum* species (Abad, 1995). According to the previous reports on essential oil compositions, *Tanacetum* species from Turkey contain α-thujone, camphor, 1,8-cineole and borneol as main components mostly but in one species with carvone is also known. These reports have shown different compositional distributions in the same species collected from different locations and between the subspecies of a species (Başer, 2001, Tepe, 2005). Our work on *Tanacetum* species also showed varying oil compositions in the same species from different locations and between different subspecies of a species.

In this present work, we have characterized the essential oil compositions of endemic plants *T. densum* ssp. *sivasicum* from Sivas-Bogrudelik and ssp. *eginense* from Sivas-Tecer Mt. and *T. cadmeum* ssp. *orientale* collected from two different locations. Essential oils obtained by Clevenger apparatus were identified by GC-MS. Percentage compositions of the separated compounds were computed by GC (FID). Essential oils of *T. cadmeum* ssp. *orientale* from two locations and *T. densum* ssp. *sivasicum* and ssp. *eginense* showed major differences in their main components respectively.

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P-47

Effects of the soil in the essential oil composition of *Eryngium campestre* L. gathered in Madrid province: Spain

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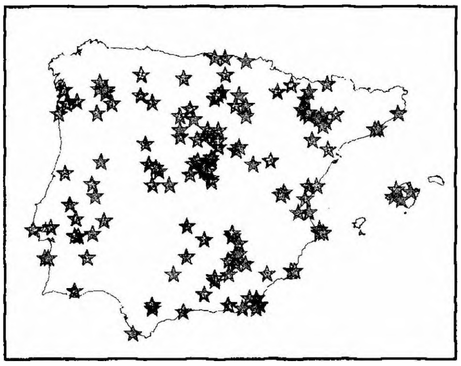
The genus *Eryngium* L. belongs to the Apiaceae family and, with about 250 species, has a cosmopolitan distribution. It grows practically all around the world with different vicariant species, most of them hemicryptophytes and geophytes although also therophyte species have been described. In Spain *Eryngium campestre* L. is a cosmopolitan species that grows over different types of soils.

The essential oil composition from the aerial parts of four populations of *Eryngium campestre* has been analysed by GC and GC/MS. The samples were gathered at the same phenological state, growing over different types of soil. The soil and regolith of the populations E.c.1 and E.c.2 is formed by gypsum and marls (alkaline soil) while the population E.c.3 was harvested in a river terrace composed by gravels, arkoses and siliciclastic sands (acid soil), finally the population E.c.4 shows intermediate conditions growing over soil formed by clays, marls, polygenic sands and limestones. Although the main compounds are always present in the different populations (flower oils: germacrene D (30.3-40.3%), β-phellandrene (0.5-22.2%), β-curcumene (0.7-22.2%), myrcene (3.0-21.7%) and *(E)*-β-farnesene (0.1-19.0%); leaf and stem oils: germacrene D (31.1-42.4%) and myrcene (0.5-23.1%)), the characteristics of the soil seem to affect to the composition. The flower oils showed a high amount of myrcene (21.7%) in the acid soil (E.c.3) while in the other populations its percentage composition is lower than 10%. On the other hand, the samples gathered over alkaline (E.c.1-2) and intermediate soils (E.c.4) showed high amounts of (*E*)-β-farnesene (0.1-19.0%) and β-curcumene (3.0-22.2%), compounds practically absent in the acid soil population. A similar pattern was detected in the leaves and stems oil, but only β-curcumene (1.9-6.1%) contained higher amounts in the alkaline and intermediate soils. A more exhaustive study should be carried out in order to confirm if the biosynthesis of these compounds could be influenced by the availability of Ca^{2+} in the soil.



Distribution map of *E. campestre* in the Iberian Peninsula.

P-48

Essential oil composition and antibactrial activity of *Meristotropis xenthioides*

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The genus Meristotropis (Fam. Papilionaceae) is represented in the flora of Iran by only one species:*M.xanthioides* vassiles (syn: *Glycyrrhiza triphylla* Fisch and C. A. Mey). Our study deals with the analysis of the volatile oil of *Meristotropis xanthioides* vassiles grown wild in Iran. The aerial parts of the genus *Meristotropis* was collected from Ashkhane, province of Khorrassane, Iran, in july 2004, at full flowering stage. The water distilled volatile oil from aerial parts of *Meristotropis xenthioides* was analyzed by GC and GC/MS. Some of the important components have been identified by using of Kovats indices and fragmentation of the components from mass spectra and GC chromatogram. Twenty two components were identified that approximately constitute more than 94% of the oil. The main constitutes of the essential oil were Myrcene (20.6%) Limonene (18.9%),and β-Caryophyllene (11.8%).Also, the antibacterial activity of essential oil in different dilutions was tested against 5 gram-positive and negative bacteria. The results of the bioassays showed that the high concentrations of the oil inhibited the growth of the all examined bacteria.

P-49

Essential oil composition and variability of *Artemisia absinthium* L. (wormwood) growing in Vilnius (Lithuania)

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Artemisia absinthium (wormwood) extracts and essential oils are used for healing of various diseases in folk medicine. Antihelmintic, antibacterial, digestive, tonic and other bioactivities are characteristic for preparations from wormwood plants. However, wormwood plants producing essential oils with high amounts of 1,8-cineole and cis (α)- and trans (β)-thujones are neurotoxic. The wormwood plants could biosynthesise various types of essential oils in different countries (Arino et al., 1999, Chialva et al., 1983, Wright, 2002, Juteau et al., 2003), while the oils have never been studied in Lithuania.

Aerial parts of *Artemisia absinthium* plants were collected in six localities during 1999-2005 and one accession at various vegetation stages. Essential oils were produced by hydro distillation and analysed by gas chromatography-mass spectrometry GC-MS (equipped with a capillary column CP-Sil 8CB, 50 m × 0.32 mm i.d., film thickness 0.25 μm). The percentage composition of the essential oils was computed from GC peak areas without using correction factors. Qualitative analysis was based on a comparison of retention times and indexes and mass spectra with corresponding data in the literature (Adams, 2001) and computer mass spectra libraries.

The first three major constituents were the following: cis- and trans-thujone, trans-sabinyl acetate, trans-sabinene hydrate, cis-chrysanthenyl acetate, β-pinene, myrcene and 1,8-cineole. The largest part of the essential oils was formed by oxygenated monoterpenes. More than eighty identified components comprised up to 95 % of total content. Principal Component Analysis was performed on the basis of the oils quantitative content and according to the main constituents the wormwood oils were distributed among chemotypes by cluster analysis.

The obtained results showed differences among the accessions of wormwood in essential oil content and its composition indicating the existence of chemical polymorphism.

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P-50

Essential oil composition from flowers and aerial parts of two St. John's Wort (*Hypericum perforatum* L.) varieties from Portugal

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Hypericum perforatum (Gutiferae) is a perennial herb commonly used as infusion whose anti-depressive effect, confirmed in pharmacological bio-assays, has driven an increasingly number of phytochemical studies. The floral tops are used as healing due to their antiseptic properties. However detailed phytochemical studies as well as studies on biological activities of their flowers are scarce. A commercial variety of *Hypericum perforatum* (Topaz) and a local ecotype of the same species, growing wild at Arcos de Valdevez, have been maintained in an experimental field from DRAEDM located in Merelim, Braga, Portugal. The essential oil obtained by hydrodistillation of a small amount of fresh flowers from the Topaz variety (<10g), harvested at the end of June, showed a complex mixture of compounds, 66 of which were identified in the commercial variety of *Hypericum perforatum* (Topaz) and 70 were identified in the local ecotype. The compounds were identified by GC-MS and quantified by GC. The sum of the absolute contents of all constituents, determined by GC, gave values somewhat higher than 1% relatively to the respective floral biomass dry weight. The identified compounds of all essential oils were grouped in monoterpene hydrocarbons (MH), oxygenated monoterpenes (MO), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (SO) and alkanes. The sesquiterpene group was the major one in the studied samples, with 37%, 35% and 49% in the essential oils of flowers, of aerial parts of the Topaz variety and of the local ecotype, respectively. Oxigenated-monoterpenes was the less represented group with less than 1% of total essential oils in the different samples. 2-Methyl octane, β-caryophyllene and *E*-β-ocimene were the major compounds in the flowers essential oils. In the essential oils of aerial parts of the Topaz variety the major ones were 2-methyl octane, γ-muurolene and β-caryophyllene. The local ecotype had the essential oil with the highest amount of germacrene D (22%) followed by 2-methyl octane and β-caryoplhyllene, as major compounds.

Keywords: *Hypericum perforatum*, essential oils, sesquiterpenes

P-51

Essential oil composition of an edemic species of Turkey: *Marrubium bourgaei* Boiss. subsp. *bourgaei* (Labiatae)

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The genus Marrubium (Labiatae) is represented by 23 taxa, 13 of which are endemic. *Marrubium bourgaei* Boiss. subsp. *bourgaei* is endemic to Turkey (Cullen, 1982; Davis et all., 1988). It has not previously been investigated. The oils of *Marrubium* species have been the subject of only a few investigations (Demirci et all., 1999; Lawrence, 1989; Nagy et al. 1998; Ball et al., 1999; Lazari et al., 1999; Weel et al., 1999; Nik et al., 2004).

Aerial parts of the plants were subjected to hydrodistillation for 3h using a Clevenger-type apparatus to produce an oil. The yield of oil was 0.4% on dry weight basis.

Essential oil of *Marrubium bourgaei* subsp. *bourgaei* from Turkey was analysed by GC and GC/MS. Seventy-eight components were characterized in total, representing 90.7 % of the oil. Hexadecanoic acid (33.3 %) and hexahydrofarnesyl acetone (6.4 %) were main constituents.

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Essential oil composition of *Artemisia biennis Willd.* and *Pulicaria undulata* (L.)C.A.Mey. Tow compositae herbs growing wild in Iran

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The genus *Artemisia* (familly Compositae, tribe Anthemideae) which contains many useful aromatic and medicinal plants comprises about 300 species in the northen hemisphere. Thirty-four species of genus *Artemisia* are found in Iran of which two are endemic: *A. melanolepis* and *A. kermanensis* .

The genus *Pulicaria* (family Compositae; tribe Inuleae) is represented in the flora of Iran by five species .The extract of the aerial parts of *P. undulata* gave two new sesquiterpene lactones, one being of a glaucolide like eudesmanolide and the second one a nor-guaianolide type (1).

The oils obtained by hydrodistillation from aerial parts of *Artemisia biennis* and *Pulicaria undulata* were analyzed by means of GC and GC/MS. The major components of the oil of *Artemisia biennis* were camphor (24.6%), artemisia ketone (11.4%) and α-pinene (10.2%).α-pinene (45.6%), 1,8-cineole (27.1%) were found to be the main constituents of the oil of *Pulicaria undulata* .

Essential oil composition of *Artemisia scoparia* Waldst. Et Kit from Iran

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The genus *Artemisia* (family, Compositae, tribe Anthemideae) which contains useful aromatic and medicinal plants consists 300 species in the northern hemisphere (1). Thirty–four species of this genus are found in Iran which two are endemic: *A.melanolepis* and *A.kermansis* (2).

In this study we report the chemical constituents of the oil of *A. scoparia* from Iran for the first time.

The oil of *A. scoparia* obtained by hydrodistillation using a Clevenger type apparatus for 3 h. The oil yield was 0.3% (W/W). The oil was characterized by mean of HP 6890/5973 GC/MS instrument. Identification of the constituents was based on comparison of their mass spectra and Kovatts retention indices (KI) with those obtained from authentic sample (3) and Wiley library mass spectra. Eighteen components were identified in the oil of *A. scoparia* which represents about 99.8% of the oil. This oil consists about 98.1% monoterpens and 1.7% sesquiterpens. Methyl chavicol (KI: 1195) (32.2%), E-β-ocimene (KI: 1050) (22.3%), Z-β-ocimene (KI: 1040) (18.3%) and limonene (KI: 1031) (11.4%) were found to be the main constituents.

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Essential oil composition of *Anthemis tinctoria* L. and *Anthemis cotula* L. from Serbia

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The genus *Anthemis* (fam. Asteraceae, tribe Anthemideae Cass.) is represented by 9 species growing wild in Serbia and Montenegro[1]. In previous phytochemical studies sesquiterpene lactones, flavonoids, acetylenes and essential oils have been reported as the main classes of the secondary metabolites in genus *Anthemis*.[2-6]

The flowering aerial parts were collected in July 2004 in Serbia: *A. tinctoria* from locality Sokolska planina and *A. cotula* from locality Resavica. The essential oils obtained by hydrodistillation were analyzed by GC and GC-MS. The aerial parts of *A. tinctoria* and *A. cotula* yielded 0.1% (v/w) of essential oil.

The main constituents in the *A. tinctoria* essential oil were elemol (8.0%), 1,8-cineole (7.2%), spathulenol (6.3%) and γ-eudesmol (5.6%), while in the essential oil of *A. cotula* the most dominant were matricaria ester (16.8%), (*E*)-β-farnesene (9.5%) and dihydromatricaria ester (8.4%).

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Essential oil composition of *Ferula szowitziana* DC. from Turkey

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Ferula L. (Apiaceae) is represented by 17 species in Turkey, seven species being endemic (Davis, 1972). *Ferula szowitziana* DC. (syn: *F. microloba* Boiss.) is distributed mainly in Inner Anatolia. To the best of our knowledge, there is no previous report on the essential oil of this species.

The aerial parts of *F. szowitziana* were collected at altitude 1120 m in Erzincan: 75 km from Erzincan to Ilic in June 2004. Voucher specimens are kept at the Herbarium of the Faculty of Education, Department of Biology of Selcuk University in Konya, Turkey (A. Duran 6523).

The plant material was hydrodistilled for 3 h using a Clevenger-type apparatus to yield 0.4 % of essential oil. The yield was calculated on dry weight basis. The oil was analysed by Agilent 6890N Network GC System-5973 Network Mass Selective Detector. An HP-Innowax FSC column (60 m x 0.25 mm i.d., film thickness 0.25 µm) was used for separation of components in the oil.

The water-distilled essential oil of *Ferula szowitziana* was analyzed by GC/MS. One-hundred and fourteen components were identified with β-eudesmol (15.5 %), α-eudesmol (14.6 %) and α-pinene (9.6 %) as the major constituents. More than half of the oil consisted of oxygenated sesquiterpenes (50.3 %) as well as sesquiterpene hydrocarbons (16.1 %).

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Essential oils composition of *Leonurus cardiaca* L. growing wild in Lithuania

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Leonurus cardiaca L.(motherwort) is growing wild in Lithuania. This plant was described in medicinal literature from X century as remedy for healing nervous and functional cardiac disorders. *L. cardiaca* herbs biosinthesised flavonoids, alkaloids, iridoids, diterpenoids, cardenolids like glycosides, tannins and other constituents in lower amounts. Among other compounds was 0.01 – 0.05 % of essential oils, The aim of the present paper is to study oils of motherwort growing wild in Lithuania.

Wild plants of *Leonurus cardiaca* L. .(motherwort) collected at full flowering in ten localities of Vilnius district in Lithuania. Essential oils produced by hydrodistillation were analysed using the GC and GC/MS methods. About the half of the oils were consisted of sesquiterpene hydrocarbons (48.8 – 62.2 %). The essential oils from fresh dried plants were of the germacrene D (26.6 – 35.1 %) chemotype. The other main constituents were β-caryophyllene (5.8 – 9.0 %) and α-humulene (6.4 – 9.2 %). Forty nine identified compounds made up 73.1 – 84.8 % of the oils.

The composition of *Leonurus cardiaca* L. oils depended on storage of the plants and the oils before analysis and contained compounds with higher boiling temperatures – phytol and caryophyllene oxide as main components.

Essential oil of *Nepeta rtanjensis* exhibits significant *in vitro* MAO B inhibition

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Nepeta rtanjensis, Diklić & Milojević (Lamiaceae), is an endemic specie of the Rtanj mt., located in the Eastern Serbia. The biological effects of this plant extracts and the essential oil have been poorly investigated until now. However, *N. rtanjensis* is related to the widespread catnip (*Nepeta cataria*), a plant famous for its insect repellent activity and intoxication of cats (Peterson and Coats, 2001). *N. cataria* is a traditional remedy for colds and flu and also have ethnomedicinal application for headache, insomnia, nervous dyspepsia and colic in children (Chevalier, 1996). These biological effects were mostly attributed to the nepetalactone, which two isomers are present in the essential oil of catnip: Z,E (*cis, trans*) and E, Z (*trans, cis*). Commercial sample of catnip essential oil contained 40% of nepetalactone and 43% of nepetalic acid (Harney et al. 1977).

The aerial parts of *N. rtanjensis* were collected in summer 2004. at the Rtanj mt. and immediately air-dried. The oil was isolated by hydro-distillation in a Clevenger-type apparatus from a powder of dried herb. Chemical composition of the oil, determined by analytical GC(FID) and GC/MS techniques (Kovacevic et al., 2005), shows predominance of Z,E-nepetalactone (79.9%). Other constituents were: E,Z-nepetalactone (6.3%), α-pinene (3.3%), δ-cadinene (2.1), germacrene D (1.8%), α-copaene (1.3%), 2-methoxy-p-cresol (1.1%), β-pinene (0.4%).

We performed *in vitro* screening of this essential oil for certain neurochemical activities, like monoamino oxidase (MAO) inhibition. Initial *in vitro* radioassays for total MAO inhibition was carried out by detecting the level of ^{14}C-tyramine degradation induced by rat microsomal MAO, while for the evaluation of partial MAO A and MAO B inhibition, their specific ^{14}C-radio-substrates were used (5-HT and β-phenylelthylamine, respectively; Vogel, 2002). The essential oil was first dissolved in DMSO and its serial dilution in water, (0.01 – 1 mg/ml), were used in the assays in duplicates, where final concentration of DMSO $\leq$ 1%.

The essential oil of *N. rtanjensis* exhibited moderate *in vitro* inhibition of total MAO activity (IC_{50}=10.93 µg/ml), as also of MAO A (IC_{50}=28.33 µg/ml), while it appeared to be a prominent MAO B inhibitor (IC_{50}=1.83 µg/ml). The therapeutic significance of MAO B inhibition is not so valuable like of MAO A inhibition, which is connected to antidepressant activity of certain drugs. Nevertheless, a proposed *in vivo* central MAO B blockade could have influence on animal behavior. The research on the essential oil of *N. rtanjensis* continues in two directions: 1) identification of the oil's active MAO B inhibiting component(s) and 2) pharmacological behavioral studies on rodents.

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Essential oil composition of *Santolina oblongifolia* Boiss. from Spain: an Iberian Peninsula endemic species

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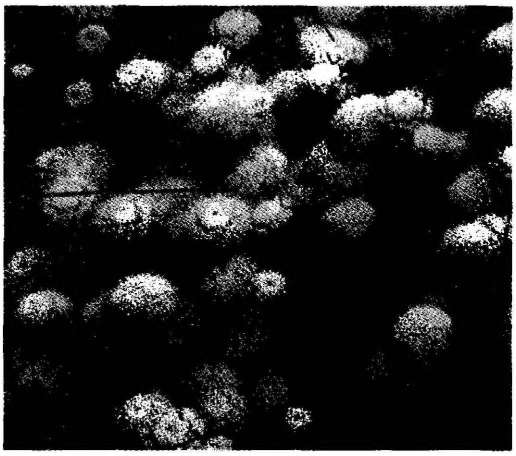
The genus *Santolina* L., which is a member of the Anthemideae tribe of the Asteraceae family contains 10 species distributed throughout the western Mediterranean countries. *Santolina oblongifolia* Boiss. is a perennial shrub endemic to the Iberian Peninsula that in Spain is widely spread in the mountains of Gredos, Bejar and Gata.

The essential oils extracted from the aerial parts of *Santolina oblongifolia* by hydrodistillation have been analysed by gas chromatography and gas chromatography coupled to mass spectrometry. The essential oil compostion is very complex, more than 60 compounds have been detected with percentage compositions always lower than 10%. The main constituents of the oil were identified as borneol (8.8%), *cis*-β-guaiene (5.9%), myrcene (5.4%), limonene (5.2%), β-oplopenone (4.7%) and β-pinene (4.2%). A peak accounting a 5.6% of TIC appears to include two unidentified compounds, with molecular weights of 220 and 236.



Santolina oblongifolia Boiss.

P-59

Essential oil composition of *Tagetes minuta* L. a new essential oil plant cultivated in Iran

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Mexican marigold (*Tagetes minuta* L.) is an annual plant belonging to the Asteraceae family. It is native to grasslands and mountain regions of south America. *Tagetes minuta* is not present in the Iranian flora. The seeds were provided from Namibia in Africa. The seeds were sown on a sandy loam soil on 4 April 2003 at the experimental station of Tarbiat Modarres University, College of Agriculture located in Tehran. Spacing in the rows is 40 cm. Plants were thinned to 15 cm within rows. Aerial parts of the plant were harvested at full flowering stage, then air dried. The essential oil was obtained by hydrodistillation. The oil was analyzed by GC and GC/MS. The main components were dihydrotageton (21.4%), α-terpineol (15.6%), (Z)-tagetone (13.1%), (E)-ocimenone (11.8%), (Z)-β-ocimene (8.3%) and spathulenol (4.8%).

P-60

Essential oil content and compositions of *Myrtus communis* L. growing in Iran

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Myrtle (*Myrtus communis* L.) is an evergreen shrub of small tree with dense foliage to 4.5 m belonging to the Myrtaceae family. It grows from south Europe to West Asia and is highly drought tolerant. The 5-6 cm lanceolate leaves are strongly scented when crushed. The white flowers are sweetly scented and appear from July to August. The scented flowers are hermaphrodite and are pollinated by bees. The ripening stage of fruits is different, depend on the climatic condition and take place in September to October. The leaves of *Myrtus communis* L. was harvested at full flowering stage from south east of Iran. The essential oil was obtained by hydrodistillation and the oil content was 1.2% (w/w) based on dry weight. The oil was analyzed by capillary GC and GC/MS. Twenty-two compounds were identified. Limonene and α-pinene constituted of 38.2% and 31.5% of the oil respectively. Other major components were 1,8-cineole (7.5%), linalyl acetate (7.1%), linalool (4.9%), geranyl acetate (1.6%) and α-terpinenyl acetate (1.3%).

Keywords: Myrtle, *Myrtus communis*, hydrosistillation, essential oil compositions, limonene, α-pinene, 1.8-cineole, linalool.

P-61

Essential oils of *Tordylium pestalozzae* Boiss., *Tordylium pustulosum* Boiss. and *Tordylium lanatum* (Boiss.) Boiss. (Umbelliferae), growing wild in Turkey

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The essential oils obtained by hydro-distillation from fruits of *Tordylium pestalozzae* Boiss., *Tordylium pustulosum* Boiss. and *Tordylium lanatum* (Boiss.) Boiss. (Umbelliferae) (Al-Eisawi and Jury, 1988; Alava, 1972) were analyzed by GC and GC/MS. In total, forty-seven compounds were characterized, representing 95.9 % of *T. pestalozzae* oil, thirty compounds were characterized, representing 98.3 % of *T. pustulosum* oil (Baser et al., 2002) and thirty-eight compounds were characterized, representing 97.2 % of the oil of *T. lanatum*. The main constituents were octyl hexanoate (56.0 %), octyl octanoate (15.7 %), octanol (14.5 %), hexadecanoic acid (6.0 %); octyl hexanoate (68.8 %), octyl 2-methylbutyrate (17.8), octanol (4.2 %); octyl hexanoate (58.8 %), octanol (21.5 %) in the oils of *T. pestalozzae*, *T. pustulosum* and *T. lanatum*, respectively.

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P-62

Essential oil of *Pluchea Quitoc* (Asteraceae)

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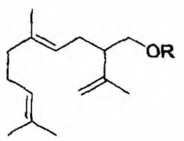
Pluchea quitoc is a large aromatic shrub found throughout of Southern Brazil (Rio Grande do Sul). Its have been used traditionally by local people as expectorant, carminative, digestive and anti-rheumatic (Guilhon, 1996). This work reports the chemical composition and antimicrobial activity of the essential oils of *Pluchea quitoc* from two different localities of the Rio Grande do Sul, Brazil (samples A and B). The qualitative and quantitative composition of the essential oils were determined by GC and CG-MS using a SE-54 and PEG-20M columns. In total 24 compounds were identified, representing 87% of sample A and 89% of sample B. These oils had a high content of sesquiterpenes, being α-gurgunene (20,5%) and *E*-sesquilavandulol alcohol (24,3%), the main components of the oil from sample A and *E*-sesquilavandulol acetate (24,1%) and α-gurgunene (20%), the major components of the oil from sample B. *E*-sesquilavandulol acetate (1), the principal constituent of oil from sample B, was isolated by preparative chromatography, characterized by NMR spectroscopy and submitted to hydrolysis basic that yield *E*-sesquilavandulol alcohol (2), the main component of sample A. To the best of our knowledge, compounds 1 and 2 are the first sesquilavandulanes derivatives found in Asteraceae family, both structurally related to compounds obtained from essential oil of *Peucedanum palustre* (Apiaceae)(Schmaus, 1989). The antimicrobial activity of isolated compounds 1 and 2 was determined by bioautography methods in a TLC bioassay. The results showed that *E*-sesquilavandulol exhibited a more potent antibacterial activity against *Staphylococcus aureus* (6.25μg), *Staphylococcus epidermidis* (50μg) and *E.coli* (50μg), while *E*-sesquilavandulol acetate was more active against *Staphylococcus aureus* (50μg).



1 - R= $COCH_3$
2 - R= H

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P-63

Essential oil constituents of two *Tagetes* species

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The species of *Tagetes* genus (Asteraceae) grow widely in warmer parts of America. *Tagetes lucida* Cav. (Mexican marigold) has an erect, not much branched habit with small yellow flower heads. *Tagetes patula* L., commonly known as French marigold, is a well-known ornamental plant widespread all over the world. It is a bushy annual and has yellow or orange capitula.

These herbs synthesize many biologically active products that can be used by the industry and the medicine. Carotenoids are pharmacological active compounds used as ophtalmologic agent and as food colorants as well. The essential oil components have antibacterial and antifungal activity. *Tagetes* species accumulate a wide range of thiophenes in their roots. These sulphurated aromatic compounds exhibit a strong biocidal activity.

The purpose of the present study was to investigate volatile compound production and composition. The essential oils of *intact* plants and *in vitro* cultures of *T. lucida* and *T. patula* were used for GC and GC-MS analyses.

In vitro T. lucida plants were maintained on solid Murashige and Skoog medium supplemented under light conditions. Genetically transformed hairy root cultures of *T. patula* were cultivated in liquid Gamborg's B5 medium (in 500 mL Erlenmeyer flask filled with 100 mL medium) on rotary shaker (140 rpm) at 23±2 °C in dark.

The essential oil was produced by steam distillation in a Clevenger apparatus for 3 hours. The oil content was measured gravimetrically. The GC-MS analyses were carried out on a Finnigan MAT GCQ mass spectrometer with ion trap analyzer. A capillary column (MDN-5S: 30 m × 0,25 mm ID × 0,25 μm film thickness) was used to fractionate the samples. The electron impact method was used to ionize the fractions. The carrier gas was high purity helium. The identification of the compounds was done by comparing the retention times and the recorded spectra with spectra known from literature and spectra of authentic standards.

The main essential oil components of the *intact* plants were found to be 'classical' terpenoids while the *in vitro T. lucida* roots, hairy roots and *intact* roots of *T. patula* produced aromatic sulphurated thiophene structures. Aerial parts of *intact* and *in vitro T. lucida* had the phenylpropanoid methylchavicol (52.8 % and 66.5 % respectively) as the main compound. The main constituent of *T. patula* capitula was β-caryophyllene (53.5 %) and the leaves had high concentrations of terpinolene (21.1 %).

The main volatile component of *T. patula* hairy roots and *intact* roots was 5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) of 28,5 and 44,0 % respectively. Other sulphurated structures were also identified, such as α-terthienyl (13.1 and 21.8 %) and 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc) of 13.3 and 6.1 %. *T. lucida in vitro* root oil was also rich in thiophenes: BBT (8.7 %) and BBTOAc (6.3 %). These thiophenes are the main secondary metabolites of the *intact* roots and can be found even in the essential oil of the *intact* plants. It is interesting to note that α-terthienyl was not detected in the essential oils of *T. lucida*.

P-64

Essential oil of *Tordylium ketenoglui* H. Duman et A. Duran (Umbelliferae) growing in Turkey

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Umbelliferae is an economically important flowering plant family known as a source of well known plants used in foods and also for the distillation of essential oils (Heywood, 1979). Flavonoids, phenylpropanoids, acetylenes, terpenoids, coumarins and essential oils are characteristic chemical groups in the family Umbelliferae (Saleh et al., 1983).

The genus *Tordylium* L. (Syn.: *Hasselquistia* L., *Condylocarpus* Hoffm., *Ainsworthia* Boiss., *Synelcosciadium* Boiss.), a member of Umbelliferae family, is represented by 15 species in Turkey (Al-Eisawi and Jury, 1988).

A few phytochemical and biological activity studies have been carried out on some *Tordylium* species (Kofinas et al., 1998, 1993). Essential oil studies on *Tordylium* species are rather limited (Kofinas et al., 1993; Baser et al., 2002).

Tordylium ketenoglui H. Duman et A. Duran (Umbelliferae, Apiaceae) is a recently described species of the Flora of Turkey (Duman, 2000).

The plant was collected from Antalya-Akseki at an altitude of 605-709 m, on May 2003. A voucher specimen was deposited at the Herbarium of the Faculty of Pharmacy of Ankara University (AEF 22992).

The essential oil obtained by hydro-distillation from fruits of *Tordylium ketenoglui* was analyzed by GC (Hewlett-Packard HP 6890 GC system with a FID detector) and GC/MS (Hewlett-Packard GC/MSD system). In total, seventy-seven compounds were characterized, representing 91.6 % of the oil. The main constituents were determined as octyl octanoate (28.9 %), octanol (11.6 %) and bornyl acetate (7.2 %).

To the best of our knowledge, this is the first report on the chemistry of *Tordylium ketenoglui*.

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P-65

Investigations on different Hungarian populations of traditionally used *Prunella vulgaris* L.

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Self-heal or *Prunella vulgaris* L. is a herbaceous, perennial herb belonging to *Lamiaceae* family. It is native to Eurasia, common in Hungary (Simon, 2000). But it is also found in North-America and Australia. In Japan flowering spikes, in China just the leaves and the stem while in Europe the whole plant is used as a drug. The only attempt to summarize the knowledge of the plant was carried out by Markova et al. in 1997 – „*Prunella vulgaris* L. – a Rediscovered Medicinal Plant"

The active constituents of the plant are the following: oleanolic and ursolic acid (triterpenes), rutin, hyperozid, campherol, quercetin (flavonoids), caffeic and rosmarinic acid (phenolic acids), coumarins, tannins, carotinoids, essential oil (0,4-0,6 %, characterised by d-camphor and d-fenchone) and prunellin. Prunellin is a polysaccharide which inhibits the replication of human immundeficiency virus-1.

Self-heal has astringent, anti-cancer, anti-HIV, diuretic, antioxidative, antibiotic and antiinflammatory properties. It is also a well-known remedy against high blood pressure. Due to these effects it has been used as an astringent for internal and external purposes since Medieval Times. Nowadays *Prunella* has an important role in modern pharmacy (during anti-HIV and anti-cancer therapies) and in food-industry (as a natural antioxidant), either.

For the purpose of getting to know this plant better as well as making a compairson on different Hungarian populations, we made a standing culture using German seeds in our experimental filed, Soroksár. Samples from two botanical gardens and from three natural habitats of the plant were also involved in our work. We studied the essential oil content (by hydrodistillation, using Clevenger apparatus), essential oil composition (by GC – HP 6890N), total phenol content (using Folin-Ciocalteau reagent) and total antioxidant capacity (by FRAP method). We tried to reveal the chemical composition of different populations of *Prunella* making the possibility to select the most perspective ones suitable for pharmacological and food-industrial usage.

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P-66

Leaves and flowers volatiles of *Cassia fistula* L.

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Cassia fistula L. (fam. Leguminosae), a semi-wild Indian Labernum also known as the Golden Shower, is widely cultivated and it is used against habitual constipation.[1]

C. fistula has significant biological activities. The methanolic extract from the seeds showed antitumor activity against Ehrlich ascites carcinoma.[2] The n-heptane extract of C. fistula leaves has been shown to have hepatoprotective activity.[3] The aqueous extract of the leaves is hypoglycaemic and used in Panamanian folkoloric medicine to treat diabetes.[4] The plant also has antifertility and hypocholesterolaemic effects.[5,6] The antipyretic, analgesic effects [7] and antitussive potential [8] of the plant have also been reported, as well as the antifungal and antibacterial activities.[9] In this work we analyzed the essential oils from fresh flowers and leaves of C. fistula, as there is no report in the literature concerning the essential oil content of this tree.

Fresh flowers and leaves of C. fistula with a slightly aromatic odor, were collected from the trees growing in Orman garden in September 2003 and authenticated by Dr. Kamal El Batanony (Professor of Taxonomy and Botany, Faculty of Science, Cairo University). The freshly collected plant material (leaves and flowers) of C. fistula were cut into small pieces and submitted to hydrodistillation by a modified Likens and Nikerson apparatus for 3 hours. Pentane (5 ml) was added to the graduated part of the apparatus to trap volatile compounds. After hydrodistillation, the n-pentane layer was separated and dried over anhydrous sodium sulphate. The obtained oils were analyzed by GC-MS.

The main components of flower oil were (E)-nerolidol (38.0%), 2-hexadecanone (17%) and heptacosane (12.8%), while the leaves volatiles consist mainly of phytol (16.1%) and hydrocarbons.

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P-67

Methyl salicylate, the one constituent of Burkina Faso *Securidaca longepedunculata* roots bark essential oil

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Securidaca longepedunculata Fers (Polygalaceae) is a shrub up to 7 meters in height which grows on deep sands and is found everywhere in savannahs and forests along river from Senegal to Southwest Africa. *S. Longepedunculata* is commonly used as traditional medicine in many parts of Africa against snake and scorpio venom as well as against a number of invertebrate pests, including insects infesting stored grain. It is also used to care malaria, hepatitis and dysentery.

Roots bark of *S. longepedunculata* from Burkina Faso have been harvested at Yalle (11°13'060'' latitude North and 1°56'392'' longitude West). The essential oil was obtained by steam distillation of the bark root during three hours with a Clevenger type system and the yield war over 0.85%. Analysis of the essential oil by gas chromatography linked to mass spectrometry (GC/MS) showed a major component accounting for over 99% of the volatile material. This was identified as methyl 2-hydroxybenzoate (methyl salicylate) by comparison of the GC retention times and mass spectrum with those of synthetic standards. The oil has also been investigated by NMR ^{1}H and ^{13}C

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P-68

Study on the essential oil of *Rhodiola rosea* L. cultivated in Finland by GC, GC/MS methods

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Rhodiola rosea L. belongs to the Crassulaceae plant family, it is widely distributed in Arctic and circumpolar mountainous regions, in high altitudes throughout Asia and Europe. *R. rosea* is a perennial plant reaches a height of 12 to 30 inches and produces yellow blossom. It has tick rhizome which has a pleasant fragrant when cut. Linnaeus renamed it from *Sedum rosea* to *Rhodiola rosea,* referring to the rose-like attar of the fresh cut rootstock.

Vikings used the herbs to enhance their physical strength and endurance. Tradicional folk medicine used *R. rosea* to increase physical endurance, work productivity, resistance to high altitude sickness and to treat fatigue, depression, anaemia, impotence, gastrointestinal ailments, infection and nervous system disorder (Shevtsov, V. A. 2003).

Extract of the *R. rosea* root were found to contain powerful adaptogen. A group of phenylpropanoid are specific to *R. rosea* : rosin, rosarin, rosavin and a phenylethanol derivates: salidrosid (György, Zs. 2004).

Rhodiola rosea L. plants have been cultivated at the Agrifood Research Centre in Mikkeli, during 1994-2004 in Finland. The slow growing plants need five growing years to reach suitable root yields.

For identification of the essential oil components, analytical gas chromatography (GC) was performed by Shimadzu GC-2010 capillary gas chromatograph apparatus at the NATURLAND Hungary Ltd. GC/MS analyses were carried out on a Finnigan MAT GCQ, Electron Ion Trap Mass Spectrometer (GC/MS) in the Semmelweis University.

The essential oil contained large amount of monoterpenoid components (97%), octen-3-on, linalool, myrtenal, myrtenol, perilla aldehyd, cumin aldehyd, cumin alcohol and high amount of geraniol 13,7% - 67,8 %. Myrtenal, myrtenol, linalool and geraniol were identified as the most important rose-like odor compounds which have been giving the pleasant rose-like scent to these Nordic rhizomes. These compounds are similar and belong to the expensive Arabic Rose Oil, which has been using for sexual desire for a long time.

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P-69

The essential oil composition of *Acroptilon repens* (L.) DC. of Turkish origin

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Acroptilon repens (L.) DC. (Russian Knapweed) from Asteraceae family (syn.*Centaurea repens* L., *C.picris* Palas ex Willd., *Serratula picris* (Willd.) Bieb., *Acroptilon picris* (Willd.) DC.) is a creeping perennial plant with black horizontal roots (Davis, 1988). Turkish local name for this plant is "kekre" (Baytop, 1994).

According to survey of the available literature, there is no report on the composition of *Acroptilon repens* (L.) DC. oil. Here, we report on the analysis of its oil for the first time.

The air-dried material was subjected to hydrodistillation for 3h using a Clevenger apparatus to obtain essential oil in trace amount. Due to the poor yield of oil, it was trapped in *n*-hexane. The oil was submitted to gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) analyses.

The composition of essential oil obtained from the aerial parts of Russian knapweed (*Acroptilon repens* (L.) DC.) (Asteraceae) was analyzed by GC (Hewlett Packard 6890 system) and GC-MS (Hewlett-Packard GCD system). Seventy-two components were identified in the essential oil of *Acroptilon repens* (L.) DC. Major constituents of the essential oil were α-copaene (22.8 %), β-caryophyllene (9.5 %), germacrene D (9.0 %), β-cubebene (7.9 %) and caryophyllene oxide (6.4 %).

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P-70

The essential oil composition of *Tanacetum macrophyllum* (Waldst. & Kit.) Schultz. Bip.

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The genus *Tanacetum* (Asteraceae) comprises various medicinally important taxa. It contains several annual and perennial species. This genus is represented by 45 species and altogether 60 taxa in Turkey (Davis, 1975; Guner et al., 2000).

In the present work, water distilled essential oil from dried aerial parts of *Tanacetum macrophyllum* (Waldst. & Kit.) Schultz. Bip., collected from the north-eastern parts of Turkey, was subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The resulting essential oil was analyzed both by gas chromatography and gas chromatography – mass spectrometry [a HP system with an Innowax fused silica column (60 m x 0.25 mm ∅, with 0.25 μm film thickness), see for analytical details (Kaya et al, 2003)].

Seventy five compounds were identified in the essential oil of *T. macrophyllum,* representing 86.5% of the total oil with 0.8% monoterpene hydrocarbons, 33.7% oxygenated monoterpenes, 8.8% sesquiterpene hydrocarbons, 41.8% oxygenated sesquiterpenes and 1.4% others, respectively. The major components were identified as *β*-eudesmol (21.4%), *cis*-chrysanthenol (12%).

To the best of our knowledge, this is the first report on the essential oil composition of *T. macrophyllum*.

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P-71

The essential oils of two endemic Iranian *Artemisia* species: *A. diffusa* and A. kopetdaghensis

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The essential oils of both species were isolated by hydrodistillation from leaves and flowers at the beginning of the flowering phase. Identification of oils constituents were analyzed by mean of GC-MS (HP 6890-HP 5973).

Twenty components of the oil of *A.diffusa* were identified and 36 of that of *A.kopetdaghensis*, amounting to 96% and 97% of the total oils, respectively.

Both oils were dominated by the monoterpene fraction, camphor being its main component (65.25% for *A . diffusa* and 59% for *A . kopetdaghenis).*

Another component found in both oil was 1,8-cineol, amounting to 10% in *A . diffusa* and to 3% in *A. kopetdaghensis.*

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P-72

Twelve species of the genus *Achillea*: phytochemical and antimicrobial studies

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We investigated the chemical composition and antimicrobial activity of the essential oils hydro-distilled from the aerial parts of four species of the section *Achillea* (*A. crithmifolia* W. et K., *A. nobilis* L., *A. asplenifolia* Vent. *and A. millefolium* L.), five species belonging to section *Filipendulinae* (*A. chrysocoma* Friv., *A. clypeolata* Sibth. et Sm., *A. coarctata* Poir., *A. depressa* Janka and *A. holosericea* Sibth. et Sm.), two from section *Anthemoideae* (*A. clavennae* L. and *A. serbica* Nyman), as well as a representative of the section *Ptarmica* (*A. lingulata* W. et K.).

The monoterpenoids (hydrocarbons and oxygenated derivatives) were the dominant group of constituents in all oils (ranging from 96.4-54.0% of the total oil) except in the oil of *A. asplenifolia* that was found to contain 70.5% of sesquiterpenoids. Borneol, camphor and 1,8-cineole were either individually or as group the most frequent major constituting compounds. The exceptions being *A. nobilis* (α-thujone, 25.7%), *A. millefolium* (β-pinene, 32.6%) and *A. asplenifolia* (sabinene 1.7% and α-terpineol 1.6%). β-sabinyl acetate (39.9%) along side with camphor (33.9%) were the characteristic feature of *A. serbica* oil. β-caryophyllene and/or caryophyllene oxide were the most abundant sesquiterpenoids in almost all investigated species but in *A. nobilis* oil (α-cadinol 4.7% and viridiflorol 3.2%).

S. aureus and *E. coli* were the most sensitive bacteria tested to the oils of *A. nobilis, A. lingulata, A. holosericea, A. clavennae* and *A. crithmifolia*. All of the microorganisms were least susceptible to the action of *A. serbica* oil.

Nonacosane (25.5% - 65.4%) was found to be the major constituent of the alkane fraction of the petrol ether extract of all species with the exception of *A. coarctata* and *A. depressa* where heptacosane dominated (23.1% and 27.9%, respectively).

Palmitic acid accounted for the biggest portion of the saturated and oleic and linolenic acids of the unsaturated fatty acids found in the methanol-chloroform extract of the investigated species.

Five bis-tetrahydrofuran lignans (epiaschantin, aschantin, epieudesmin, sesartemin and yangambin) and apigenin-7-*O*-glucoside were isolated from the ether : methanol : hexane (1:1:1) extract of *A. lingulata*.

The ether : methanol : hexane (1:1:1) extract of *A. clavennae* yielded apigenine, centaureidine, 1-deoxy-1α-peroxyrupicolin A, 1-deoxy-1α-peroxyrupicolin B, rupicolin A and rupicolin B.

From the extract (methylene chloride : methanol = 1 : 1) of the roots of *A. holosericea* we isolated the following hydroxycinnamoyl conjugates: 3,5-di-*O*-[*E*]-caffeoylquinic acid, 3-*O*-[*E*]-feruloyl-5-*O*-[*E*]-caffeoylquinic acid, 4-*O*-[*E*]-feruloyl-5-*O*-[*E*]-caffeoylquinic acid, 5-*O*-[*E*]-caffeoylquinic acid and eicosanyl-*trans*-*p*-coumarate.

The extract of *A. clavennae* showed strong antifungal activity against *C. albicans* and *A. niger*.

P-73

Variation of volatile sulfur compounds among Iranian garlic ecotypes

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The essential oil of garlic is greatly appreciated throughout the world because of its flavouring properties as well as medicinal values. There are a number of organosulfur compounds (mainly allicin) responsible for the characteristic flavour and medicinal activity of these species. Thermal decomposition of allicin gives origin to a variety of volatile sulfide compounds, namely 2-propenyl (allyl) derivatives (Avato *et al*, 1998).

The total amount of these compounds depends on variety, agronomical and environmental condition. On the other hand a series of different ecotypes have been established over time showing great morphological and phytochemical diversity with highly variable sulfur-containing constituents. Clonal selection of garlic with suitable content of sulfur-containing constituents and agronomical traits is desirable for large-scale culture and drug production (Baghalian *et al*, 2005). It is thus desirable to determine the relationship between ecological parameters and content of sulfur compounds in garlic (Ueda *et al*, 1991). The goal of this study is to identify the volatile sulfur compounds existing in ecotypes various origins and unique ecotypes with potential industrial value.

In this study the essential oils of 24 garlic ecotypes collected from main cultivation areas in Iran were extracted and investigated by means of GC-MS (Agilent GC-6890N with a MS-5973N detector).

Cluster analysis was conducted using unweighted pair-group method arithmetic average (UPGMA) and the ecotypes in each cluster were analyzed for basic statistics. The information on clusters will assist in looking extensively for more ecotypes with similar characters. The entries were grouped into four clusters with 2, 9, 11 and 2 ecotypes in clusters I, II, III and IV, respectively. Mean value along with standard deviation for each cluster revealed that all ecotypes were high in allyl methyl trisulfide. Diallyl disulfide and diallyl trisufide were the major compounds in all ecotypes with the exception of cluster IV for diallyl disulfide and cluster I for diallyl trisufide. Significant positive correlations were detected for diallyl trisulfide and diallyl tetrasulfid as well as for allyl sulfide and diallyl disulfide (r =0.498 and 0.415 respectively). The results showed that all the ecotypes contain high amounts of the active principal allicin and also there was no significant correlation between the ecology condition and organosulfur compounds.

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P-74

Volatile constituents of *Ajuga tomentella (Boiss)*, a Labiatae plant growing in Iran

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The genus Ajuga represent four species in Iran. A.austro-iranica, A.Chamaecistus, A.comata, A.orientalis.

In this investigation, we study on the essential oil of Ajuga tomentella that grows wild in Iran, for the first time.

The oil of Ajuga tomentella optained by the hydrodistillation from the aerial parts of the plant using a Clevenger type apparatus for 3 h.The oil yield was 0.18%(W/W).Water distilled essential oil was analyzed by a HP 6890/5973 GC/MS instrument .Identification of the constituents was based on comparison of their mass spectra and Kovatts retention indices (KI) with those optained from authentic sample (3) and Wiley library mass spectra.

Fifty components were identified in the oil of Ajuga tomentella which represents about 85.4%of the oil.of A.tomentella ,were identified .β-pinene (10.5%),α-thujene (5.9%) and linalool (5.4%) were found to be the major compounds.

The oil characterized by β-pineen (KI:980)(10.5%), α-thujene (KI:1102)(0.45%), linalool (KI:1098)(5.4%) as the main constituents.

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P-75

Volatile constituents of *Levisticum officinale* Koch. and *Seseli libanotis* (L.) W. D. Koch var. armeniacum Bordz. two Umbelliferae herbs growing wild in Iran

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The genus *Leivisticum* is represented in the flora of Iran by only one species, *L. officinale* Koch. This plant has long been cultivated in Europe and Northern-America. Flavonoids have been identified in solvent extracts of the aerial parts of *L. officinale*. Chemical studies on some *Seseli* species have resulted, cumarins, sesquiterpenes and phenyl propanoids.

Water distilled volatile oils from aerial parts of *Levisticum officinale* Koch. and *Seseli libanotis* (L.) W. D. Koch var. armeniacum Bordz., two Umbelliferae species, were analyzed by GC and GC / MS.

The aerial parts of two Umbelliferae species were collected during the flowering stage at the following places: *L. officinale* in Hezar mountains, in Province of Kerman and *S. libanotis* from Yosh, Chalous road, Province of Mazandaran, Iran, both in June 2002.

Twentry-three compounds were identified in the oil of *L. officinale* representing 86.9% of the total oil with α- terpinyl acetate (40.5%) and β-phellandrene (16.7%) as the major constituents. Other notable constituents were γ- terpinene (7.8%) and sabinene (6.5%). The oil of *L. officinale* was characterized by large amounts of monoterpenes (85.8%) and the sesquiterpene fraction of the oil was relativerly small, representing only 0.3% of the total oil. Composition of the oil obtained from dry leaves of *L. offticinale* from Finland (1992), previously reported to contain α- terpinyl acetate and β-phellandrene, as the major components, agree with our research.

Twenty- five components in the oil of *S. libanotis,* which represented about 83.6% of the total oil, were identified. The oil of *S. libanotis* constisted of six monoterpene hydrocarbons (19.6%), three oxygenated monoterpenes (6.6%), eight sesquiterpene hydrocarbones (6.0%) and eight oxygenated sesquiterpenes (51.4%). The major component of this oil was acorenone (35.5%). Other notable constituents were limonene (7.2%), α-pinene (7.1%) and caryophyllene oxide (6.1%). In our previous investigation, the oil obtained from *S. trotuosum*(2003) were rich in monoterpenes (85.4%) with α-pinene (21.2%), β- phellandrene (14.9%), β-pinene (14.2%) and sabinene (13.4%) and poor in sesquiterpenes (8.1%), while in *S. libanotis* oil sesquiterpenes (57.4%) prodominated than monoterpenes (26.2%).

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P-76

Volatile constituents of *Phlomis persica* Boiss. from Iran

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The genus *Phlomis* is represented by 24 species grown in Iran, which 10 of them are endemic (Rechinger, 1982). The essential oil and ethanol extract of *Ph. fruticosa* L. showed antibacterial, antifungal and antimutagenic activity (Ristic, 2000). The essential oil of *Ph. lanata* had a moderate invitro activity against gram positive and gram negative and stronger activity against pathogenic fungi (Couladis, 2000). *Phlomis persica* Boiss. (syn: *Ph. cymifera* Boiss) is an endemic plant grown in north, west and center of Iran.

In this study the aerial parts of the plant were air-dried at ambient temperature in the shade and hydrodistilled by using a Clevenger-type apparatus for 5 hours. The yield of oil was 0.01%(w/w). The essential oil of *Phlomis persica* Boiss. was analyzed by GC and GC/MS. Fifty-two components were identified in the oil. 6,10,14-trimethyl-pentadecan-2-one (24.2%), germacrene-D (12.9%) and germacrene-B (6.6%) were the main compounds of the oil of *phlomis persica*.

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Volatiles of *Hypericum bupleuroides* Griseb

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The genus *Hypericum* of the Guttiferae family is represented with around 450 species of herbs, shrubs and trees all over the world. Eighty-nine *Hypericum* species are present in Turkey of which forty three are endemic as recorded in the Flora (Güner et al., 2000; Ernst, 2003).

Hypericum sp. have a wide range of uses such as a dye, flavoring, food and as a medicine. Folk uses of various preparations are for treating wounds, infections, digestive disorders, etc. (Baytop, 1999).

Hypericum bupleuroides Griseb. used in this study was collected from the North-Eastern region of Turkey. The aerial parts were subjected to microdistillation for the isolation of volatiles, which were subsequently analyzed by GC and GC/MS. An HP-Innowax FSC column (60 m x 0.25 mm, with 0.25 µm film thickness) was used under previously described conditions (Baser et al., 2001). The investigation resulted in the characterization of 30 components, representing 92% of the total volatiles. Sesquiterpenes such as β-sesquiphellandrene (33.2%) and β-caryophyllene (20.2%) were assigned as major compounds.

The microdistillation method enabled fast and efficient isolation of volatiles from a small (500 mg) amount of plant material.

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P-78

Volatile constituents of *Thymus migricus* Klokov & Desj. Shost, a Labiatae plants growing in Iran

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The genus *Thymus* represents fourteen species in Iran which four are endemic: *T. trautvetteri, T. percicus, T. daenensis* and *T. carmanicus* (1, 2).

In this investigation, we study on the essential oil of *T. migricus* that grows wild in Iran, for the first time.

The oil of *T. migricus* obtained by hydrodistillation from the aerial parts of the plant using a Clevenger type apparatus for 3 h. The oil yield was 0.15% (W/W). Water distilled essential oil was analyzed by a HP 6890/5973 GC/MS instrument. Identification of the constituents was based on comparison of their mass spectra and Kovatts retention indices (KI) with those obtained from authentic sample (3) and Wiley library mass spectra.

Nighteen components were identified in the oil of *T. migricus* which represents about 99.5% of the oil. This oil consists about 97.4% monoterpens and 2.1% sesquiterpens. The oil characterized by thymol (KI: 1290) (56.0%), p-cymene (KI: 1026) (16.2%), γ-terpinene (KI: 1062) (9.1%), 1.8-cineole (KI: 1033) (3.0%) and α-terpinene (KI: 1018) (2.7%) as the main constituents.

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P-79

Volatile constituents of an Argentinean *Nothofagus* species

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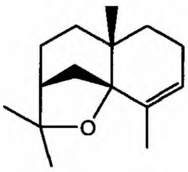
The beeches of the genus *Nothofagus* are widely distributed within the southern hemisphere of the world with occurrence in temperate as well in tropical regions. Here we report about the examination of the main aroma components of a leave specimen from Argentina.

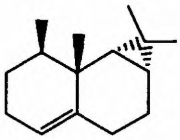
The analysis was carried out by means of GC-MS and NMR after chromatography (FCC, PHPLC) of an ether crude extract of the dried and ground material.

The comparison of the mass spectra of the several fractions from FCC with reference spectra[1]-[3] showed that α-agarofuran (1) was the characteristic aroma component. In addition, calarene (2), aromadendrene (3), eremophilene (4), premnaspirodiene (5) and guaia-6,9-dien-4β-ol (6) could be identified as minor constituents (see structures below). Besides some peaks of high abundance with a retention time larger than 35 minutes, belonging to not further examined alkenes from waxes etc., two peaks showed characteristic chalcone fragmentation patterns.[4][5] It could be concluded that these patterns were in accordance with the structures of 2',6'-dihydroxy-4'-methoxychalcone (7a) and 2',4',6'-trihydroxychalcone (7b), respectively.

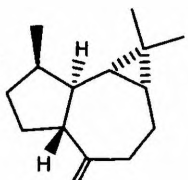
Our investigations reveal that ether extracts of the Argentinean *Nothofagus* sp. serve as a good source of aroma components, especially α-agarofuran. The isolation can easily be done by normal open-column chromatography.



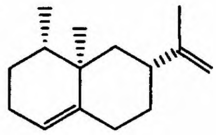
(1) α-Agarofuran



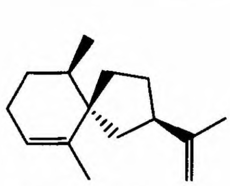
(2) Calarene



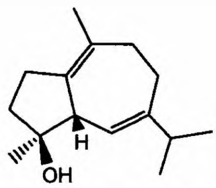
(3) Aromadendrene



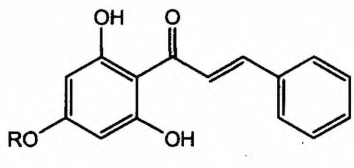
(4) Eremophilene



(5) Premnaspirodiene



(6) Guaia-6,9-dien-4β-ol



(7a) R=CH_3
(7b) R=H

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P-80

Volatile constituents of *Stachys pubesces* ten, a Labiatae plant growing in Iran

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The genus Stachys represents thirty four species in Iran which thirteen are endemic: *St.veroniciformis*, *St.persepolitana*, *St.Koelzii*, *St.pilifera*, *St.laxa*, *St.obtusicrena*, *St.subaphylla*, *St.Benthamiana*, *St.asterocalyx*, *St.kermanshahensis*, *St.acerosa*, *St.lucheri*, *St.ixods*.

In this investigation, we study on the essential oil of Stachys pubesces that grows wild in Iran, for the first time.

The oil of *Stachys pubesces* optained by the hydrodistillation from the aerial parts of the plant using a Clevenger type apparatus for 3 h. The oil yield was 0.15%(W/W).Water distilled essential oil was analyzed by a HP 6890/5973 GC/MS instrument .Identification of the constituents was based on comparison of their mass spectra and Kovats retention indices (KI) with those optained from authentic sample (3) and Wiley library mass spectra.

Fourty Seven components were identified in the oil of *Stachys pubesces* which represents about 93.4%of the oil.of *S.pubesces* ,were identified .β-pinene (10.0%),α- pinene (5.8%) and germacrene D(13.1%) were found to be the major compounds.

The oil characterized by β-pinene (KI:980)(9.6%),α- pinene (KI:939)(5.8%), germacrene- D (KI:1480)(13.1%) as the main constituents.

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P-81

Yield and essential oil composition of japanese Perilla (*Perilla frutescens* L.) genotypes under Hungarian conditions

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Perilla frutescens (*Lamiaceae*) – or Chinese Basil is a traditional and widely used plant in South-South East Asia. In the traditional Chinese Medicine the leaves of *Perilla* are used to cure bronchitis and asthma. Acccording to the newest research the essential oil of the plant has a very strong antiallergic effect. In Hungary it is used as an ornamental annual plant.

In 2004 we tested three commercial items of *Perilla frutescens* in open field experiments. We examined the following cuntivars: one of simple green leaves (P1), one of green, scallop-edged ones (P2) and one type of red leaves (P3). The yield of herbage and dried leaves, and the accumulation level of the essential oil has been evaluated. The composition of the essential oil was analysed by GC method.

We obtained significant differences between the three *Perilla*- types: the yield of dried leaves/ plant of P1 and P2 was much higher than the yield of the red P3 taxon. The accumulation of essential oil showed a similar tendence. We measured the highest essential oil content in P1 genotype.

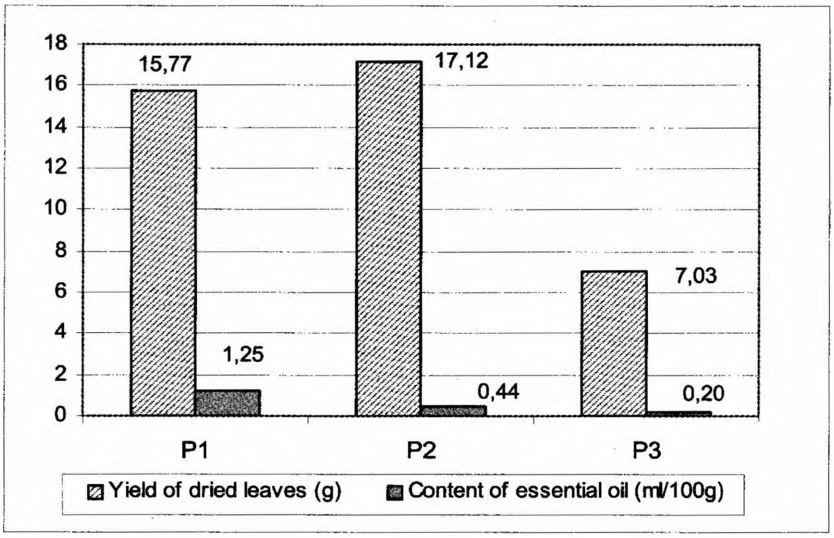

Figure 1.: Yield of dried leaves/plant and essential oil content of the examined *Perilla frutescens* genotypes

The main component of the examined cultivars' essential oil was perilla- aldehyde in each oil.

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Production, extraction and utilization of essential oils

P-82

Changes of chemical and microbiological quality of marjoram cultivars by steam treatment for reduction of the microbial level

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Spices are added frequently to thermally untreated food (e.g. chips, curds, cheese). The microbial level in these spices must be particularly low. The steam treatment commonly applied in Germany cannot be used without problems for marjoram, because it does not only cause a reduction of the microbial level, but also changes of quantity and composition of the essential oil are possible. This could have influence on the sensory quality.

Therefore a careful steam treatment, which reduces the microbial count and keeps the oil content and the sensory quality at the same time, should be used.

Air dried samples of three marjoram cultivars were available, a new developed hybrid form, the cultivar "Marcelka" and an industrial marjoram sample. The microbial and chemical initial levels of these samples were investigated, likewise the levels after standard steam treatment and "soft" steam treatment (lower temperature and shorter treatment time).

Both steam treatments reduce strongly the total microbial level, the coliform bacteria, the yeast-like fungi and the mould fungi.

The oil content is reduced in the soft variant by approx. 0.3 %, in the standard variant by approx. 0.5 %.

The essential oils were isolated by extraction with isooctane and subsequently investigated by GC.

It has been found that the influence of steam treatment results above all in a decrease of sabinene and cis-sabinenhydrate acetate in the oils, whereas the contents of cis-sabinenhydrate and terpinen-4-ol increase. These changes are particularly large when the standard variant is applied.

After the steam treatment a clear colour change is observed in each case. A sensory change could not be determined.

P-83

Comparative analysis of the oil and supercritical CO_2 extract of *Cymbopogon citratus* Stapf.

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Cymbopogon citratus Stapf., (Gramineae) is commonly know as lemon grass, growing spontaneously in tropical regions. It is widely used as essential ingredient in Asian cuisine due to its sharp lemon flavor, in India a tea prepared from lemon grass is used as a sedative for central nervous system and the roots are used as chewing sticks or rubbed on the teeth for cleaning purposes. It is used for medicinal purposes in a variety of traditional preparations. The essential oil of lemon grass has been used to treat a wide variety of health conditions such as acne, athlete's foot, excessive perspiration, flatulence, muscle aches, oily skin and scabies.

Bioactivity studies have shown that various components in the essential oil possess antimicrobical, antifungal, antibacterial and mosquito repellent activity. Velluti et al.(2004), reported that this essential oil inhibited growth of *Fusarium verticillioides* in maize. Wannissorn et al.(1996), reported that citral was the major active component of lemon grass oil. Citral is a mixture of bioactive isomers neral and geranial. High-quality lemon grass essential oil is composed primarily of citral (>75%) and other isolated active components are limonene, citronellal, β-myrcene and geraniol.

Dried leaves of lemon grass were used as a matrix for supercritical extraction of essential oil with CO_2. The objective of this study was to analyze the influence of the extraction pressure on the yield and composition of the oil. A series of experiments were carried out, for 360 min, at 50°C and at different pressures: 90, 100, 110 and 120 bar.

Extraction conditions were chosen to maximize citral content in the essential oil. The collected extracts were analyzed by GC-MS and their composition were compared with that of the essential oil isolated by hydrodistillation. At higher solvent density the extract aspect changes passing from characteristic yellow essential oil to yellowish semi-solid mass because of the extraction of high molecular mass compounds. The optimum conditions for citral extraction were 90 bar and 50°C . Indeed at these conditions citral represent more than 68% of the essential oil and the extraction yield was 0.65% while the yield obtained from hydrodistillation was 0.43% with a content of citral of 73%.

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P-84

Composition of the essential oils of *Angelica sylvestris* L. var. *sylvestris* isolated from the fruits by different isolation techniques

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The genus *Angelica* L. (Umbelliferae) encompasses 80 species spread in the northern hemisphere (1). It is represented in Turkey by two varieties of *Angelica sylvestris* L. (2). The oils of two varieties, *A. sylvestris* L. var. *vulgaris* Lalem. and *A. sylvestris* L. var. *elatior* Wahlenb. were found to be rich in monoterpenes with α-pinene and β-phellandrene as predominant constituents (3,4). The oil of *A. sylvestris* var. *sylvestris* has not previously been investigated. In order to achieve a thorough characterization of the volatile components present in the fruits of *A. sylvestris* var. *sylvestris*, different techniques were employed for the isolation of the oil, namely hydrodistillation (HD), microdistillation (MD) and micro-steam distillation – solid-phase microextraction (MSD-SPME).

The essential oils of the fruits of *Angelica sylvestris* var. *sylvestris* obtained using different techniques were analyzed by GC and GC/MS. The composition of the oils depended on the isolation method employed. α-Pinene (25.6%, 36.2% and 9.2%, respectively), β-phellandrene (9.1%, 9.9% and 3.2%), bornyl acetate (7.3%, 4.3% and 6.9%), limonene (5.6%, 4.3% and 2.1%), myrcene (4.4%, 4.0% and 1.3%), camphene (3.9%, 4.7% and 1.2%), α-chamigrene (3.4%, 4.4% and 9.1%) and β-sesquiphellandrene (2.5%, 3.8% and 8.7%) were found as main constituents in the oils. *p*-Cresol (6.5%), *epi*-α-bisabolol (5.6%), (Z)-β-farnesene (5.5%), naphthalene (4.4%), daucene (3.1%), amorpha-4,11-diene (3.1%) and γ-muurolene (2.5%) were also among the main constituents of the oil isolated by MSD-SPME.

The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices.

Generally, the overall composition of the oils, isolated using different techniques, appeared to be the same. There was no significant qualitative difference detected, although the percentage compositions for some components varied significantly.

The oil composition of *A. sylvestris* var. *sylvestris* was comparable with the oils of other varieties reported in literature.

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P-86

Effects of cooking on cedren, zingiberen and curcumen content in ginger

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Ginger, the underground stem, or rhizome, of the plant *Zingiber officinale* has been used as a medicine in Asian, Indian, and Arabic herbal traditions since ancient times. In addition to these medicinal uses, ginger continues to be valued around the world as an important cooking spice and is believed to help different health problems (the common cold, flu-like symptoms, headaches etc.). Ginger is native to Asia where its use as a culinary spice spans at least 4,400 years.

In this study, the content of three essential oil constituents - cedren, zingiberen and curcumen were evaluated in the edible portions of fresh rhizome of *Zingiber officinale* before and after cooking. All analyses were performed by GC/MS after extraction with mixture methanol/water. Low-pressure boiling (conventional cooking), high-pressure boiling and wok cooking were the three domestic cooking processes used in this work. All preparations were performed in the same quantities of water.

The highest loss during cooking processes was detected for cedrene, in average 57 % (conventional boiling 58 %, high-pressure boiling 55 %, wok 57 %). Conventional boiling led to the same loss of zingiberen and curcumen (29 %) as well as wok cooking (27 % of loss for both compounds). The similar situation was observed in the case of conventional boiling where the loss of zingiberen and curcumen was almost equal (21 and 23 % respectively).

These results show no considerable differences among the three treatments in their influence on cedren, zingiberen or curcumen content in ginger.

P-87

Essential oil compositions of Turkish oil rose (*Rosa damascena Mill.*) products

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Oil rose (*Rosa damascena* Mill.) is the main source of the rose oil and other materials including concrete, absolute and rose water, the most important commercial products especially in the flavour and fragrance industry. Rose oil is produced by water steam distillation of fresh rose flowers. Concrete is a waxy product extracted from fresh rose flowers with volatile solvents. Absolute and bioabsolute are alcohol extracts of the concretes from fresh and residue flowers, respectively. Rose water is a by-product obtained during the distillation of rose flowers.

The aim of the presented study is to examine the essential oil compositions of rose oil, concrete, absolute, bioabsolute and rose water in order to determinate the differences among them. The analyses of essential oils were performed by GC/MS. In the rose oil, 16 essential oil components were determined and the most abundant components were citronellol, geraniol and nonadecane as 39.3, 16.3 and 11.6%, respectively. On the other hand, 8 essential oil components were found in the rose concrete and they were phenylethyl alcohol (55.3%), nonadecane (11.4%), heneicosane (10.5%), citronellol (8.1%), geraniol (5.6%), 1-nonadecane (2.2%), nerol (1.4%), α-pinene (0.8%). Absolute and bioabsolute had similar essential oil components with different percentages. The main component in the absolute and bioabsolute was phenylethyl alcohol with percentages of 48.6% and 21.5%, respectively. The essential oil from rose water was extracted with two different solvent. Hexane and diethylether extractions exhibited different composition. While hexane extraction gave geraniol and citronellol as the most abundant components, phenylethyl alchohol was the highest component found in the diethylether extraction. As a result, it was found differences among oil rose products with respect to essential oil composition.

Keywords: Rose oil, rose concrete, absolute, rose water, essential oil composition.

P-88

Essential oil flavored yoghurt: physicochemical, microbiological and sensory properties

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For a long time, yoghurt by itself has been recognized as a healthy food, due to the beneficial action of its viable bacteria that compete with pathogenic bacteria for nutrients and space (Tamime, 1985; Staffolo 2004). The product is accepted by consumers because of its flavour and aroma, mainly acetaldehyde and texture. Several researches have been conducted about enhancing the sensory characteristics of yoghurt by supplementation of some additives (Kumar and Mishra, 2004; Shukla and Jain 1991).

Yoghurts were manufactured from pasteurized evaporated milk by using different essential oils at three different concentrations (basil, savory and coriander; 10, 50,100 µl) as flavoring agents. They were compared with plain yoghurt manufactured from the same milk as control. There were greater differences between the control and essential oil flavored ones with respect to acidity and syneresis. Basil oil added yoghurt samples had lower acidity levels. The syneresis values of all samples ranged between 15.28%-20.28%. Syneresis increased with higher amounts of basil and coriander oils. Addition of essential oil did not affect viscosity of yoghurt samples significantly. The microbiological quality of essential oil containing yoghurt samples was found better than that of control sample and the yoghurt starter bacteria were in abundance. The sensory panel identified significant differences ($p<0.01$) between essential oil containing and control yoghurt in terms of odor and taste attributes.

Essential oils in yoghurt appear to be promising for the low Coliform bacteria growth. Total bacteria and molds-yeast counts were similar to control. Basil and coriander oils added samples had lower, savory oil added samples showed higher LAB numbers than control. Savory oil in yoghurt is worth to pay attention for the higher LAB growth than other oil added and control samples, lower syneresis and higher sensory scores. Savory oil added yoghurt was the most acceptable for the taste on sensory evaluation, followed by basil and coriander oil. Increasing essential oil amounts were found to be unacceptable for both odor and taste characteristics.

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P-89

Essential oils from the forestry sector in India

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Forest land plays a vital role mainly in the country like India where 23% of land is covered by forest. Looking at expanded demand from Flavour and Fragrance Industry, forest based aromatic crops have better future. Forest land can be utilized for cultivation of aromatic crops which are sacred and has tremendous demand because of its oriental fragrance. Nagarmotha oil (*Cyperus Scariousus*), rosa oil/ jamrosa oil, jatamansi oil (*Nardostachys Jatamansi)*, vetiver oil *(Vetiveria Zizanoides*), *Artemesia annua* oil, bursear oil (Linaloe-berry), juniper berry oil, valerian oil, lavender oil & many others are examples of aromatic crops which can be effectively grown on forest land.

Many of these oils are very unique oils aroma of which is impossible to create synthetically eg. nagarmotha oil has aroma of sandalwood, vetiver and agarwood. Since sandalwood and agarwood has became extinct so nagarmotha oil has great opportunities for oriental perfumery and only in India nagarmotha is herb is found as wild weed.

Since all above are from natural source, all these are an important oils from India for which efforts are being carried out on mega scale. One major important factor is that if the crops are cultivated on forest land, they will be 100% organic in nature without any pesticide or fertilizer so more effective for therapeutic purpose. It is found that the oil produced from the crops cultivated using modern synthetic fertilizers and pesticides are inferior in quality when compared with oil produced from plant cultivated using natural manure. Use of modern synthetic fertilizers and pesticides in harmful for cultivation of all aromatics and medicinal plants. Also such cultivation will be a source of income generation for natives/ tribal people and the essential oils produced this way for the country and world market will be 100% wild and organic.

P-90

Hydroextraction of essential oil from *Lavandin Abrialis*

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The isolation of essential oil from Lavandin Abrialis (Lavandula Angustifolia M. x Latifolia M.) was performed using saturated steam as extraction agent at pilot scale. The yield and efficiency were determined for different conditions of operation.

The chemical composition of essential oil was determined by GC/MS, agree to UNE standards. The glandular trichomes distribution and geometry were observed by SEM, before and after the process. The purposes were to know the effect of steam and determining the presence of natural barriers to the isolation.

The bed porosity and the flow of floral water were defined as the process parameters. A set of experiments was established with these parameters. Fig. 1 presents the yield of essential oil vs. extraction time, for three different flows of floral water, at constant bed porosity. The output rate increased agrees with the floral water flow. Fig. 2 shows the homogenous distribution and spherical geometry of glandular trichomes in the flowers.

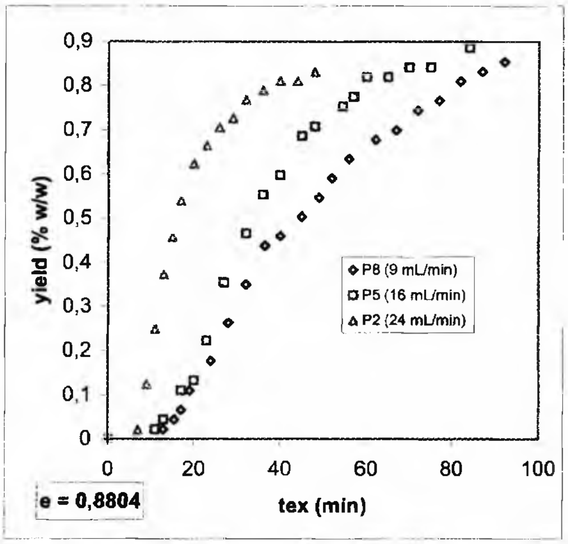

Fig. 1: Curves of yield vs. extraction time.

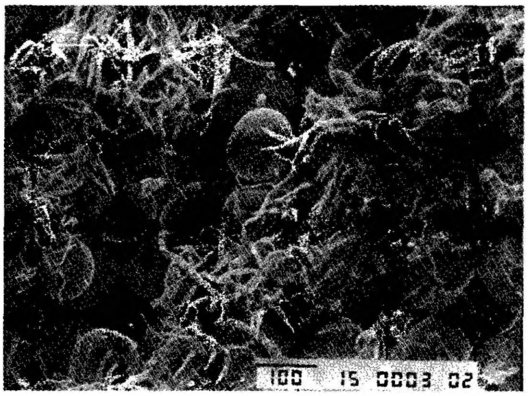

Fig. 2: Photography by SEM of Lavandin Abrialis flower.

The essential oil of Lavandin Abrialis was obtained with an average yield of 0,96% w/w, in wet base. The medium flow of floral water (16 mL/min) and minimum bed porosity (0,85) achieved the larger yields. High amounts of 1,8-cineole (4,4%) and camphor (8,5%) facilitated the identification to this oil by GC/MS.

P-91

Saffron (*Crocus sativus*) green leaves odorous volatiles investigation

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Saffron is a high-value spice obtained from dried Crocus sativus L. stigmas. It is widely used as a condiment for its delicate flavour and intense color. Although the production of 1 kg of saffron, induces the growing of more than 1.5 tones of green leaves, only few studies have been carried out on Crocus sativas leaves. Especially the aroma of the leaves has not yet been subject of detailed investigation. During May 2003, green leaves from the Quercy area (France) have been collected. Leaves have been extracted by two different methods. Solvent maceration has been made in diethyl ether to characterize leaves volatiles and in hexane as industrial process, giving concrete. Essential oil and aromatic waters were obtained by hydrodistillation. Samples were analysed by GC/MS and by GC/O for concrete in order to identify and determine the olfactory impact of aroma compounds. The main compounds identified by GC/MS in hydrodistillation extracts are 2-[5H]-furanone (6.5% of total area) and 2-hexenal (3.9%). The study of key-odorous compounds of solvent extracts shows that the strongest odour is the "honey" one given by phenylethyl alcohol.

P-92

Isolation of essential oil and supercritical carbon dioxide extract of fennel seeds (*Foeniculum vulgare* Mill.) from Montenegro

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Fennel (*Foeniculum vulgare* Mill.) is biennial or short-lived perennial herb, depending on the variety, belonging to Apiaceae family and is native to the Mediterranean region. Fennel is known since antiquity as a medicinal and aromatic herb, commonly used to flavour liqueurs, breads, fishes, salads and cheeses. The fennel seeds essential oil is used as an ingredient of cosmetic and pharmaceutical products for its balsamic, cardiotonic, laxative, digestive, lactogogue and tonic properties.

Ground fennel (*Foeniculum vulgare* Mill.) seeds (particle diameter size of 0.9 mm), growing wild in Montenegro, were extracted by hydrodistillation as well as with supercritical carbon dioxide (SC-CO_2) in order to determine yield, composition and organoleptic characteristics of obtained extracts.

The extracted material was characterized by gas chromatography using flame ionization and mas specrometic detection. In the SC-CO_2 extracts as well in the hydrodistilled oil the major compounds were *trans*-anethole (68.59-75.01%) and (61.99%), methyl chavicol (5.09-9.10%) and (4.90%), fenchone (8.40-14.65%) and (20.25%), respectively.

A comparison between the hydrodistilled essential oil obtained by hydrodistillation and SC-CO_2 extracts showed that in latter technique some undesired higher molecular weight compounds (waxes) were co-extracted with essential oil.

With pressure varied from 80 to 150 bar and temperature varied from 313K to 330K, it was found that, for the selected herb material, the optimal conditions of SC-CO_2 extraction (the high percentage of *trans*-anethole, with significant content of fenchone and reduced content of methyl chavicol and co-extracted cuticular waxes) are: pressure, 100 bar; temperature, 40°C; extraction time, 120 min.

The organoleptic tests confirmed that hydrodistilled oil possessed a less intense fennel seed aroma then extracts obtained by SC-CO_2.

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P-93

Steam distillation in pilot plant scale of the essential oil of *Origanum vulgare* L.

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Genetic, environmental, climatic and soil conditions strongly effect the quality of essential oils. The chemical composition of essential oils and relative amounts of their compounds depend additionally on the manufacturing process itself and the duration of the steam distillation [1].

Essential oils of the herb *Origanum vulgare L.*, all grown in three habitats in Lower Austria, were analysed. The essential oils were obtained using a hundred litres distillation plant of the type Herba-tec TWE 250-2000.

During the distillation process samples were taken in order to investigate the changes of essential oil composition within a steam distillation process. The samples were subjected to gas chromatographic investigation (GC/MS and GC/FID).

Progression and development of the qualitative constitution and the relative amounts of the most important components were examined. These are important factors for the application of the essential oils in pharmaceutics, cosmetics and feed. The results provide the possibility to determine the optimised distillation time with the highest relative amount of the main compounds. The oils were found to be rich in carvacrol and they contain minor amounts of the two monoterpene hydrocarbons γ - terpinene and p-cymene, the biosynthetic precursors of thymol and carvacrol [2].

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P-94

Study of combined SAFE-MIXXOR and MIXXOR-SAFE extractors for obtention of representative aromatic extracts: application to green tea (*Camelia sinensis*) infusions and processed chanterelles (*Cantharellius cibarius*)

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The enrichment of odor-active volatiles from complex food matrices remains a challenge to flavor chemists interested in identifying and quantifying food odorants. To reach this goal, sophisticated techniques for carefully separating volatile aroma compounds from non volatile ones were developed during the last decades. Since few years, a compact and versatile distillation unit called SAFE (Solvent Assisted Flavor Evaporation), based on an high vacuum ($5x10^{-3}$ Pa) transfert of volatiles from aqueous or raw solvent extracts was commercially available. In order to reinforce the versatility of such method, a combination of SAFE to MIXXOR device (a commercial liquid-liquid extractor combining a mixer separator piston to a reservoir) was proposed as it allowed to obtained flavor solvent extracts ready for analysis. The SAFE-MIXXOR and MIXXOR-SAFE combinations in which SAFE was respectively used for obtention of concentrated aromatic waters and purified solvent extracts, were applied on two kinds of food products : an aqueous (green tea infusion) and a solid ones (processed chanterelles), hexane and methylene chloride being used as solvent. In order to evaluate their flavor representativity, the 2 final aromatic extracts per products were analysed by GC/MS and GC/O. If the MIXXOR-SAFE combination was more time consuming than the SAFE-MIXXOR ones, it allowed to obtain higher concentrations of key flavor compounds both for infusion and mushroom, demonstrating its great efficiency in purification of solvent extracts.

P-95

Supercritical fluid extraction of lipophilic phenoloids in *Filipendula ulmaria*

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Filipendula ulmaria L. Maxim known as meadowsweet in a well-known medicinal plant, which is used for the moderate analgesic, antiinflammatory and antipyretic activity. Its lipophilic and hydrophilic compounds have significant antioxidant activity and the salicylates in *Filipendula* are the primary active model ingredients for Aspirin ®. *Filipendulae herba* is officinal in the European Pharmacopoeia (Ph. Eur. 5.) and the Hungarian Pharmacopoeia (Ph. Hg. VIII).

As various chemical substances are involved in the bioactivity to obtain high quality extracts of meadowsweet has a great value. The aim of this work was to examine the effect of sample preparation, extraction and separation conditions on the yield and composition of *Filipendulae herba,- leaf, -stem, -flower* and to compare the yield and composition of various extracts.

Traditional extraction methods, such as n-hexane Soxhlet extraction, steam distillation (at pH neutral and acidic) and supercritical fluid extraction (SFE) with carbon dioxide and carbon dioxide plus ethylalcohol modifier (10%) were used. Lipophilic phenoloids were studied by thin- layer chromatography, gas cromatography, UV-spectroscopy and gas chromatography / mass spectrometry. The non specific scavanger activities were studied by chemiluminometric technique.

It was concluded that lipophilic phenoloids such as methylsalicylate and salicylaldehyde are present predominately in glycosilated from and can be distilled at acidic pH.

The accumulation of salicylates in the various plant parts was significantly different. The main component - contrary to the literature- was always methylsalicylate in the volatile oils and extracts and salicylic acid was also detected in aged plant extracts. Supercitical fluid extraction proved to be true alternative to the conventional procedures. To obtain optimal composition of extract carbon dioxide plus modifier was an extractant which resulted the quantitative dissolution of lipophilic salicilates. Both the volatile oils and SFE extracts showed remarkable scavanger activity.

The work was supported by OTKA TS 049849 and GVOP- 3. 1. 1. 2004-05-0397/3.0

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P-96

The stability of Australian tea tree oil (*Melaleuca alternifolia)* individually and in combination with topical O/W emulsion

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The oil of the tea tree (*Melaleuca alternifolia*) is a popular and effective natural ingredient in cosmeceuticals. It is a complex mixture composed of approximately 100 monoterpene and sesquiterpene hydrocarbons and alcohols. It contains mainly terpin-4-ol.

The aim of this study is to identify the main constituents in the tea tree oil by gas chromatography-mass spectroscopy and monitor the stability of these constituents under certain conditions. A comparison between the stability of the main constituents of the oil individually and in combination with the emulsion has also been shown in this study.

Pure tea tree oil and 1% (w/w) oil in water (O/W) emulsions were stored at different temperatures (25, 30, 35, 40 and 45°C) for a period of three months to monitor their stability. Steam distillation has been used to re-extract the oil from the emulsion. The composition of the oil samples was determined by gas chromatography.

Biological background of essential oil accumulation

P-97

Chemical composition and variation of the essential oil of wild Sardinian *Helichrysum italicum* G. Don ssp. *microphyllum* (Willd) Nym during plant blooming time

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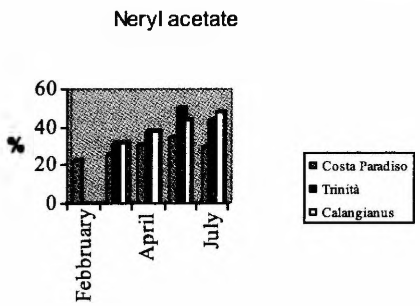
Helychrysum (*H.*) is a aromatic plant belonging to *Asteraceae* family, 16 species of *H.* are spontaneous in Europe and in particularly *H. italicum* G. Don ssp. *microphyllum* (Willd) Nym is the more diffuses in Sardinia island (Italy). In ours previous papers[1-3] we rescribed the essential oil composition of Corsican and Sardinian *H. italicum* ssp. *microphyllum* and we compared these oils together and also with that growing in Toscana (Italy).

In the present work we studied the variation induced on essential oil by the different vegetation time in three different Sardinian stations of north Sardinia (Costa Paradiso at 100 mslm, Trinità at 400 mslm and Calangianus at 700 mslm).

The fresh plant material was submitted to hydro distillation. For separation and quantitations a Hewlett Packard 5890 gas chromatography interfaced with an work station was used equipped with a capillary column AT-5 (length 60m, film thickness 0.25µm, i.d. 0.25 mm) and chromatographed under the following conditions: carrier gas, helium; oven 50°C, increasing by 3°C/min to 135°C (1min) and by 5°C/min to 225°C (5min) and than by 5°C/min to 260°C (10min); detector and injector, 280°C. The oils were analyzed without dilution and analysed also using a GC/MS Hewlett Packard G1800B-GCD System using the same conditions and column as that used for GC analyses.

Considering all the analyzed stations fifty-five constituents accounting between 96.5% and 83.0% of total volatile components of essential oils have been identified and quantified. Among the identified constituent those present in highest percentage were neryl acetate, linalool, 5-eudesmen-11-ol, neryl propionate and limonene. The more representative are neryl acetale and 5-eudesmen-11-ol; this second compound showed a trend that seems to be jointed with neryl acetate in fact it is higher were neryl acetate showed a decrease. Limonene is surely important in recognize essence from Costa Paradiso in which it reach 7%. The total of oxygenated compounds showed in every sample and at every time to be the preponderant group and that its higher concentration is in June.



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P-98

Chemical diversity of single oil glands of clary sage (*Salvia sclarea* L.)

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The glandular trichomes of clary sage (*Salvia sclarea* L., Lamiaceae) were analysed to look for intraindividual chemical variation. The calyx trichomes show many stalked and few sessile secretory glands with different size and shape. The different oil gland types were punctured with a polydimethylsiloxane-coated fused silica fibre and the content of the gland was directly transferred to GC/MS. The single oil gland analysis with this SPME method (Grassi et al., 2004) was compared with extracts of leaves, calyces and flowers as well as with the essential oil of clary sage.

Preliminary results show differences of the essential oil compounds on different aerial parts. The most striking feature however, was the conspicuous chemical difference between the two oil gland types on the calyx. According to the infrequent occurrence of sessile calyx oil trichomes, the main compounds of these glands almost do not appear in the extract of calyces.

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P-99

Comparative investigations on wild growing chamomile (*Matricaria recutita* L.) populations of different origin

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We compared 8 populations of chamomile originating from different Hungarian habitats, both at the original growing areas and as experimental populations cultivated in the same environment at the research field of the department in Budapest, Soroksár. We examined the height of the plants, the diameter of the flowers, content and main components of the essential oil as well as the content of flavonoids. Aim of the investigations was the evaluation of Hungarian wild growing genotypes in order of the development of a new, special quality cultivar.

The population originating from Transdanubia could be separated from the ones originating from the Great Plain ('Hortobágy') according to both morphological traits and active ingredients (Figures 4-5.). The single population of 'Polgár' can not be integrated in either group, it seems to be some kind of transition.

The populations from Transdanubia showed more intensive growth and bigger flower size than the others, both in the original areas and in cultivation. Their essential oil content was by about 40% higher too, than that of the populations from 'Hortobágy'. The proportion of bisabolol-oxide I. (34-43 %) and of chamazulene (7-15 %) are quite high, however the proportion of α- bisabolol was low (7-9 %). The materials from 'Hortobágy', in the contrary, showed a high proportion of α- bisabolol (45-58 %). Thus, according to the aims of the utilisation, we have two chemotypes for cultivar selection.

The environment and the vegetation year modified several characteristics of each populations (growth intensity, spectrum of essential oil and flavonoids), which calls the attention to the importance of interaction between variety and environment.

We propose the definition and standardisation of the actual quality of chamomile as „Hungaricum" (special Hungarian product), because the collected material in Hungary seems to be rather diverse concerned both morphological features and active ingredients.

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P-100

DNA-Barcoding of a plant extract

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DNA was successfully extracted from minor amounts of remaining intact plant cells of an ethanolic spissum extract of *Echinacea* sp.

Although the DNA extracted was not quantifiable, a short fragment from a DNA region often used in molecular plant taxonomy was amplified using the same primers in two consecutive PCR reactions. The amplification product was purified and sequenced. The sequence comparison to published sequences revealed the unambiguous identification of the plant DNA in the extract as originating from the genus *Echinacea* sp. with the closest similarity to other *Echinacea* species. Closely related Asteraceae from the same tribe of the Heliantheae were following in similarity.

The sequence of the plant extract differed to *Echinacea purpurea* in only one nucleotide, to *E. paradoxa* and *E. pallida* in only two nucleotides, respectively. These small differences between the *Echinacea* species do not justify an unambiguous determination of the species in the extract yet, but with increasing sequence information authenticity testing will be gaining better separation capacity.

P-101

Essential oils from *Cannabis sativa* L.

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Hemp (*Cannabis sativa* L.) is an annual species, native of central Asia and spread in Europe and Africa, source of hundreds of biological active compounds such as cannabinoids, terpenoids, flavonoids and polyunsaturated fatty acids. Hemp essential oil, with its unique smell is, at present, used in cosmetic and perfume products, aromatherapy and as beer flavouring agent. Moreover it is traditionally employed as anti-inflammatory in the respiratory and digestive tracts and some its components possess recognized biological properties. In particular, myrcene is a potent analgesic (Rao et al., 1990), 1,8-cineole increases cerebral blood flow and enhances cortical activity (Nasel et al., 1994) and limonene inhibits the growth of many species of fungi and bacteria and as well as α,β-pinene, α-terpineol and borneol possesses repellent effects against many insects. For these aspects, it has the potential to be more exploited in different applications.

In our research, five hemp cultivars, three dioic (Carmagnola, Dioica 88 and Fibranova) and two monoic (Epsilon and Futura), cultivated as fibre crops were also evaluated for the essential oil yields and compositions of their inflorescences. The aim of this work was to deep the knowledge on hemp essential oils and to obtain preliminary information on the possible exploitation the inflorescences, unused in fibre production, as source of a value-added for these cultivars.

The oils, obtained by steam distillation and characterized by GC-MS, were made up by the same pool of components with α-pinene (from 9.5 to 16.3%), myrcene (from 14.6 to 20.9%) and β-caryophyllene (from 10.3 to 24.6%) as main constituents followed by α-pinene, limonene, trans ocimene, terpinolene and α-humulene. The cultivars showed marked quantitative differences. The oil from Carmagnola had the highest content of myrcene, that from Fibranova was characterized by the higher amounts of β-caryophyllene and α–humulene and that from Epsilon was the richest in α-pinene and terpinolene and possessed also a high content of myrcene.

The cultivar Fibranova showed the highest oil yield (0.28%) whereas Epsilon the lowest one (0.13%).

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P-102

Essential oils of six different genotypes of *Origanum vulgare* L.

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Genetic conditions and scale of the distillation process strongly determine the quality of essential oils and their possible use in cosmetics, pharmaceuticals and feed. It is well known, that the distribution of chemical components as a function of distillation time differs appreciably between the Origanum species even within the same taxon. Essential oil of the herb *Origanum vulgare L.* has been characterized as thymol chemotype [1]. In other cases the relative percentages of carvacrol and thymol were almost equal [2]. In contrast to that, carvacrol instead of thymol was determined as main compound in oregano oils by other researchers [3] [4].

Essential oils of six different genotypes of oregano, all grown by organic farming in one habitat in the northeast of Styria (Austria), were analysed. The essential oils were obtained using a hundred litres distillation plant of the type Herba-tec TWE 250-2000 and a ten litres distillation plant of the type UMWEX 100-1000.

In the end of the distillation processes samples were taken to investigate the relative amounts of main compounds of the different genotypes. Samples were subjected to gas chromatographic investigation (GC/MS and GC/FID). The results provide the possibility to determine the genotype with the highest relative amount of the main compound(s) and differences in the chemical composition between the two distillation methods. The oils were found to be rich in carvacrol and they contain minor amounts of the two monoterpene hydrocarbons γ-terpinene and p-cymene, the biosynthetic precursors of thymol and carvacrol [5].

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P-103

Essential oil yield and composition of organically grown thyme (*Thymus vulgaris* L.) from different origins

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Thyme (*Thymus vulgaris* L.) is an aromatic perennial evergreen shrub, belonging to the family Lamiaceae. The leaves are dark green with lilac flowers. It is a popular herb since ancient times and has a number of important uses. The essential oil of thyme has antiseptic, antioxidative, insecticidal, anaesthetic and food-preserving properties. It is used in perfumery for the spicy, leathery notes and as a flavoring agent in foods and oral hygiene products.

It is native in various subspecies in central and southern Europe, the Mediterranean Coast and Asia Minor. The plant is now widely cultivated in various European countries, India, Turkey, Israel, Egypt, Morocco, Argentina, Russia, East Africa and North America.

Dried leaves from Argentina, Egypt, Poland, Spain and fresh plants from America from two different cutting periods were obtained and essential oil isolated by hydro distillation from fresh and dried plants (leaves and stem), fresh leaves, dried leaves and dried stems. The yield of essential oil ranged from 1.7% to 3.33%. The oils were analyzed by GC and GC/MS. Thymol was found to be the major constituent (15.78% to 58.49%). The Spanish oil had 69.86% carvacrol and 15.78% thymol. In all the other oils carvacrol ranged from 1.81% to 6.71%. The other major constituent p-cymene ranged from 1.51% to 28.04%.

P-104

Evaluation of active compounds in *Satureja montana* L.

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Satureja montana L. (perennial savory) is an essential oil containing species, belonging to the familiy Lamiaceae. It can be utilised by many ways, in the therapy it is applied against bronchitis, furthermore as antitussive, carminative and anthelmintic agent. In the folk medicine uses for diuretic, stomachic and antiseptic properties are mentioned. As condiment, it is similar to annual savory (Satureja hortensis) however, somewhat more spicy.

It is known in Europe, most significant cultivation is noticed in Germany, France, great-Britain and Bulgaria.

The species has several subspecies: *S. montana* ssp. *montana*; ssp. *variegata* (Host.) P.V.Ball, ssp. Kitaibelii (Wierzb.); ssp. taurica (Velen.). This savory species is suitable for essential oil production. The content of the shoots is high, 1,5-2,5%. In France, the essential oil is listed among the 9 most important oils concerning their antibacterial effect.

Main essential oil compounds are: carvacrol, terpinene, cymene, linalool, borneol, carvon, thymol, α and β-pinene.

Comparison of the composition of essential oil of *S. montana* and *S. hortensis*

Species	carvacrol	terpi-nene	cymene	linalool	carvone	Borneol	thymol	pinene
S. montana	55,31	9,54	8,31	1,01	5,46	2,02	0,12	1,12
S. hortensisi	24,12	47,01	3,33	0,31	1,05	0,32	0,14	3,01

The cultivar 'Bokroska' is a state registrated variety in Hungary since 1999. Its characteristics are:
Essential oil is highest at the beginning of flowering, generally 1,6-1,7 %.

Main essential oil compounds (ess. oil %) in dried herb: carvacrol (28%),cymene (27%), γ-terpinene (4,3%), dipentene (14%), borneol and ketons. Besides, it contains: bitter substances (00,4%), alcohols (10%).

Essential oil production (ml/100g) of S. montana 'Bokroska' during the vegetation period

Beginning of July	Middle of August	End of September
1,61%	0,72%	0,63

Breeders of the variety are T. Szeprethy, E. Németh, K. Halasz, J. Bernáth.

P-105

Evaluation of ornamental oregano cultivars (*Origanum vulgare* L.) from the aspects of aesthetics and essential oils content

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Origanum vulgare L. is extremely variabile in appearance and in chemical composition.

Oregano is perennial species, sometimes mistaken for other species in the genus and it is common in France. Several species in this complexed taxonomy genus are used as spices around the Mediterranean basin.

Phytopharmaceutical products are based on the flowering tops of oregano and designed for oral use (Bruneton, 1999).

Minimum content of essential oil is 0,1% (Český farmaceutický kodex, 1993).

Organo is important ornamental plant, e.g. cultivars ´Aureum´, ´Gold Splash´ and ´Variegated´.

The six cultivars (72 plant) where evaluated from the aestetic point of view and the volume of essential oils.

Into the aestetical evaluation are involved: growth stages of plant (beginning of flowering, full flowering, end of floweringt, end of vegetation period and hibernation ability), morphologic features of plant and weight durability of blossoms.

Amount of the essential oil is determinated after Czech Pharmaceutical Codex 2002 by vapour destilation. Average amount of essential oils content is 3,15 ml per 1000 g of dried plant material this means 0,315 %.

Following ornamental cultivars contained important quantity of essential oils: ´Variegated´ (3,3-4,66 ml.kg^{-1}) and ´Gold Splash´ (2,85-4,8 ml. kg^{-1}).

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P-106

For a better genepi (*Artemisia umbelliformis* Lam.)

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During two years, Mediplant, in collaboration with the Swiss Agricultural Research Center and the Haute Ecole Valaisanne has determined the influence of the harvesting stage on the quality of the genepi floral stems. The best harvesting period is short and the optimal quality is obtained at the beginning of flowering.

Genepi is the name of five species of alpine plants, all of them are protected in Switzerland and picking is prohibited. The floral stems are mainly used to produce liquors. The part of wild harvesting and the part of adulteration with other aromatic species are unknown. In the early 1990s, the Swiss Agricultural Research Center of Changins begun the domestication and the selection of genepi. One species, the white genepi (*Artemisia umbelliformis*), showed a good behaviour in culture but it must be planted over 1000 meters asl. to grow correctly. Four varieties were selected within different origins of white genepi, each of them has a distinct chemotype and is tolerant to wormwood rust. Actually, commercial cultures are grown in Switzerland, Italy and France. They have unfortunately shown great variations in the quality of floral stems. Their contents of essential oil (aromatic compounds) and of costunolide (bitter compound) were irregular. In order to elucidate the sources of variation, a trial has been conducted during two years near the locality of Liddes (VS, 1400 m. asl.). Harvests were made every week from the button stage (before blooming) until the post flowering stage (1 month later). The essential oil was obtained by water distillation of dry plants and its quality was analysed by GC-MS. The costunolide was measured using HPLC after an extraction with ASE (Accelerated Solvent Extractor).

Maximal concentrations of essential oil (1.76%) and of costunolide (3%) were recorded at the beginning of flowering (opening of the first capitulum). The essential oil content decreased quickly and lost 30 to 60% one week later (full flowering stage) and 70% at the end of flowering (2 to 3 weeks after the beginning of flowering). The content of costunolide showed the same variation as that of the essential oil. The chemical composition of the essential oil remained similar during all the flowering stages.

The yield of dry floral stems per m^2 increased continually to double between the beginning and the post flowering stage (40 g/m^2 until 80 g/m^2). The woody parts of the floral stems doubled also to reach 25 to 30% of total weight at the end of flowering. Only the non woody parts contained essential oil, but costunolide is present in all the different organs of the floral stem. The results were similar during the two years of evaluation.

In conclusion, the best harvesting date state at the beginning of flowering. Producers have to be correctly paid for the best quality because the yield at the beginning of flowering is smaller but of higher quality than that at the post flowering stage.

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Micromorphology of trichomes and composition of essential oil of *Vitex ferruginea* Schumach. & Thonn.

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Vitex ferruginea is a taxonomically complex species distributed through the African subtropical habitats. The morphology and distribution of the glandular trichomes of *V. ferruginea* from Mozambique, as well as the chemical composition of the essential oil, were studied. Scanning electron microscopic studies on vegetative and reproductive organs of the plant revealed non-glandular trichomes and two types of glandular ones. Type I - a sessile trichome with a large head and type II – a shortly pedunculated with a globular head. On the leaves these two glandular types are present, whereas on the reproductive organs only the sessile ones are observed. The essential oils from two samples growing in Matutuíne (near Maputo) were analyzed by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). The oils were characterized by high amounts of sesquiterpene hydrocarbons. Some quantitative differences were found with regards to the major constituents of the oils from the two populations: germacrene D (26.8% *vs* 35.0%), α-humulene (9.32% *vs* 1.9%), *E*-caryophyllene (6.9% *vs* 4.0%), β-phellandrene (3.0% *vs* 10.7%) and limonene (1.8% *vs* 6.1%).

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Pistacia lentiscus L. annual essential oil variations in different Sardinian stations

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Pistacia lentiscus L. is an evergreen shrub of the family *Anarcadiaceae*. This dioecious species can reach 3m in height and grows wild in arid areas and is characteristic of Mediterranean countries In Corsica and in Sardinia it is widely distributed over all the littoral. The essential oil of *Pistacia lentiscus* is obtained by hydro distillation of leaves, fruits or from a trunk exudates called mastic gum. In Corsica, not jet in Sardinia, it is commercialised by local producers. Several studies concerning the chemical composition of *P. lentiscus* oil and solvent extracts have been reported[1]. Only a few studies have reported on the chemical composition of *P. lentiscus* leaf oil and they concerned plants from various origins (Spain, France, Italy, Egypt, Israel, etc.) and a intensive study was made on essential oil of *P. lentiscus* growing in Corse[2]. In the present work we considered the annual variation of essential oil of wild *P. lentiscus* growing in two different station Porto Ferro on the East coast(10 mslm) and Monte Bianchino (300 mslm) close to Sassari.

To obtained the essential oil the lives were submitted to hydro distillation. For separation and quantitations a Hewlett Packard 5890 gas chromatography interfaced with an work station was used equipped with a capillary column AT-5 (length 60m, film thickness 0.25µm, i.d. 0.25 mm) and chromatographed under the following conditions: carrier gas, helium; oven 50°C, increasing by 3°C/min to 135°C (1min) and by 5°C/min to 225°C (5min) and than by 5°C/min to 260°C (10min); detector and injector, 280°C. The oils were analyzed without dilution and analysed also using a GC/MS Hewlett Packard G1800B-GCD System using the same conditions and column as that used for GC analyses. This allowed as to identified sixty-six constituents of which only very few reach the 1% (9-10) and that β-myrcene is low in Monte Bianchino (ecepted in April) with a high content of α-pinene(24-5%). In Porto Ferro Myrcene reached 44% in March and presents also a good content of limonene and phellandrane. From this study was possible to note that the pathway of this compounds is very influenced from the climatic and environmental conditions. The essential oil composition change for the different soil substrate, during the vegetative period and with the climatic conditions. In particularly the two station deferred for the presence of two different compounds in April: in Monte Bianchino station we found limonene and in Porto Ferro samples myrcene. This last one population might be considered a myrcene ecotype instead of Monte Bianchino in which we have the predominance of α-pinene and terpinen-4-ol. To obtained the essential oil the lives were submitted to hydro distillation.

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P-109

Secreting structures and secretion in *Stachys recta* L. ssp. *recta* and *S. recta* ssp. *recta* var. *serpentini* (Fiori) Fiori

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Stachys recta, a perennial herb widespread all over the central and south Europe, is a highly variable species containing many subordinate taxa (Ball, 1972). Variable characters are the shape and dimension of leaf and calyx and the presence of glandular and non glandular trichomes. Within the species *S. recta* ssp.*recta* Pignatti (1982) reported the presence of the var. *serpentine* (Fiori) Fiori, typical endemism of serpentine soils in northern Appennines (Italy). These plants bear narrow leaves and appear very different from those of *S. recta* ssp. *recta* growing on non serpentine soils in conterminous areas.

In this work we examine the glandular trichomes and the essential oil composition of specimens of *S. recta* ssp.*recta* growing on calcareous soil at Monte Morello (Florence) and plants of *S. recta* var. *serpentini* gathered on serpentine soil near Gabbro (Leghorn).

Both the plants present two types of capitate trichomes (Type A and type B) on leaves, stems, calices and corollas. Type A presents a basal epidermal cell, a stalk cell and a head of four cells with a small subcuticular space. The histochemical stains do not characterise clearly the type of secretion; the ultrastructural observations indicate a polysaccharidic, but also polyphenolic and perhaps essential oil secretion. Type B is similar in shape to the previous trichomes, but the head presents only two secreting cells with a well developed subcuticular space. The histochemical and ultrastructural observations clearly indicate an essential oil secretion. On the calyx also several long capitate trichomes with multicellular head, are present. Their secretion is clearly lipophile, with probably small quantities of essential oil and other unknown compounds.

The essential oils of leaves and flowers of both the taxa examined were obtained by hydrodistillation in a Clavenger apparatus and their composition was determined by means of GC-MS analysis. The main components in leaves and inflorescences of *S. recta* ssp. *recta* is cis-muurola-4(14), 5diene (21.9% and 35.9% respectively); other important components of the leaves are 1-octen-3-ol (19.7%), β-caryophyllene (11.2%), hexadecanoic acid (13.3%). The same components, but with different percentage, are the most important also in the inflorescences: β-caryophyllene (27.3%), 1-octen-3-ol (3.3%) and hexadecanoic acid (3.8%). In the var. *serpentini* the main components of leaves and inflorescences are γ-cadinene (15.6% and 13.8 % respectively) and epi-α-muurolol (12,6% and 13,3%), γ-amorphene (8,6% and 9.8 % respectively) and hexadecanoic acid (9.9%and 6.2% respectively).

The study of the trichomes shows a good similarity between the plants examined. Concerning the essential oil composition, in both taxa derivatives from muurolol and other unusual components, are present. In each taxon, the essential oil composition in leaves and inflorescences, is similar.

In conclusion, there are more similarities than differences between the two taxa, irrespective of morphological and ecological differences.

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Study of the essential oils from the flowers and fruits of *Scandix iberica* Bieb. growing in Turkey

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The genus *Scandix* belongs to the Umbelliferae family and is represented by eight species and nine taxa in the Flora of Turkey. *Scandix iberica* Bieb. is a annual herb, ± glabrous 10-20(35) cm in height and much branched. Rays (3) 4-9, sepals absent, petals white, outer clearly radiant. Fruit linear, ± terete, glabrous to densely strigose, with a long beak that is always ciliate. It grows on limestone slopes, grassy slopes, steppe and cultivated land and at 500-2000 m altitudes and widespread in Anatolia.

S. iberica was collected in May 2004 from Eskişehir province of Turkey. To identify essential oils content the plant materials, flowers (A) and fruits (B), (~1 g) were distilled using the MicroDistiller® (Eppendorf, Germany) system. The composition of the oils were analyzed by GC, using a Hewlett Packard 6890 system and GC–MS, using a Hewlett-Packard GCD system. Library search was carried out using the commercial Wiley GC/MS Library, MassFinder and the in-house Baser Library of Essential Oil Constituents.

A total of twenty-nine and twenty-seven compounds were identified representing 99.3% and 99.4% of the flower and fruit oils, respectively. The main constituent was found to be methyl chavicol (85.8-90.5 %) in both cases.

Although we could not find any previous work about *S. iberica*, papers exist on the chemical composition of *Scandix australis* L. (Velasco-Negueruela et al., 1991) and *Scandix australis* L. subsp. *grandiflora* (L.) Thell. (Tümen and Baser, 1997). Our results generally agree with them in that the oil of *S.australis* contained trans-anethole (86%) and methyl chavicol (8.47%) and the oil of *S.australis* subsp. *grandiflora* was methyl chavicol (95.9%).

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P-111

The role of environmental factors in the occurrence of different indigenous thyme (*Thymus*) taxa in the Carpathian Basin

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Thyme species indigenous in the Hungarian flora – *Thymus glabrescens* Willd., *T. pannonicus* All., *T. praecox* L., *T. pulegioides* L. and *T. serpyllum* L. - are practically named *T. serpyllum* L. as medicinal plants. Flowering shoots of any native taxa can be used for the production of *Serpylli herba,* quoted in the Pharmacopoeas, without marking the exact name of the species. All the five Hungarian collective species involve 2-3 microspecies and numerous infraspecific taxa. On the basis of their diverse occurrence and complex taxonomy, we supposed a considerable chemical variability among and within species.

We are aimed at determining the degree of chemical diversity of the indigenous thyme species occurring among different ecological and coenological circumstances. Habitats were selected according to the presence of thyme populations and diverse environmental conditions in the Carpathian Basin.

The base rocks found in the case of Hungarian thyme (*T. pannonicus*) were limestone of dachstein type, dolomite, sand, loess and sandstone of Hárs Hill type. Populations of this species exist on chernozhem, rendzina, bare and reddish-brown forest soils. Essential oil content of the samples collected, changed between 0.14-1.70 ml/100 g. Beside the known chemotypes (thymol, thymol+p-cymene, p-cymene) of Hungarian thyme, we have shown the geraniol-germacrene-D and the linalyl-acetate ones for the first time.

Populations of *T. praecox* have been found in the Buda Hills and on the Tétényi Plateau on different substrates (dolomite, sarmathian and dachstein limestones) with rendzina and bare soils. Drug samples of this species contained only 0.01-0.13 ml/100 g of volatile oil. Both chemovarieties found by us –the geraniol+germacrene-D and the β-caryophyllene+germacrene-D - were unknown till now.

In the case of *T. pulegioides* a population existing on sandstone (essential oil content: 0.178 ml/100 g) and five Transsylvanian ones, grow on andesite (essential oil content: 0.169- 1.049 ml/100 g) were studied. The latter ones represented the carvacrol chemovariety.

Wild populations of *T. glabrescens* were examined in the Bakony on limestone, in the Börzsöny Hills on andesite and in the Medves Hills on basalt. The volatile oil level of its samples changed significantly (0.05-2.14 ml/100 g), the best drug quality could be obtained on carbonate containing soils. Beside the known geraniol and thymol chemotypes, a spathulenolic one was also determined.

One population of *T. serpyllum* was found in the Bakony Hills, where 0.43 ml/100 g was measured.

According to the data obtained, a considerable level of infraspecific and interspecific diversity have been shown. This means that the drug quality of collected wild thyme samples is likely to be quite variable.

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Variation of essential oil of a typical Sardinian *Citrus* fruits called Pompia

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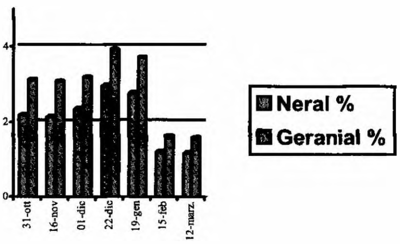
Citrus is a common term and genus of flowering plant in the family *Rutaceae*, originating in tropical and subtropical southeast Asia. The genus contains three species and numerous natural and cultivated origin hybrids, including commercially important fruit such as the orange, lemon, grapefruit, lime and tangerine. The taxonomy of the genus is complex, but recent genetic evidence (see e.g. external link cited below) supports the presence of only three species, *C. maxima, C. medica* and *C. reticulata*, with all the other taxa previously accepted as species being of hybrid origin between these three. They are large evergreen shrubs or small trees, reaching 5-15 m tall. *Citrus* fruits are notable for their fragrance and most are juice-laden In Sardinia grown some particular varieties of *Citrus* but one of these is very interesting; its name is "Pompia" it might be an hybrid between a grapefruit and a Citron[1] and grown only in a very restricted area in Siniscola and Nuoro zone. This fruit is known because it is used in the preparation of traditional recipes; its origin is not known but there are some news since 1760. There are no studies on its essential oil composition and very few on the agronomic aspect[2].



The essential oil was obtained by hydro distillation. For separation and quantitations a Hewlett Packard 5890 gas chromatography interfaced with an work station was used equipped with a capillary column AT-5 (length 60m, film thickness 0.25µm, i.d. 0.25 mm) and chromatographed under the following conditions: carrier gas, helium; oven 50°C, increasing by 3°C/min to 135°C (1min) and by 5°C/min to 225°C (5min) and than by 5°C/min to 260°C (10min); detector and injector, 280°C. The oils were analyzed without dilution and analysed also using a GC/MS Hewlett Packard G1800B-GCD System using the same conditions and column as that used for GC analysesThe essential oil is characterized by the presence of 41 constituents of which less than a half reached 0.1%. At a first analyse this oil seems to be very similar to that of lemon essential oil.

We found a good content of citral with a maximum of 6.8% in December and is very evident the predominance of geranial on neral. Also β-myrcene is present in a good percentage (2%). Limonene is around 87% and didn't showed big variability during the analised frutification time.

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P-113

Variation in essential oil content and composition of *Artemisia scoparia* Waldst. & Kit at different plant growth stages

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The genus *Artemisia* is one of the largest in the *Asteraceae* family. 34 species of this genus are found wild all over Iran. The aerial parts of well known species, *A. scoparia* are used for their Hypolipidaemic and hypoglycemic effects. Because of its choler tic, anti-inflammatory and diuretic activity *A. scoparia* is used in the treatment of hepatitis. Fresh leaves are consumed as vegetable and dried leaves are used as spice by local peoples. Here, variation in the quantity and quality of the essential oil of *A. scoparia* at different developmental growth stages including vegetative, floral budding and flowering are reported. The oils were obtained by hydrodistillation of air-dried samples. The yields of oils (w/w %) in different stages were in the order of: flowering (0.9%)> floral budding (0.7%)> vegetative (0.4%). The oils were analyzed by GC and GC-MS. In total, 27, 26, 20 constituents were identified and quantified in vegetative, floral budding and flowering, representing 98.9 %, 99.3% and 99% of the oil, respectively. α- and β-thujone and 1,8- cineol were the main compounds in all samples. α-thujone, was lower in vegetative stage and increased in the subsequent harvesting times to reach maximum in flowering. In contrast, β-thujone, was higher in vegetative stage and decreased during flowering.

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Effect of different locations on the chemical composition of essential oils of laurel (*Laurus nobilis* L.) leaves groving wild in Turkey

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Laurel (*Laurus nobilis* L.) is a well known medicinal plant and spice of the Lauraceae family. It is the laurel or bay laurel. The leaf contains approximately 1-3 % essential oil with cineole (30-70 %), Linalool and engenol. Seldom used in pharmacy, the bay leaf may claim "digestive" indications: traditionally used to treat digestive symptoms such as epigastric bloating, impaired digestion, eructations and flatulence. It is chiefly a spice in culinary art; the essential oil is used in food technology. The essential oil components of *Laurus nobilis* gathered from seven different locations of Turkey were identitied by GC/MS. The oil yields on a dry weight basis ranged betveen 1.4% to 2.6%. Among the major component was 1,8- cineole (51,73-68,48%), other predominant were α-terpinyl acetate (4,04-9,87 %), sabinene (4,44-7,75%), α-pinene (2,93-4,89 %), β-pinene (2.58-3,91%), terpin-4-01 (1,33-3,24 %) and α-terpineol (0,95-3,05 %), Minor qualitative and major quantitative variations of some compound, were determined with respect to localities. As a result, 1,8-cineole content of these oils had singnificantly higher than those of other constituents of *L. nobilis*. Which are usually considered as natural sources of this compound used in the flavor and fragrance industry.

In Turkey folk medicine for remedies, the leaves have been used topically for relieving rheumatic pains, stomachic, astringent, carminative, diaphoretic, digestion, eructations, flatulence, stimulant, emetic, emme nagogue and insect repellent. The observed differences may be probably due to different enviromental and genetic factors, different chemotypes and the nutritional status of the plants as well as other factors that can influence the oil composition.

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Production aspects of volatiles

P-115

Baccharis dracunculifolia DC. agronomical yields and essential oil composition

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The aim of this project was to study the biomass yield and essential oil composition from one access of *B. dracunculifolia,* a species that is under studies at CPQBA, Campinas-SP (Brazil). Due to its popular use as anti-inflammatory activity (also found in the green propolis produced by bees where epidermis leaves of this species are present), we proposed an evaluation of its essential oil trying to characterize the composition of two fractions during the steam distillation.

The seeds for producing seedlings were sown on 18/06/2003. The seedlings were transferred to the field on 12/12/2003, with 1,0 x 0,5m spacing. The plants were cut in September, 2004 at 50cm high. The harvest was done on 01/04/05 collecting only the parts where leaves were present in 28 plants from which the thicker stems were removed. The essential oil was sampled in the first and in the second hours of distillation. The total yield of each fraction was calculated in percentage on dry basis. The oil was analyzed by GC-MS (Gas chromatography/mass spectrometry) equipped with a ZEBRON ZB5 column (30mm x 0.25 mm x 0.25 μm). Chromatographic conditions: column temperature 55° (0min) - 20°C/min - 120°(0min) - 1,5°C/min - 150°(0min) - 20°C/min - 180°(5min); injector 220°C and detector 250°C. The identification of components in the oil was based on retention indices relative to n-alkanes (Adams, 1995) computer matching with the Wiley library and by comparison of the spectra date in the literature (Queiroga et. al., 1996).

The harvested area, at 8 months from the first cut, produced 600g of fresh leaves/plant. The essential oil of *B. dracunculifolia* presents few monoterpenes and several sesquiterpenes. The essential oil yield in the first hour was present as 0,08% (dry basis) and in the second extraction 0,27% (dry basis). Monoterpenes were the major compounds during the first hour (61,3%) and only 37,4% in the second hour of extraction process.

A significant decrease from the first to the second hour of extraction is noticed for the monoterpenes: α-pinene, β-pinene and Limonene, 24.3 to 41.6%. In relation to the sesquiterpenes we observed an increase up to 50% in: *trans*-caryophyllene, germacrene D and bicyclogermacrene (52.7 to 57.7%). The most polar sesquiterpenes presented different variations: δ-cadinene and nerolidol increasing 10% and 24%, respectively; almost no variation occurred to spathulenol (0,2%).

This work provides important parameters to establish the best extraction time considering the aims of the cultivation. In this case, the oil obtained at 1 hour of distillation is rich in monoterpenes while in the second hour mainly sesquiterpenes are obtained. Therefore, by controlling the extraction time, one can reach the oil composition wished for their purposes.

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P-116

Concentration of heavy metals in a essential oil from an aromatic plant used in phytoremediation

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Plants growing on metal contaminated soils can concentrate high levels of heavy metals in their roots and shoots. Interest in the mechanism of metal accumulation in plants, has led to the development of phytoremediation (Reichman. 2002).

In Argentina dredged sediments of the bottom of contaminated rivers and their disposal over soils, require attention. Finding some heavy metal tolerant crops (Zheljazkov y Nielsen, 1996) which final products is not contaminated could be one alternative for solving the problem in these areas. The essential oil of *Mentha arvensis* is extensively used in flavor, fragrance and pharmaceutical industries (Rajeswara Rao, 1999). So, the aim of this work is to characterize the production of this specie in areas with contaminated dredged sediments and evaluate its possible use as heavy metals accumulator.

A cultivar of *Mentha arvensis* is implanted in different treatments: T_1: Control (Soil), T_2: Soil+ Sediment, T_3:Soil + Sediment + Pb 1000mg/kg, T_4: Soil + Sediment + Pb 1400mg/kg, T_5: Soil + Sediment + Cd 15 mg/kg, T_6: Soil + Sediment + Cd 45 mg/kg, T_7: Soil + Sediment + Zn 750mg/kg, T_8: Soil + Sediment + Zn 1900mg/kg,

The treatments were replicated three times in completely randomized design. Analysis of variance was carried out and Tukey value at the 5% level was used to separate treatments mean

The plants were harvested when reaching full flowering. Then biomass was recorded and essential oil was extracted from leaves and inflorescences and its yield was determined by hidro-distillation, in a Clevenger apparatus (Gil et al. 2000) Heavy was determined by atomic absorption spectrometry. No significant differences were found in the concentration of essential oil among treatments. The heavy metal concentrations in the essential oils were lowers than the critical levels. Addition of sediment increases the aerial biomass, except in Zn treatment.

The metal levels in the tissues of this plant reflected metal levels in the soil. So this plant could be extract heavy metals from these polluted areas without contamination of the end product.

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P-117

Effect of development stage at harvest on the composition and yield of volatile terpenes of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare* ssp. *hirtum*)

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Aromatic plants are well known for their excellent odour/flavour, anti-microbial and antioxidant activity that are due to volatile compounds (VOCs)[1]. Essential oils from aromatic plants are widely used as flavourings, food preservative agents, in cosmetics and in medicines. The yield and chemical composition of VOCs in aromatic plants depends on a number of factors, such as growing conditions, development stage at harvest and genotype. Thyme and oregano are used in foods, where the most important VOCs are thymol and carvacrol. These terpenes are highly antibiotic and at the same time the major contributors to the pleasant odour of the plants. Very little information exists on how the development stage at harvest and re-harvesting the same plants affects the chemical composition and yield of VOCs in thyme and oregano.

Methods: Thyme and oregano were grown under similar field conditions in 2003 and plant material was harvested at five different development stages during the growing season from June to August and again two times in September and October (re-harvest). The upper 10 cm of shoots in thyme were harvested and the hole above ground shoot in oregano was harvested. VOCs were extracted with dichloromethane and quantified by GC and identified by GC-MS using authentic standards.

Results: The profile of the VOCs of thyme was similar to that of oregano in the major VOCs but at different concentrations. The major VOCs in thyme (T) and oregano (O), at the flowering stage (50–75% open flowers), were thymol (T: 61.9%; O: 1.4%), carvacrol (T: 2.8%; O: 75.8%), *p*-cymene (T: 12.1%; O: 4.7%), γ-terpinene (T:10.2%; O: 8.0%), myrcene (T: 2.3%; O: 2.5%), α-pinene (T: 1.8%; O: 1.8%), linalool (T: 3.0%; O: 0%), β-caryophyllene (T: 4.0%; O: 1.9%). Yields of VOCs varied from 3–15 kg/ha in thyme and from 75–165 kg/ha in oregano. The profile of the VOCs did not change during the different development stages, although significant differences in the concentrations of the major VOCs were observed. In particular re-harvesting of thyme in September affected the relative abundance of thymol and its biosynthetic precursors *p*-cymene and γ-terpinene compared to harvesting at the flowering stage in June. The yields of VOCs in re-harvested thyme were not significantly affected, because the concentration of VOCs was much higher in re-harvested material. The present investigation shows that it is possible to optimise the yield and the quality of VOCs and hence the yield and quality of essential oil of thyme and oregano by harvesting the plant material at the right development stage.

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P-118

Effect of different level of vermicompost, vermiwash and chemical fertilizers on growth, seed yield and essential oils content of sweet basil (*Ocimum basilicum* L.)

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There is a world-wide effort to eliminate the amount of chemicals used in agriculture. There are several adverse effects of chemical fertilizers such as nitrate and cadmium accumulation in plants and underground water pollution. Sweet basil (*Ocimum basilicum)* is a multi-purpose medicinal herb. It contains volatile oil with eugenol, methyl eugenol, carvacrol and caryophylene. Basil essential oils have long been used to flavor food, dental and oral products, in fragrances and traditional medicines.

In this research we conducted a pot experiment with aim to verify the influence of different level of vermin-compost (zero,5,10,15,20 and 25 %), vermi-wash spraying, complete chemical fertilizer and Phosin® on the plant. Fresh and dry herb yield, plant height, leaf area /plant, days need to flowering, seed yield/plant and essential oils content of the plant were measured. This experiment had done in randomized complete block with 9 treatments and 7 replications.

The results showed that different levels of vermicompost had significant effects on growth, development and yield of sweet basil. The highest fresh and dry herb yield (22.85 and 4.05 g/plant) detected in 20% vermicompost treatment and the lowest one (5.13 and 0.77 g/plant) found in chemical fertilizers treatment. There are not significant differences between control and chemical fertilizers treatment as fresh and dry herb yield as concerned. The results also showed that vermicompost significantly increased essential oil content of the plant. The highest content of essential oil(1.2 % DWB) found in the 15% vermicompost and the lowest one(0.31 %DWB) produced in control plot. The results of experiment about the effect of treatments on the seed production shown that the highest amount of seed production (2.31 g/plant) was found in 25% vermicompost treatment which has a significant difference with control (0.063 g/plant) and other treatments. Therefore 20%vermicompost is the best treatment in organic culture of sweet basil.

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P-119

Evaluation of essential oils to control postharvest disease of strawberries: Antifungal activity of essential oil of *Artemisia sieberi* against *Botrytis cinerea* Pers

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The aerial parts of *Artemisia* sieberi were collected at full flowering stage and their essential oil obtained by means of hydro-distillation. Chemical components of the oil were identified by GC-MS. The major components of the oil of were β–thujone (19.79%), α–thujone (19.55%), camphor (19.55%), verbenol (9.69%), p-mentha-1,5-dien-8-ol (6.39) and davanone (5.79). Antifungal activity of the oil was evaluated against mycelia growth of *Botrytis cinerea* (gray mold) as fruit rot agent of strawberries. The essential oil completely inhibited *B. cinerea* growth in vitro but doesn't effectively reduced the incidence of *B. cinerea* after being applied directly over the inoculated wound at 1000 μl/l.

Keywords: Artemisia sieberi, essential oil, antifungal, post harvest disease, Strawberries, Botrytis cinerea Pers.

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Comparison of greenhouse and open field cultivated basil, parsley, dill and leaf coriander using hydrodistillation and SPME

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Basil, parsley, dill and leaf coriander were cultivated in the greenhouse (Joroinen) and in the open field (Karila) and samples were collected once a month from the greenhouse and at the end of the outdoor growing season from the open field. The samples were frozen and stored at –20°C. The aim was to compare the aroma content and composition of plants grown under different conditions. Because the number of samples was high we also wanted to compare conventional hydrodistillation plus GC-MS analysis to a direct method, i.e. SPME (Solid Phase Micro Extraction) + GC-MS analysis.

About 100 g of the samples were used for hydrodistillation in the European Pharmacopoiea apparatus. The samples were homogenised using an Ultra-Turrax and distilled for 2 h with hexane as solvent trap. Different SPME conditions were tested on the basis of the experiments by Richter *et al.* (2004) and Tammela *et al.* (2003); extraction temperature (35, 40, 50, 60, 70, 80, 90 and 100 °C), extraction time (10 and 15 min) and sample size (50, 100, 200 and 300 mg). The conditions chosen were: extraction temperature 100 °C, extraction time 10 min and sample size 200 mg. A polydimethylsiloxane fiber was used and the desorption was done by inserting it into the injection chamber of the GC (240°C) for 1 min and a splitless injection was made. The column used was Stabilwax and the oven temperature was programmed: 50 (1 min)- 6°/min – 240 °C.

The composition of basil, which was of a methyl eugenol type, was studied both from the hydrodistilled oil and by SPME. From the distilled oil 43 components and from the extraction 37 components were identified, in both cases accounting for over 90% of the total. Interestingly, the content of the main component methyl eugenol seemed to be higher in the extracts than in the distilled oils.

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P-121

Effect of multiple stresses on the production and composition of essential oil in Tagetes minuta l.

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Environmental and ecological conditions may affect the productivity of aromatics plant. Variations in the environment influence the production of secondary metabolites, which is frequently increased by both biotic and abiotic stress (Coley *et al.*,1985; Chapin *et al.*,1987; Waterman and Male, 1989; Coleman and Jones, 1991; Herms and Mattson,1992). Differences in the response to stresses among chemotype can be expected as a result of the process of adaptation to climate and local habitat (Gil *et al*, 2000; 2002). Within this context our objetive was to study the impact of biotic stresses (interspecific competence and herbivory) on the production of biomass and essential oil in three chemotypes of *Tagetes minuta* L. (Mexican marigold). During two years, we laid a complete randomized block design factorial experiments with five replicates, in the Agronomy Faculty of the University of Buenos Aires, Argentina. We tested three chemotypes (BA, SA y ME), two levels of herbivory (*Meloydogine* spp.) and two levels of interspecific competence (with and whithout competence). Experimental units were harvested when reaching full flowering. Then biomass was recorded and essential oil was extracted from leaves and inflorescences and its yield was determined by hidro-distillation, in Clevenger apparatus. Qualitative analysis was performed on a Perkin Elmer Autosystem Gas Chromatograph/Q-Mass 910 Mass Spectrometer. Biomass and essential oil yield were analysed with ANOVA and composition data were analysed by multivariate statistical procedure. The chemotypes were affected by the interspecific competence in both years. Increment of aereal biomass was detected in the second year in response to herbivory in every chemotypes. ME and SA chemotype showed a more stable behavior in the concentration of essential oil than the BA chemotype in response to herbivory and competence. Changes in the yield and oil composition were observed in response of multiples stresses. Based on these results we argue that in order to develop management practices aimed to improve the essential oil yield is important to know the relationships between environmental conditions at which the crops are grown and the production of essential oil.

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P-122

Effects of mycorrhization on the essential oil content and composition of aroma components of marjoram *(Majorana hortensis)*, thyme *(Thymus vulgaris L.)* and caraway *(Carum carvi L.)*

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Many plant species live in symbiotic interaction with mycorrhizal fungi. This symbiosis between plants and soil fungi is known as mycorrhization. The fungus settles on the roots and, together with the plant, forms a large fine root system. This is beneficial for the plant as it improves the plant uptake of nutrients and water. Among a lot of positive effects of mycorrhization such as increased biomass production or enhancement of plant resistance the influence of arbuscular mycorrhizal fungi on concentration and quality of essential oils and related compounds in herbal and medicinal plants have been investigated.

Marjoram, caraway and thyme have been cultivated with and without the use of a mycorrhiza inoculum in greenhouse trials as well as in field experiments in conventional and in ecological cultivation.

Greenhouse trials and field experiments were evaluated on the one hand by total content of essential oil after steam distillation or total content of SPME extracted aroma giving compounds and on the other hand by composition of the aroma components after SPME-Gaschromatography. For the identification of the aroma active compounds mass spectrometric detection was performed.

The results of the comparative experiments with mycorrhized and non-mycorrhized plants showed some interesting influences of arbuscular mycorrhizal fungi on amount and composition of essential oils and related components. In some cases mycorrhization increased the amount of essential oils. Effects of mycorrhization on the composition of aroma giving components have also been detected in some cultivation experiments. Furthermore, influences of mycorrhization depend on the mycorrhiza inoculum (fungal species) used.

In conclusion can be stated, that a selective use of mycorrhization in agriculture could positively affect the amounts and compositions of enriching plant ingredients.

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Effect of some hormonal treatments on *Grindelia camporum*

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Natural plant products are utilized, either directly or indirectly in the cosmetic, food, agrochemical and chemical industries. *Grindelia* is a vigorous resin producer plant which holds promise as a new economical and medicinal plant for the arid lands of Egypt. Its flower head contains about 20% resin, leaves 14% and stem 2% (Mahmoud, 2002).

Resins are complex mixtures of different chemical types of substances which occur as exudates from plants (Tyler et al., 1977). Natural resins are still used for varnishes, incense, turpentine and as mixture in pharmaceuticals (Mahmoud, 1993). They are also useful for plastic, all-weather plywood, paints, softy glass and waterproofing clothes.

This investigation was carried out in the Horticulture Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, during two successive seasons (1999/2000 and 2000/2001). The objective of this study is to identify the effect of some growth regulators (Ethryl and CCC) at rates of 0, 5000, 10000 and 15000 ppm as well as auxin (IAA) at rates of 0, 500, 1000 and 1500 ppm on vegetative growth, flowering and the resin content of *Grindelia camporum*.

Plant length, fresh and dry mass decreased by increasing Ethryl, CCC and IAA concentrations. IAA application caused a noticeable increase in number of branches/plant in comparison to the untreated plants, especially in high concentration.

Maximum reduction in number of flowers and fresh and dry mass of flowers were observed when 10000 or 15000 ppm CCC or high Ethryl or high IAA doses were applied.

Crude resin(CR), acid number(AN) and resin acid percentage(RA %) in both herb and flowers was increased, due to the increase of CCC, Ethryl and IAA concentrations. When the concentration of IAA, Ethryl and Cycocel was raised to their maximum level the resin percentage was greatly improved being significantly higher as measured in the control.

Increasing the concentration level of the growth regulators the acid number improved markedly. The moderate CCC and Ethryl concentrations of 10000 ppm or IAA of 1000 ppm were the most effective on acid number. Ethryl hormone promoted the acid number level more intensively than that of CCC or IAA, in both growing seasons.

There is a relation between the increasing of growth retardant level and the notable increase in the RA% in both herb and flowers of *Grindelia*. The maximum value of RA% occurred in plants treated with concentration of 15000 ppm of CCC or Ethryl and with dose of 1500 ppm of IAA in both growing seasons. Ethryl hormone was ideal for increasing RA% in flowers while IAA was the best for rising resin acid content in the herb.

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P-124

Effect of sowing date and plant density on seed yield, quantity and quality of active substance in fennel (*Foeniculum vulgare* Mill)

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Fennel is herbaceous, perennial plant belonging to the Apiaceae family. The seeds have an active substance called "Essential oil" and the most important constituent in the essential oil is Anethole. The main aim of this investigation is to find out the effect of sowing date and plant density on the seed yield and quantity and quality of active substance of fennel. This experiment was done in factorial experiment in the base of randomized complete blocks design with nine treatments and three replications at the Khojir Medicinal Plants Research Station, Jahad Keshavarzi of Tehran. The field experiment was started in the spring of 1998. In this experiment, the effects of three levels of sowing date (25 March, 4 and 14 April) and three levels of plant density (50×20, 50×30 and 50×50 cm) was studied. The measured characteristics in this investigation were seed yield per hectare, content of essential oil in seed, essential oil yield per hectare, anethole percentage in essential oil and anethole yield per hectare. The means of treatments was assessed by Duncan test(5%).

The results showed that sowing date had only a significant effect on the seed yield per hectare. Also plant density had significant effect on the essential oil yield, anethole yield and seed yield per hectare.

According to the results of this investigation the best time for sowing is 25 March and the most suitable plant density is 50×20 cm.

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Effect of sowing times monoculture and mixed cultures on the yield and secondary metabolites of St. John's wort and chamomile cultivars

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St. John's wort (*Hypericum perforatum* L.) and chamomile (*Matricaria recutita* L.) are important medicinal plants, which used for hypericin, chamazulene and other secondary metabolites. The effects of 6 sowing times (4 April, 4 May and 4 June, 4 October, 4 November and 4 December), monoculture and mixed cultures and different types of cultivars (St. John's wort: Topaz and Riger and chamomile: Bona and Goral) on plant growth, yield components and the hypericin and chamazulene content were conducted during 2002-2004. The competitive ability and yield of St. John's wort and chamomile cultivars was investigated in a de Wit replacement series. Results showed that sowing times had significant effects on St. John' flowers, plant height, tillering and hepericin content. The highest number of height, flowers and tillering were obtained on 4 October. Also, the highest hepericin content was obtained in mixed culture, October 4 sowing time and Topaz cultivar. Results showed that sowing dates had significant effects on chamomile flower size, flower yield and the number of flowers per plant. Also the highest content of chamazulene were obtained on 4 October when the production of flowers were at high level. The highest chamazulene content and flower yield was obtained in pure culture and Goral cultivar.

Keywords: St. John's wort, Chamomile, Sowing times Mixed cultures, Secondary metabolites

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Effect of weather conditions on essential oil content in thyme (Thymus vulgaris L.)

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Thyme (*Thymus vulgaris* L.) is one of the important medicinal plant cultivated in Poland. Herb of thyme (*Herba Thymi*) collected in the flowering time is the raw material. In Poland, thyme cultivar "Sloneczko" is widely introduced into cultivation. This cultivar gives yield 55 dt/ha of dry herb; the content of essential oil – 1,6%, 90% of flowers are male fertile, yield of seed app. 100kg/ha. Beside its medical value, thyme is commonly used as a spice. When the maintenance breeding of cultivar "Sloneczko" was done, the different content of essential oil in years was observed: from 1,3 to 2,8%. This results has induced the author to detailed analysis of correlation between the weather conditions and essential oil content.

In 1998 – 2003 the experiment was taken in the plant-breeding nursery of cultivar "Sloneczko" in Plewiska near Poznan. Each year, the 20 samples of herb from two-year-old individual plants were taken. The herb was dried in the controlled temperature of 25 – 28°C. Essential oil was obtained by hydro-distillation in Deryng's apparatus following the method of Polish Pharmacopea VI. To evaluate effect of weather conditions, the average day temperature, total sum of sunshine hours and total sum of rainfall of 31 days before herb harvest were analyzed. The comparison of the mean content of essential oil was done by using analysis of variance on level of 0,05. Significance of correlation was verified by test t-Student on level 0,05.

Table 1. Essential oil content in thyme herb and selected weather condition in 1998 - 2003

Weather conditions/ years	1998	1999	2000	2001	2002	2003
Mean day temperature [°C}	14,35	14,26	16,8	14,7	16,7	15,6
Sunshine [hours]	288,3	272,0	321,0	252,3	253,8	256,3
Rainfall [mm]	27,2	57,6	27,8	20,5	65,1	77,2
Ess. oil content [%]	1,74 a	1,51 a	2,49 b	1,82 b	1,96 b	2,01 c

The obtained results showed that content of essential oil in thyme herb was highly significantly different in the investigated years. Analysis of the effect of weather conditions on essential oil content showed that there was a significant positive correlation between essential oil content and the mean day temperature, while the correlation between essential oil content and other two weather factors was no found.

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New volatile terpenoids of chamomile *In Vivo* and *In Vitro*

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The effect of *Chamomilla recutita* (L.) Rauschert is made up by several groups of active substances, among which the terpenoids and polyins of the inflorescences have the greatest importance.

Among wild chamomile populations in Hungary, a population was found in the area of Szabadkígyós containing significant amounts (48 %) of α-bisabolol in the inflorescences-oil. The intact roots synthetised no α-bisabolol but the sesquiterpene alcohol β-eudesmol as new compound was identified by our group. Gas chromatographical standard addition and GC-MS methods were used to identify the oil components.

It was identified on three types of stationary phases by GC. The confirmation of identity was carried out by comparison of mass spectra with those reported in the literature and reference compound. The percentage evaluation of the oil component was made by area normalisation, on the basis of three parallel measurements.

To keep the genom of *Szabadkígyós* wild type having high (-)-α-bisabolol content, we used biotechnological methods. GC and GC-MS studies showed that organised chamomile cultures generated the most important terpenoid and polyin compounds characteristic of the mother plant. We identified berkheyaradulene, geranyl-isovalerate and cedrol as new components in these *in vitro* cultures. The sterile roots of organised culture contained also β-eudesmol, wich was firstly identified from the intact roots by us.

Sterile organised cultures were infected by microinjection of *Agrobacterium rhizogenes*. By naturally occuring gene-transformation Ri plasmids of *Agrobacterium* integrate into the plant genome, thereby inducing the formation of hairy roots. Clones possessing the best growing and biosynthetical potential were multiplied for phytochemical investigations. The amount of terpenoid and polyin compounds in genetically transformed cultured was compared with that of *in vivo* plants.The main component of *hairy root* cultures was tr-β-farnesene and in addition a new compound: α- selinene was identified.

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Production of essential oil in hairy root cultures of valerian and Korean angelica

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Agrobacterium rhizogenes is a genus of gram-negative soil bacteria belonging to the Rhizobiaceae. *A. rhizogenes* can transfer T-DNA, excised from Ri (root inducing)-plasmids several hundred kb in size, from the bacterial to the plant cell. It is the causal agent of 'hairy root' diseases in plants and has been used for the production of hairy root cultures from a multitude of species. Hairy root cultures from plants have attracted considerable attention because of their genetic and biochemical stability, rapid growth rate and ability to synthesize secondary products (at levels comparable to the original plants.

Thus hairy root cultures could possibly serve as a potential system to study biosynthesis of important essential oils from roots of several herbs. Valeriana (*Valeriana officinalis* L). and Korean Angelica (*Angelica gigas*) produce essential oils from its roots. However, there have been no reports about essential oil production in hairy root culture of these herbs. We report on the production of essential oil in hairy root cultures of Korean Angelica and Valeriana .

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P-129

Effect of the irrigation methods on the yield and quality of lovage (*Levisticum officinale* KOCH.) crude drugs

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The yield and the drug quality is playing very important role in the medicinal plant production. Some production technological factors, which are influencing the drug yield and quality, were investigated by the researchers (Galambosi and Szebeni-Galambosi, 1992; Mheen, 1997). However the effect of the irrigation has not been examined yet. Because of this reasons the aim of our study was to examine the lovage crude drug yield and quality depend on different irrigation methods (drop irrigation and spray irrigation).

The one-year-old experimental populations of *Levisticum officinale* KOCH were grown under open field conditions at the Experimental Station of the Department in Budapest in 2002 and 2003. We applied two irrigation methods, drop and spray irrigation beside the non-irrigated control plots in two agrotechnical variation (directly sowed and planted stands). The plant height, fresh and dry mass of the leaf and root, the yield of the crude drugs and the essential oil content and composition were measured. Essential oil content of crude drugs was determined by hydrodistillation. The main components of the oil were analysed by capillary gas-chromatography using standards for identification.

According to our results both irrigation methods improved the root yield with 28-75%, compared with the yield of the control plots. The same effect could be detected in the case of the essential oil content, one and half times more essential oil were measured in the irrigated plots: 0,3 ml/100g d.w. in the control plot and in the irrigated plots 0,4-0,5 ml/100g d.w. in 2002; 0,3-0,4 ml/100g d.w. in 2003, respectively. Comparing the irrigation methods, in the case of drop irrigation we got better root yield results, that could be explained by the bigger amount of water. The leaf yield also improved, however, it was not as significantly as in the case of the root yield. Comparing the essential oil content in crude drugs originated from the irrigated and non-irrigated plots, significant differences could not been detected.

It can be ascertained, that under Hungarian ecological circumstances lovage requires irrigation to achive a good drug yield and active agent content,. However, the optimal water level of lovage is probably above the used amount (700 mm/vegetation year) irrigation water. On the other side some biological factors such as *Ramularia* leaf spot (*Ramularia levistici* OUD.) can reduce the yield significantly due to spray irrigation.

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Biological activity

P-130

A comparative study on inhibiting activity of essential oils from several herbal plants against *Blastoschizomyces capitatus*

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The essential oils from *Eukalyptus golbulus* (Myrtaceae), *Pinus sylvestris* (Pinaceae), *Santalum album* (Santalaceae), *Styrax tonkinensis* (Styracaceae) *and Thymus vulgaris* (Labiatae) which have been recommended for the treatment of dermatological fungal infections in aromatherapy and complementary medicines were analysed and compared by GC-MS. The inhibiting activities of the oils against *Blastoschizomyces capitatus,* an yeast like pathogenic fungus forming hyphae and blastosore which is relatively not common but sometimes causes severe mycosis in mouth, bronchia, lung and intestinal systems, were investigated by broth dilution method and disk diffusion test.

GC-MS: Hewlett-Packard 6890 GC, Hewlett-Packard 5973 MSD, HP-5 capillary column (30m x 250um x 0.25um), Temp. prog.: 70°C(5min, 3°C/min)-180-270°C (20°C /min, 10min), Carrier Gas: He (1.0ml/min), EI:70 eV, CI:200, Solvent Delay: 5min.

Culture: *Blastoschizomyces capitatus* KCCM50270 was subdivided from Korean Culture Center for Microorganisms (KCCM). They are cultured in YM broth or malt extract liquid medium 48 hours at 25°C.The turbidity of the cell suspension measured at 600 nm is adjusted with media to match that of a 0.5 McFarland barium sulfated standard.

1,8-Cineol, one of the well known biostatic compounds, was dominantly contained in the oil of *E. globulus* (54.02 %) while benzoic acid and thymol was the representing compound in case of *S. tonkinensis* which have been used as antiseptics and disinfectants in various preparation forms. The contents of these compounds were 60.24% and 25%, respectively. The essential oil from *S. album,* which is also one of the promising sources for antimicrobial drug development, contained much amount of alpha-santalol (36.14%). In *P. sivestris* oil, endo-bornyl acetate and alpha-pinene showed the highest content, 8.78% and 8.16% respectively. These results supported the possibility for the broad antimicrobial activity including antifungal activity. The oils from *T. vulgaris* and *E. golbulus* showed the strongest activity. They were still effective at concentration of $\leq$ 0.125 %.. The minimal fungistatic concentration of *S. tonkinensis* oil was 0.25% while the oil form *P. sylvestris* exhibited moderated activity. The essential oil from *S. album* showed did not inhibit the fungal growth at 20%. The standard compounds, thymol and benzoic acid, which are the main components of *T. vulgaris* and *S. tonkinensis* also exhibited strong inhibition as expected.

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P-131

Antibacterial activity of *Cuminum cyminum* L. essential oil

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Cuminum cyminum L. (Family Apiaceae), commonly known as "cumin" is widely cultivated in the Sudan. Cumin is in highly demand by national and international markets. The fruits are used in the traditional medicine of Sudan as carminative and to treat hoarseness as well as stomach pain (EL-Kamali and Khalid, 1996).

Cuminaldehyde was found in *C. cyminum* essential oil (Opdyke, 1973). The fruits of cumin gives a volatile oil which possesses high antibacterial activity against eight pathogenic bacteria causing infections in the human body (Singh et al., 2002).

In this study the essential oil of Sudanese variety was extracted from the fruits and tested for its antibacterial activity against *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella paratyphi* A & B, *Salmonella typhi* and *Klebsiella pneumoniae.*

The antibacterial activity of the oil was investigated by well diffusion technique on Muller-Hinton agar was found to be oil concentration dependent. The ethanol oil solution showed activity even at the dilution of 1:75 to all used clinical strains of bacteria except *P. aeruginosa*. The maximum activity against gram-positive bacteria *S. aureus* and *B. subtilis*. Reference antibiotic discs (Ampiclox, Tetrecycline, Chloramphenicol, Gentamicin, Oxfloxin and Ciprofaxicin) were used for comparison. This study shows the potential for replacement of synthetic preservative by the use of natural essential oil of cumin.

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P-132

Antifungal activities of essential oils from *Glehnia littoralis*

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Glehnia littoralis, one of the important traditional medicines, which has been used as for the treatment of various respiratory disease and pruritus by various dermal infections as well. As active compounds of the roots of this plant furanocoumarins, polyines and essential oils have been reported. The raw leaves of this plant also are eaten as a seasoned vegetable and for protection of the palsy.

Trichophyton is a fungal species that causes superficial mycoses commonly known as tinea infections in various areas in humans and other animals. Ketoconazole is one of the commonly used antifungal drugs administered orally for the treatment of both superficial and deep infections caused by *Trichophyton.* However, the unpleasant side effects of this drug include nausea, abdominal pain and itching and its toxicity limits its therapeutic use in many cases. Moreover, the therapeutic response may be slow and thus inappropriate for treatment of patients with severe or rapidly progressive mycoses. In many cases, the efficacy of antifungal therapeutics is poor in immuno-suppressed patients and in the treatment of meningitis.

In this study the composition of essential oils in the leaves of *G. littoralis* was analyzed by gas chromatography-mass spectrometry and the antifungal activities of essential oils were evaluated against five *Trichophyton* species. Additionally, the synergistic effects of the oils in combination with ketoconazole were tested by the checkerboard titer test.

The essential oil fraction and its main components showed significant inhibition of the tested *Trichophyton* fungi, with minimal inhibitor concentrations (MICs) in the range of 16-32 mg/ml. The results suggest that activities of this oil are based mainly on the contents of α-pinene (22.17%), the next prominent component of the oil fraction, while the first main components β-pinene (57.83%) have relatively mild activity. The MICs of α-pinene and β-pinene were 1-4 mg/ml and 4-32 mg/ml, respectively. Additionally the *Glehnia* oil fraction and its main components as well, exhibited significant synergism with ketoconazole against *Trichophyton rubrum.*

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P-133

Antifungal activity of essential oil of *Artemisia aucheri* on soilborn pathogens

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Artemisia aucheri Boiss. is an endemic species distributed in desert area of Iran. Aerial parts of *A. aucheri* at flowering stage, were collected from Bajestan-saride (Khorasan province). The essential oil obtained by means of hydro-distillation and its chemical components were identified by GC-MS. The essential oil of *A. aucheri* was rich in linalool (44.1%), gernyl acetate (10.74%),), citral (9.73 %) and z-citral (7.73 %) as the major compounds. The essential oils and some of their major compounds were tested for their antifungal activities against four soil-born pathogens include *Rhizoctonia solani*, *Macrophomina phaseolina* ‹*Phytophtora citrophtora* and *Pythium sp*. The essential oils of *A. aucheri* exhibited considerable inhibitory effects against all tested fungi While its major components demonstrated various degrees of growth inhibition.

Keywords: *Artemisia aucheri,* Iran, essential oil, linalool soilborn pathogens

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Anti-inflammatory, antiulcer and antioxidant activity of the essential oil from the root of *Carlina acanthifolia* ssp. *utzka*

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Carlina acanthifolia All. ssp. *utzka* (Hacq.) Meusel & Kästner (Asteraceae) is perennial species with leaf rosette, without stem and singular capitula, up to 10 cm in diameter. Root of this plant was collected in August 2003 at Mt. Suva planina (E Serbia). Essential oil was isolated from dried, powdered plant material by hydrodistillation, according to the Procedure III of the Yugoslav Pharmacopoeia IV. The essential oil yield was 1.0% (w/w). Benzyl 2-furylacetylene (*carlina* oxide) was identified as the main component of the oil (91.49%) (1). Isolated oil was investigated for its anti-inflammatory, antiulcer and antioxidant activity.

In assessing anti-inflammatory and anti-ulcer activity the oil in different concentrations (pure oil, 50% and 10% in absolute ethanol) was administered p.o. in doses of 0.25, 0.5 and 1.0 ml/kg. The carrageenan-induced rat paw oedema test was used for screening the anti-inflammatory activity (2). Indomethacin (8 mg/kg p.o.) was used as a reference drug. In all applied concentrations the essential oil significantly reduced the carrageenan-induced rat paw oedema in a dose-dependent manner (see Table). Antiulcer activity was investigated by using ethanol-induced stress ulcer in rats with ranitidine as a reference antiulcer drug. Absolute ethanol (1 ml/rat) caused large gastric lesions (gastric damage score of 5.9±1.14 at the 8-point scale) (3). Pretreatment of animals with the essential oil, 60 min before ethanol, like ranitidine, significantly reduced its ulcerogenic action (see Table).

Essential oil showed strong antioxidant activity. DPPH radical was dose dependently scavenged (IC_{50}=13.7 µl/ml). Inhibition of OH radical and lipid peroxidation reached maximum (42.13 and 65.89%, respectively) at 2.5 µl/ml (4-6).

Essential oil conc. (%)	Anti-inflammatory effect (%)			Gastric damage score		
	Dose (ml/kg)			Dose (ml/kg)		
	1	0.5	0.25	1	0.5	0.25
100	64.46±12.13	61.22±10.72	49.08±10.88	1.15±0.78	2.00±1.77	2.50±1.51
50	62.19±8.22	57.01±7.24	44.10±7.74	1.20±2.14	2.40±2.85	4.90±2.82
10	57.11±11.65	54.65±8.82	40.91±6.22	3.50±2.06	4.00±2.76	3.85±2.27
Ref. drugs	Indomethacin (8mg/kg p.o.): 73.38±39.23			Ranitidine (20 mg/kg p.o.): 1.77±1.08		

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P-135

Antioxidant activity of the essential oils of *Thymus* species from Section *Thymus* grown in Portugal

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Seven Sections can be considered in the genus *Thymus*: *Hyphodromi*, *Mastichina*, *Micantes*, *Piperella*, *Pseudothymbra*, *Serpyllum* and *Thymus* (Salgueiro, 1994). According to Morales (1986), based on the morphological characteristics of species, two subsections are considered in Section *Thymus*: Subsection *Thymus* and Subsection *Thymastra*. In Portugal, three species of *Thymus* belonging to Subsection *Thymus* can be found: *Th. carnosus* Boiss., *Th. zygis* Loefl. ex L. subsp. *zygis* and *Th. zygis* Loefl. ex L. subsp. *sylvestris* (Hoffmanns. & Link) Brot. ex Coutinho. *Th. capitellatus* Hoffmanns. & Link and *Th. camphoratus* Hoffmanns. & Link are the two species of to the Subsection *Thymastra* grown in Portugal.

Populations of *Thymus capitellatus* and *Th. camphoratus* collected in different places show a large chemical polymorphism, as well as the *Thymus* species of Subsection *Thymus* (Salgueiro, 1994). Nevertheless, with the exception of *Th. carnosus* oils, the essential oil composition can be used to differentiate the Subsection *Thymus* from that of *Thymastra*, since Subsection *Thymus* species show thymol and/or carvacrol type oils, in contrast to the oils from Subsection *Thymastra*, dominated by 1,8-cineole, borneol/1,8-cineole or linalol chemotypes.

The main goal of this work was the determination and comparison of the antioxidant ability of the oils of *Thymus* species from Section *Thymus*, using the modified thiobarbituric acid reactive species (TBARS) assay, with egg yolk as lipid-rich media.

Th. zygis subsp. *zygis* oil showed higher capacity than the remaining oils to inhibit lipid oxidation, with antioxidant index always ≥80%. For *Th. zygis* subsp. *sylvestris* oils the antioxidant index ranged from 69% to 83%, the latter value being only reached at the concentration of 1000mg/L. The oils isolated from *Th. capitellatus*, *Th. camphoratus* and *Th. carnosus* showed always lower percentages of antioxidant index (30-70%). These differences can be partly explained by the presence of higher concentrations of phenolic compounds in the oils of *Th. zygis* subsp. *zygis* and *Th. zygis* subsp. *sylvestris*, which is not common in the oils of the remaining *Thymus* species from Section *Thymus*.

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Antioxidant and scavenger activity of essential oil from *Allium ursinum* L.

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Other previous studies showed that many *Allium* (*Alliaceae*) plants other than *A. sativum* and *A. cepa* are of great importance owing their versatile uses as flavoring agents, antioxidants, fragrance and therapeutics (Štajner et al., 2003; 2004). In the present study we investigated the antioxidative properties of bulb, leaf and stalk of *Allium ursinum* L. Activities of antioxidant enzymes (superoxide dismutase, catalase, peroxidase, glutathione peroxidase), quantities of malonyldialdehyde, superoxide and hydroxyl radicals and reduced glutathione and also the content of total flavonoids, chlorophylls a and b, carotenoids, vitamin C and soluble proteins were determined.

Our results indicate that extracts from all plant organs exhibited antioxidant activity. The highest antioxidant activity was observed in the leaves. Furthermore, ESR signal od PBN-OH radical adducts in the presence of leaves phosphate buffer (pH 7) extract was reduced for 87.61%.

The content of essential oils of *Allium ursinum* L. expressed in in percentage was 2.67 %. 23 components were detected. Among all identifies components (total % of identified compounds was 83.57%), predominant (60,85%) were volatile sulphur organic compounds.The sample contained main constituents phosphorodithioic acid (34.70%), dimethyl-trisulfide (8.68%), methyl-2-propenyl disulfide (7.47%), di-2-propenildisulfide (3.62%), 3,3-thiobis-1-propene (2.62%), 2,2-thiobis propane (2.37%) and 1,3-dithiolane-2-thione (2.09%).

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P-137

Antioxidant effects of the essential oils of different parts of *Cupressus arizonica* and *Pinus sp*

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Use of antioxidant is a mean of reducing rancidity of fats and oils in food stuffs. Although synthetic antioxidants, (e.g., BHA and BHT) are effective, concern about possible adverse effects is increasing. Essential oils have been qualified as natural antioxidants and proposed as potential substitutes of synthetic antioxidants. The aim of the present study was to evaluate the antioxidant properties of the essential oils of different parts of *Cupressus arizonia* and *Pinus sp.* Also to find out which components of the essential oils contribute to this effect.

Fresh leaves and fruits of these plants were steam distilled and volatile oils were analyzed by GC-MS. The essential oils and several standards like α-pinene, β-pinene, myrecene, sabinene, limonene, δ-3-carene, γ-terpinene and terpinolene were tested for a possible antioxidant activity. Different methods of rapid TLC screening, DPPH assay, desoxyribose assay, assay for site-specific reaction and non-enzymatic lipid peroxidation were used to evaluate their antioxidant activity. Positive controls were quercetin, vitamin E, vitamin C, dimethylsulfoxide (DMSO) and Buthylhydroxytoluene (BHT).

The main components of these essential oils were α-pinene, β-pinene, myrcene, β-caryophyllene and Δ-3-carene. In rapid TLC screening, the tested compounds mainly showed antioxidant activity. In DPPH assay the strongest effects were shown by the oil of leaves and fruits of *C. arizonia.* In deoxyribose degradation assay test, most of the tested compounds showed some antioxidant effects. In non-enzymatic lipid peroxidation test, most antioxidant activity was shown by fruits of *C. arizonica.*

Finally it seems oils of *Cupressus arizonica* and *Pinus sp,* main components of the oils and positive controls have a different antioxidant effect when they were tested by different methods.

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P-138

Antimicrobial activities of essential oils from *Artemisia* spp.

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This study was performed to investigate antimicrobial activity of essential oils extracted from *Artemisia* spp. Artemisia can be considered as one of the most important Korean medicinal herbs for digestion, gender diseases, asthma, etc. Also, importance of Artemisia is increasing continuously for antimicrobial, antiviral and antioxidant activities of its essential oil. *Artemisia* spp. which is local cultivar of Korea collected in Ganghwa R&D Center experimental field and distilled the essential oil by Steam Distillation Extraction Method.

For investigating antimicrobial activities used four bacterium strains; *Candida albicans* KCTC7965, *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27853. The essential oil of Artemisia showed very high antimicrobial activities against four strains harmful to human. Antimicrobial activities were increased in over 90% by adding 0.5% of essential oils extracted from *Artemisia* spp. The results of this study suggested that *Artemisia* spp. be a promising agent against noxious microbe.

Table 1. Antimicrobial activity of *Artemisia* spp. essential oil against *Candida albicans*

Concentration of essential oil (%)	Control	Survival activity (cfu/㎖)		
		15 min.	30 min.	60 min.
0.25	1.1×10^5	$1.0\times10^{5\,B}$ $(9.1)^a$	9.0×10^4 (18.2)	3.2×10^4 (70.9)
0.50	1.1×10^5	7.6×10^4 (24.0)	6.5×10^4 (35.0)	4.4×10^3 (95.6)

[a]: Reduction rate(%)= (A-B)/A×100 A; primary cell number($1<9\times10^5$cfu/㎖), B; number of after treatment

Table 2. Antimicrobial activity of *Artemisia* spp. essential oil against *Staphylococcus aureus*

Concentration of essential oil (%)	Control	Survival activity (cfu/㎖)		
		15 min.	30 min.	60 min.
0.25	4.2×10^5	$3.8\times10^{5\,B}$ $(9.5)^a$	2.4×10^5 (42.9)	6.5×10^3 (98.4)
0.50	3.9×10^5	3.3×10^5 (15.4)	1.5×10^5 (61.5)	4.8×10^3 (98.8)

[a]: Reduction rate(%)= (A-B)/A×100 A; primary cell number($1<9\times10^5$cfu/㎖), B; number of after treatment

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Anti-*Streptococcus* activities of essential oils from *Thymus magnus* against antibiotic-resistant strains

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The rapid increase in the development and variety of antimicrobial resistance is one of the greatest challenges threatening the future of the human race. Furthermore, increased case of therapy and excessive application has accelerated the resistance of microorganisms against specific antibiotics and in many cases, multiple drugs (Oluwatuyi *et al.*, 2004). Especially, the emerging resistance of strains causing respiratory infections, especially community-acquired pneumonia, is a serious problem worldwide. In particular, the treatment of *Streptococcus pneumonia* infection is currently hampered by increasing antibiotic-resistance (Esposito and Principi, 2002). In this study, the *in vitro* inhibitory activities of essential oils from *T. magnus* as well as their main constituents are evaluated against antibiotic-resistant strains of *Streptococcus pneumoniae.* Moreover, we determine the capacity of the oils to modulate the resistance of *S. pneumoniae* to norfloxacin, one of the quinolone antibiotics commonly used for the treatment of various *Staphylococcus* infections.

To evaluate the activity the broth dilution method and disk diffusion test were used in this study. The checkerboard titer test is used to determine the synergism between the *Thymus* oils and antibiotics.

As the results, the essential oil fraction of *T. magnus* and its main components displayed notably significant inhibition activity against both antibiotic-susceptible and resistant strains. The differential minimum inhibiting concentration (MIC) values obtained imply that the oil fraction and its main component, thymol, display distinct patterns of activity against the tested species. Moreover, the disk diffusion test disclosed that these inhibitory activities are dose-dependent. Data from the checkerboard titer test confirmed synergism between norfloxacin and *T. magnus* oil or thymol, particularly against norfloxacin-resistant strains of *S. aureus.*

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P-140

Antioxidant activity of *Nepeta nuda* ssp. *nuda* essential oil from Greece

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Nepeta L. is a genus of annual or perennial herbs found in temperate Europe, Asia, North Africa, in mountains of tropical Africa and comprises of approximately 250 species.[1] Many *Nepeta* species have been investigated for their oil constituents.

Nepeta nuda L. is a common species of this genus found throughout Europe. The flowering aerial parts of *Nepeta nuda* L. ssp. *nuda*, growing in Mt Parnassos were collected in August 2003 in Greece.

Air-dried leaves (sample A) and verticillasters (sample B), were subjected separately to hydrodistillation in a Clevenger-type apparatus for 3h. Oil composition was analyzed by means of GC-MS.

The dominant constituent in the verticillasters oil was the 4aα,7α,7aβ-nepetalactone (75.7%). The main metabolites of the leaves oil were 1,8-cineole (16.8%), 4aα,7α,7aβ- nepetalactone (24.7%) and caryophyllene oxide (16.3 %).

The free radical scavenging capacity (RSC) was evaluated by measuring the scavenging activity of the essential oils on the 2,2-diphenyl-1-pycrylhydrazile (DPPH). The influence of essential oils (2.13, 1.065 and 0.425μg/ml) on Fe^{2+}/ascorbate induced lipid peroxidation (LP) in lecithin liposomes (comparing with 0.5M BHT as positive control) was evaluated by TBA-test. Examined essential oils were able to reduce DPPH radicals form into the neutral DPPH-H and this activity was in dose-dependent manner. However, essential oils didn't reach 50% of neutralization. Both examined essential oils exhibited inhibition of LP. The verticillasters essential oil expressed significant effects on LP (in range from 41.18 to 44.44%), comparing to BHT (37.04%). On contrary, the leaf essential oil exhibited pro-oxidant activity (-22.9%) in highest concentration (2.13μg/ml).[2,3,4]

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P-141

Antioxidant capacity of some *Lamiaceae* species

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Antioxidant capacity is due to the interaction of several different compounds (polyphenoles, essential oil components, vitamins, microelements etc.). These components are present in the most parts of plants, but their amounts depend on several parameters such as species, the developmental stage, weather conditions and other abiotic stress effects. Extraction also influences the measured antioxidant parameters, since each component is characterized by different solubility: some of them are water-soluble, while others are non-polar compounds.

During this experiment the antioxidant capacity of three *Lamiaceae* species (*Rosmarinus officinalis* L., *Salvia officinalis* L. and *Thymus vulgaris* L.) was examined during the whole vegetation period. The aim of this study was to determine changes in the total antioxidant capacity measured in aqueous and ethanolic extracts, during the development of three rosemary clones ('Harmat', 'Salem' and 'Horvát') and three selected populations of sage ('SN', 'SP', 'SR') and thyme ('TV2', 'TV4', 'TV14').

The plant material of the experiment was grown in Soroksár, Hungary. Ten cm long shoots of two-year old sage and thyme (sowing) and newly planted rosemary plants (half-woody cuts) were collected during the vegetation (2003). Aqueous and ethanolic extracts were prepared from the dried and grounded drugs. The total antioxidant capacity were determined in spectrophotometrical way by FRAP method (Ferric Reducing Ability of Plasma) at $\lambda = 593$ nm from the filtered extracts. Calibration curves obtained by ascorbic acid were used for the calculation.

Our measurements revealed differences in the antioxidant capacity among the species, among the rosemary clones, among the thyme and sage selected populations and between the extraction methods.

Sage had the highest antioxidant capacity from all of the three species. Aqueous extracts performed favourable results in most cases. It indicates that most of the characteristic antioxidant compounds were putatively water-soluble. Among the rosemary clones, total antioxidant capacities of 'Salem' and 'Horvát' were similar but higher as compared to those of the 'Harmat'. Otherwise, they performed sharp fluctuations while the 'Harmat' could be characterized by nearly constant values during the whole vegetation. The selected sage populations showed high antioxidant capacity, but the highest value (2.78 AA mg/ml) was obtained at the end of June in the new shoots of 'SN'.

From the selected thyme populations, the 'TV4' had the best properties. It possessed the highest and the most constant results during the whole vegetation. Changes of the total antioxidant capacity were very similar in case of the'TV2' and 'TV14'.

Differences and changes of the total antioxidant activities can be a consequence of several reasons, such as genetically encoded properties, environmental factors (heat, solar radiation etc.), different levels of tolerance, or various secondary metabolic processes at the sampling time.The results show that for preparing herb teas, it is worth choosing those clones and selected populations of the given species, which had best antioxidant properties.

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Bunium persicum essential oils suppressed potato sprouting in storage

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Black Zira (*Bunium persicum* Boiss.) is a native medicinal plant of Iran. It belongs to *Apiaceae* family and its fruits contain high level of essential oils. The idea of using an essential oil or naturally occurring substances as sprouting inhibitors for potatoes has become a global goal for scientists. There are some literatures about the effects of carvone rich medicinal plants on potato sprouting (Doster Haven,1992) but there is any report about the *Bunium persicum* effects on post-harvest physiology of potato.

Essential oil content of Black Zira fruits were indentified by Clevenger apparatus. In this research the effects of *Bunium persicum* essential oils and its by-product on post harvest physiology of potato was investigated. Seven treatments (including 0.2, 0.5, 1% essential oils, diluted bunium water, fumigation, residue after distillation and control) with 4 replications were compared in a randomized complete block design. Several factors such as sprouting, weight loss, transpiration, appearance and flesh firmness of treated potatoes were evaluated and measured.

Results shown that *Bunium persicum* seeds contained near 9% essential oil(v/w dry weight basis) and the main constituents was cuminaldehyde, p-cymene, farnesol and γ-terpinene. Results also shown that the *Bunium persicum* essential oils had a significant effect on post harvest physiology of potato and inhibit tuber sprouting completely. The best treatment was fumigation of essential oils that decreased sprouting to zero and weight loss to 1.2 % in comparison with control (15 sprout/tuber and 3.64 % weight loss respectively). Therefore *Bunium persicum* essential oils is an strong potato sprout suppressor that don't contain carvon and need more attention for dissolving post harvest problems of horticultural crops in the future.

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P-143

Chemical composition and antioxidant activity of myrtle (*Myrtus communis* L.) leaf oil from Montenegro

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Myrtle (*Myrtus communis* L., Myrtaceaea) is well known aromatic plant, growing spontaneously throughout Mediterranean area. In the folk medicine this plant is used for the treatments of various medical conditions such are bronchial infections, catarrh, chronic cough, cold, flu, infections diseases. In Serbia & Montenegro myrtle is distributed through Montenegro coastline. The chemical composition of myrtle oil varied in dependence of plant origin. Most of the oils have a high content of 1,8-cineole and among them two main chemotypes can be distinguished, depending of presence or the lack of myrtenyl acetate, as well as, ratio of α-pinene (Lawrence, 1993; Bradesi et al., 1997). As far as now, comprehensive data on myrtle oil from Serbia and Montenegro, have not been published. Therefore, here we report on chemical composition of myrtle oils from six localities in Montenegro and their antioxidant activity, measuring in different *in vitro* systems.

In the oil twenty nine compounds have been identified (89.8-95%), with 1,8-cineole (10.7-19.3) and myrtenyl acetate (10.2-18.5%) as dominant. Beside, them, significant amount of linalool (8.4-22.1%), α-pinene (0.5-9.4%), geranylacetate (4.8-7.1%) and linalyl acetate (3.8-6.5%) were found. In all sample, except one, 1,8-cineole had highest ratio. Comparing to literature data, analyzed oil can be distinguished with relative small amount of α-pinene and appreciable content of linalool.

The essential oil exhibited strong scavenger activity on DPPH radical (EC_{50}=5.99-6.24μL/ml), which was higher or similar to commercial antioxidants (BHA, BHT, PG). Furthermore, myrtle oil inhibited Fe^{2+}/ascorbate induced lipid peroxidation in liposomes for maximum 28% (c=1.7μL/ml). The present study, suggest that myrtle oil besides its fragrance and flavor properties, posses strong antioxidant activity and can be used as natural antioxidant in various food and cosmetic products.

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P-144

Chemical composition and antibacterial activity of the essential oil of *Phagnalon saxatile* (L.) Cass. (Asteraceae) growing wild in Southern Italy

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The genus *Phagnalon* belongs to the subtribe Gnaphaliinae (tribe Gnaphalieae, Asteraceae) and is represented by about 30 species worldwide distributed, six of which are typical of the Mediterranean region. Phytochemical investigations on this species are mainly devoted to the study of *Phagnalon rupestre* (L.) DC. (Bicchi, 1975; Góngora., 2002) and report the presence of *n*-paraffins and fatty substances, essential oil, flavonoids, glycosides. The plant is used in the popular medicine and a variety of extracts were examined. Among the Bedouins of the Negev desert the bark of *P. rupestre* is widely used to induce deliberate burns for the healing of various ailments, while in the Palestinian area the whole plant is used to treat asthma, headache and as anesthetic for toothache. The aqueous and ethanolic extracts of the plant showed antimicrobial activity against both positive and negative bacteria and *C. albicans*. *Phagnalon saxatile* (L.) Cass. is one of the five suffruticous chamaephyte species growing wild in Italy, mostly in Southern Italy (Pignatti, 1982). A survey of literature did not reveal any reference to previous work on the essential oil of this or other species of *Phagnalon*. Therefore, continuing our studies on the volatile components from plants growing wild in Southern Italy (Senatore, 2004), in this paper we report for the first time the essential oil composition of *Phagnalon saxatile* (L.) Cass. Aerial parts of *Phagnalon saxatile* (L.) Cass. were collected in May 2004, in the Parco Nazionale del Cilento (Salerno province, Southern Italy). Dried aerial parts of the plant were subjected to hydrodistillation according to the standard procedure reported in the *European Pharmacopoeia*. The dry material yielded 0.11% of a pale yellow oil having a typical smell. The oil components were quantified and identified as previously described (Senatore, 2004). Sesquiterpenes represent the 21.8% of the oil; in the oil were also present fatty acids (22.6%) and waxes (19%).The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), using. eight bacteria species selected as representative of the class of Gram+ and Gram.

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P-145

Chemical composition and antioxidant activities of the essential oils from *Lavandula luisieri*, *L. pedunculata* and *L. viridis* grown in Portugal

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Lavandula spp. are Lamiaceae species that have been used either as dried plants or as essential oils for a large number of therapeutic as well as cosmetics purposes (Cavanagh and Wilkinson, 2002). In Portugal, mainly in southern Portugal, *L. luisieri*, *L. pedunculata* and *L. viridis* can be found growing wild in uncultivated fields. The chemical composition of their essential oils as well as their antioxidant activities has not been evaluated. In this way, it was our purpose to compare the chemical composition of the essential oils from two populations of *L. luisieri* collected in different places of Algarve (Salir and praia de Faro), two populations of *L. pedunculata* also from Algarve (Carriagem and Faro) and one population of *L. viridis* from Vale das Éguas (Algarve).

The oils were isolated by hydrodistillation, for 4h, from dried material, collected during the flowering phase, using a Clevenger-type apparatus and the chemical composition was further analysed by GC and GC-MS. The antioxidant activity was measured through the modified thiobarbituric acid reactive species (TBARS) assay, using egg yolk as lipid-rich media.

Qualitative and quantitative differences in some components were detected in the essential oils of the studied *Lavandula* species. The oils isolated from both *L. luisieri* populations were dominated by 1,8-cineole (26% in both cases). *L. viridis* had also high amounts of 1,8-cineole (33%) but along with high levels of camphor (20%), whereas *L. pedunculata* oils were predominantly constituted by fenchone (42-44%) and camphor (35-36%).

Concerning the antioxidant activity, all *Lavandula* oil samples showed lower capacity to prevent oxidation than the synthetic antioxidants BHT and BHA. The best antioxidant index percentages (AI%) were generally observed at 500mg/L, the antioxidant indexes decreasing from this concentration up to the highest concentration tested (1000 mg/L). At 500 mg/L, the percentages observed ranged from 51-54 % (*L. luisieri* oil from praia de Faro and *L. viridis* oil, respectively) to 61-66 % (*L. pedunculata* oil from Carriagem and *L. luisieri* oil from Salir, respectively). The percentages values found for BHA were always superior to 80 %, being even superior for BHT, exceeding always 90 %.

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P-146

Composition and antimicrobial activity of *Chenopodium votrys* essential oil from Greece

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The Chenopodiaceae is a large family of mainly perennial herbs widely distributed in temperate and subtropical saline habitats.[1] *Chenopodium* L. genus, a member of Chenopodiaceae family, comprises of *c.* 100 species.[2] *Chenopodium* species have been used for a long time in folk medicine.[3]

In our continuing research on Greek aromatic plants we analysed the essential oil of *Chenopodium botrys* L., a plant with a peculiar fragrant odor similar to pine resin.

C. botrys was collected during the flowering stage at Karpenisi (Perfecture Fokida, Greece) in August 2003. The essential oil was obtained from the dried aerial parts by hydrodistillation in a Clevenger-type apparatus and analyzed by means of GC-MS.

The main metabolites of the oil were elemol acetate (16.3%), elemol (14.1%), botrydiol (11.0%), α-chenopodiol (9.5%) and selina-3,11-dien-6-α-ol (6.1 %).

The essential oil showed no significant activity against the tested Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and the Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*). The oil showed a moderate activity against the mycetes *Candida albicans* and *Aspergillus niger*, whereas it was very active against the tested dermatophytes *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum canis*.

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Composition and antimicrobial activities of different distillation samples of the essential oil of *Origanum heracleoticum* from Bulgaria

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In continuation of our research work of combined interpretation of analytical and biological data of various aroma samples (essential oils, extracts and pure compounds) using gas chromatographic-spectroscopic (GC and GC-MS) and olfactoric method as well as antimicrobial tests (e.g. Jirovetz, 2003 and 2004, Schmidt, 2004), 7 samples obtained after different distillation times of the essential oil of *O. heracleoticum* from Bulgaria were investigated.

All the samples were found to be rich in carvacrol (sample 1: 18.8%, 2: 33.3%, 3: 51.8%, 4: 56.6%, 5: 72.3%, 6: 56.5%, 7: 65.7%), *p*-cymene (1: 42.0%, 2: 23.5%, 3: 16.3%, 4: 14.0%, 5: 3.8%, 6: 8.6%, 7: 12.8%) and γ-terpinene (1: 18.2%, 2: 20.7%, 3: 15.3%, 4: 11.7%, 5: 2.1%, 6: 7.7%, 7: 12.4%).

Using two different agar assessment methods (diffusion and dilution) the seven samples showed remarkable activity (reference component eugenol) against the microorganisms: gram-(+)-bacterium *Staphylococcus aureus* ATCC 6538P, gram-(-)-bakteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* G 28 and *Klebsiella pneumoniae* as well as against the yeast *Candida albicans* ATCC 10231, but with more significant IZ-data (agar diffusion method) in correlation to the MIC-data (agar dilution method).

Because of the characteristic, pleasant aroma and antimicrobial effects of all samples, these *O. heracleoticum* essential oils from Bulgaria and their compositions may be useful for various applications in medicine, pharmacy, perfumery, cosmetics and for food flavouring.

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P-149

Composition and antibacterial activity of the essential oils of *Semenovia dichotoma* (Boiss.) Manden., *Johreniopsis seseloides* (C.A.Mey.) M.Pimen. and *Bunium cylindricum* (Boiss, et Hohen.) Drude., three Umbelliferae herbs growing wild in Iran

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Comparison of the chemical composition and antibacterial activity of the essential oils obtained from the aerial parts of *Semenovia dichotoma* (Boiss.) Manden., which is endemic to Iran, *Johreniopsis seseloides* (C.A.Mey.) M.Pimen. and *Bunium cylindricum* (Boiss. et Hohen.) Drude., were carried out. The oils were obtained by water – distillation and were analayzed by GC and GC/MS.

Caryophyllene oxide (25.5%) and β- pinene (10.9%) were the main components in the oil of *S. dichotoma.*

The oil of *J. seseloides* was found to contain β- pinene (14.9%) and germacrene D (11.1%) as the major constituents.

The major components found in the oil of B.cylindricum were myristicin (43.1%), β- phellandrene (20.0%), β- pinene (15.6%) and α-pinene (10.7%).

Antimicrobial activity was determined by measurement of growth inhibitory zone.

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Composition and antimicrobial activity of the essential oils of two endemic species from Turkey: *Sideritis cilicica* Boiss. & Bal. and *Sideritis bilgerana* P.H. Davis

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The genus *Sideritis* (Lamiaceae) is represented by 46 species and 55 taxa in Turkey, 42 taxa being endemic (Davis, 1988). These species are generally known as "dag cayi, yayla cayi" and are extensively used as herbal tea and folk medicine in Turkey (Baytop, 1999). They are used as anti-inflammatory, nervous system stimulant, antispasmodic, carminative, analgesic, antitussive, stomachic and anticonvulsant (Kırımer, 2004; Baytop, 1999).

The essential oils of *Sideritis cilicica* and *S. bilgerana* were analyzed both by GC and GC/MS to determine their constituents. As a result of GC and GC/MS analyses, 40 and 81 components were identified representing 98% of the total for both *S. cilicica* and *S. bilgerana* oils, respectively. GC/MS analyses of the oils have revealed the occurrence of β-pinene (39%, 48%) and α-pinene (28%, 32%) as the main constituents *S. cilicica* and *S. bilgerana* respectively. β˜phellandrene (20%) was also characterized as a main component in the oil of *S. cilicica*. Antimicrobial assay on the essential oils showed moderate to significant inhibitory effect on human pathogenic bacteria from 0.125 to 0.5 mg/ml. Significantly enough, *S. cilicica* oil showed promising activity close to that of the standard antimicrobial agent Chloramphenicol, against MRSA having MIC value of 0.125 mg/ml.

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P-151

The determination of antibacterial effects of some plants of Labiateae growing naturally around Şirnak-Silopi

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In this research, the essential oils and the ethanol extracts of *Thymbra spicata* var. *spicata* L., *Cyclotrichium stamineum* (Boiss. & Hohen.) Manden.& Scheng., *Teucrium polium* (Stapf Brig.), *Salvia russellii* Bentham and *Mentha longifolia* L. Hudson var. *calliantha*, plants were tested against *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 29998, *Salmonella cholerasuis* ATCC 14028, *Enterococcus faecalis* RSKK 97008, *Streptococcus mutans* NCTC 10449 and *Sarcinia lutea* ATCC 9341, by using the disc diffusion method.

The oils of *T. spicata* var. *spicata* and *C. stamineum* were effective on *S. aureus*, *B. cereus*, *E. coli* and *S. cholerasuis* strains, whereas the oils of *S. russellii* and *M. longifolia* var. *calliantha* were effective on *S. aureus, B. cereus, E. coli, S. cholerasuis* and *E. faecalis* strains, on the other hand, the oil of *T. polium* was effective on *S. aureus, B. cereus, E. coli* and *E. faecalis* strains. When the essential oil discs were used together with the control antibiotic discs (Chloramphenicol 30 µg) for the antibacteriological tests, *S. lutea* and *S. mutans* bacteria did not show any reproduction performances in their petries. The extract of *T. spicata* var. *spicata* was effective on *S. aureus, E. coli, S. mutans* and *S. lutea* strains, while the extracts of *C. stamineum, S. russellii* and *T. polium* were effective on *S. mutans* strain and than, the extract of *M. longifolia* var. *calliantha* was effective on *E. coli* strain. The essential oils were found more effective than the extracts. These results confirm the possibility of using these plants in food systems, medicine and pharmacy.

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P-152

Composition and microbiological activity of *Micromeria kosaninii* essential oil

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Micromeria kosaninii is Macedonian endemic plant that grows in clefts of rocks on the mountain Galicica.

The volatile constituents, obtained by hydrodistilation of the dried aboveground parts of M. kosaninii in full bloom stage in a yield of 0.1%, were investigated for the first time using GC/MS. Twenty-two components were identified accounting for 76.2% out of the total quantity of the oil. Only 3 compounds have the content higher than 5%: caryophyllene oxide (18.8%), caryophyllene (6.9%) and borneol (6.2%), reaching together 31.9% of the oil. The identified sesquiterpenoids (45%) were present in higher quantity than monoterpenoids (31.2%).

A disk diffusion method was used for the evaluation of the antimicrobial activity of this oil against a panel of microorganisms (bacteria: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella enteritidis* fungi: *Aspergillus niger*). The solution of oil in ethanol (1:20) showed approximately equal week activity against all microorganisms except *Salmonella enteritidis* which is the most resistant to the oil. This is a surprising result because caryophyllene oxide, dominant compound (18.8%) of the oil, is known as powerful antimicrobial agent.

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Essential oil and antioxidant activity of *Asaraum europaeum* L. (Aristolochiaceae) from Serbia

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Aarum europaeum L. (Aristolochiaceae) has a long history of herbal use. The root leaves and stems are cathartic, diaphoretic, emetic, stimulant and tonic. Owing to its odoriferous properties this plant is utilized both in phytotherapy and in the alimentary industry for food and beverage spicing and preparing. Essential oil with ginger aroma, is concentrated in the asarum rhizome and is known to contain aromatic volatile compounds such are asarones and eugenols. However, utilization of asarum in beverage and food stuff is limited, since asarone, in particularly β-asarone is susceptible of carcinogenic action (Oprean et al., 1998). Besides essential oil in the *Asarum* genus phenolic compounds, such are mono- and di-glycosides of kaempferol and quercetin have been recognized.

In the present study we investigated the composition and antioxidant activity of essential oils obtained from the rhizome of two different population of *Asarum europaeum*. Besides, antioxidant activity of leaves extracts was also evaluated. The obtained results show great difference in oil composition between two populations. Thus, plants collected from North Serbia (mountain Fruska Gora in Vojvodina) are characterized with high content of α-asarone and those collected from the East Serbia (mountain Goc), with only trace of α-asarone, but with huge amount of trans-methylisoeugenol (90.6%). The other oil constituents were also different. Since in the first one menthone, menthol and spathulenol were found in considerable amount, in the second one, besides trans-methylisoeugenol, only bornylacetate was found more than 1%.

Both, essential oil and extracts, exhibit high antioxidant activity, which was measured in different *in vitro* systems and compared with commercial antioxidants: BHA, BHT and PG. EtOAc leaf extract and essential oil are found to be most potent antioxidant, with higher free radical scavenging capacity and higher redox potential than commercial antioxidants.

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P-154

Essentail oil composition and antibacterial activity of the *Perovskia abrotanoides* Karel (Labiatae) growing in Central Iran

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There are a lots of genus of the "Labiatae" family included in Iran and one of the most famous species is *Perovskia abrotanoides*. Traditional medical application of this plant is very important because of several effective ingredients, which has antibacterial properties. *P. abrotanoides* is an herb used to treat leishmaniasis in Iranian folk-medicine tradition. Another application of the essential oil of this plant is in perfumery industry.

The chemical variation of the essential oils of different plant organs such as flowers, leaves, stems and roots of *P. abrotanoides* Karel from Kashan area (Isfahan province, central Iran) have been studied. The essential oils were isolated by hydrodistillation in July and December. Analyses of the oils by GC and GC-MS spectrometry were performed. From 28 identified compounds in July and 26 compounds in December α-cadinol, borneol, camphor, cathin and 1,8-cineole were the common major components of the oils. Essential oils showed antimicrobial activity against *Microsporum gypseum*, *Candida albicans* (yeast), *Aspergillus fumigatus* (fungi) and *Salmonella typhi* (Gram negative bacteria).

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P-155

Glandular trichomes morphology, essential oil composition and its biological activity in *Plectranthus neochilus* (Lamiaceae)

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Five morphological types of glandular trichomes are described in *Plectranthus neochilus* vegetative ad reproductive organs. Peltate and short-stalked capitate trichomes are similar to the previously reported to Lamiaceae, although in *Plectranthus* genus the peltate trichomes have a characteristic orange to brownish colour. Long-stalked capitate trichomes possess a two-to-three celled stalk of variable length and a unicellular bulb-shaped head, which develops a large subcuticular space where secretion accumulates temporarily. Digitiform trichomes, also present on the leaves, do not show a clear distinction between the apical glandular cell and the subsidiary cells. Conoidal trichomes, which are an unusual type of glandular trichomes with a two-to-three celled stalk and a long unicellular head, occur exclusively on the reproductive organs, particularly in the calyx and the corolla. This type of trichomes has only been reported in Lamiaceae for this genus (Ascensão *et al.* 1998, Ascensão *et al.* 1999). Histochemical characterization of the secretion products showed that peltate and long-stalked capitate trichomes produce the bulk of the essential oils whereas short-stalked capitate trichomes, as well as digitiform and conoidal trichomes produce mainly polysaccharides.

P. neochilus essential oils were obtained by hydrodistillation and by distillation-extraction from leaves and flowers and were analyzed by GC and GC-MS. α-Thujene (11-13%), α-pinene (7-8%), β-caryophyllene (7-8%) and α-terpenyl acetate (5-6%) were the dominant components of the essential oils isolated from the leaves plus stems and from the flowers. The essential oils were tested for antioxidant activity using TBARS and DPPH methods and the antimicrobial activity was determined using the agar diffusion method. Strains of *Salmonella spp.*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocitogenes*, *Helicobacter pylori*, and a strain of the yeast *Saccharomyces cerevisiae*, were tested. All the assayed essential oils revealed a low antioxidant and antimicrobial activity.

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P-156

Inhibiting activities of essential oils from *Allium* species against *Trichophyton* fungus

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Allicin, a well-known compound of *Allium* spp., is so unstable that upon generation it readily changes into other compounds. Thus, allicin has not been conclusively proven to be responsible for the known health benefits of garlic. Moreover, recent characterization of the pharmacokinetics and metabolism of organosulfur compounds in garlic has revealed that allicin is not biologically active in the body. Therefore, compounds in *Allium* spp. other than allicin may very well be responsible for the beneficial activities.

In order to develop stable and safe antifungal agents from natural products (daily foodstuffs in particular), the composition and antifungal activities of the essential oils from *A. sativum* for. *pekinense*, *A. cepa and A. fistulosum* against *Trichophyton* spp. were investigated and compared in this study. In addition, in an attempt to achieve a more powerful and safer therapy, the combined effects of *Allium* oils and their main components with ketoconazole were determined by checkerboard titer tests and isobolograms constructed with combined MICs.

Among the tested oils, *A. sativum* for. *pekinense* oil exhibited the strongest inhibition of growth of *T. rubrum, T. erinacei and T. soudanense* with MICs (minimum inhibiting concentrations) of 64 ug/ml, while the activities of *A. cepa* and *A. fistulosum* were relatively mild. The inhibiting activities of the oils on Sabouraud agar plates were dose dependent against *Trichophyton* species. Additionally, these oils showed significant synergistic antifungal activity when combined with ketoconazole in the checkerboard titer test and disk diffusion test.

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P-157

Inhibition of acetylcholinesterase activity by essential oils

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Reversible inhibitors of cholinesterase are currently used in clinical trials examining the treatment of Alzheimer's disease. The treatment of Alzheimer's disease was based on inhibition of the acetylcholinesterase (AChE), which hydrolyses acetylcholine, increasing acetylcholine available for transmission at the cholinergistic synapse. Some of acetylcholinesterase inhibitor has been found naturally occurring in plants. Recently, galantamine, amaryllidaceae alkaloid, have shown effective results for Alzheimer's disease and safety of treatment. The effects of *Salvia lavandulaefolia* Vhal (Spanish sage) essential oil and some of its constituent terpenoids on human erythrocyte acetylcholinesterase have been reported, too. In our previous study, the inhibition of AChE by monoterpenoids with a *p*-menthane skeleton, essential oils of *Mentha* species[14], volatile αβ-unsaturated ketones and essential oil from *Citrus paradisi* were reported.

In this study, we investigated the inhibition of acetylcholinesterase (AChE) activity by 17 kinds of bicyclic monoterpenoids. Bicyclic monoterpenoids are contained in many kinds of essential oils. Inhibition of AChE was measured by the colorimetric method. 3.1.1. and 4.1.0 bicyclic hydrocarbons with allylic methyl group showed strong inhibition. (+) and (–)- α-pinene and (+)-3-carene were potent inhibitor of AChE. 3.1.1. and 2.2.1. bicyclic alcohols and ketones showed weak inhibition. 3.1.1. and 4.1.0 bicyclic hydrocarbons with allylic methyl group were found to be uncompetitive inhibitors.

P-158

Inhibiton of the resistant strains of *Streptococcus pneumoniae* by the essential oils components of *Glehnia littoralis*

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To develop a new strategy from plant Koran plant resources for dealing with the current situation regarding the rapid increase in antibiotic-resistant pathogen, the in vitro inhibitory activities of essential oils from the young leaves of *Glehnia littoralis* as well as its main constituents were evaluated against susceptible and resistant species of *Streptococcus pneumoniae.*

Clinically isolated antibiotic-susceptible and resistant strains of *S.pneumoniae* (n=5) were obtained from the Culture Collection of Antibiotic Resistant Microbes (CCARM) and the Korean Culture Center of Microoganisms (KCCM) and subcultured on Mueller Hinton II agar plates with 5% sheep blood or Trypticase soy agar plates. A range of two-fold dilutions (160-0.125 mg/ml) of essential oils in medium containing 2% Tween-80 was prepared. The oil suspensions (100 µl) were added to 96-well plates. Antibiotics were similarly diluted in DMSO to generate a series of concentrations, ranging from 100 to 0.78 µg/ml per well. The turbidity of the bacterial suspensions was measured at 600 nm and adjusted with medium to match the 0.5 McFarland standard (10^5-10^6 colony forming units/ml). Next, 100 µl bacterial culture was inoculated into each well and plates incubated at 36°C for 24 hours.

The essential oil fraction of *G. littoralis* and its main components, β-pinene and α-pinene, exhibited significant inhibitory activities against the antibiotic-susceptible and resistant strains of *S. pneumoniae,* with MICs(minimum inhibiting concentrations) ranging from 4.0 mg/ml to 16 mg/ml. No remarkable differences were evident between the susceptible and resistant strains. Moreover, the disk diffusion test disclosed that these inhibitory activities are dose-dependent. Furthermore, data from the checkerboard titer test with FICIs (fractional inhibiting concentration indices) from 0.15 to 0.28 indicated significant synergism between norfloxacin and α-pinene, or β-pinene in activity against the tested *S. pneumoniae* strains.

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P-159

Investigation on antioxidant properties and inhibition of MAO Activity of the essential oils from the fruits of *Athamanta turbith* ssp. *hungarica* and *A. turbith* ssp. *haynaldii*

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In this study, essential oils of mature fruits of *Athamanta turbith* (L.) Brot. ssp. *hungarica* (Borbás) Tutin (sample 1) and *A. turbith* ssp. *haynaldii* (Borbás & Uechtr.) Tutin (1) (sample 2) were investigated in *vitro* on antioxidant properties and inhibition of MAO activity.

The essential oils were isolated from the powdered fruits by hydrodistillation, according to the procedure of the European Pharmacopoeia 4 (2), using n-hexane as a collecting solvent. In previous researches, thirty-eight compounds were identified in the oil of *A. turbith* ssp. *hungarica* and thirteen compounds in the oil of *A. turbith* ssp. *haynaldii*. It was found that the major component in both oils was myristicin (3).

In order to investigate the antioxidant properties of the oils, the effect on lipid peroxidation and radical scavenging activity (RSC) were examined. The investigation of RSC was performed against 2,2-diphenil-1-picrylhydrazyl radical (DPPH) and OH radical.

For measuring the inhibitory effect on Fe^{2+}/ascorbate induced lipid peroxidation (LP) in liposomes, TBA test was used (4). The samples were applied at concentrations from 0.125 up to 2.5 µl/ml. Both oils reached maximum of inhibition at the concentration of 1.25 µl/ml (16.29 and 12.08%, for sample 1 and sample 2, respectively).

When reduced into DPPH-H, DPPH radical changes color from violet to yellow and RSC is measured spectrophotometricly (5). Both oils exhibited dose-dependent and significant activity: IC_{50}=10.9 µl/ml for sample 1 and IC_{50}=38.8 µl/ml for sample 2.

OH radical, generated in Fe^{3+}-EDTA-H_2O_2 system, induces degradation of 2-deoxyribose into TBA-reactive substances. OH radical scavenging activity of the examined oils was measured spectrophotometricly, using TBA test (6). Both samples reached maximum of inhibition at the concentration of 0.5 µl/ml: 50.47% for sample 1 and 50.42% for sample 2.

In vitro radioassays of MAO A and MAO B inhibition were performed by ^{14}C-5-HT and ^{14}C-β-phenyelthylamine, respectively (7). Both oils exhibited insignificant influence on MAO A, as well as on MAO B inhibition. For *A. turbith* ssp. *hungarica* oil IC_{50} values were 3.86 mg/ml for MAO A and 1.38 mg/ml for MAO B inhibition. The oil of *A. turbith* ssp. *haynaldii* gave following results: IC_{50}=4.62 mg/ml for MAO A and IC_{50}=1.82 mg/ml for MAO B.

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P-160

Investigation of GC-MS and antioxidant activity of *Rosa damascena* essential oil and extract from Iran, population gilan province

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Rosa damascena Mill (Rosaceae) usually grows in Kashan and uses for preparation of essential oil and rose water, but the plant grows in Gilan province near Rasht city has beautiful pink or white flower with pleasure smell and the people of Gilan use the petal flowers as a food flavour and heart tonic. Because there in not any report about the composition of essential oil and flower properties of this plant which grows in Gilan, we decided to study about it. The essential oil of fresh petals of R.damascena (400g) was prepared by hydro distillation with a Clevenger type apparatus for 4-5 h. and 0.8 ml (0.2%, V/W) volatile oil was obtained. Dehydrated essential oil on anhydrous sodium sulfate was analyzed by GC-MS on HP-5 and DB1 columns, 27 and 17 compounds were obtained which 86.2 and 97.4% of them were identified respectively.

α-Pinene, 1,8-cineol, β-phenyl-ethylformate, β-citronellol, eugenol, linalool, nerol, geraniol and some hydrocarbons were identified by two columns.

Hydro alcoholic extract (20water-80alcohol) of fresh petals (400 g) were prepared by percolation and liquid extract was dried under vacuum and low temperature. The dried extract was washed with chloroform and the antioxidant activity of remaining extract (31.4 g) and essential oil were determined with lipid peroxidation (FTC, ferric amonium thiocyanate) and radical scavenging (DPPH, 2,2-diphenyl-1-picrylhydrazyl) methods.

Essential oil and extract with DPPH assay were showed IC50=2.64 μg/ml and IC50=2.24 μg/ml after 30 min. respectively. They were showed stronger activity than BHA with IC50=5.07 μg/ml after 30 min.

Essential oil and extract with FTC assay were showed IC50=23 μg/ml and IC50=520 μg/ml after 72h.respectively and their antioxidant activity was a little weaker than BHA which was showed IC50=10 μg/ml after 72h.

The results were showed the essential oil and extract of R. damascena have more radical scavenging activity than lipid peroxidation activity.

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In vivo antidepressant potential of the essential oil of *Nepeta rtanjensis*

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Nepetalactone is among the prevalent components of the essential oils isolated from the *Nepeta* species. It exists in two isomers: Z,E (cis, trans) and E,Z (trans, cis). The latter form dominates in catnip (*N. cataria*), notorious for intoxication of cats and insect repellent activity (Peterson and Coats, 2001). The Z,E isomer is overabundant in *N. rtanjensis*, Diklić & Milojević (Lamiaceae), endemic specie of the Eastern Serbia mountains. The biological effects of this plant extracts and the essential oil has been poorly investigated until now. Otherwise, the pharmacology of catnip was moderately explored and some behavioral tests on rodents found that acute treatment with catnip increased open field locomotion and stereotypy, while the nepetalactone was suggested as the active principle (Massoco et al., 1995). Therefore, we found interest to perform some pharmacological *in vitro* and *in vivo* tests on the essential oil of *N. rtanjensis,* regarding its potential neuro/psycho-activity.

The essential oil was isolated by hydro-distillation in a Clevenger-type apparatus from a powder of dried aerial parts of *N. rtanjensis* herb (collected in summer 2004. at the Rtanj mt., Serbia). Chemical composition of the oil, determined by analytical GC(FID) and GC/MS techniques (Kovacevic et al., 2005), shows abundance of Z,E-nepetalactone (79.9%) versus E,Z-nepetalactone (6.3%). A variant of the original forced swimming test (FST) was used as a test for antidepressant activity (Tomic et al., 2005). Male adult CBA mice were randomly divided into 4 groups of 6 animals each, which were individually forced to swim for 10 min in an open glass cylinder containing fresh tap water, 24 h before the treatment. Each group received *i.p.* injection (2.5 ml/kg) of either imipramine: 10 mg/kg, essential oil (in 5 % DMSO in physiological saline) of *N. rtanjensis*: 25 or 50 mg/kg, or vehicle. 60 min after the injection, each single animal was placed into the water and, from the second minute onward, its immobility was rated during 5-min-period. The animal that kept floating, with only essential movements to keep its head above the water, was considered immobile.

The FST reveals that both doses of the essential oil induced certain reductions of the immobility score, which was near the level of significance only for the 50 mg/kg dose. This effect appeared to be faint when compared to the decreased immobility score following official antidepressant drug, imipramine. We have detected previously a marked *in vitro* MAO inhibiting potency of the essential oil of *N. rtanjensis*. The enzyme blockade was about 20 times stronger for MAO B, than for MAO A. However, it is prominent that only reversible MAO A inhibition is a base of the therapeutic activity of some antidepressant drugs. Thus the results of FST here, together with such MAO inhibiting profile, suggest that the essential oil of *N. rtanjensis* does not have significant antidepressant capacity.

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P-162

In-vitro activities of essential oils from *Ostericum koreanum* against antibiotic-resistant *Salmonella* spp.

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Plant essential oils are well-known sources for development of new natural antimicrobial agents. In many cases, the activities of the essential oils are distinctly lower than the antibiotics from microbiological sources or synthetic drugs. *Ostericum koreanum* (Umbelliferae) is a perennial herb, which has been used in Korean traditional medicines for treatment of common cold and for relieving of rheumatic pains or headaches as one of the pungent and warm exterior relieving drugs. This plant, one of the rich sources of essential oil, is widely distributed in wild and cultivated in Korea.

Increased case of therapy and excessive application has accelerated the resistance of microorganisms against specific antibiotics and in many cases, multiple drugs. Especially, the cases of illness in human and animals by antibiotic-resistant organisms through the consumption of processed or unprocessed foods which are contaminated with them by increased application of antimicrobials in veterinary. *Salmonella* species comprise one of the common pathogenic bacterial groups causing food-borne diseases.

To develop a new effective and safe therapeutics for infections by antibiotic-resistant bacteria, the essential oil fraction of *Ostericum koreanum* was analyzed by GC-MS. Inhibiting activities of this oil and its main component were tested by broth dilution assay and disk diffusion test against one antibiotic-susceptible and two resistant strains of *Salmonella enteritidis* and *S. typhimurium*, respectively. Additionally the combination effects of essential oil and antibiotics were determined by checkerboard titer test.

As the results of GC-MS analysis of this oil, fifty eight compounds were identified, including its main components, α-pinene (42.12%), p-cresol (17.99%) and 4-methylacetophenone (7.90 %). The essential oil of *O. koreanum* and its main components showed significant susceptibility against the tested antibiotics-susceptible strains as well as against the resistant strains of the two *Salmonella species* with MICs (minimum inhibiting concentrations) ranging 2 mg/ml to 16 mg/ml. The anti-*Salmonella* activities were dose dependent in this experiment. Additionally, from the results of checkerboard titer tests the significant combination effects of streptomycin and *O. koreanum* oil or cresol, one of the main components of this oil, against the two streptomycin resistant strains of *S. typhimurium* were confirmed with FICIs ranging from 0.12 to 0.37.

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Shin, S. *et al.* 2004. In vitro activities of essential oils from *Thymus magnus* against some antibiotic-resistant pathogens. Proceedings of the Convention of the Pharmaceutical Society of Korea (8 April, 2005) Seoul, Korea, 275-276.

P-164

Study on essential oils of Korean *Thymus* species as an anti-*Trichophyton* agent

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Thymus species are well-known sources of antimicrobial essential oils and vary tremendously in composition depending on the plant source. *T. quinquecostatus* Celakov and *T. magnus* Nakai (Labiatae) contain high percentages of thymol. They are native to Korea and they have been used as diaphoretics, carminative, etc in traditional medicine. In this study the antifungal activities of the essential oils from *T. quinquecostatus* and *T. magnus* were evaluated against eight common pathogenic fungi.

The compositions of the essential oils were analyzed and compared by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The essential oils and their main constituents were evaluated for antifungal activity against eight common pathogenic fungi. By the broth dilution method and the checkerboard titer test, the antifungal activities of the two oils and their main components were evaluated against *Aspergillus niger* KCCM 11239, *A. flavus* KCCM 11453, *Candida albicans* KCCM 11282, *C. utilis* KCCM 11356, *Cryptococcus neoformans* KCCM 50564, *Trichosporon mucoides* KCCM 50570, *Trichophyton rubrum* ATCC 6345 and *Blastoschyzomyces capitatus* KCCM 50270.

Both of two *Thymus* oils showed significant inhibition of the tested fungi, with minimal inhibitor concentrations (MICs) and minimal fungicidal concentrations (MFCs) in the range of 0.04-0.39 mg/ml and 0.19-0.78 mg/ml, respectively. The antifungal activity of the oil of *T. quinquecostatus* was slightly more active than that of *T. magnus.* The results suggest that activities of the two oils are based mainly on the contents of thymol (41.7 and 39.8%) and carvacrol (4.0 and 3.6%) in *T. quinquecostatus* and *T. magnus,* respectively, while the next main components, p-cymene and γ-terpinene, have relatively mild activity. The two *Thymus* oils and thymol as well, exhibited synergism with ketoconazole against *Trichophyton rubrum*, which showed the highest susceptibility to these oils.

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P-168

The essential oil components from *Allium monanthum* as anti-*Streptococcus* agent

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Allium monanthum is one of the favorite wild vegetables, which has a unique fragrance. This has been collected in the early spring on the field in Korea. The whole plant parts including bulbs and roots are used as condiments and vegetables. It has anti-microbial, anti-cathartic, hematinic and sedative activities. Allicin, a well-known compound of *Allium* spp., is so unstable that upon generation it readily changes into other compounds. Thus, allicin has not been conclusively proven to be responsible for the known health benefits of garlic. Moreover, recent characterization of the pharmacokinetics and metabolism of organosulfur compounds in garlic has revealed that allicin is not biologically active in the body, compounds in *Allium* spp. Other components of the plant may very well be responsible for the beneficial activities. In this study we analyzed the essential oil from *A. monanthum* by GC-MS and evaluated its inhibiting activity by the broth dilution method and disk diffusion test against one antibiotics-susceptible and two resistant strains of *Streptococcus pneumoniae*.

GC-MS: 5% Phenyl methyl siloxane, I T.: 50℃, 50-180 °C: 2 °C /min, FT: 230 °C.

MIC: The antimicrobial activity was tested with trypticase soy agar with 5% sheep blood.Disk diffusion test: The culture suspension of strains were added to trypticase soy agar with 5% defibirinated sheep blood plate and distributed uniformly. Sterile paper discs (8mm) were wetted with 50 μℓ of each dilution of the essential oil fraction of *A. monanthum*, allicin, allyldislufide, allylsulfide, oxacillin and erythromycin. They were cultivated at 37℃ for 24 hours.

As the results, more than eighty compounds were identified in this oil, including its main component, dimethyl trisulfide (21.74%), dimethyl tetrasulfide (12.26%) and methlyl propyl trisulfide (10.68%). The essential oil of *A. monanthum* showed mild susceptibility against the tested antibiotics-susceptible strain of *S. pneumoniae* as well as against the resistant strains with MICs ranging from 8mg/ml to 32mg/ml. However, in checkerboard titer test it showed strong synergism with oxacillin with FICIs (fractional inhibiting concentration indices) of 0.37. The anti-*Streptococcus* activities were dose dependent.

Reference;

S, Shin, MS, Pyun, 2003. Effects of essential oils from Allium plants against *Trichophyton* species. Proceedings of the 12th World Congress of Food Science and Technology. (16-20 July) Chicago, USA

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The essential oils in herbal plants of *Mentha* species cultivated in Korea

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Essential oils are one of the most promising groups of natural compounds for the development of safer antifungal agents. However, their poor absorption from the human intestine and relatively mild antifungal activity compared to commercial, synthetic antifungal drugs may ultimately limit their clinical application in the treatment of systemic fungal infections. In this study, the composition and antifungal activities of the essential oils of the *Mentha* species, *Mentha piperita* (peppermint), *M. spicata* (spearmint), *M. piperita* var. *citrata* (eaudecolognemint), *M. pulegium* (pennyroyalmint), *M. rotundifolia* (applemint) and *M. suaveolens* 'Variegata' (pineapplemint), which are at present in Korea massively cultivated for consumption as one of the favorite herbal plants, were investigated and compared.

The essential oils were extracted by steam distillation and analyzed by GC-MS using Hewlett-Packard 6890 GC and Hewlett-Packard 5973 MSD. The conditions of the analysis were as follows. Carrier gas: He (1ml/min), Column: HP-5 (30mx0.25mmx0.25μm) capillary column, Temperature: 70°C, 5min, 70°C-180°C, 2°C /min, 180°C-250°C, 20°C/min, 180°C, 10min, Injector: 250°C, Detector (FID): 280°C, Splitless. Antifungal activities of the oils were investigated by broth dilution method against *Aspergillus niger, Candida utilis* and *C. tropicalis.*

As the results, it was found there was tremendous diversity in composition of essential oils according to the species. Lianlyl acetate (39.5%) and linalool (5.05%) were major components of the oil of *M .piperita*, whereas *M. pulegium* oil contained relatively high amounts of pulegone (33.52%) and neomenthol (7.63%). As expected the main components of *M. spicata* was carvone (57.88%). Carveol, neodihydrocarveol and cis-dihydrocavone were the next representing compounds of this plant. Like as in M. piperita, lianalyl acetate (54.27%) and linalool (8.20%) were also the prominent compounds present in *M.piperita var. citrata.* In the essential oil from *M. rotundifolia* and *M. suaveolens "Variegata"*, piperiton oxide was identified as major components. Especially, the oil of *M. suaveolens "Variegata"* contained a significant amount of eugenol and carvacrol, the well-known potential antimicrobial essential oil compounds. The tested essential oils significantly inhibited growth of *C. tropicalis* and to a lesser extent that of *A. flavus* and *C. utilis,* with MICs (minimal inhibitory concentrations) in the range 0.31 – 5.00 mg/ml.

References:

Shin, S. *et al.* 2005. Studies on essential oils from Mentha species in Korea as new antifungal agents. Proceedings of the 5th International Symposium on Antimicrobial Agents and Resistance, Asian-Pacific Research Foundation for Infectious Diseases. (27-29 April, 2005) Seoul, Korea, 286.

P-170

The examination of hydro alcoholic extract of *white cabbage* in prevention and treatment of peptic ulcer caused by aspirin in rats

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Introduction and objectives: Ulcer peptic is a multifactorial trend caused by blood vessels alterations, mucus secretion and gastric acid biochemistric condition of gastrointestinal tract. Aspirin (non-steroidial anti inflammation) with high curative effects can lead to mucus lesions in stomach. The lesion is caused by delay in stomach mucus rebuilding, increased free radicals and prostaglandins (PG_{I_2}, PG_{E_2}) producing prohibition. Prostaglandins produce from *arachidonic acid* (A.A). Arachidonic acid is a *unsatureated fatty acid* with twenty number carbon. Arachidonic Acid (AA) is an essential fatty acid.

This acid has been synthesis from *phospholipids* by phospholipase A_2 in cellmembrane.

Phospholipase A_2 Inhibited by lipocortin & steroids but *cycloxygenase* (Cox) Inhibited by aspirin & NSAIDs. Arachidonic Acid converted to endoproxide by Cox, endoproxide is precourser for prostaglandins and prostcyclin (PG_{I_2}). There are two kinds Cox: Cox_I and Cox_{II}. Cox_I has physiologic effect and Cox_{II} is pathologist effect. Aspirin and old NSAIDs Inhibited Cox_I but new NSAIDs inhibited Cox_{II}.

In traditional medicine, there are different applications for white cabbage however; the most important one is in digestive pains.

White cabbage acts adverse Aspirin and old NSAIDs, perhaps this plant acts. Like new NSAIDs (e.g. celebrax).

Procedure: In this research 48 Rats were kept hungry for 48 hours and as a result ulcer peptide appeared with aspirin suspension in standard condition. For a group of rats an hour before prescribing aspirin, different amounts of edible cabbage's extract were used. For the other group of Rats the same were used but this time after prescribing aspirin. In both of the conditions, animal's stomach were brought out and opened through the large curvature and lesion signs were assessed according to J. sore criterion.

Then statistical considerations were preformed using variation factor and T. student test under SPSS program.

Findings showed that white cabbage's extract in the amount of 100 mg/kg has the most effect to prevent from peptic ulcer caused by aspirin ($P<0.01$) comparing with 200 mg/kg of the same as the most effective dose used to cure peptic ulcer ($P<0.05$).

Conclusion: white cabbage can be used to prevent and cure the peptic ulcer. However, it would be more effective if it is used as a prevention way.

One of the component of white cabbage is *Jafernic acid* (J.a) and seems competatives with *Cox Enzyme*. In futher will produce will produce drug from whithe cabbage (Jafernic acid). This drug is arachidonic acid mimethic.

Our research group will report and introduce this drug (Jafernic acid) at 37th congress of *essential oil.*

Keywords: Rat, Prevention, Cure, Aspirin, Peptic ulcer, White cabbage

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Faculty of Horticultural Sciences, Department of Medicinal and Aromatic Plants, Budapest

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SCIENTIFIC PROGRAMME

4-7 September, Budapest, Hungary

36th International Symposium on Essential Oils Scientific Programme

Sunday	**September 4, 2005**
14.00 – 19.00	Registration
19.00 – 21.30	**Welcome reception at Hotel Bara**
Monday	**September 5, 2005**
9.00 – 9.30	**Welcome Addresses** Zs. Harnos, vice rector of Corvinus University of Budapest J. Bernáth, chairman of the local organising committee
Chair:	**D. Joulain and J. Bernáth** In memory of Wilfried A. König, Anders Baerheim-Svedensen and Peter Weyerstahl
9.40 – 10.20	*Plenary Lecture* **K.-H. Kubeczka:** 15 years research on volatile secondary metabolites a tribute to Wilfried A. König
10.20 – 10.25	**J. Karlsen:** Remembering Anders Baerheim-Svedensen
10.25 – 10.30	**C. Bicchi:** Remembering Peter Weyerstahl
10.30 – 11.00	Coffee break
11.00 – 11.20	**P. Rubiolo:** HS-SPME-GC-PCA as a tool to classify different chemotypes of chamomile flowerheads (*Matricaria recutita* L.)
11.20 – 11.40	**F. David:** Determination of suspected allergens in non-volatile matrices
11.40 – 12.00	**L. Mondello:** New chromatographic methods for the analysis of essential oils
12.00 – 12.20	**É. Lemberkovics:** Influence of technological and biological conditions on essential oil Composition
12.20 – 14.00	Lunch break
Chair:	**K.-H. Kubeczka and A. N. Figueiredo**
14.00 – 14.50	*Plenary Lecture* **É. Németh:** Essential oil compounds as chemotaxonomic markers
14.50 – 15.10	**K. H. C. Baser:** Essential oils of the genus Calamintha in Turkey
15.10 – 15.30	**Y. Asakawa:** Volatile components of *Polygonum punctatum* var. *punctatum* and two ferns, *Thelypteris hispidula* and Brechnum fluviatile: Distribution of polygodial and their related compounds
15.30 – 15.50	**D. Joulain:** New insight in the knowledge of the qualitative and quantitative composition of oakmoss resinoids
15.50 – 16.10	Coffee break
Chair:	**K. H. C. Baser and B. M. Lawrence**
16.10 – 16.30	**F. Pank:** Essential oil content of crossing progenies of annual and perennial caraway (*Carum carvi* L.)
16.30 – 16.50	**N. Baldovini:** New necrodane monoterpenoids from *Lavandula luisieri* essential oil
16.50 – 17.10	**J. Lazarevic:** Chemotaxonomically significant 2-ethyl substituted fatty acids from *Stachys milanii* Petrović (*Lamiaceae*)
17.10 17.30	**N. Radulovic:** A detailed study of the essential oils of a new chemotype of *Tanacetum vulgare* L. from Serbia
17.30 17.50	**Y. Fan:** Study on the volatile components and the other higher polar components in *Amomum villosum Lour*
17.50 18.10	**K. Karlova:** Variability of the content of chamazulene in essential oil during *Achillea collina* Becker ex. Rchb. ´Alba´ ontogenesis

Tuesday	**September 6, 2005**
Chair:	**G. Buchbauer and L. Mondello**
9.00 – 9.50	*Plenary Lecture* **R. Adams:** The genus *Juniperus*, evolution, biogeography and essential oils
9.50 – 10.10	**Cs. Fodor:** Hungarian essential oil production: past, present and future
10.10 – 10.30	**M. Eikani:** Superheated water extraction of essential oil-bearing materials: An experimental study
10.30 – 11.00	Coffee break
11.00 – 11.20	**Y. Noma:** New Metabolic Pathways of β-Pinene and Related Compounds by *Aspergillus niger*
11.20 11.40	**T. Akenga:** Composition of essential oils of some aromatic plants growing in Kenya and their potential on the development on the essential oil industry
11.40 12.00	**A. Thosar:** Planning scheme for introducing new aromatic plants in India P atchouli (*Pogestemon cablin*) a case study
12.00 12.20	**A. Arabhosseini:** Effect of drying on the essential oil in tarragon (*Artemisia dracunculus* L.) leaves
12.20 14.00	Lunch break
Chair:	**C. Bicchi and F. Pank**
14.00 14.50	*Plenary Lecture* **J. Novak:** Genetics of monoterpenes in the genera *Origanum* and *Salvia*
14.50 15.10	**K. Dusek:** *Lavandula officinalis* L. selection of optimal essential oil producing type
15.10 15.30	**H. Baydar:** Essential oils of *Origanum*, *Thymus*, *Satureja* and *Thymbra* species growing wild in Lakes region in Turkey
15.30 15.50	**J. Palá-Paúl:** Essential oil composition of a new *Eryngium species* from Australia: *Eryngium rosulatum* P.W. Michael ined
15.50 16.30	Coffee break
16.30 18.00	**Poster Session**
Wednesday	**September 7, 2005**
Chair:	**Y. Asakawa and R. Adams**
9.00 9.50	*Plenary Lecture* **W. Jaeger:** Metabolism of terpenoids: impact on flavour and biological activity
9.50 10.10	**I. Rasooli:** Chemical composition, antifungal and anti-aflatoxin properties of essential oils of *Thymus eriocalyx* and *Thymus x-porlock* with special reference to ultrastructural alterations of *Aspergillus parasiticus*
10.10 10.30	**L. Jirovetz:** Purity and antimicrobial activities of geraniol and various geranyl derivatives
10.30 11.00	Coffee break
11.00 11.20	**H. Ibrahim:** Traditional utilisation of aromatic plants in Peninsular Malaysia
11.20 11.40	**J. Filippi:** Volatile constituents and antimicrobial activity of Corsican *Achillea ligustica* essential oil
11.40 12.20	**Closing Session**
12.20 14.00	Lunch break
14.00	**Excursion to Bugac - Banquet**