41st INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

Wroclaw, Poland 2010

Wrocław
University
of Technology

Wrocław University
of Environmental
and Life Sciences

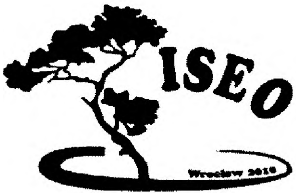
WROCLAW UNIVERSITY
COLLEGE OF
PHYSIOTHERAPY

POLISH CHEMICAL
SOCIETY

100 YEARS
OF TECHNICAL UNIVERSITIES
IN WROCLAW

UNDER AUSPICES

41st International Symposium on Essential Oils



PROGRAMME AND BOOK OF ABSTRACTS

September 5-8, 2010, Wroclaw, Poland

Organization:
Wrocław University of Technology
Wrocław University of Environmental and Life Sciences
Wrocław University College of Physiotherapy

Editors:

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Czesław Wawrzeńczyk	Wrocław University of Environmental and Life Sciences

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Welcome

For the second time the International Symposium on Essential Oils is held in Wroclaw. In 2001 the Symposium was organized by Wroclaw University of Technology and Wroclaw University of Environmental and Life Sciences. This year Wroclaw University College of Physiotherapy joined to the Organizing Committee.

We expect that this year 150-200 scientists from all over the world will attend the Symposium. This scientific program of the Symposium as you can read in the Book of Abstracts is reflection of very broad interests of participants. One can find the papers concern the isolation of essential oils, their analyses and results of studies on biological activities. For the first time the section on asymmetric synthesis, biotransformation and syntheses of valuable compounds was also created. We hope that such broad area of scientific activity of participants will be a good platform for exchanging of scientific information and encouraging partnership between laboratories working with essential oils.

On behalf of the Organizing Committee of ISEO 2010 we would like to wish all participants valuable inspirations during the lectures and poster sessions stimulating scientific discussions and nice stay in beautiful and friendly city of Wroclaw.

Czesław Wawrzeńczyk
Wroclaw University of Environmental
and Life Sciences

Stanisław Lochyński
Wroclaw University of Technology

ISEO

Wroclaw 2010

GENERAL INFORMATIONS

Symposium venue

The symposium will be organized at the Wrocław University of Technology Congress Centre
ul. Z. Janiszewskiego 8, 50-372 Wrocław

Organizing committee

Chairpersons

Stanisław Lochyński	*Wrocław University of Technology*
Czesław Wawrzeńczyk	*Wrocław University of Environmental and Life Sciences*

Members

Andrzej Czamara	*Wrocław University College of Physiotherapy*
Anna Gliszczyńska	*Wrocław University of Environmental and Life Sciences*
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Renata Kuriata	*Wrocław University of Technology*
Jolanta Pękala	*Wrocław University of Technology*
Damian Smuga	*Wrocław University of Environmental and Life Sciences*
Małgorzata Smuga	*Wrocław University of Environmental and Life Sciences*
Daniel Strub	*Wrocław University of Technology*
Antoni Szumny	*Wrocław University of Environmental and Life Sciences*

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Chlodwig Franz	*University of Veterinary Medicine of Vienna, A*
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Daniel Joulain	*Grasse, F*
Jan Karlsen	*University Oslo, NOR*
Karl-Heinz Kubeczka	*Margetshoechheim, GER*
Brian Lawrence	*Winston-Salem, USA*
Luigi Mondello	*University of Messina, IT*
Johannes Novak	*University of Veterinary Medicine, Vienna, A*
Éva Németh	*Corvinus University of Budapest, HUN*
Patrizia Rubiolo	*Universtity of Torino, IT*
Johannes J. C. Scheffer	*University of Leiden, NL*
Elisabeth Stahl-Biskup	*University of Hamburg, GER*

Symposium secretariat and assistance

Secretariat will be opened in **Main Building (A-1)** and **D-20** as follows:

Building A-1

Wybrzeże Stanisława Wyspiańskiego 27, 50-370 Wrocław:

Sunday, 5th September	15:00-19:00

Building D-20

ul. Janiszewskiego 8, 50-372 Wrocław

Monday, 6th September	8:00 – 18:00
Tuesday, 7th September	8:00 – 18:00
Wednesday, 8th September	8:00 – 14:30

Registration

Conference materials and name badges can be obtained at the registration desk which will be opened in **Main Building (A-1)** and **D-20** as follows:

Building A-1

Sunday, 5th September	15:00 – 17:00

Building D-20

Monday, 6th September	8:00 – 9:00
Tuesday, 7th September	8:00 – 9:00

Assistants and conference secretariat

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

Name badges

Participants and accompanying persons are obliged to wear the conference name badges on all conference events.

Opening lecture and concert

Opening lecture and concert will be held in the main building of Wrocław University of Technology at Wybrzeże Wyspiańskiego 27 (building A-1) on Sunday, 5th September 2010, from 17:00 to 19:00.

Opening ceremony

Opening Ceremony will take place in lecture hall (10AC) in symposium venue (building D-20) on Monday, 6th September from 9:00 to 9:30.

Speaker's instructions

Speakers are requested to copy their presentation the day prior their speech **before** the session starts. It is crucial that speakers are on time and that they keep their presentation within the allotted time scheduled. The plenary lectures are foreseen in **60 minutes** including discussion. The time for oral presentations is **20 minutes** including discussion. The lecture room is equipped with PC computer and a ceiling mount projector. PowerPoint software will be provided. The file format preferred for the presentations is .ppt for Office 2003 or pdf. There will be **no possibility** of using MAC file format. An audiovisual technician will be available for assistance in the conference hall.

Poster sessions

Poster sessions will take place in poster exhibit area in building D-20. The posters should be placed on certain day in the morning and removed just after the end of the session. Maximum size of a poster is **841x1189 [mm]** (A0 format).

1st Poster Session A	Monday, 6th September	16:30 - 18:00
2nd Poster Sessions B-D	Tuesday, 7th September	16:00 - 17:30

Language

The conference language is English.

Lunches and refreshments

Lunches are included in the participant's registration fee and will be served in building D-20 as follows:

Monday, 6th September	13:00 – 14:30	Building D-20
Tuesday, 7th September	12:30 – 14:00	Building D-20
Wednesday, 8th September	12:30 – 14:00	Building D-20

Social events

Get-together party

Date	Sunday, 5th September 2010
Time	19:00 – 21:00
Place	Main Building (A-1) foyer Wybrzeże Wyspiańskiego 27

Symposium dinner

Date	Tuesday, 7th September 2010
Time	19:30 – 23:00
Place	Restaurant in the Mercure-Panorama Hotel Pl. Dominikański 1

City guided tour

Date	Wednesday, 8th September 2010
Time	14:30 – 19:30
Place	Meeting point: in front of building D-20 ul. Z. Janiszewskiego 8

Insurance and liability

Neither the organizers nor Wrocław University of Technology take any responsibility for injury or damage involving persons or property during the conference. Participants are advised to make their own arrangements with respect to health, travel and property insurance before they depart from their country.

Conference website

www.iseo2010.wsf.wroc.pl

Exhibitors

Exhibition site will be located in building D-20 foyer near the posters area.

Emergency phone numbers

Mobile phones:

112 - police, fire department, emergency

Stationary phones:

999 – Emergency
998 – Fire Department
997 – Police
981 – Road Assistance
986 – Municipal Police

Scientific Programme

Sunday September 5, 2010
Main Building A-1, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27

15.00 – 17.00 **Registration**

17.00 – 19:00 **Opening lecture**
History of 40 years ISEO meetings
K.-H. Kubeczka
Permanent scientific committee, Germany

Concert

19.00 – 21:00 **Get- together party** *(Main Building A-1 foyer)*

Monday September 6, 2010
Building D-20, Wrocław University of Technology, ul. Z. Janiszewskiego 8

9.00 – 9.30 **Opening Ceremony**

9.30 – 10.30 **Plenary lecture**
Bryophytes: Chemical Diversity, Synthesis and Biotechnology (PL-1)
Y. Asakawa
Tokushima Bunri University, Japan

10.30 – 11.10 **Oral presentations**
Terpenoids of 18-21 Million Years Old Japanese Fossil Conifer Plants (OP-1)
A. Ludwiczuk, K. Terada, N. Iwashita, Y. Asakawa
Tokushima Bunri University, Japan; Medical University of Lublin, Poland

Essential Oil Bearing Plants from Nigeria (OP-2)
I.A. Ogunwande
Lagos State University, Nigeria

11.10 – 11.40 **Coffee Break**

11.40 – 13.00 **Oral presentations**
Research into Salvia Essential Oils of Turkey: Recent Results (OP-3)
K.H.C. Başer, B. Demirci
Anadolu University, Turkey

The diketonic constituents of *Helichrysum italicum* (OP-4)
N. Baldovini, D.A. Lanfranchi
Université de Nice-Sophia Antipolis, France

Variability of thujone and other essential oil components of sage (*Salvia officinalis* L.) during ontogenesis (OP-5)
É. Németh, J. Bernáth, S. Sárosi, K. Szabó
Corvinus University of Budapest, Hungary

High Efficient One Pot Production of Hydrogenated Compounds by Some Fungi (OP-6)
Y. Noma, Y. Asakawa
Tokushima Bunri University, Japan

13.00 – 14.30 **Lunch**

14.30 – 15.30 **Plenary lecture**
Essential Oils: Sensory and Instrumental Research Must Go Together (PL-2)
Á.A. Carbonell Barrachina
Universidad Miguel Hernández, Spain

15.30 – 16.10 **Oral presentations**
Application of SPME for the rapid control of spices aroma (OP-7)
R. Zawirska-Wojtasiak, E. Wojtowicz
Poznan University of Life Sciences, Poland

Criteria for the identification in nature of flavouring substances (OP-8)
T. Cachet
International Organization of the Flavor Industry, Belgium

16.10 – 16.30 **Coffee Break**

16.30 – 18.00 **Poster Presentation - Session A**

Tuesday September 7, 2010
Building D-20, Wrocław University of Technology, ul. Z. Janiszewskiego 8

9.00 – 10.00 **Plenary lecture**
New trends in the analysis of the volatile fraction of plant matrices (PL-3)
C. Bicchi, P. Rubiolo
Dipartimento di Scienza e Tecnologia del Farmaco, Italy

10.00 – 11.00 **Oral presentations**
Pinewood nematode (*Bursaphelenchus xylophilus*) inoculated *Pinus pinaster* and *Pinus pinea*: time-course study of volatiles and enantiomeric evaluation (OP-9)
A.S. Lima, M.D. Mendes, P. Barbosa, D.A. Geraldes, L.S. Dias, M. Mota, J.G. Barroso, L.G. Pedro, A.C. Figueiredo
Universidade de Lisboa, Portugal

Advanced Gas Ghromatographic Techniques for the Quali-Quantitative Analysis of Essential Oils: Fast GC-qMS (20,000 a.m.u./sec) and Multidimensional MDGC-qMS (OP-10)
D. Sciarrone, G. Purcaro, P.Q. Tranchida, C. Ragonese, P. Dugo, G. Dugo, L. Mondello
Università di Messina, Italy

Chemical variation in the leaf essential oils of Australian Corymbia spp. (OP-11)
M.A. Deseo, K.M. Shepherd, M. Abasolo, M. Shepherd, D.J. Lee
Southern Cross University, Australia

11.00 – 11.30 **Coffee Break**

11.30 – 12.30 **Oral presentations**

Essential oil yield-composition hypothesis: could the oil yield give the first insight into its chemical composition? (OP-12)
N. Radulović, P. Blagojević
University of Niš, Serbia

Application of Histochemistry in the Qualitative and Quantitative Identification of Major Monoterpenoid Phenols in Essential Oil Producing Plants (OP-13)
S.H. El-Ahmady
Ain Shams University, Egypt

Application of two-dimensional comprehensive gas chromatography time-of-flight mass spectrometry (GCxGC-TOF MS) in challenging analyses of essential oils (OP-14)
T. Kovalczuk, J. Zrostíková, J. Schůrek
LECO Corp. Application Laboratory Prague, Czech Republic

12.30 – 14.00 **Lunch**

14.00 – 15.00 **Plenary lecture**

Cosmetology: Ageing and Ageing of Elastic Tissue –„Juvenile" Hormones (PL-4)
A. Milewicz
Medical University of Wrocław, Poland

15.00 – 15.40 **Oral presentations**

Biological Activities of Liverworts (Hepaticae) Growing in Turkey (OP-15)
A. Tosun, E. Küpeli Akkol, A. Ludwiczuk, H. Özenoğlu Kiremit, P. Tomek, Y. Asakawa
Ankara University, Turkey

Solubilization of Essential Oils in Surfactants Solutions (OP-16)
B.W. Domagalska
Academy of Cosmetics and Health Care, Poland

15.40 – 16.00 **Coffee Break**

16.00 – 17.30 **Poster Presentation - Session B-D**

17.30 **Meeting time**

19.30 – 23:00 **Symposium dinner** *(Mercure-Panorama Hotel, Pl. Dominikański 1)*

Wednesday September 8, 2010
Building D-20, Wrocław University of Technology, ul. Z. Janiszewskiego 8

9.00 – 10.00 **Plenary lecture**

Biocatalytic methods for the preparation of the single enantiomers of chiral biologically active molecules (PL-5)
E. Brenna
Politecnico di Milano, Italy

10.00 – 11.00	**Young Scientist Session** Biotransformations of (+)-nootkatone by fungal strains and the biological activity of its derivatives (YSS-1) A. Gliszczyńska, C. Wawrzeńczyk *Wroclaw University of Environmental and Life Sciences, Poland* Conmaculatin — a new alkaloid from the essential oil of *Conium maculatum* L. (YSS-2) N. Radulović, N. Djordjević, M. Denić *University of Niš, Serbia* Biological control formulations incorporating essential oils' components (YSS-3) S. Heuskin, S. Lorge, J.P. Wathelet, F. Verheggen, G. Lognay *University of Liege, Gembloux Agro-Bio Tech, Belgium*
11.00 – 11.30	**Coffee Break**
11.30 – 12.10	**Young Scientist Session** Comparison of the composition of essential oils and hydrolate volatiles of *Pinus sylvestris* L., *Rosa rugosa* Thumb. and *Melissa officinalis* L. (YSS-4) A. Maciag, M. Woźniak, D. Kalemba *Technical University of Lodz, Poland* Comparative toxicities of a wild Plant essential oil and blends of its major constituents on mortality and fecundity of spider mites (Acari: Tetranychidae) (YSS-5) S. Attia, K.L. Grissa, Z. Ghrabi, A.C. Mailleux, G. Lognay, T. Hance *Institut National Agronomique de Tunisie, Tunisia; Université Catholique de Louvain, Belgium.*
12.10 – 12.30	**Closing remarks**
12.30 – 14.00	**Lunch**
14.30 – 19:30	**City guided tour** *(meeting point: in front of building D-20)*

Abbreviations

PL	Plenary Lecture
OP	Oral Presentation
YSS	Young Scientist Session
PP	Poster Presentation

OPENING LECTURE

History of 40 years ISEO meetings

Karl-Heinz Kubeczka
Permanent scientific committee, Margetshoechheim, Germany
e-mail: kubeczka@t-online.de

During the very first meeting in 1969 at the University of Leiden in the Netherlands a few enthusiastic scientists came together and discussed analytical and associated problems concerning essential oil research. They decided to meet in the following years annually in connection with the GA (Society Medicinal Plant Research) in order to save travelling costs, because all participants of the first meeting were members of this society.

In 1971, scientists from the essential oil industry joined the meetings and furnished substantial contributions. In the following years the number of participants increased step by step and the more familiar meetings grew up to real symposia with lectures and poster presentations. The results from the meetings 1976 to 1978, 1979 to 1980, and 1984 has been published as books (1,2,3).

Progressively the scope of the meetings has broadened. Although the methods and techniques of essential oil analysis including multidimensional gas chromatography, LC and HPLC and the structure elucidation of the oil constituents by MS, IR, and NMR spectroscopy have been the main topics of the symposia, additionally further topics such as biogenesis and accumulation of secondary products, the distribution of essential oils in the plant kingdom, essential oil formation by tissue cultures, the biological role of terpene glycosides, cytology and physiology of glands in essential oil bearing plants, antimicrobial activities of essential oils, quality control and standardisation of essential oils and data processing by means of computers have been discussed.

During the last 10 to 20 years additional analytical techniques have been discussed in detail such as determination of the enantiomeric composition of oil constituents, comprehensive multidimensional separation techniques in capillary gas chromatography and HPLC, fast and ultra-fast gas and liquid chromatography, different head-space techniques, the use of ATR-IR and Raman methods for analytical characterisation of aromatic plants, authentication of essentials and flavour components by online coupled multidimensional GC and isotope ratio mass spectrometry (MDGC-IRMS), but also toxicological and safety aspects of essential oils and their constituents including national and EU regulations. The lecture will be supplemented by numerous photos of the meetings in the past.

References

[1] Kubeczka, K. H. (ed.): "Vorkommen und Analytik ätherischer Öle", Georg Thieme Verlag, Stuttgart **1979**.
[2] Kubeczka, K. H. (ed.): "Ätherische Öle, Analytik, Physiologie, Zusammensetzung", Georg Thieme Verlag, Stuttgart – New York **1982**.
[3] Baerheim Svendsen and J. J. C. Scheffer (eds.): "Essential Oils and Aromatic Plants", Martinus Nijhoff / Dr W. Junk Publishers, Dordrecht, Boston, Lancaster **1985**.

ISEO Records 1969 – 2010

1st	1969	Leiden, Netherlands
2nd	1971	Freiburg i. Br., Germany
3rd	1972	Helsinki, Finland
4th	1973	Freiburg i. Br., Germany
5th	1974	Freiburg i. Br., Germany
6th	1975	Leiden, Netherlands
7th	1976	Würzburg, Germany
8th	1977	Freiburg i. Br., Germany
9th	1978	Münster, Germany
10th	1979	Würzburg, Germany
11th	1980	Groningen, Netherlands
12th	1981	Marburg, Germany
13th	1982	Würzburg, Germany
14th	1983	Freising – Weihenstephan, Germany
15th	1984	Leiden, Netherlands
16th	1985	Holminden/Neuhaus, Germany
17th	1986	Bevensen, Germany
18th	1987	Hordwijkerhout, Netherlands
19th	1988	Zürich – Greifensee, Switzerland
20th	1989	Würzburg, Germany
21st	1990	Lahti, Finland
22nd	1991	St. Vincent, Italy
23rd	1992	Auchincruive, Scotland
24th	1993	Berlin, Germany
25th	1994	Grasse, France
26th	1995	Hamburg, Germany
27th	1996	Vienna, Austria
28th	1997	Eskisehir, Turkey
29th	1998	Frankfurt, Germany
30th	1999	Leipzig, Germany
31st	2000	Hamburg, Germany
32nd	2001	Wrocław, Poland
33rd	2002	Lisbon, Portugal
34th	2003	Würzburg, Germany
35th	2004	Giardini Naxos, Italy
36th	2005	Budapest, Hungary
37th	2006	Grasse, France
38th	2007	Graz, Austria
39th	2008	Quedlinburg, Germany
40th	2009	Savigliano, Italy
41st	2010	Wrocław, Poland

PLENARY LECTURES

Bryophytes: Chemical Diversity, Synthesis and Biotechnology

Yoshinori Asakawa
Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan
e-mail: asakawa@ph.bunri-u.ac.jp

The present paper concerns with bio- and chemical diversity of the Marchantiophyta, the isolation, structure elucidation of terpenoids, aromatic compounds and lipids of the bryophytes as well as their biological activity and chemosystematics, and the chemical relationships between algae, bryophytes and pteridophytes will be discussed. The bryophytes are taxonomically placed between the algae and the pteridophytes and there are 25000 species: Bryophyta (14000 species), Marchantiophyta (=liverworts) (6000) (Fig.1) and Anthocerotae (300). Almost all liverworts possess beautiful cellular oil bodies (Fig. 2). Over several hundred new compounds have been isolated from bryophytes. 33 new carbon skeletal terpenoids have been found in liverworts. Liverworts are also rich sources of various types of bis-bibenzyl (**1**) which are one of the most characteristic compounds from the liverworts. The most interesting chemistry of liverworts is that most of sesqui- and diterpenoids are enantiomers of those found in higher plants. Some of the isolated compounds from liverworts show characteristic scents, pungency and bitterness, allergenic contact dermatitis, cytotoxicity, anti-HIV and DNA polymerase β inhibition, antimicrobial and antifungal activity, insect antifeedant and mortality, nematocidal activity, superoxide anion radical release, 5-lipoxygenase, calmodulin, hyaluronidase, cyclooxygenase and NO production inhibitory activity, and plant growth inhibition, neurotrophic and muscle relaxing activity, cardiotonic, piscicidal and anti-obesity activity [1-5].

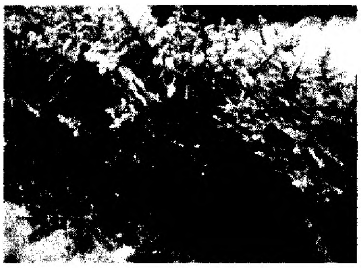
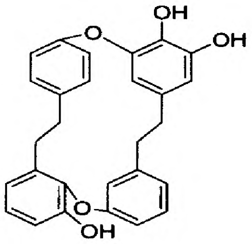


Fig. 1. *Ptychanthus striatus*



Marchantin A (**1**)

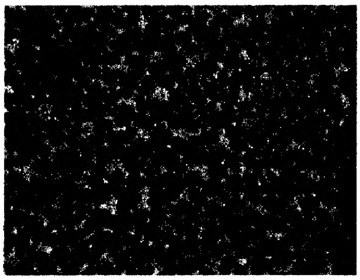


Fig. 2. Oil bodies of *Frullania* sp.

Although the morphological classification of liverworts is difficult, because of their small gametophytes, it has been demonstrated that the secondary metabolites, such as terpenoids and aromatics in their cellular oil bodies can assist in their taxonomic differentiation. Knowledge of chemical constituents of liverworts might serve to delineate not only chemical, but also evolutionary relationships within the Marchantiophyta at the genus or family level [1-3,5]. The hemisyntheses of a few diterpenoids and microbial biotransformation of several sesquiterpenoids isolated from liverworts are also discussed.

References

[1] Asakawa, Y. Chemical Constituents of Hepaticae. In *Progress in the Chemistry of Organic Natural Products.* Vol. 42, 1-285 (**1982**).
[2] Asakawa, Y. Chemical Constituents of the Bryophytes.*ibid.*Vol. 65, 1-618 (**1995**).
[3] Asakawa, Y., Ludwiczuk, A., Nagashima, F. Chemical Constituents of
Bryophytes: Bio- and Chemical Diversity, Biological Activity and Chemosystematics. *Ibid.* Vol. 93 (in preparation).
[4] Asakawa, Y. *Curr. Pharm. Design.* 14, 3067-3088 (**2008**).
[5] Asakawa, Y. *et al..Heterocyles* 77, 99-150 (**2009**).

Essential Oils: Sensory and Instrumental Research Must Go Together

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The analytical procedure for obtaining and characterising essential oils from plants or spices comprises two steps: i) extraction and ii) analysis. The standard protocol for the isolation of essential oils starts with an extracting step, in which different techniques are used, including, for instance steam distillation (Clevenger or Dering apparatus), simultaneous-distillation-extraction (Likens-Nickerson system), vacuum-distillation, etc. The extraction techniques have been drastically improved during the last years, with new techniques significantly improving the quality of the essential oil, for instance by using supercritical fluids (e.g. CO_2), and/or reducing the extraction time (e.g., the use of focused-microwave to generate water vapour can reduce the extraction of rosemary essential oil from 60 min down to 10 min). After isolation of the essential oils from the plant material, solvent, if used, can be evaporated and oils are ready for the characterisation of their volatile composition, especially by gas chromatography (GC) with help of different detectors, mass spectrometry (MS) being the most popular one. The combination "GC-MS" provides researchers with great results and very detailed information on the volatile composition of essential oils. However and even after the use of these highly sophisticated analytical techniques, it is impossible to state, without any doubt, that the quality of the essential oil under analysis/study is of the outmost quality. In many cases, or at least in some cases, researchers involved in the study of essential oils are able to detect, through their sense of smell, differences in essential oils that are not easy to predict just by studying their volatile profiles. Sometimes, volatile compounds can be found in very low concentrations or are unexpected in the oils under study and their presence may not be detected only by using analytical protocols (e.g., off-flavours). Other times, residues of organic solvents, bad adjustment or conditions of the GC system (e.g. old column, low maintenance of detector, etc.), and/or presence of compounds in very high concentrations, can mask the presence of key odorants, which can be present in very low concentrations, but at the same time have very low odour thresholds (detection and/or identification), and therefore can play a predominant role in the oils odour profiles. Consequently, some other techniques are required to improve the knowledge researchers have about essential oils; at this time is when sensory evaluation comes into play. The only way of being completely sure that the distilled or extracted essential oil is free of off-flavours and it is of the expected quality is to "smell" it. However, smelling can be conducted just by the operator doing the analytical analyses (without any training on sensory evaluation) or by an appropriate panel (depending on the objectives, a trained or consumer panel should be used). The first choice (operator) will lead us to subjective results and they will depend heavily on the opinion of just one person. On the other hand, the use of a sensory panel will lead us to objective results based on trained people, who have been subjected to proper selection, training and validation tests, or even to subjective results but based on the opinion of a target segment of consumers.

Sensory tests can be classified into three main categories: i) discriminative, ii) descriptive, and iii) affective. In the first two tests, trained panels are required while in the third one, a consumer (non-trained) panel is used. *Discriminative tests* can be selected when the objective is, for instance, to evaluate whether a new distillation technique yields essential oils with exactly the same quality than the previously used system. An example of this type of tests is the triangle test, in which three samples are presented to the panellists (two of them are equal) and they must decide which sample is the different one. *Descriptive tests* are much more informative but the training of a descriptive panel requires much more time, effort (especially money investment) and product knowledge (composition, manufacturing unit operations, etc.) than for a discriminative panel. An example of descriptive tests could be the Quantitative Sensory Analysis, which describes for instance the flavour profile of dry rosemary samples using attributes such as fresh rosemary, vegetable, pine, herbaceous, balsamic, spice, etc. This particular test is very useful and can be applied to establish relationships among sensory properties/attributes with parameters from the chemical analyses, for instances concentrations of individual volatile compounds or even chemical families (ketones, aldehydes, pyrazines, terpenes, etc.). Finally, if essential oils are going to be used for manufacturing of foods, cosmetics, etc., the opinion of the final consumers is of the outmost importance because they finally will decide whether to buy or not the products. The tests in which the subjective opinions of consumers are sought are called *affective or hedonic tests*. This buying decision will directly affect the success of our product in the market and the survival or not of our company. Therefore, asking consumers whether they like or dislike our essential oils or, even better, the product containing the essential oil is a very good idea to ensure the success of the developed product.

Essential oils have gained wide interest for both the general population and the scientific community, and their main applications are related to the manufacturing of cosmetics (e.g. perfumes), drugs (e.g. insect repellents), and foods (e.g. functional products). Different applications of essential oils in food products manufacturing can be found in the recent literature, for instance use as edible coating of fruits and as functional ingredient in meat products. Many authors have studied the effects of the application of essential oils from different plants/spices to increase the stability and shelf life of meat products. However, no always the essential oils with a higher antioxidant activity, higher total phenolic content (e.g. clove) can be applied to the products, for instance, Bologna sausage, because consumers rejected the flavour of the final products. The too intense and unexpected flavour of the clove essential oil made its use inappropriate for Spanish or Italian meat products. Therefore, if a company launching clove-treated meat products only considers instrumental or analytical parameters, such as antioxidant activity, their product could be a good choice for health-concerned consumers; however, the lack of using sensory tests will lead them to the bankrupt because consumers will not like and buy their products. In this particular case, first the antioxidant activity and other related analytical parameters should be evaluated in different essential oils under study (clove, rosemary, sage, etc.). Then, affective tests should be conducted, using a consumer panel, to study the preference and degree of satisfaction of the potential consumers of these products. If the affected tests rendered positive results, then the development of new food products is finished; however, if the results are not 100% positive, descriptive analysis should be conducted, using a trained panel, to understand which parameters from the essential oil treated food is leading to the product rejection. Finally, essential oils are also used in the formulation of insect repellents, for instance mosquitoes. If the repellent product developed has a disgusting smell, no matter how good its ingredients are in repelling mosquitoes, because nobody will buy and used them.

It can be concluded that sensory analysis will be needed to i) completely characterise essential oils (together with instrumental analyses) and ii) determine the acceptance of consumers of products developed using essential oil-based products, including foods, cosmetics and even drugs.

New trends in the analysis of the volatile fraction of plant matrices

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The plant volatile fraction is an important marker diagnostic not only of their origin and quality, but also of the compositions of other even-non-volatile fractions. The term volatile fraction is a framework involving a range of approaches and/or techniques, which produce samples that, while they may have different compositions, are representative of the volatiles characterising a vegetable matrix, e.g. headspace, essential oils, flavours, fragrances, aromas and extracts prepared through specific techniques. The study of the volatile fraction requires analytical methods and technologies able not only to evaluate its composition exhaustively but also to monitor variations of the volatile profile and to detect trace components characterizing the plant investigated. As a consequence, analytical set-up based on sampling techniques where recovery over time of the components of interest is maximized and analytical techniques reducing analysis time to a minimum are necessary to satisfy the ever increasing request of control analysis of plants to be applied in the food, cosmetic and pharmaceutical fields.

The overwhelming evolution that has taken place in analysis over the last decades has strongly influenced the strategy to be adopted in this field, also thanks to the introduction of a group of new approaches, in particular:

- solventless sample preparation techniques and in particular of headspace sampling based on High Concentration Capability Headspace Techniques (HCC-HS e.g. HS-SPME, HSSE, STE, SE-HSSE);
- fast GC and Fast-GC-Fast-MS and enantioselective GC (ES-GC) and ES-GC-MS analysis;
- new analytical techniques such as comprehensive GCxGC;
- new operative strategies based on approaches (e.g. Analytical Decision Marker (ADM)) or technologies (e.g. Total Analysis Systems (TAS)) developed for other fields (environmental pollution, oils and fats, petrol, etc.) and applied to plant analysis;
- data elaboration strategies producing a higher level of information (metabolite profiling).

This lecture will consist of two parts: a) the first one includes a short overview on the advancement of analytical techniques and on how they can influence strategies and approaches to study the plant volatile fraction; b) the second part discusses more in detail enantiomer GC and GC-MS separation using cyclodextrins as chjral selectors.

Cosmetology: Ageing and Ageing of Elastic Tissue –„Juvenile" Hormones

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One of the most remarkable manifestations of ageing is the evaluation of the „body frame", with loss of tissue elasticity together with a decrease in skeleton and muscle mass density and an increased incidence of osteoporosis fractures. Prevention of this frailty and loss of body elasticity is the major challenge. Interestingly, bone and elastic tissues are mainly formed during development and early adult growth , with a low replacement rate during adulthood.

Aging is accompanied by several age- related diseases, most of them involving tissue disorganization associated with manifestation of hard and soft connective tissue ageing.

It has long been described that the imbalance between formed and resorbed bone is more pronounced with age. This bone turnover is increased at tissue level, while at the same time more bone is removed than subsequently replaced because of osteoblast dysfunction at the cellular level. Besides, calcium is poorly conserved in the body and if it is not re-adsorbed, it is withdrawn from bone, which contains 99% of the body's capital.

Different mechanisms related to vitamin D metabolism explain the high frequency of negative calcium balance in the elderly. It should be noted that muscle mass and bone density are directly related. Women have been shown to be more sensitive than men, perhaps because they have lower level of serum calcitonin, a potent anti-resorptive hormone.

The degeneration of elastic tissues, which are built during development, have consequences for birthing, regulation of pulse pressure atherosclerosis, aneurysmus, hypertension, emphysema, skin wrinkles, degeneration of intervertebral ligaments and degeneration of the Bruch's macula membrane.

Dysfunction of elastic tissues is due to the lack of elastic fibre components (more than 30 proteins are involved in functional elastogenesis) and/or to modifications of elastic fibers (calcification, lipidation , oxidation or glycation). Degradation of elastic fibers is considered, as whole, as factor amplifying the inflammation process and the degeneration-dependent ageing processes.

It is notable that normal ageing and ageing of elastic tissues display common features among the major parameters affecting the maximal lifespan potential (such as the beneficial influence of dietary restriction or the post-translational modifications). Therefore, elastic tissues can be considered as relevant biomarkers of ageing.

It is postulated that one of the major cause of the aging process is the decrease in so-called „juvenile" hormones such as melatonin, growth hormone, dehydroepiandrosterone and its sulphate, estradiol and testosterone. Changes in the endocrine system, including the menopause in women , androgen deficiency in men, loss of skeletal and muscle mass, skin thickness, decrease in growth hormone serum concentrations and increased incidence of type 2 diabetes and thyroid disorders are more common in older individuals. Normal ageing and ageing of elastic tissues display common features among the major parameters affecting the maximal lifespan potential (MLSP) – growth hormone, IGF-1, sex hormones and corresponding downstream signaling factors are potential regulators of MLSP and neoelastogenesis in adult tissues. Elastogenesis mainly occurs until the end of the second decade of life. Then, some genes encoding elastic fibre components are still active (elastin, fibrillin 1), but mRNA stability can be greatly decreased (elastin, fibrillin-1) while others, though necessary, are strongly decreased (elastin cross – linking lysil oxidases) and elastogenesis in adults becomes low or inefficient. Furthermore, in adult tissue, the three-dimensional organization of the numerous elastic fibre components is defective. Among the potential candidates known to re-induce elastic fibres, IGF-1 is recognized to be the most potent at the tissue levels. Others, such as growth hormone, DHEA, sex hormones, TGF-beta, retinoic acid or glucocorticosteroids, are also mentioned, but their effects are still controversial. However, TGF-beta and growth hormone have been demonstrated to be very efficient in inducing elastin synthesis in several models including cutis laxa fibroblasts. Different disorders affecting the formation and evaluation of elastic fibers are due to defective gene expressions or to increased proteolysis and may be regarded as good models of elastic tissues accelerated ageing.

The prevention of frailty and loss of body elasticity is a major challenge; healthy nutrition, physical activity and hormonal therapy, if indicated, can be recommended. Non-pharmacological therapy is focused on climacteric syndrome in women and men, and the results are controversial.

Biocatalytic methods for the preparation of the single enantiomers of chiral biologically active molecules

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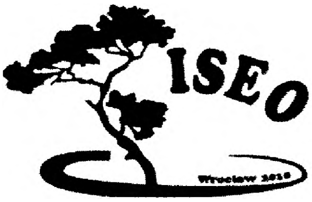
In the last decade the need of biologically active enantiopure compounds for practical applications has been growing increasingly, because the way they act within living organisms depends largely on their absolute configuration. Most of the molecular components of living beings, e.g. carbohydrates and proteins, are chiral, thus they may have different interactions with the enantiomers of chiral compounds, such as drugs, flavours, fragrances, and agrochemicals.

The biological effects of chiral pharmaceuticals have been widely investigated: it has been established that chirality plays a fundamental role both in the pharmacokinetic and in the pharmacodynamic phase. The consequence is that one enantiomer may be therapeutically active (the "eutomer"), and the other one (the "distomer") may show no or toxic side effects. The investigation of the olfactory properties of odorous compounds have highlighted that enantiomers of chiral odorants, flavours or fragrances, can differ either in odour quality, i.e. they can elicit different odour sensations, or in odour intensity. One enantiomer can be nicer and/or more potent than the other one. As for chiral agrochemicals, it may happen that one enantiomer is more persistent in the environment than the other, that one is more target-active than the other, or one is completely inactive, and simply adds an extra pollution load to the environment.

The leading idea is that whenever it is beneficial and profitable, racemic mixtures of chiral compounds that are going to interact with living beings have to be substituted by the stereoisomers, which are effectively responsible for the biological effect.

Synthetic chemists are requested to optimise efficient and highly selective procedures for the preparation of enantioenriched chiral compounds. Among the possible synthetic strategies biocatalysed methods are of great significance and potential, because the enzyme catalysts are highly chemo-, regio-, and stereoselective, and usually avoid protection-deprotection steps. The processes have low energy requirements because of their ambient temperature and pressure conditions. Enzymes are renewable resources and also biodegradable, thus presenting a valuable tool for more environmentally friendly transformations.

The lecture will show the application of enzyme-catalysed methods for the synthesis of the real biologically active stereoisomers of chiral compounds, with particular reference to odorous molecules which are employed in fine and functional perfumery.



ORAL PRESENTATIONS

Terpenoids of 18-21 Million Years Old Japanese Fossil Conifer Plants

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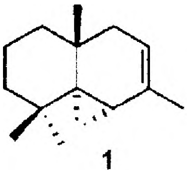
The Noto Peninsula of Ishikawa Prefecture, Japan is one of the rich locations of fossil woods created in the Miocene epoch. Our interest is to identify the species of such fossil plants not only by morphology but also by the biomolecules preserved in ca. 20 million years. Recently we collected two silified woods (No. F1B and F2A) in the two different location as mentioned above. We suggested that both specimens were identified as *Taxodioxylon cunninghamioides* by morphological characters and it was closely related to the present *Cunninghamia and Glyptostrobus* of Cupressaceae *sensu lato* (Taxodiaceae). In order to confirm the presence of biomolecules in these samples, they are carefully crushed by iron hammer and then fine powders obtained were extracted with diethyl ether and chloroform for two weeks. Solvents were evaporated at room temp. at 1 atm to give the crude extracts which were further analyzed by GC/MS.

From F2A, sesquiterpenoids, thujopsene (**1**), widdrol (**2**), 7-*epi*-*α*-cedrene (**3**) as well as cadalene (**4**), and daucalene (**5**) which is the degradated product from thujopsene (**1**) were identified together with an unidentified oxygenated sesquiterpene $[M]^+$ 234 base peak (*m/z* 219) as the major components. The presence of the same sesquiterpenoids (**1**, **2**, **4**) and $[M]^+$ 234 base peak (*m/z* 219) as seen in F2A have also been confirmed in F1B sample. Both samples showed strong sulfuric smell. Indeed, they contained a large amount of S_8, especially in chloroform extracts. Surprisingly both samples preserve a very small amount of monoterpene alcohols, like borneol, *α*-terpineol and verbenone which have been found in a number of the present conifer trees. The Cupressaceae family is divided into two types: *Chamaecyparis* and *Cryptomeria*. The former type belongs to *Chamaecyparis, Thujopsis and Juniperus* and the latter does *Cryptomeria, Taxodium, Metasequoia, Sequoia, Sequoiadendron, Glyptostrobus, Cumminghamia and Thuja*. From the above chemical profiles, it is quite clear that the present silified woods are not *Cryptomeria* like plant since the both crude extracts did not contain the abietane diterpenes and their degradated components. It is noteworthy that both fossils preserved thujopsene (**1**) and its degradated product (**5**) and widdrol (**2**). Thujopsene (**1**) has been identified as the major component in the present *Thujopsis dolabrata* together with widdrol as a minor component. *Chamaecyparis obtusa* also contains thujopsene (**1**) and widdrol (**2**) as minor component. The present chemical data suggests that the silified woods created in the Miocene epoch in the Noto Peninsula might belong to the Cupressaceae.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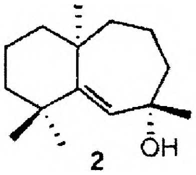

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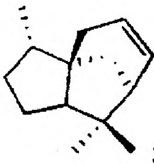
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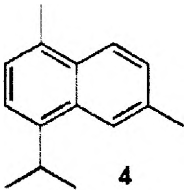
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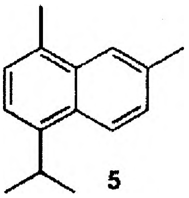
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Essential Oil Bearing Plants from Nigeria

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Nigeria is blessed with abundant flora many of which have not been exploited for their chemical constituents; medicinal, food and industrial potentials. These plants are either introduced or endemic. Many of such trees and herbs are aromatic and therefore have potential for the production of essential oils. In our laboratory, we have investigated essential oil yields, chemical compositions and biological potentials of a wide range of aromatic plants and herbs from Nigeria [1-6]. This paper will discuss on the chemical constituents and biological potential of essential oils obtained from five of such plants. The oil samples were obtained from the plant species by hydrodistillation in an all glass Clevenger-type apparatus and subsequently analysed for their constituents by means of gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). The quantitatively significant constituents of the leaf oil of *Cassia alata* (Linn.) Roxb., (Fabaceae) were 1, 8-cineole (39.8%), β-caryophyllene (19.1%) and caryophyllene oxide (12.7%). Limonene (5.2%), germacrene D (5.5%) and α-selinene (5.4%) constituted the other significant terpenoid compounds present in the oil. The oil also displayed significant anti-inflammatory activities. The volatile oil of *Peristrophe bicalyculata* (Retz) Nees (Acanthaceae) had β-caryophyllene (33.9%), α-zingiberene (10.4%), germacrene D and globulol (ca. 5.0%) as compounds occurring in abundance. Moreover, the oil exhibited cytotoxicity to the MCF-7 (human breast tumor) cells and MDA-MB-468 (human breast tumor) cells. The aerial parts of *Borreria verticillata* (L.) G. F.W. Mey., (Rubiaceae) posses' an abundance of phytol (56.3%) and 1, 8-cineole (20.4%) with sizeable proportion of α-pinene (7.1%) and *p*-cymene (4.0%). Both the oils of *P. bicalyculata* and *B. verticillata* displayed promising *in-vitro* antimicrobial activities to the tested micro-organisms (MIC 12.5-22.3μg/mL). The major compounds identified in the leaf essential oil of *Casuarina equisetifolia* L., (Casuarinaceae) were pentadecanal (32.0%) and 1, 8-cineole (13.1%), with significant amount of apiole (7.2%), α-phellandrene (7.0%) and α-terpinene (6.9%), while the fruit oil was dominated by caryophyllene oxide (11.7%), *trans*-linalool oxide (11.5%), 1, 8-cineole (9.7%), α-terpineol (8.8%) and α-pinene (8.5%). The main compounds identified from the leaf oil of *Ficus elastica* Roxb. ex Hornem., (Moraceae) were 6, 10, 14-trimethyl-2-pentadecanone (25.9%), geranyl acetone (9.9%), heneicosene (8.4%) and 1, 8-cineole (8.2%). The result reinforces the presence of 6, 10, 14-trimethyl-2-pentadecanone as a chemical marker of the essential oils of Nigerian grown *Ficus* species [7]. The antioxidants and antimicrobial activities of *C. equisetifolia* and *F. elastica* would be mention.

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Research into Salvia Essential Oils of Turkey: Recent Results

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The genus Salvia L. (Lamiaceae) is represented in Turkey by 95 species, of which 48 are endemic. As part of a project involving a revision of the genus Salvia we are investigating the chemical composition of Salvia species. In the course of the present work, hydrodistillation of the following Salvia taxa have been investigated by gas chromatography/mass spectromety (GC/MS) and gas chromatography-flame ionization detector (GC-FID): Salvia aethiopis L., S. anatolica Hamzaoglu et A. Duran, S. aramiensis Rech. fil., S. aucheri Bentham var. aucheri, S. aucheri Bentham var. canescens, S. ballsiana (Rech. fil.) Hedge, S. cadmica Boiss., S. ceratophylla L., S. cryptantha Montbret and Aucher ex Bentham., S. cyanescens Boiss. & Bal., S. divaricata Montbret & Aucher ex Bentham, S. euphratica Montbret et Aucher ex Bentham: var. euphratica and var. leiocalycina (rech. Fil) Hedge, S. fruticosa Mill., S. glutinosa L., S. hedgeana Dönmez, S. heldreichiana Boiss. ex Bentham, S. huberi Hedge, S. hydrangea DC. ex Benth., S. kronenburgii Rech. fil., S. limbata C.A.Meyer, S. macrochylamys Boiss. & Kotschy, S. nydeggeri Hub.-Mor., S. pachystachys Trautv., S. pisidica Boiss. & Heldr. ex Benth., S. pseudeuphratica Rech. fil., S. recognita Fisch. & Mey., S. rosifolia Sm., S. russellii Benth., S. sclarea L., S. smyrnaea Boiss., S. wiedemannii Boiss., S.tomentosa Mill., S. verticillata L. subsp. amasiaca (Freyn & Bornm.) Bornm.

Chemical compositions ofthe essential oils of these species will be presented and chemodiversity of Salvia essential oils of Turkey will be discussed based on the previously published data (1).

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The diketonic constituents of *Helichrysum italicum*

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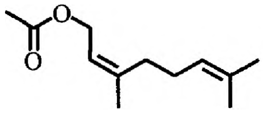
Helichrysum italicum ssp. *italicum* (Asteraceae) is widespread all over the Mediterranean basin. It is sometimes called "curry plant" because of the typical strong spicy smell of its leaves. In the flavour & fragrance industry, this species is used for the production of absolute and essential oil. The composition of the essential oil has been extensively studied, and the main constituent is usually neryl acetate **1**. Despite some controversies, it appears that the characteristic spicy odor of the plant is due to non terpenic 2-methyl-1,3-diketones **2** & **3** which are formally acylated derivatives of pentan-3-one **4** [1] and 4,7-dimethyl-oct-6-en-3-one **5** [2] and have never been observed in other species.



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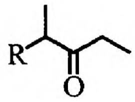

2 : R = R'CO
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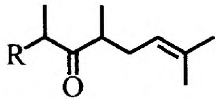

3 : R = R'CO
5 : R = H

Up to now, around ten of such diketones could be identified in the essential oil, but it seems possible that trace amounts of other homologous diketones are also present in the plant. Moreover, the individual contribution of each diketone to the global odor profile of the fresh plant is still unknown. In order to answer to these questions, we synthetised a series of diketones by acylation of **4** and **5**, and studied the distribution of these compounds in the absolute, the essential oil, as well as in the headspace of the fresh wild plant. The olfactive impact of these constituents was determined with the help of GC/O experiments.

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Variability of thujone and other essential oil components of sage (*Salvia officinalis* L.) during ontogenesis

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Salvia officinalis L. is one of the "evergreen" medicinal plants, popular in several national cousines as a spice and gaining importance also as natural antioxidant. Flavouring characteristics are connected to the essential oil, but at the same time its thujone content has been evaluated controversial [1]. Results on the quantitative and qualitative changes of essential oil during phonological phases are scarce. Besides, they either refer to calendar date which is not reproducible concerning ontogenetic effects; or are mixing the effect of ontogenesis and morphological differentiation [2, 3]. The aim of our study was to clear up the accumulation characteristics of most important compounds for quality assurance.

Experiments were carried out in 2009 at two growing sites (Budapest and Vácrátót) in Hungary in perennial sage populations /without cultivar name/. The fully developed leaves were sampled in vegetative stage, budding stage, full flowering and at green seed stage. During full flowering also the flowers were harvested for comparison. Essential oil was distilled after drying according to PhEur, its components determined by GC-MS based on NIST spectrum library and retention indices.

While the essential oil content of the leaves decreased continuously during phenophases (1,71-1,11%), the majority of the components did not show ontogenesis-dependent changes concerning their proportions in the oil. The main compound α- thujon proved to be relatively stable (CV < 20 %). Fluctuations without firm tendencies were detected in the relative percentages of β-thujone (3,3-6,8%), camphor (3,4-13,%), β-caryophyllene (3,0-5,9%) and viridiflorol (9,9-12,4%). The proportion of 1,8-cineole is showing an increasing (10,8-16,2%) while that of α-humulene a decreasing (11,9-6,4%) tendency. Among the identified 41 compounds, the ratio of total sesquiterpenes is slightly decreasing.

Differences between flowers and leaves are more characteristic. Beside the higher accumulation level of the total oil, flowers contain more of 1,8-cineole and viridiflorol. On the contrary by about 50-70% more α-thujone, β-pinene, camphor and by 20% more α-humulene was measured in the leaves.

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High Efficient One Pot Production of Hydrogenated Compounds by Some Fungi

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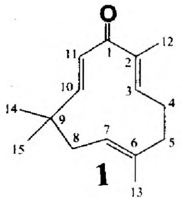
In the continuing study on the microbial biotransformation of terpnenoids and related compounds[1-3] we report the highly efficient one pot production of hydrogenated compounds from zerumbone (1) by *Fusarium culmorum*, β-cyclocostunolide (2) by *Aspergillus cellulosae* IFO4040 and chalcone (3) by 8 kinds of *Aspergillus* spp. including *A. niger* TBUYN-2.

Microorganisms were cultivated in the Czapek-peptone medium at 30oC for 3 days. After full growth of microorganisms 100mg (200ml medium) of 1 was added to the cultured medium of *F. culmorum*. In case of 2, 125mg (200ml medium) was added in the cultured medium of *A. cellulosae*. In case of 3, 20g (200ml medium) was added in the cultured medium of 8 kinds of Aspergillus spp. Biotransformation was carried out for 7-10 days. Each cultured broth was filtrated, and the resulting broth and the mycelium were extracted with ether, and then with ethyl acetate. These extracts were chromatographed on silica gel to give the metabolites whose structures were elucidated by means of spectroscopic analysis (1D and 2D NMR).

Compounds 1, 2 and 3 were stereospecifically hydrogenated to give 4, 5 and 6, respectively. Compounds 4-6 were abnormally folded in the mycelium of microorganisms as a crystal after biotransformation. This phenomenon was observed only in the limited substrates like curcumin, β-carotenoid and sesquiterpene with an α-methyl-γ-butyrolactone.



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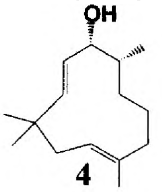

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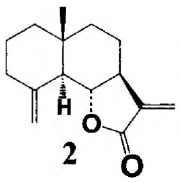

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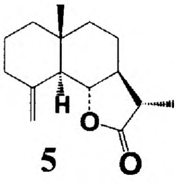

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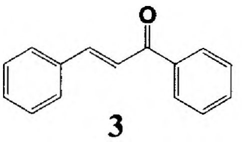

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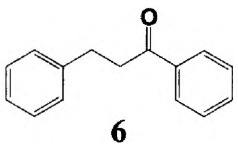

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Application of SPME for the rapid control of spices aroma

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The steam-distillation method traditionally used for aroma isolation prior to GC separation is very efficient in the case of essential oils, although it is time-consuming. Recently solid phase microextraction (SPME) proved to be a very sensitive and also a low-cost, rapid method. It has been utilized for the separation of volatile organic compounds in various food but also for herbs and their essential oils such as dill and caraway, sage, thyme, valerian, basil, oregano, bay and also rosemary.

SPME was used to examine quality and quantity as well as enantiomeric ratio of volatiles in several herbs like dill seeds, rosemary, black cumin, marjoram, thyme. Quantitative measurement was performed on the basis of elaborated calibration curves for odor-active components of these herbs. The correlation of SPME and traditional distillation was calculated. In most cases like carvone in dill seeds and caraway, eucalyptol in rosemary or thymol in thyme the correlation was very high, however not for all the compounds. The concentration of volatiles and their losses during decontamination process of some herbs (caraway, marjoram, coriander, thyme) were also determined by SPME method.

The enantiomeric ratio for chiral compounds was measured by both methods SPME and distillation. The very similar results were obtained. The analysis of variance of the data for a given method (SPME, distillation) did not show significant differences. The correlation coefficient for the estimated compounds in the case of rosemary was not lower than 0.96. Application of this method might be of importance in fast testing when chirality of main odorants is used as a criterion of aroma authenticity.

In the next step SPME was used to examine quality of microencapsulated powders of herbs aroma compounds. It appears useful not only for measuring total aroma content but also, what is more important, for the surface aroma, which is crucial for microencapsulated aromas.

It may be concluded that SPME is suitable, simple and fast method for monitoring herbs aroma quality particularly during technological process.

Criteria for the identification in nature of flavouring substances

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The new Codex Alimentarius Guidelines for the Use of Flavourings (CAC/GL 66-2008) provide principles for the safe use of flavourings and establishes practices that do not mislead the consumer. The guidelines also contain a set of new definitions for flavourings, including what it takes to consider a flavouring substance "natural".

Under the leadership of the "Working Group on Methods of Analysis" (WGMA), composed of industry and association scientists, the International Organization of the Flavor Industry (IOFI) has maintained a programme for the evaluation of the validity of identifications in nature of flavouring substances.

Determining the "nature-identical" status of flavouring substances has been the driver for this programme as until recently it was an important regulatory and labelling consideration in many regions of the globe. However, when referring to flavourings obtained by synthetic means, the new Codex Guidelines do not define "nature-identical" anymore. Instead, in line with general developments in the flavour world, more attention is given to defining "natural" flavourings. For a substance to be considered natural a Codex requirement is that it has been identified/detected in a natural material of animal or vegetable origin. Fortunately, the historic IOFI programme related to the identifications in nature in the context of the "nature-identical" status can now also address the Codex needs as well.

The most important source of information of this programme remains the scientific literature related to the identification of novel flavouring substances in various food products.

The WGMA has published criteria to be met before a decision on the identification status can be made [1,2]. In practice this means that any particular substance must have its identity confirmed by at least two analytical methods, e.g. comparison of chromatographic and spectroscopic data with those of an authentic sample.

In this paper we will discuss the decision criteria and provide examples of recent decisions by the WGMA regarding acceptance or non-acceptance of reported identifications. A general overview of the work of the WGMA will also be given.

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OP-9

Pinewood nematode (*Bursaphelenchus xylophilus*) inoculated *Pinus pinaster* and *Pinus pinea*: time-course study of volatiles and enantiomeric evaluation

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The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is the causal agent of pine wilt disease (PWD), spread by insect vectors, the cerambycid beetles, *Monochamus* spp. PWN is considered one of the most damaging pests and pathogens, of conifer forests worldwide [1].

Previous characterization of the volatiles from two-years-old healthy *Pinus pinaster* and *Pinus pinea* inoculated with the PWN, showed no major differences between the essential oils from healthy plants (HP) and from mechanically wounded uninoculated (C) and inoculated (In) individuals, from both *P. pinaster* and *P. pinea*, 3, 5 and 7 weeks, after inoculation [2]. For this reason, a time-course study was carried out to evaluate essential oil composition 48h to 2 weeks after nematode inoculation. Moreover, the enantiomeric ratio of optically active main oil components from both *Pinus* species was also evaluated. The inoculation procedure, the essential oil extraction and analysis was as in [2].

Although in general terms no relevant qualitative and quantitative differences between HP, C and In oils were found (Table 1), and no new compounds were detected in C or In oils, some quantitative differences were detected during the time-course study at early stages after mechanical wounding and *B. xylophilus* inoculation. In general, but with different ratios, the (-) forms of the main compounds reported in Table 1 were dominant. Macroscopically, only *P. pinaster* individuals exhibited pine wilting.

Table 1. Range of main volatile components (%) isolated from *Pinus pinaster* and *Pinus pinea* HP, C and In plants.

	Pinus pinaster			***Pinus pinea***		
	HP	**C**	**In**	**HP**	**C**	**In**
α-Pinene	25-42	23-40	21-62	11-13	6-14	7-12
β-Pinene	36-52	33-54	23-56	15-17	14-25	13-21
Limonene	2-3	2-3	2-4	54-61	50-63	54-60

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Advanced Gas Ghromatographic Techniques for the Quali-Quantitative Analysis of Essential Oils: Fast GC-qMS (20,000 a.m.u./sec) and Multidimensional MDGC-qMS

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Essential oils are mainly used in food and perfume industries, while several isolated components are also employed for their pharmacological and anti-microbial properties and they can be classified as moderately to highly complex samples. It is well known that analytical time costs may be very high in standard GC applications. In the last few years, fast GC methods have been shown to be suitable for routine laboratory work, even if the coupling with mass spectrometry detection has been a serious problem due to the fast acquisition rate required to achieve a reliable reconstruction of the chromatographic profile. Time of flight mass spectrometry (ToF) has been used since its very high acquisition frequency but the high cost of the instrumentation has strongly limited its diffusion in control quality labs. Quadrupole MS systems are less expensive but are characterized by much lower spectra production rates, because the mass analyzer scans individual ion groups on a *m/z* basis. In the last decade a faster quadrupole mass spectrometer (10,000 a.m.u.) has been introduced enabling the correct identification of unknown compounds, and in some cases, generating a sufficient number of data points for correct peak reconstruction with some limitation due to peak width [1]. In this concern, a new rapid-scanning quadrupole mass spectrometer (qMS) under fast GC conditions, will be presented. The innovative qMS system is characterized by a scan speed of 20,000 amu/sec, and a spectral acquisition frequency of 50 Hz, using a normal mass range. A comparison will be made with the fast GC-MS results obtained employing an established "10,000 amu/sec" qMS instrument.

Nevertheless, a single capillary GC column often proves to be insufficient for complete separation of all the compounds of interest, and therefore there has always been a strong interest in the research of more powerful separation methods. To overcome this problem, many advanced analytical solutions have been studied concerning multidimensional (MDGC) approaches. These chromatographic techniques can be considered innovative methods, only quite recently developed. Since their introduction to the chromatographic community, these techniques have been used in several fields and have gained an excellent reputation as valuable and powerful analytical tools.

The development of a series of methods [2-5] 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. The present contribution is focussed on the most advanced fast and multidimensional chromatographic techniques, coupled with qMS, today employed in essential oil analysis. A series of applications on different essential oil samples will be described in order to demonstrate the effectiveness of these approaches.

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Chemical variation in the leaf essential oils of Australian *Corymbia* spp.

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This study looks into the chemical variation in the leaf essential oil of Australian *Corymbia* species with the goal of establishing the volatile composition as a differentiating parameter of hybrids among and within the species. Leaves from six *Corymbia* species were collected from Grafton Agricultural Research and Advisory Station (GARAS) in northern NSW, Australia, which included four species of the native spotted gums *C. citriodora* subsp. *citriodora* (CCC), *C. citriodora* subsp. *variegata* (CCV), *C. henryi* (CH) and *C. maculata* (CM); *C. torelliana* (CT); and a *Corymbia* complex hybrid (Ctx) with *C. torelliana* and a putative hybrid that was likely to be CT x CH as parents.

The essential oils were steam distilled with cohabation and analysed by GCMS. The leaf oil profiles showed distinguishing characteristics between species. CCC showed a high citronellal content (87%), which distinguished it from the other species that did not contain this monoterpene. CH and CCV are closely related taxonomically and the presence of α- and β-eudesmol in both species at relatively similar proportion (11 to 14%) differentiated these two from the other species. CH had high 1,8-cineole content (31%), which was not present in CCV; and CCV contained 61% α-pinene, which was very low in CH (1%). CM, which is the most distant in the phylogenetic classification of the spotted gums, contained more sesquiterpenes than monoterpenes with 38% of an unidentified sesquiterpene and 12% of 1,8-cineole.

CT showed a more complex chromatographic profile among the native species. More sesquiterpene compounds were present but the major compound is the monoterpene α-pinene (17%). The hybrid species Ctx also showed leaf volatile composition ranging from mono- to sesquiterpenes with 1,8-cineole (16%), terpinene (15%) and *p*-cymene (10%) as major components. There are common terpene components observed between Ctx and the native species CH and CM but principal components analysis showed that Ctx is closer to CH.

Essential oil yield-composition hypothesis: could the oil yield give the first insight into its chemical composition?

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Numerous analysis of the essential oils composition, performed within our research group, and of the appropriate data from the literature pointed our attention to a very frequent correspondence between oil yield and its composition (classes of constituents). A related (yield-composition) hypothesis considering a possible link between oil yield and composition has already been proposed: the oil-poor species of the family Lamiaceae produce essential oils rich in hydrocarbons, with germacrene D often being one of the predominant components [1]. All of this provoked us to further test this class-yield hypothesis by means of multivariate statistical analysis (MVA) of the class distribution (MT - monoterpenoids, ST - sesquiterpenoids, FP - phenylpropanoids, K - carotenoid derived compounds, MK - fatty acids and related compounds, PAC – polyacetylenes, C_6C_2 and C_6C_1 shikimate metabolites) of oil constituents (in percentages; original variables without any recalculation) of 47 different, randomly chosen, plant taxa (divided into two groups), characterized by a wide range of essential oil yields (from 0.002 to 4.5%). The obtained results strongly suggest that the main volatile classes of the oil rich species (yields >0.1%) are MT and PP, while oil-poor species (yields much lower than 0.1%) are dominated by FAD, FAE and/or CD volatiles. Plant species that could be considered as border line cases in respect to the essential oil production (yields ~0.1%) are more heterogenic, as they could have ST, or also MT, FAE, FAD and CD as one of the main classes of volatiles. Moreover, obtained results pointed out to the fact that comparison of plant species, especially those belonging to the same family or genus, using essential oil yield (i.e. ability of the species to produce volatile metabolites) as a criterion, could provide a fast (comparing to time consuming identification of oil constituents) insight into their mutual (evolutionary) relations.

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Application of Histochemistry in the Qualitative and Quantitative Identification of Major Monoterpenoid Phenols in Essential Oil Producing Plants

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Histochemical methods, in combination with various microscopic imaging techniques, have been significantly utilized to study the essential oil secretion in plants [1]. We are interested in implementing a technique developed for the detection of monoterpene phenols using the reagent 4-nitrosophenol in conc. H_2SO_4 [2]. The plant material selected for this study consisted of the leaves obtained from five Lamiaceae species, *Thymus vulgaris, Thymus capitatus, Origanum syracum, Plectranthus barbatus, Mentha piperita* and one *Asteraceae species, Eriocephalus africanus.* These species except for *Mentha sp.* were chosen for their high content of thymol and/ or carvacrol which produce colored indophenols of red and green respectively. In this study, we successfully establish a method for quantifying these compounds which was verified by the analysis of the corresponding plant leaf oils using GC and GC-MS techniques. The micrographs produced for all species show ideal colour reactions except for *Mentha piperita,* devoid of monterpene phenols, which was used as a negative control for the study. This method could serve for a rapid screening of specific oil components that could help distinguish several plant chemotypes in the field, hence saving time spent in laboratory analyses.

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Application of two-dimensional comprehensive gas chromatography time-of-flight mass spectrometry (GCxGC-TOF MS) in challenging analyses of essential oils

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The unique degree of spectral continuity across a chromatographic peak provided by time-of-flight mass spectrometer (TOF MS) has allowed the development of several revolutionary software algorithms. The peak find algorithm effectively locates the position of all peaks in the chromatogram including multiple components in complex co-elutions. The deconvolution algorithm effectively resolves the mixed mass spectra of the co-elution into accurate individual mass spectra for each analyte, including the accurate distribution of signal from masses shared by several components in the co-elution.

Comprehensive two-dimensional gas chromatography (GCxGC) is an advanced technique, which drastically increases chromatographic separation by applying two independent separation mechanisms (e.g. volatility x polarity) for each compound in the analyzed sample. In our experiments GCxGC coupled to TOF MS was applied for the profiling of composition of different Essentials oils.

Essential oils are complex mixtures that can contain hundreds of different compounds. Traditional analyses have been done with one-dimensional GC. The increase in detectability, resolution, and peak capacity offered by GCxGC-TOF MS allows for the potential to characterize a complex sample in a single analysis.

In this contribution the results of the GCxGC-TOF MS analysis of Bulgarian rose oil prepared by different extraction set-ups will be shown and compared to one-dimensional setup under the same conditions. Also the results of different column configurations in GCxGC analyses will be demonstrated with special attention to chiral columns utilization.

OP-15

Biological Activities of Liverworts (Hepaticae) Growing in Turkey

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Nature is the rich source of bioactive secondary metabolites with a great variability of biological effects. The liverworts are the interesting examples of the spore forming plants with distinguish chemical diversity of the secondary metabolites and wide range of biological activities. Liverworts (Hepaticae) are estimated with ~8000 species in 380 genera and 74 families and traditionally separated two morphologically defined classes: Jungermanniopsida and Marchantiopsida [1]. They are considered to be oldest terrestrial plants although no strong scientific evidences have appeared in the literature [2]. Liverworts contain a wide variety of terpenoids and aromatic compounds which elaborates the characteristic cellular oil bodies, and occasionally produce their own peculiar dimeric compounds such as bisbenzyls, dimeric isocuparens having interesting biological activities [3-5].

Recently, we focused on to study with Turkish liverworts either with their chemical constituents or biological activities as well as the other spore forming plants like ferns, mushrooms, mosses grows in Turkey. This current study is deal with GC/MS analysis of the chemical constituents obtained from ethereal extracts of Riccia fluitans L. (Ricciaceae), Porella cordaeana (Huebener) Moore (Porellaceae), Porella platyphylla (L.) Pfeiff. (Porellaceae), Corsinia coriandrina (Spreng.) Lindb. (Corsiniaceae), Mannia androgyna (L.) A. Evans (Aytoniaceae), Reboulia hemisphaerica (L.) Raddi (Aytoniaceae), Plagiochasma rupestre (J.R. Forst et G. Forst) Steph. (Aytoniaceae), Targionia hypophylla L. (Targioniaceae), Conocephalum conicum (L.) Dumort. (Conocephalaceae). The major components of the species will be given in this study. Moreover, cytotoxic activity on ethereal extracts obtained from Turkish liverworts were determined according to the MTT and for this purpose, HeLa, Sp2, YAC-1 cell lines were used. The most active extracts were found, and the results will be presented as % inhibitions rates. On the other hand, the ethereal extracts from the mentioned species given above were evaluated for their in vivo anti-inflammatory and antinociceptive activities. Carrageenan-induced hind paw edema for the anti-inflammatory activity and, p-benzoquinone-induced abdominal constriction test for the antinociceptive activity [6] were used in this study. The extracts of Reboulia hemisphaerica and Plagiochasma rupestre showed the highest activity without inducing any gastric damage for these tests. During the acute toxicity evaluation neither death nor gastric bleeding were observed for any of the plant extracts

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Solubilization of Essential Oils in Surfactants Solutions

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Essential oils (EOS) are important additives in cosmetics, food and pharmaceuticals as well as their components in household cleaning products. EOS are well soluble is various organic solvents but insoluble in water which is basic solvent according to green chemistry principles. To overcome this problem we can use surfactant/co-surfactant solution as the solubilization system [1]. Obtained microemulsions offer features that are attractive for cosmetic, pharmaceutical and food applications, principally, thermodynamic stability, spontaneous formation, transparency and low viscosity [2].

The purpose of this work is to study the influence of cosurfactant (isopropyl and ethyl alcohol, propylene and butylene glycol) as well as nonionic surfactant (Oleth-20) addition on the shape and size of one phase region (oil in water microemulsion) in the Polysorbate 20 (P20)/Mandarin oil/water system. The size of the isotropic region increases as a function of the cosurfactant content but shrank when isopropyl alcohol is replaced with propylene glycol. Addition of Oleth -20 leads to very viscous gel systems. The maximum amount of solubized mandarin oil is 14 % (for oil in water system).

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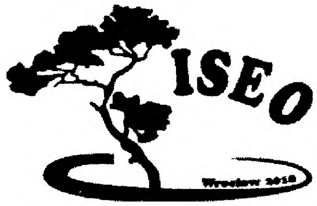

YOUNG SCIENTIST SESSION

Biotransformations of (+)-nootkatone by fungal strains and the biological activity of its derivatives

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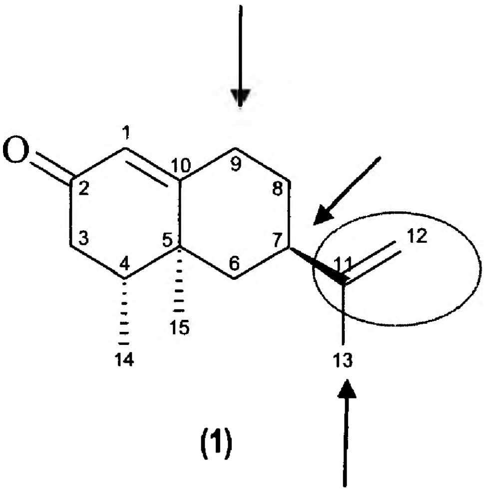
Biotransformations of natural products are very good way to obtain many valuable products that have economical potential to perfume, food and pharmaceutical industries [1,2]. It offers significant advantages over conventional chemical methods. Microbial transformations are also applied for studying the metabolism of natural products. In these aspects the major aim of our studies was the synthesis of new active derivatives of natural sesquiterpene ketone – (+)-nootkatone (**1**). Here we report the microbial functionalization of (+)-nootkatone (**1**) by the fungal cultures: *Botrytis cinerea*, *Didymosphaeria igniaria*, *Aspergillus ochraceus*, *Aspergillus niger MB*, *Chaetomium* sp. and *Fusarium culmorum*. Results of biotransformations of (+)-nootkatone (**1**) allowed us to propose their metabolic pathways in the culture of fungal strains studied. The products of epoxidation of double bond between C11-C12 and hydroxylations of allylic positions at C7, C9 and C13 were isolated from the reaction mixtures. In the next step of our studies the antiproliferative and antifeedant activity not only (+)-nootkatone (**1**) but also its oxyderivatives was investigated.



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Conmaculatin — a new alkaloid from the essential oil of *Conium maculatum* L.

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Conium maculatum L. (hemlock) is a highly poisonous perennial herbaceous flowering plant in the family Apiaceae, native to Europe and the Mediterranean region. In ancient Greece, hemlock was used to poison condemned prisoners. The most famous victim of hemlock poisoning is the philosopher Socrates [1]. Identification of an unknown volatile (RI 1205) constituent of the hydrodistilled fruit essential oil, as well as of the fruit and both leaf and flower alkaline solvent extracts, of the plant species *Conium maculatum* L. (Apiaceae) was achieved in this work. The resemblance of mass spectral fragmentation patterns of coniine (2-propylpiperidine) and of the unknown compound, along with the increment of the retention index of *ca.* 200 units, suggested the possible identity of the compound – 2-pentylpiperidine. 2-Pentylpiperidine was synthesized in 11 reaction steps with the overall yield of 12% starting from cyclohexanol. Gas co-chromatography of the obtained standard with the fruit essential oil of *C. maculatum* unequivocally corroborated the original assumption. This compound, named conmaculatin, can be regarded as a new naturally occurring secondary plant metabolite (alkaloid) representing a dihomolog of coniine (one "acetate" unit extra). The structural elucidation of the target molecule, as well as of the synthetic intermediates, and some by-products, was achieved by detailed spectral analysis (1D and 2D NMR, IR, MS). Along with 2-pentylpiperidine, nine of these represent newly synthesized and characterized compounds.

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Biological control formulations incorporating essential oils' components

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E-β-Farnesene, the alarm pheromone of many aphid species [1] and β-caryophyllene, recently identified as one of the possible component of the aggregation pheromones of the Asian ladybeetles Harmonia axyridis Pallas [2], are considered as two sesquiterpenes attractive for aphids' predators and parasitoids, Episyrphus balteatus De Geer [3,4] and Aphidius ervi Haliday [5], respectively.

In the present research, alginate gel beads formulations were optimised as semiochemical slow-release devices acting in biological control. The sesquiterpenes used in the formulations were purified by flash chromatography fractionation of essential oils of Matricaria chamomilla L. and Nepeta cataria L., for obtaining E-β-farnesene and β-caryophyllene respectively. The purities of the fractions were determined by means of a fast gas chromatograph with a method optimised for a good resolution of terpenes [6] in less than five minutes.

The formulations were evaluated in terms of volatile release capacity, protection efficiency of sesquiterpenes against oxidation, and biological activity towards Episyrphus balteatus and Aphidius ervi.

The experiments lead to the conclusion that the semiochemical formulations are efficient as biological control devices considering the results obtained with biological tests led on predators and parasitoids. Moreover, the sesquiterpenes are more protected when formulated in alginate beads. The devices allow also a slow-release of semiochemicals during at least 40 days depending on physico-chemical parameters (temperature, relative humidity).

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Comparison of the composition of essential oils and hydrolate volatiles of *Pinus sylvestris* L., *Rosa rugosa* Thumb. and *Melissa officinalis* L.

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Essential oils and hydrolates are obtained in the same process of industrial hydro- or steamdistillation of plant material. Both of these products are mixtures of volatile compounds. Essential oils contain the volatiles in pure from. Hydrolates are water containing small amounts of fragrant compounds. Essential oils have been well known and used, e.g. in perfumery, aromatherapy and as food and cosmetic additives. Recently, hyrdolates that revealed some similarities to essential oils in composition and influence on human, are more and more popular and used together with essential oils or instead of them.

The aim of our research was comparison of essential oil and respective hydrolate composition. Both products were obtained at the same process of industrial hydrodistillation from three plant materials: rose flowers (*Rosa rugosa* Thunb.), pine needles (*Pinus sylvestris* L.) and lemon balm herb (*Melissa officinalis* L.). Volatiles from hydrolates were isolated by three different methods: liquid-liquid extraction (LLE), solid phase extraction (SPE) and headspace-solid phase microextraction (HS-SPME). Essential oils and hydrolate volatiles were analyzed by GC-FID-MS.

LLE and SPE were efficient methods of volatile isolation and gave similar quantitative and qualitative results. The content of volatile compounds from hydrolates amounted to about 320 mg/L, 110 mg/L and 290 mg/L for rose, pine and melissa hydrolate, respectively.

Composition of essential oil and hydrolate obtained from the same plant material differed considerably. The main constituents of pine oil were mono- and sesquiterpene hydrocarbons (α-pinene, car-3-ene, δ-cadinene, β-caryophyllene, α-muurolene and germacrene D). The major group of pine hydrolate constituents were oxygenated mono- and sesquiterpenes, mainly alcohols, e.g. terpinen-4-ol, α-terpineol and *p*-cymen-9-ol.

Long chain hydrocarbons (above 25%) and monoterpene alcohols: citronellol and geraniol and their acetates (above 7%) were the main groups of rose oil constituents while β-phenylethanol and methyleugenol dominated as rose hydrolate components. Monoterpene alcohols: geraniol, citronellol, nerol and linalool as well as oxygenated sesquiterpenes: α-bisabolol oxide A, α-cadinol and α-muurolol) were also identified in rose hydrolate.

Lemon balm oil, likewise rose oil was rich in oxygenated monoterpenes, mainly aldehydes: citronellal, geranial and neral, and sesquiterpene hydrocarbons: (*E*)-*β*-caryophyllene and germacrene D. The main volatiles isolated from lemon balm hydrolate were geranial, neral, citronellol, citronellyl acetate and geraniol.

From our investigations new ways of hydrolate applications might result. Hydrolates (by-product of essential oils distillation) can become new valuable raw-materials.

Comparative toxicities of a wild Plant essential oil and blends of its major constituents on mortality and fecundity of spider mites (Acari: Tetranychidae)

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The phytophagous two-spotted spider mite Tetranychus urticae Koch is a ubiquitous species, present worldwide on a large variety of plant families [1]. Since T. urticae resistance to acaricides spread rapidly, biological control tactics are crucial to manage spider mite populations [2]. In this respect, plant-derived essential oil products are a good alternative as they are, in general, considered as minimum-risk pesticides [3]. In that context, laboratory experiments were conducted to assess the toxicity of essential oil of Pteranthus dichotomus a wild-growing plant of Tunisia on females and eggs of the two spotted spider mite T. urticae (Koch).

Essential oil was distillated from Fresh leaves from P. dichotomus and was sprayed on groups of adult females. The susceptibility of these females to P. dichotomus essential oil was tested. A series of dilutions were used to bracket the dose-response range. Tests proved that female mortality increased with essential oil concentration with DL_{50} value of 75µl/l. The percentage of egg-laying inhibition is prominent with doses of 5, 10 and 20µl/l compared to control, while at the same concentrations no mortality was observed.

The analysis of P. dichotomus essential using GC-MS revealed presence of 10 major constituents: alpha-thujene, alpha-pinene, sabinene, Myrcene, 3-carene, ocimene, terpinene-4-ol, pulegone, eugenol and β-eudesmol. For a comprehensive evaluation of the potential of P. dichotomus essential oil as acaricidal, individual and blends activity of these constituents were tested against T. urticae female. Toxicity of blends of different components indicated significant differences among the active and inactive components, with the presence of all constituents necessary to have toxicity near to that of whole P. dichotomus oil. The results showed that natural oil of P. dichotomus and some of its constituents have potential for development as botanical acaricide, at least against T. urticae.

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POSTER PRESENTATIONS

SESSION A

Chemical composition of essential oils

Variation of the composition of *Asarum canadensis* essential oil cultivated under forest canopy

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Asarum canadensis, Wild Ginger or Canada Snakeroot, are a common plant of the maple grove of the center and south-east of Canada (1). The rhizome has a strong flavour of ginger and contains a very aromatic essential oil, which is used in aromatherapy or natural medicine and perfumery (2).

The analyses of the oils were carried out by gas chromatography (GC) and were confirmed by gas chromatography coupled with mass spectrometry (3, 4). The chemical composition of the essential oils of the seedlings of asaret transplanted in maple groves at La Pocatière, Cap-St-Ignace, Pohénégamook and Grondines were followed during three years. The harvest of some plants was carried out to evaluate the growth of the plant during each season as well as the composition of the essential oil of the rhizome.

The chemical compositions of the essential oil of the rhizomes are mainly monoterpenes such as methyl eugenol and linalool. The characteristic of this essential oil as well as the very aromatic notes are conferred by geranyl, bornyl, neryl and linalyl acetate. Methyl eugenol varies from 53% to 73% and the linalool varies from 3.9% to 17.4% according to the age of the plant, the site and the rate of light received.

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Chemical Composition and Antibacterial Activity of Blue Cypress Essential Oil, *Callitris intratropica*

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The wood and bark of *Callitris intratropica* has long been used for medical purposes by the native people of Northern Australia and it is also applied as an insect repellent when burned in a fire [1]. The light blue colour of the steam distilled oil from pine wood chips leads to the assumption of the presence of azulene derivatives or precursors in this oil which are also found in quite larger amounts in the dark blue chamomile, yarrow and in wormwood oil [2]. A chromatographic analysis of the essential *C. intratropica* wood oil from Australia using GC/MS and GC/FID on different capillary columns resulted in the identification of guaiol (13.5%), bulnesol (9.7%) and the β-selinene (7.3%) as main compounds. The antibacterial activity of this oil was tested on 3 Gram-positive and 8 Gram-negative bacteria using agar serial dilution method [3]. The blue cypress oil was effective against the all used strains and most against the Gram-positive bacteria *Brochothrix thermosphacta* and *Staphylococcus aureus* (2 types).

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Composition of essential oil and hydrolate from lemon balm (*Melissa officinalis* L.)

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Melissa L. from mint family (Lamiaceae) is a small genus represented in Europe by three subspecies *M. officinalis* subsp. *officinalis*, *M. officinalis* subsp. *altissima* and *M. officinalis* subsp. *indora*. *Melissa officinalis* L. (lemon balm) is native to the Mediterranean region but now naturalized through Europe and northern Africa and also found as garden escape in North America. In Poland there are several areas where lemon balm is cultivated.

Melissa oil is obtained by hydrodistillation of the leaves or the herb. Low yield of the production process causes the high price of this oil. During distillation except melissa oil, hydrolate is also obtained. These two products can be used in cosmetics, pharmaceuticals and food [1-2].

In our investigation the composition of essential oil and hydrolate from *Melissa officinalis* L. cultivated in Poland was analyzed. Both products were obtained in the same process of industrial steamdistillation. From 500 kg of fresh melissa herb 150 mL of the oil and 130 L of hydrolate were produced.

Essential oil and volatiles isolated from hydrolate by three methods (diethyl ether extraction, SPE, HS-SPME) were analyzed by GC-FID-MS.

The content of volatiles in hydrolate determined by extraction was 289 mg/L. Three isolation methods gave the same qualitative results. Significant differences were observed between composition of *M. officinalis* oil and hydrolate. The main group of essential oil components were monoterpene aldehydes (citronellal 39.6%, geranial 13.5% and neral 8.8%) and sesquiterpene hydrocarbons ((*E*)-β-caryophyllene 9.7% and germacrene D 5.5%). Monoterpene alcohols (citronellol 6.9% and geraniol 0.9%) and their acetates (0.1% and 0.6%, respectively) were also found.

The main volatiles identified in hydrolate extract were geranial (7.6%), neral (5.9%), citronellol (4.6%), citronellyl acetate (7.1%) and geraniol (2.1%).

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Essential oil from different parts and *in vitro* shoots of *Telekia speciosa*

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Yellow oxeye (*Telekia speciosa* (Schreb.) Baumg) is a perennial plant native to Southeastern Europe and Asia Minor. It is the only species belonging to the genus *Telekia* Baumg. Although *T. speciosa* is mostly known as an ornamental not medicinal species, biological activity of secondary metabolites produced by the plant have gained some interest. Sesquiterpene lactones telekin and 2,3-dihydroaromaticin have shown marked antiproliferative activity against human cancer cell lines *in vitro* [1]. Moreover, 2,3-dihydroaromaticin is a potent inhibitor of lipopolisaccharide induced nitric oxide synthesis and a moderate inhibitor of transcription factor NF-κB activation [2, 3].

The essential oil from underground parts of *T. speciosa* have been studied previously and only isoalantolactone (95%) was mentioned as its constituent [4]. The aim of this work was to investigate the chemical composition of the essential oil of aerial and underground parts as well as *in vitro* shoots of this species.

Leaves, flowers and stems of *Telekia speciosa* contained 0.10%, 0.19% and 0.04% (wt./wt.) of essential oil, respectively. The amount of volatiles in the roots (0.41%) was similar to that found in *in vitro* cultured shoots (0.44%).Essential oil was analyzed by GC-FID-MS, leading to the determination of 112 compounds.

The qualitative composition of essential oil from examined plant parts was similar, but we have noticed big differences between quantitative composition of aerial parts, underground parts and *in vitro* samples of *T. speciosa*. The main volatiles of leaf oil were: (*E,E*)-farnesol (21.2%) and (*E*)-nerolidol (17.9%), while isoalantolactone was predominant component of root (62.3%) and flower oil (23.0%). Numerous thymol derivatives were also found, many of them were detected as pairs of homologues. These compounds constituted 4-5% of leaf, root and stem oil of yellow oxeye, and 25-28% of flower and *in vitro* shoot oil. 10-Isobutyryloxy-8,9-epoxythymolisobutyrate was the main thymol derivative in flower oil (20.5%) and *in vitro* culture oil (20.2%).

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Essential oil and hydrolate from *Chamaemelum nobile* (L.) All. flowers

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Chamaemelum nobile (L.) All. (syn. *Anthemis nobilis* L.) also called Roman chamomile, belongs to Asteraceae family and is one of the 130 species within the genus. It is a small, perennial herb, up to 25 cm high, native to northern Europe and West Asia. The Roman chamomile flowerheads are important medicinal material. Infusion from flowerheads are used: for internal use – as a relaxing tea, in digestive problems, colic and in fever, for external use – to treat rashes and irritated skin.

Essential oil from Roman chamomile flowerheads is pale blue liquid (turning yellow on keeping) with a warm, sweet, fruity-herbaceous scent and is used mainly in cosmetic and perfumer industry [1,2]. It is also used in aromatherapy and has got sedative, antidiuretic, anti-inflammatory, antibacterial and antifungal properties. The dry flowers contain from 0.3 to 1.5% of essential oil. The main components of the oil are esters of angelic, isobutyric and tiglic acids, with α-pinene, pinocarvone and *trans*-pinocarveol [1,2,3].

Essential oil and hydrolate from Roman chamomile was obtained from 5 kg of fresh, full blooming flowerheads, collected in August 2009 from organic farm in northern Poland, near Puck. In the same step of industrial steam-distillation (90 min), 8 ml of the essential oil and 15 l of hydrolate (collected in 3 fractions of 5 l every 30 minutes) were obtained. The constituents of Roman chamomile hydrolate were isolated by extraction with diethyl ether and analysed by GC-FID-MS. The content of plant volatiles in hydrolate decreased from 440 mg/l in the first fraction to 71 mg/l in the third fraction.

The main constituents of the essential oil were isobutyl angelate, 2-methylprop-3-enyl angelate, 3-methylpentyl angelate, isoamyl tiglate, α-pinene, *trans*-pinocarveol, isoamyl angelate, pinocarvone and 2-methylbutyl isobutyrate. The main constituents of hydrolate were *trans*-pinocarveol, pinocarvone, 2-hydroxy-2-methylbut-3-enyl angelate and isobutyric acid.

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Determination of the chemical composition of the essential oil from *Pinus sibirica* nuts

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Pine nut (Pinus sibirica) oil is widely used in medicine to tread burns, boils, psoriasis, stomach ulcers, and other diseases. Furthermore pine nut oil can normalize the blood lipid spectrum, has cholesterol-lowering activity, assists the reduction of surplus body mass, as a result corrects the risk factors, lowers arterial pressure etc. [1].

The aim of presented work was to develop effective ways of obtaining oil from P. sibirica nuts and an attempt to analyze the product by GC-MS. In order to obtain oil from pine nuts three extraction methods were used: a Soxhlet apparatus extraction, maceration, and ultrasonic extraction. Three solvent were used for the extraction: *n*-hexane, dichloromethane and methanol. Afterward, the nuts oil was analyzed using at first TLC and then the GC-MS.

Extraction in a Soxhlet apparatus using n-hexane as solvent allowed to obtain the largest amount of oil from the nuts. Polyunsaturated long-chain alcohols, polyunsaturated hydrocarbons and polyunsaturated fatty acids were indentified in pine nut oil. Z,Z,Z-6,9,12-octadecatrienoic acid and Z,Z,Z-8,11,14-eicosatrienoic acid were identified in pine nut oil, and they belong to the group of essentials fatty acids (EFA).

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Essential oil of *Phellodendron lavallei* Dode

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Phellodendron lavallei Dode (*Rutaceae*) is a medium sized, deciduous tree with thick, corky bark, leathery pinnate leaves, yellowish-green flowers and small, black, aromatic fruits. It is native to east Asia. In Europe and North America it is planted in parks as an ornamental tree because of attractive appearance, high resistance to frost, drought, insects and pollution.

Essential oils from fruits, flowers and leaves of *P. lavallei* were investigated. The plant material was collected in Rogow arboretum, flowers in June, leaves in July, ripe fruits in October 2008. Fresh flowers, fresh leaves and fresh ripe crushed fruits were separately hydrodistilled. The essential oils were obtained in yields 1.20% from fruits, 0.10% from flowers and 0.02% from leaves.

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

About 70 components were identified in the oils. Fruit oil and flower oil contained mainly monoterpene hydrocarbons (92% and 96% respectively). The principal constituents of the fruit oil were myrcene (48.9%) and limonene (41.0%), followed by *β*-caryophyllene (2.4%), germacrene D (2.4%) and *α*-phellandrene (1.0%). The main constituents of the flower oil were: myrcene (44.8%), limonene (43.2%), (*E*)-*β*-ocimene (4.1%), *α*-pinene (2.4%), *allo*-ocimene (1.3%). The leaf oil contained monoterpene hydrocarbons (65%), sesquiterpene hydrocarbons (16%) and aliphatic hydrocarbons (10%). Its main constituents were: limonene (27.0%), myrcene (22.1%), heneicosane (7.8%), (*E,E*)-*α*-farnesene (5.6%), *α*-pinene (5.0%), (*Z*)-*β*-ocimene (4.1%), (*E*)-*β*-ocimene (3.2%) and germacrene D (3.2%).

New data of the essential oils of new aromatic species Artemisia from Mongolian Gobi

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The genus Artemisia (Asteraceae) is represented in the flora Outer Mongolia by 103-106 species [1-3]. A literature was searched that the chemical composition of essential oils not been results of previous study. The hydro distilled essential oils obtained dried plant materials were analyzed by GC-MS. An abstract of results in as follows:

Species of Artemisia	Collection place	Main compounds (%)
A.davajamtzii Darji	Dzungaryn Gobi, Baytag-Bogd (september)	1.8-cineol (28.8)., α-thujone (12.9)., camphor (8.53)., β- thujone (6.8)
A.caespitosa Ldb	Southern-Gobi, Gurvan-saikhan (september)	1.8-cineol (31.1)., α-thujone (7.3)., camphor (12.6)., terpin-4-ol (6.8)
A.subchrysolepis Filat	Dzungaryn Gobi, Khovd-Bulgan (september)	camphor (35.9)., β- thujone (20.8)., 1.8-cineol (11.8)., α-thujone (14.9)., camphene (5.5)
A.gorjaevii Poljak	Mongolian Altai, Khovd-Bulgan	camphor (46.5)., β-thujone (9.0)., 1.8-cineol (17.1)., α-thujone (5.3)
A.schischkinii Krasch	Dzungaryn Gobi, Khovd-Bulgan (september)	camphor (56.7)., 1.8-cineol (14.5)., α-thujone (15.4)., sabinene (7.4)
A.mongolica Fisch.ex.Nakai	Middle Khalkh-Gobi (august)	1.8-cineol (14.6)., (Z)-β-ocimene (14.6)., camphor (7.1)
A.gobica (Krasch) Grub	Southern-Gobi, Ush-Mahkhan (september)	camphor (21.2)., 1.8-cineol (15.3)., α-thujone (12.8)., terpin-4-ol (5.2)
A.intricata Franch	Gobi-Gurvan-saikhan, Southern-Gobi (august)	camphor (50.0)., 1.8-cineol (15.4)., camphene (5.1)
A.ordasica Krasch	Eastern-Gobi, Southern-Gobi (september)	(Z)-β-ocimene (19.1)., β-pinene (18.4)., limonene (8.2)., trans-sabinene hydrate (7.8)., α-pinene (7.1)., sabinene (6.7)., p-cymene (5.7)
A.saposhnikova Krasch	Southern-Gobi of Mongolia (september)	β-pinene (18.9)., limonene (9.8)., (Z)-β-ocimene (8.7)., α-pinene (7.5)., p-cymene (6.9)

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New linear ketone from *Chamaemelum mixtum* essential oil

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Chamaemelum mixtum (syn. *Anthemis mixta*) belongs to the *Anthemideae* tribe and *Asteraceae* family, it is a species growing wild on the coastal zones of West Europe and Mediterranean [1]. In Morocco, it is cultivated for its essential oil which was used in folk medicine like aphrodisiac and anxiolytic. To our knowledge, only one study deals with the chemical composition of *C. mixtum* essential oil from Morocco [2]. Among the 50 components identified santolina alcohol (27.3 %) and germacrene D (10.2 %), were reported as main components.

The aim of this work was to investigate Corsican essential oil of *C. mixtum* and to demonstrate the complementarities of analytical techniques like GC, GC/MS-(IE), GC/MS-(CI) and NMR spectroscopy (^{1}H, ^{13}C, 2D experiments) for the identification of a new natural compound.

After analyses of *C. mixtum* essential oil using GC and GC/MS-EI, 77 components which accounted for 80.1% of the total amount were identified. The main components were yomogy alcohol (16.2%), santolina alcohol (12.5%) and artemisia alcohol (12%). However a major compound (9.9 %) remained unidentified because its absence of the mass spectra libraries.

The unknown compound was first isolated by successive column chromatography then its structural characterization was carried out. PCI-CH_4 and -NH_3 mass spectra of unknown compound allowed establishing the molecular mass and suggesting a $C_{17}H_{30}O$ linear ketone. ^{13}C-NMR and DEPT spectra confirmed the occurrence of a ketone with $C_{17}H_{30}O$ formula and show us the presence of two double bonds: one in terminal position and the other in the indeterminate position. The specific alpha-cleavage from ketone gave a huge peak on the EI-MS which allowed us to locate the position of the carbonyl group on the carbon chain. Finally, 2D-NMR spectroscopy (HMBC, HSQC and COSY) gave us complete structure of a new natural compound never described in the literature (figure 1).

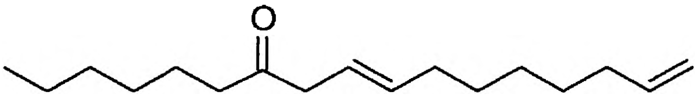

Figure 1. Heptadeca-9,16-dien-7-one, a new linear ketone from *C. mixtum*.

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Chemical investigations of *Xanthium italicum* Moretti essential oil from Corsica

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Xanthium genus belongs to the Asteraceae family and includes 25 species widespread all around the world. In Corsica, Xanthium genus is represented by three species: X. spinosum, X. strumarium and X. italicum (syn: X. strumarium subsp. italicum) [1]. X. italicum (Italian cocklebur) is an annual plant, 30 to 90 cm high with thorny fruits which grows in humid field [2]. The leaves of X. italicum were used in South America and Central America folk medicine to cure carcinoma [3]. Several studies reported the occurrence of sesquiterpene lactones belonging to the xanthanolide group, identified in solvent extracts from the aerial part and the roots of the plant [4-6]. To our knowledge, the chemical composition of X. italicum essential oil was never studied.

The aim of the work was to investigate the chemical composition of the essential oils of X. italicum harvested in different localities of Corsica. The strategy carried out required the pooling of all Corsican oils in a mixture called 擟orsican collective oil66, in order to investigate all CC-fractions and sub-fractions by a combination of techniques including GC/RI and GC-MS.

Integrated analysis of X. italicum oils allowed the identification of 110 components accounting for 90.1 to 95.2% of the total oils. The main components were limonene (16-37.4 %), germacrene D (1.3-15.9 %), borneol (0.3-15.1 %), α-humulene (0.7-14.2 %) and α-bisabolol (0.5-19.0%). Our study showed that Corsican X. italicum oils were qualitatively rather similar but differed by the relative amount of their major components. With the aid of principal component analysis, oil samples could be divided in three clusters discriminated by (i) the amount of limonene which was higher (26.0-37.6%) for the samples of cluster I, (ii) the amount of α-bisabolol which was higher in the oils of cluster II (11.0-19.0 %) and iii) the average amount of borneol which was higher in the oils of cluster III (13.5-15.0 %).

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Evolution of volatile composition during fruit ripening of *Citrus medica* L. var. corsican

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The revival of the market for Corsican citron liqueur and new informations about the medicinal properties of citron essential oil [1] have led us to study the evolution of volatile composition during fruit ripening of *Citrus medica* L. var. *corsican*. To our knowledge, little attention has been paid to assay the chemical variability of *Citrus medica* L. var. *corsican* [2,3] and no data are available on volatile fraction of corresponding alcoholic extracts. The study of influence of fruit maturity on volatile composition could be used by commercial producers to select the most appropriate phytochemical traits and to improve the quality of Citron alcoholic beverages (liqueur and eau-de-vie).

1500 fruits were selected at the bud stage on 100 trees cultivated in the same climatic and cultural conditions. From July to January, twenty fruits were monthly sampled, weighed and measured (height and width). In addition, the fruit maturity was established using the methodology reported by Ometto [4] and modified as follows. Devices were used to measure air temperature every 15 minutes throughout the fruit ripening. Changes in temperature during the day allowed to determine the accumulated growing degree-days (GDD), that were calculated using minimum air temperature (Tm) and maximum air temperature (TM), with a lower base temperature (Tb) of 12.8°C and an upper threshold temperature (TB) of 36°C.

The chemical composition of essential oils and extracts from peels was carried out by GC and GC/MS. In both matrices, the chromatographic profiles characterized by 29 compounds were qualitatively and quantitatively similar. Indeed, the major components were limonene, γ-terpinene, nerol, neral, geraniol and geranial. However, quantitative differences were reported during fruit maturation; the essential oil and alcoholic extract compositions evolved in the same way. Thus, the samples harvested on July exhibited the lower concentration in γ-terpinene and the higher concentration in oxygenated monoterpenes whereas the volatile fraction of samples from August to January was qualitatively and quantitatively closed.

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Quality assessment of Corsican chestnut honey (*Castanea sativa*) using melissopalynological and volatile composition analysis

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Corsica Island is characterized by the richness of polliniferous and melliferous resources which provide honey productions throughout the year. The diversity of Corsican honeys is recognized by "Protected Designation of Origin" denomination: "Miel de Corse-Mele di Corsica", divided into six categories. Data analysis of melissopalynological and sensorial characteristics has been used to characterize the geographical and botanical origins of Corsican Honeys [1]. The honeys dominated by *Castanea sativa* are therefore defined as one of the major types: "miels de la châtaigneraie". In Corsica, as in many other European countries, chestnut is one of the best sources of nectar and pollen for honeybees [2], at the beginning of summer. However, chestnut is widespread in the whole island as mixed grove around villages or dense forests from 600 to 900m.

The aim of this work was to establish the first typology of volatiles from Corsican chestnut honeys. The relationship between volatile fractions of honey and those of *Castanea sativa* nectar was also determined.

In the current study, 50 Corsican Chestnut honeys from 2003 to 2009, representative by their geographical distribution were selected according to their melissopalynological profile. Thus, two distinct types of pollen spectra dominated by *Castanea sativa* were reported corresponding to "monofloral" honeys or more complex honeys. Furthermore, analysis of pollen spectrum has allowed certifying their geographical origin by highlignting the typical species associations which distinguishes them from other European chestnut honeys [1, 3, 4].

The volatile compositions of honey samples and *Castanea sativa* nectar were studied using HS-SPME, GC and GC-MS. The volatile fraction of honey was characterized by 39 components amounting to 68.1-89.4% of the total composition. The main compounds were 2-aminoacetophenone, benzaldehyde, acetophenone, furan-3-carboxaldehyde, octanoic acid and nonanoic acid. Finally, the nectar from *Castanea sativa* flowers harvested on eight Corsican locations showed acetophenone, nonanal, methyl salicylate and linalool as main components.

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Characteristic Volatile Components of Japanese Sour Citrus Fruits, Yuzu, Sudachi and Kabosu

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There are several unique sour citrus fruits with pleasant flavour in Japan, which are processed into vinegar and used as seasonings and dressings. In this study, volatile components of both peel and juice of three major Japanese sour citrus fruits, yuzu (*Citrus junos* Sieb. ex Tanaka), sudachi (*Citrus sudachi* Hort. ex Shirai) and kabosu (*Citrus sphaerocarpa* Hort. ex Tanaka) were investigated using solvent assisted flavor evaporation technique following solvent extraction, respectively.

Limonene was the most abundant monoterpene hydrocarbons in all the extracts, followed by γ-terpinene and *β*-phellandrene in yuzu and sudachi, myrcene in kabosu. Mintsulfide was newly identified in juice extracts from yuzu and kabosu. Among oxygenated components of these extracts, linalool was the most abundant in both peel and juice of yuzu and peel of sudachi, while both peel and juice of kabosu revealed the presence of more saturated aliphatic aldehydes. Wine lactone and rose oxide were identified in all the extracts, which haven't been previously reported to occur in these citrus species.

The odor active constituents of each oxygenated fraction were revealed by aroma extract dilution analysis. As regards yuzu, linalool, (6*Z*,8*E*)-6,8,10-undecatrien-3-one and (*Z*)-methyl epijasmonate showed high flavor dilution factors in peel, while linalool, (6*Z*,8*E*)-6,8,10-undecatrien-3-one and wine lactone in juice. As for sudachi, linalool, (6*Z*,8*E*)-6,8,10-undecatrien-3-one, (2*E*)-4,5-epoxy-2-decenal, wine lactone and *β*-citronellol in peel, while wine lactone, (2*E*)-4,5-epoxy-2-decenal, ethyl butyrate, *cis*-rose oxide and (*Z*)-1,5-octadien-3-one in juice. And as for kabosu, linalool, (2*E*)-4,5-epoxy-2-decenal, (*Z*)-4-decenal, octanal, geraniol and wine lactone in peel, while wine lactone, linalool and eugenol in juice.

The high enantiomeric excesses of linalool were determined using multidimensional chiral GC/MS, resulting in the fact that (*R*)-enantiomer was predominant in yuzu and (*S*)-enantiomer in sudachi on the contrary. Only (3*S*,3a*S*,7a*R*)-enantiomer of wine lactone was detected in these citrus species, which gave intense sweet and coconut-like aroma [1].

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Essential oil composition of *Teucrium divaricatum* Sieb. subsp. *villosum* growing wild in Lebanon

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Teucrium divaricatum Sieb. subsp. *villosum* (Celak.) Rech. fil. is a subshrub that belongs to the section Chamaedrys Schreber. It is known in traditional medicine in Lebanon as the infusion of the plant in flower is mainly used as a stomachic. In case of fever and common cold, the infusion is used orally but also in association with inhalation of the steam. It is mixed with boiled water and sugar to form a refreshing beverage. The leaves are used in cooking as a spice. Externally, the infusion of the plant is used as cicatrisant.[1] *T. divaricatum* is characterized by the presence of neo-clerodane diterpenoids[2] and essential oils[3]. The subspecies *divaricatum* was showed to exert antiulcer activity in rats. A survey of the literature revealed that no studies on the chemical composition of the essential oil of *T. divaricatum* ssp. *villosum* growing in Lebanon had been undertaken. For these reasons, we have reputed that it may be of a certain importance the study of volatile components of *T. divaricatum* and the evaluation of the antimicrobial properties of this species.

The oil from air-dried and ground aerial parts of plants was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus as described before[4]. The sample yielded 0.27% of yellowish oil (w/w), with a pleasant smell. The oil was dried over anhydrous sodium sulphate and stored under N_2 at +4°C in the dark until tested and analysed.

The analysis of volatile constituents by GC and GC-MS led to the identification of 60 compounds representing 94.5% of the total oil. On the whole, the oil was constituted mainly by sesquiterpenes (64.6%), particularly sesquiterpene hydrocarbons (50.0%). In this fraction (E)-caryophyllene (30.1%) was the most abundant. Among the 9 oxygenated sesquiterpenoids, caryophyllene oxide was present in good amount (6.1%). The in vitro activity of the essential oil against some microorganisms in comparison with chloramphenicol by the broth dilution method was determined. The oil exhibited a good activity as inhibitor of growth of Gram + bacteria.

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Composition of the essential oils in the different plant parts of wild parsnip (*Pastinaca sativa*) from the urban area of Vienna, Austria

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The area of the city of Vienna (Austria) is in the range of four phytogeographical regions, harbouring plants with central European, subcontinental, pannonic and submediterranean distribution. A research project funded by the City of Vienna is investigating the variability and biodiversity of aromatic plants in this region.

Wild parsnip (*Pastinaca sativa* L.) is a very common species from the Apiaceae family distributed troughout Europe, growing mainly on fresh, rich meadows. Uses in folk medicine include the treatment of fever and diseases of the lung, digestive system and the kidney [1]. Cultivated forms with an enlarged tap root (var. *sativa*) are used as vegetable.

The present work reports the composition of the essential oils from the different plant organs from plants collected at two distinct sites in the urban area of Vienna, Austria.

The root oil was dominated by myristicin (50%) and α-terpinolene (36%). Myristicin was also the main oil compound in the stems (64 and 40%) followed by palmitolactone (hexadecanolide) (21 and 36%). One of the leaf oils was very high in myristicin (67%) with palmitolactone (8%) and Z-β-ocimene (6%) as minor compounds while the other leaf oil displayed myristicin (21%), palmitolactone (21%), octylacetate (22%) and octylbutanoate (19%) in approximately equal proportions. The fruit oils were characterised by octylacetate (41 and 43%) and octylbutanoate (36 and 32%). Hexylbutanoate (4-5%), myristicin (5-8%) and palmitolactone (2-5%) were minor compound of the fruit oils. The main compounds reported here were already documented for *P. sativa* but in comparison to previous studies [2-4], the present oils appeared low monoterpenes.

Parsnip is a species which produces furocoumarins [2], but these compounds were not present in the distilled oils.

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Chemotaxonomy of Serbian Teucrium species inferred from essential oil chemical composition: the case of Teucrium scordium L. subsp. scordioides (Schreb.) Maire & Petitm (Lamiaceae)

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Teucrium scordium subsp. scordioides is a perennial plant of the Lamiaceae family, growing in South and South-East Europe, Middle East and North Africa on moist places and in swamps [1]. Phytochemists paid very little attention to this taxon. There are two reports on this subject and concern the obsolete detection, but not the identification of alkaloids [2] and the more recent screening work on polyphenols [3], while the essential oil has never been studied previously.

Volatile constituents of T. scordium ssp. scordioides, T. polium and T. montanum were investigated by GC and GC-MS. A total of 297 constituents were identified, representing 89.8-98.4% of the oils. The oils of T. polium and T. montanum consisted mainly of sesquiterpenes (64.3 and 72.7%, respectively), with germacrene D (31.0%) and δ-cadinene (8.1%) as the main constituents, respectively. On the contrary, menthofuran (11.9%), a monoterpene predominated in the oil of T. scordium subsp. scordioides, and this clearly distinguished this species from the rest of the Teucrium taxa investigated up to date.

Chemistry of the volatiles of eight taxa from Serbia and Montenegro belonging to this taxonomically difficult genus [4–6] were compared using multivariate statistical analysis. Namely, the significance of variation in essential oil production among the investigated species provided support for the differentiations of Teucrium taxa at both infrageneric and infraspecific level. The volatile composition of investigated Teucrium species was in agreement with the conclusions of Tzakou [7], who emphasized that, "even though the main components vary, the sesquiterpene fraction is usually the dominant one". A taxon that seems not to follow this rule is T. scordium subsp. scordioides. This is the case where chemistry provides more convincing evidence for taxa delimitations than the classical morphological approach, and perhaps the balance tipping argument in favor for T. scordioides rather than T. scordium subsp. scordioides.

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New fragranyl esters from the essential oil of *Tanacetum coccineum* (Willd.) Grierson

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We have recently come across an essential oil sample of an ornamental plant, *Tanacetum coccineum* (Willd.) Grierson, belonging to the Compositae plant family and through a routine GC-MS analysis tentatively identified the two major contributors of the oil as fragranol and fragranyl acetate. A literature survey conducted revealed that this rather rare irregular plant monoterpenol occurs only in the Compositae (except for two papers dealing with the volatiles of *Geranium tuberosum* [1] and *Stachys recta* [2]). More precisely speaking, this alcohol was reported only for three genera within the Asteraceae- *Achillea* [3-9], *Artemisia* [10-12] and *Tanacetum* [13-14], taxonomically highly mutually related. Column chromatography of a portion of the oil provided pure samples of the aforementioned alcohol and its acetate, and made possible their structure elucidation by ^{1}H and ^{13}C NMR. A more detailed GC-MS analysis of the oil, pointed out to the presence of a series of fragranyl esters, namely the formate, propanoate, butanoate, 2-methylpropanoate, pentanoate, 2-methylbutanoate, 3-methylbutanoate, hexanoate, heptanoate, 6-methylheptanoate, octanoate, nonanoate and benzoate. The last five compounds represent new natural products. The structure of fragranyl benzoate was confirmed by synthesis. The treatment of fragranol with benzoyl chloride in dry pyridine afforded the new ester in sufficient quantity to allow full spectral characterization (^{1}H and ^{13}C-NMR, IR, UV, MS). The identity of all other esters was confirmed by means of gas co-chromatography of the oil with samples of the authentic esters acquired through microreactions of another batch of fragranol (obtained through the alkaline hydrolysis of fragranyl acetate) and the corresponding acids and *N,N'*-dicyclohexylcarbodiimide in the presence of 4-dimethylaminopyridine in dry dichloromethane. It is important to note that neither the epimer of fragranol (grandisol), that is also believed to be a plant constituent [3], nor its derivatives were detected in our oil.

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Volatile constituents of the essential oils of *Satureja kitaibelii*

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Herein, we present the results of GC and GC-MS analysis of the chemical composition of the essential oils of *S. kitaibelii* fresh above-ground parts (endemic species of the Balkan Peninsula) taken from the locality of Visočka Ržana (Stara planina), Devojački grob (Suva planina) and Sićevačka klisura (surrounding of Niš, Serbia). The yield of the essential oils of the fresh over-ground parts of *S. kitaibelii* was 0.06 % (middle value) and indicates that this plant species of the genus *Satureja* is the low content oil-type species. In the essential oils of the fresh above-ground parts of the analyzed populations 181 components were identified (79 in trace amount), which represents 95.52-99.51 % of the oil. The monoterpenes (approximately 3/4 of the oil) were the dominant compound class in the analyzed oils. The ratio of oxygenated monoterpenes and monoterpenes hydrocarbons was approximately 4 : 1. The quantity of sesquiterpenes was 1/5 of the oils. Geraniol (1/3 of the oil), was the predominant component of the investigated essential oils. Limonene, linalool and caryophyllene-oxide were the dominant components in the significant quantities, while geranyl-acetate was dominant in relatively significant quantity in the oil of the population on the locality of Sićevačka klisura. Our results suggest that *S. kitaibelii* oil should be classified in so called monoterpene-alcohol type, particularly taking into account the fact that the analyzed populations contain large quantities of geraniol. This work, for the first time, presents composition of the essential oils of *S. kitaibelii* in the phase of blossoming from mentioned localities.

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A detailed analysis of essential oils of *Lamium garganicum* L. and *Lamium maculatum* L. from Serbia

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The genus *Lamium* L. (Lamiaceae) comprises about 40 species, distributed in Europe, Asia and Africa [1]. Eight species of *Lamium* are reported in Flora of Serbia [2]. The chemical composition of the oils obtained from two *Lamium* wild-growing species: *L. garganicum* and *L. maculatum* was determined by GC and GC/MS. Oil constituents were identified by comparison of their linear retention indices (relative to C_7-C_{28} alkanes on the HP-5MS column) with literature values [3] and their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3, and a homemade MS library with the spectra corresponding to pure substances and components of known essential oils, and wherever possible, by co-injection with an authentic sample. GC (FID) analysis was carried out under the same experimental conditions using the same column as described for the GC/MS. The percentage composition of the oils was computed from the GC peak areas without any corrections. *Lamium garganicum* had a total of 172 compounds identifiable being 5% of monoterpenoids, 34.2% of sesquiterpenoids, 18.9% of diterpenoids, 34.2% of fatty acid derived compounds and 5.2% of other molecules. *Lamium maculatum* oil sample had a total of 99 compounds identifiable being 1% of monoterpenoids, 72.2% of sesquiterpenoids, 6.6% of diterpenoids, 15.5% of fatty acid derived compounds and 2.4% of other molecules. Essential oil chemistry of *L. garganicum* and *L. maculatum* have been studied previously [4,5], however, this report gives more detailed composition on volatiles profile. Additionally, this would be the first report on the diterpenoid fraction detected in *Lamium* oil sample.

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Characterisation of volatile profile of *Stachys recta* L. subsp. *baldaccii* (K. Malý) Hayek var. *chrysophaea* (Pančić) Hayek from Serbia

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Stachys L., one of the largest genera of the Lamiaceae family, contains more than 270 species. This subcosmopolitan genus has two main centers of diversity in the Old Word area [1]. One is confined to S. and E. Anatolia, Caucasia, N.W. Iran and N. Iraq, and the other to the Balkan Peninsula. Serbia is an area that is not very rich in taxa belonging to the genus, however, eight species are recognized as Balkan, or even narrower regions, endemics [2]. *Stachys recta* L. subsp. *baldaccii* var. *chrysophaea* (*sin. S. chrysophaea* Pančić) is Balkan endemic that almost exclusively grows on serpentine bedrock. It belongs to polymorphous *Stachys recta* complex in which numerous taxa are recognised. The systematics of this group is difficult, since the diversity is caused by a prominent taxa variability and their ecological plasticity. The implied subspecies volatile constituents have never been investigated previously, and here we report on the chemical composition of the hydrodistilled essential oil. The GC and GC-MS analysis of the oil resulted in the identification of 159 components. The major constituents were 1-octen-3-ol (12.4%), (*Z*)-3-hexenol (8.5%), (*E*)-phytol (8.3%) and myristicin (6.9%). The fatty acid derived compounds represented nearly one half of the oil (47.1%), while the terpenoid fraction made up a third (25.8%), and the carotenoid derived compounds only 2.2% of the oil. Additionally, principal component analysis and agglomerative hierarchical clustering of the up to now available data (essential oil volatiles) on the taxa belonging to *Stachys recta* complex have been preformed in order to observe potentially distinctive characters at subspecies level.

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PP-A21

The volatile profile of *Hypericum annulatum* Moris (Hypericaceae) essential oil from Serbia*

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Hypericum L. (Guttiferae/Clusiaceae/Hypericaceae) is a genus represented by ca. 400 species, widespread in warm-temperate areas throughout the world, as well as on the Balkan Peninsula [1]. Plants of the genus *Hypericum* have traditionally been used as highly esteemed medicinal plants [2]. In this study, chemical composition of the essential oils of European taxa belonging to the *Adenosepalum* Spach section (*Hypericum annulatum* Moris is a member of this section according to Robson[3] and Flora Europaea[4]) or collected from the same region (southeastern Serbia) as *H. annulatum* were compared. The essential oil of fresh aerial parts of *H. annulatum* obtained by hydrodistillation was analyzed by GC and GC/MS. One hundred and sixteen constituents identified accounted for 98.9% of the total oil. The major components of the oil were: α-pinene (56.3%), (*E*)-β-ocimene (10.2%), undecane (6.3%), myrcene (4.3%) and β-pinene (4.2%). The oil was characterized by a large amount of terpenoids unevenly distributed among mono- (79.5%) and sesquiterpenoids (8.6%). Monoterpene hydrocarbons, dominated by molecules possessing pinane (60.7%) and acyclic (14.5%) skeletons, had the highest over all contribution (77.0%). The oxygenated monoterpene fraction amounted to only 2.5%. The distribution of volatile compounds within sesquiterpene fraction was also in favor of hydrocarbons compared to oxygenated derivatives (7.9 and 0.7 %, respectively). To the best of our knowledge the essential oil of *H. annulatum* has not been studied previously. The volatile profile of *H. annulatum* differs significantly from that of the rest of the taxa belonging to the section *Adenosepalum* as well as from previously investigated *Hypericum* species growing in the same region (southeastern Serbia) [5].

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Volatiles of four *Parmeliaceae* species growing on the same host tree

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The volatiles of Parmeliaceae lichens: *Hypogymnia physodes*, *Evernia prunastri*, *Flavoparmelia caperata* and *Parmelia sulcata*, growing on the same host tree (Prunus sp.), on the same locality and harvested at the same time were analyzed by GC and GC-MS. All obtained chromatograms of the examined species showed only few prominent peaks corresponding to components present in significant amounts (i.e. more than 10%): E. prunastri 4, both H. physodes and P. sulcata 3 and F. caperata 2 constituents. Orcinol and atraric acid are the major compounds of E. prunastri extract detected in approximately equal quantities comprising together 55.1%. Orcinol derivatives with the aldehyde group in the vicinity of the two phenolic groups, atranol and methyl haematommate, were also present but in smaller quantities, as well as the orcinol monomethyl ether. Among coupled phenolic compounds only the dibenzofurane derivate usnic acid was identified.

Beside atraric acid, which was present in all examined samples in considerable amounts, H. physodes extract was composed of olivetol (33.5%), olivetonide (15.7%) and olivetonic acid (7.7%). Usnic acid was not detected in this extract. However it dominated among the F. caperata volatiles representing approximately two thirds of the extract (63.2%).

P. sulcata extract differ from the other by a high content of vitamin E (24.7%) and three sterols comprising together 16.9%. Methyl ester of β-orinolcarboxylic acid (atraric acid) was the most abundant mono-aromatic compound followed by atranol.

Even growing on the same substrate and under same conditions, lichen species under investigation showed different GC-MS profiles indicated that synthesis of identified volatiles was genetically determined and could be used for their characterisation.

Keywords: Hypogymnia physodes; Evernia prunastri; Flavoparmelia caperata; Parmelia sulcata; usnic acid; olivetol; atraric acid; orcinol

Chemical Characterisation of Hungarian Wild Chamomile (*Matricaria recutita* L.) Populations

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In order to evaluate the quality parameters and to prepare the preservation of the genetic resources of chamomile, 50 wild growing populations in the characteristic Hungarian collection areas (meadows, pastures, ploughland, etc.) were examined in spring of 2009.

We measured both the total flavonoid content of the dried flowers, and their mucilage content by swelling value according to PhEur [1]. The essential oil content was determined by water distillation and the component spectrum by GC-MS method based on standard compounds and retention indices.

The results indicated a considerable chemical variability of the Hungarian populations.

Total flavonoid content varied between 0.94 and 2.28% (CV=22%), while the most frequent values were found between 1.25 and 1.75% (in 56% of the populations).

The swelling value proved to be between 15.8 and 80.8 (CV=41%), however, most abundantly between 20 and 40.

Accumulation rate of the essential oil of the different populations varied between 0.30 and 0.88 g/100g (CV=21%), while the samples (62%) showed 0.5 - 0.7g/100g. Only two populations were not able to fulfill the minimum requirements (0,4%) of PhEur.

The major compound of the essential oil was bisabolol-oxide A in 56% of the populations. The second most frequent main componund was α-bisabolol, in 36% of the populations. In about 20% of them, α-bisabolol and its oxides were found approximately in equal proportions.

The ratio of bisabolol-oxide A varied between 0.6-56.5% of the oil, however, most frequently it was found in a range of 10-40% (CV=60%). Only a single population was free of this compound.

The proportion of α-bisabolol varied between 6.8-71.3% of the oil (CV=53%), but most often found values lay in the range of 10-30%.

The marginal values of chamazulene were 5.4 and 19.7% of the oil (CV=27%), but 58% of the the populations showed values between 8-12%.

Proportion of bisabolol-oxide B generally lay between 2.1 and 22% of the oil (CV=45%), however the majority (42%) of the populations contained 6-12%.

Other components identified in the essential oils as â-farnesene (1-6.3%), cis-dicycloether (3,9-23,3%), trans-dicycloether, germacrene-D, bicyclogermacrene, epi-â-bisabolol, alloaromadendrene, nerolidol, l-epi-cubenol, â-eudesmol showed less correlation with the origin of the sample.

This high degree of chemical variability confirms the importance of quality assurance. For this reason, EU origin protection has been achieved for the special Hungarian quality chamomile product "Puszta gold" (wilding plain chamomile flower).

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Essential oil composition of SELECTED *Thymus vulgaris* L. clones belonging to five chemotypes

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Garden thyme (*Thymus vulgaris* L.) is an aromatic herb originating from the West-Mediterranean. Six chemotypes has already been described in southern France, in natural habitats, based on the chief monoterpene compound of the essential oil [1]. The essential oil (*Thymi aetheroleum*) and the drug (*Thymi herba- Ph. Hg.* VIII.: min.1.2 ml/100g essential oil), being rich in phenolic monoterpenes (thymol, carvacrol) is used for therapeutic purposes as well as in the food industry, where the characteristic thymol-like aroma is also expected [2]. The significance of the other essential oil varieties of garden thyme was only partly examined.

In our work, ten selected clones of garden thyme, propagated by softwood cuttings in the autumn of 2007, were studied. Their parent populations had been established by seeds, originating from seed exchange, at our Research Station in 2002. As a result of the several years' evaluation of these populations, we have found a considerable level of chemical polymorphism. Finally, ten accessions of high (2-4 ml/100g) essential oil producing ability were selected and classified into five chemotypes [3]. Concerning the dominant monoterpene of the essential oil, clones TV2, TV17, TV135 and 'Deutscher Winter' (used as a control) represented the thymol ('T') chemotype, while No. TV121 and TV127 were involved in the linalool ('L') one. Clone TV143 was grouped into the α-terpineol/ α-terpinyl acetate ('A') chemotype, while TV132 was the member of the carvacrol ('C') one. At clones TV 107 and TV 115, geraniol ('G') and geranyl acetate were shown to be the chief monoterpenes in the volatile oil.

The selected clones produced fairly high levels of essential oil (mean: 2.32 ml/100g, min.: 1.54 ml/100g, max.: 3.70 ml/100g) in their second growing season (2009). The vegetative propagation provided quite homogenous essential oil composition within clones, as it has been proven by evaluating GC/MS data of individual samples.

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Chemical Composition of Essential Oils from Leaves and Stem of *Parkia biglobosa*

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The constituents of the essential oils of leaf and stem of *Parkia biglobosa* (Mimosaceae) were determined by GC-MS. Forty four (41) compounds, constituting 83.44% of the oil, were identified in the leaves. The major constituents of the leaf oil were limonene (15.96%), hexadecanoic acid (12.48%), farnesene (10.21%), b-fenchylalcohol (9.18%) and b-pinene (5.72%). Thirteen (13) compounds, representing 91.84% of the oil were identified in the stem. The essential oil of the stem was dominated by 3-furan acetic acid (81.39%). A comparison of the compounds of the oils revealed that the volatile compounds of the leaf oil is distinctly different from that of the stem oil. However, limonene, hexadecanoic acid and caryophyllene oxide were the common compounds identified in the leaf and stem essential oils. This is the first report of the essential oil compounds of *P. biglobosa.*

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Chemical and morphological variability from Portuguese *Mentha aquatica* L. progeny accessions

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Mentha aquatica L. is one of five basic Eurasian and African species from genus Mentha, a very complex genus with taxonomically difficulties. The goal of this work was to chemically characterize a water mint germplasm collection based on volatile oil constituents.

The progeny flowering aerial parts from eight accessions of M. aquatica collected from wild plants grown in Northern Portugal, were evaluated for essential oil yield and composition variability. This germplasm was evaluated for 42 morphological descriptors [1] and the cluster analysis of five accessions from this germplasm was performed.

The essential oils were isolated by hydrodistillation and analyzed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) and the percentage composition of the essential oils was used to determine the relationship between the different oil samples by cluster analysis, as previously reported [2].

The essential oil yield from the eight samples analysed ranged between 0.1-1.1% (v/f.w.).

Essential oils analysis defined three main clusters poorly correlated with each other. One cluster (C1) was composed of six samples that were menthofuran rich (72-85%), a second group of one sample (C2) was cis-pinocamphone/ limonene/ β-pinene rich (33%, 23% and 20%, respectively) and the other one sample group (C3) was linalool/ linalyl acetate rich (36% and 28%, respectively).

Morphological descriptors cluster analysis showed similar results with two subgroups and an accession, probably a hybrid, grouped separately.

In variable amounts (5-89%), menthofuran has been found present in the oils of M. aquatica L. var. aquatica, whereas M. aquatica L. var. citrata oils are characterized by being linalool (23-55%) and linalyl acetate (34-61%) rich [3]. Based on the essential oil composition, plants from cluster C1 were considered M. aquatica L. var. aquatica and the individual from C3, M. aquatica L. var. citrata. Cluster C2 plant is considered a hybrid yet to be more deeply investigated.

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Chemical composition of the essential oil from *Lippia triplinervis* Gardner (Verbenaceae) from Minas Gerais, Brazil

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The genus *Lippia* (Verbenaceae) comprises about 200 species occurring mainly in Central and South America, as well as in some areas of Tropical Africa [1]. One of the main diversity centers of the genus is located at the "Cadeia do Espinhaço" Mountains, in the State of Minas Gerais, Brazil [2]. During a field trip for plant collecting in the mountains of Aiuruoca (Minas Gerais State) we found a strongly aromatic shrub, growing above the height of 1.800 m. The plant was collected and classified as *Lippia triplinervis* Gardner. As part of our study on Verbenaceae species occurring in Brazil, the volatile oil from fresh leaves of *L. triplinervis* was obtained by hydrodistillation in a Clevenger type apparatus for 2 h, yielding 1.4% of a yellow essential oil, which was analyzed by GC-FID using an Agilent 7890A gas chromatograph fitted with a 5%-phenyl-95%-methylsilicone capillary column (HP5, 30m X 0.32mm X 0.25μm), oven temperature program from 60°C to 240°C at 3°C/min, split injection (1:20, 250°C, 1μL of a 1% solution in dichloromethane) and hydrogen as the carrier gas (1.5mL/min). GC-MS was performed using the same conditions in an Agilent 5973N (EIMS, 70eV), but helium (1.0mL/min) as the carrier gas and a HP5-MS column (30m X 0.25mm X 0.25μm). For identification, both mass spectra and linear retention indices were considered. Fifty-five compounds were detected in the essential oil, of which the major components were myrcenone (57.7%), ipsenone (11.4%), (*E*)-calamenene (4.8%), myrcene (2.6%). Apart from the presence of ipsenone, the chemical composition of this oil closely resembles that of *Lippia lacunosa* previously studied by our group, which had a strong mango-like aroma [1]. The essential oil from *L. triplinervis* displayed similar sensory characteristics. To the best of our knowledge, this is the first report on the chemical composition of *Lippia triplinervis* essential oil.

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Influence of genetic materials and seeding density on chamomile essential oil production

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The only genetic material used for chamomile (Chamomilla recutita (L.) Rauschert) production in the South Brazil is the cultivar Mandirituba. Besides the limitations regarding the floral heads and essential oil yield of this cultivar, phytosanitary problems have also been observed. The objective of this work was to compare genetic materials from Holland, Denmark and Brazil and to evaluate the effect of seeding density on floral heads development and essential oil production, both yield and quality. The experiments were carried out at field conditions in Piraquara, Paraná State, Brazil. The essential oil was obtained by hydrodistillation in a Clevenger apparatus and the oil composition was analyzed by gas chromatography (GC/FID) and mass spectrometry (GC/MS). Genetic materials from Holland and Brazil presented statistically higher dry mass accumulation than materials from Denmark. However, the material from Brazil showed a higher essential oil yield (4.7 $\mu L \cdot g^{-1}$, dried mass) and productivity (2.40 $L \cdot ha^{-1}$). The genetic material from Denmark and Brazil showed similar essential oil composition. The best seeding density was 1.0 $kg \cdot ha^{-1}$ for cultivar Brazil and the use of 3.0 $kg \cdot ha^{-1}$ reduced plant development due to the high plant competition.

Essential Oil Composition of *Salvia nemorosa* L. (Labiatae) Growing in Turkey

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Labiatae, a well known family with strongly aromatic plants, is composed of more than 500 species growing widespread in the world [1]. The genus *Salvia* L. belongs to Labiatae (Lamiaceae) family, and is represented by 86 species in *Flora of Turkey* [2]. *Salvia* species have various uses, and the essential oils have been used in foods, drugs, and perfumery industries. *Salvia nemorosa* L. is a widespread perennial herb growing in eastern Anatolia, the leaves of *S. nemorosa* have been used in Turkish medicine, as cataplasms to stop bleeding [3].

The essential oil obtained by hydrodistillation of aerial parts of *Salvia nemorosa* L. was analyzed by GC/MS (Agilent 6890 N GC-MSD system). In total, twenty compounds were characterized, representing 85.3 % of the oil. The main constituents were determined as caryophyllene oxide (23.6 %), spathulenol (23.2 %) and *β*-caryophyllene (12.0 %). In this study, the essential oil composition of *S. nemorosa* was discussed with the previous work on the plant that grows in Iran and northern Serbia [4, 5] according to the literature data.

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Composition of Essential Oils of Two Endemic *Anthemis* Species from Turkey

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The genus *Anthemis* (Asteraceae) is represented in Turkey by 50 species, 27 being endemic and altogether 80 taxa. Dried aerial parts of *Anthemis pestalozzae* Boiss. and A. *rosea* Sm. subsp. *cannea* (Boiss.) Grierson were micro distilled and the oils obtained were analysed simultaneously by gas chromatography-mass spectrometry (GC/MS).

Main componenets were characterizied as follows:

Anthemis pestalozzae: b-caryophyllene (26.3 %), 1,8-cineole(10.6 %), linalool (5.0 %), tricosane (4.4 %), a-humulene (4.0 %)

Anthemis rosea subsp. *cannea*: sabinene (13.1 %), borneol (9.0 %), b-caryophyllene (8.0 %), b-pinene (8.0 %), 1,8-cineole (7.1 %)

To the best of own knowledge this is the fist report on the chemistry of these species.

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PP-A31

Composition of the Essential Oils of *Artemisia annua* L. and *Artemisia vulgaris* L. from Turkey

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Artemisia L. (Asteraceae) is represented in Turkey by 22 species (1). *A.annua* is also known locally as "Peygamber süpürgesi" and used internally as 2-3% infusion against dysentery and tuberculosis, and externally to cure carbuncle. *A. vulgaris*, which is known as "ayvadana" has similar effects but weaker. It has been used as stomachic, tonic, antipyretic, diuretic, anthelmintic and emmenagogue. There activities have been reported to occur in higher doses (2). Water distilled oil from aerial parts of *Artemisia annua* L. and *Artemisia vulgaris* L. (Asteraceae) collected from different localities in Turkey were analyzed by GC and GC/MS. Aerial parts were subjected to water distillation for 3h using a Clevenger-type apparatus to yield oils in the following percentages: 0.5% and 1.2% for *Artemisia annua* L. and *Artemisia vulgaris* L., respectively. Dihydrotagetone (41.9 %), Z- β- ocimene (24.8 %), trans-tagetone (10.5 %) and limonene (7.1 %) were detected as main constituents in the oil of *Artemisia vulgaris*; artemisia ketone (61.8 %), camphor (10.3 %) and 1,8-cineole (5.1 %) were found as main constituents in the oil of *Artemisia annua*.

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The effect of indol-3-acetic acid and gibberellic acid on composition the essential oil of *Lavandula angustifulia Mill*

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The genus Lavandula belongs to the family Lamiaceae and it comprises of more than 30 species of which only very few are commercially used: Lavandula angustifolia Mill. Lavender is native to the western Mediterranean region, primarily in the Pyrenees and other mountains in northern Spain and is cultivated extensively [1].

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

Aerial parts of lavender plants treated with indol-3-acetic acid (150 µM) and gibberellic acid (100 µM) as 3×48 hrs interval. After a week aerial parts of plants were harvested and dried in the dark with air stream .The essential oil of dried aerial parts were obtained by hydrodistillation and investigated by GC/MS.The essential oil content was increased in all treatments. The percentage of 1,8-cineole in lavender essential oil showed a increase by treatments. The concentration of 1,8-cineole was 19.05%, in control condition and 24.57%, 30.41% in indol-3-acetic acid and gibberellic acid conditions respectively.

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Chemical composition of the essential oil of C. sinensis cv. valencia and quantitative structure-retention relationship study for prediction of retention indices by multiple linear regression

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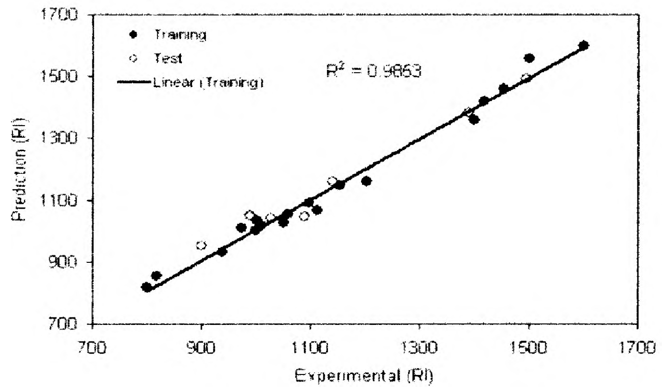
The chemical composition of the volatile fraction obtained by head-space solid phase microextraction (HS-SPME), single drop microextraction (SDME) and the essential oil obtained by cold expression (CE) from the aerial parts of C. sinensis cv. valencia were analyzed by employing GC-MS. The main components were limonene (61.34%, 68.27%, 90.50%), myrcene (17.55%, 12.35%, 2.50%), sabinene (6.50%, 7.62%, 0.5%) and α-pinene (0%, 6.65%, 1.4%) respectively obtained by HS-SPME, SDME and CE. Then quantitative structure-retention relationship (QSRR) study for prediction of retention indices of the compounds were developed by application of the structural descriptors and multiple linear regression (MLR) method. QSRRs are statistical models which quantify the relationship between the structure of a molecule and its chromatographic retention index, enabling prediction of the retention indices of novel compounds. A simple model with low standard errors and high correlation coefficients was selected. The results illustrated that the linear techniques such as MLR combined with a successful variable selection procedure are capable to generate an efficient QSRR model for predicting the retention indices of different compounds. This model, with high statistical significance (R2calibration=0.983, R2prediction=0.970, Q2LOO=0.962, Q2LGO=0.936, REP(%)=3.00), could be used adequately for the prediction and description of the retention indices of the volatile compounds.



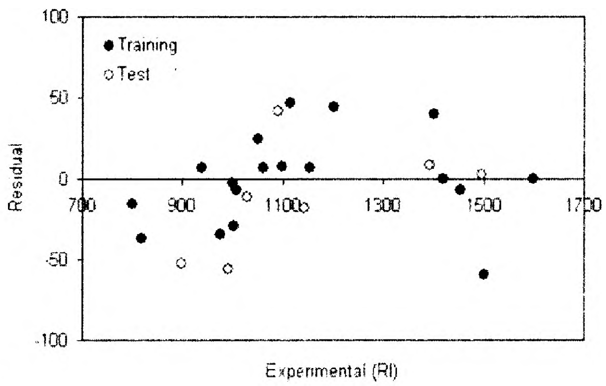


Fig. 1. The predicted RI values by the MLR modeling vs. the experimental RI values
Fig. 2. Plot of the residuals against the experimental values of the retention indices

Chemical composition of the essential oil separated from the stems of *Prangos serpentinica* (RECH.F., AELL. ESFAND.) HERNNSTADT&HEYN in the marginal parts of Semnan province by using gas chromatography-mass spectrometry

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Prangos genus of Umbelliferae family involves about 30 species and its various species have been widely distributed worldwide [1]. In Iran we can find about 50 species of Umbelliferae family plants. *Prangos* is frequently used as a powerful tropical medical plant and as an attractive alternative for chemical drugs with harmful side effects. The common pharmaceutical and clinical applications of various *Prangos* species are as emulient, carminative, tonic antiflatulent, anthelmintic, antifungal and antibacterial agents [2-4]. A literature survey reveals that analysis of the essential oils of different species of *Prangos* has attracted a growing interest [5-7]. However, identification and comparison of the constituent components of *the stems of Prangos serpentinica* (RECH.F., AELL. ESFAND.) HERNNSTADT&HEYN by hydrodistillation is the main purpose of this work for the first time. The volatile oil was obtained as a clear yellowish color liquid involving 0.35 weight percent. Under the optimized experimental conditions, injection of 2 micro liter parts of the oil yielded a series containing 37 components comprising 97.4% of the oil structure. The major components of the oil were α-pinene (31.1%), δ-3-carene (10.5%), germacrene D (7.4%), δ-guaiene (4.2%), δ-cadinene (4.2%), and α-humulene (3.8%).

Key words: Prangos serpentinica (RECH.F., AELL. ESFAND.) HERNNSTADT&HEYN, GC-MS, hydrodistillation, α-pinene

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Essential Oil Composition Aerial Parts of Perovskia Abrotanoides Karel in Shahkuh Mountaine, North of Iran

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Perovskia abrotanoides Karel. is one of the most important medicinal plants , with wild dispersal in cold dry mountainous rangeland in north east of Iran .It has been used by the rural people with another medicine herbs as a tonic , antiseptic , anti inflammation , rheumatic pain , expel worms and treatment leishmaniosis especially with combination to *Artemisia sieberi, Artemisia annua* and *Thymus carmanicus* .

Flowering aerial parts , were collected in August 2008 in two 2300 m Shahkuh . The essential oil was obtained by water distillation and analyzed by GC and GC/MS method . verbenone (25.1%), terpinolene (22.6 %), sabinene (12.2 %), myrcene ,(3.5%), were the most components of essential oil .

These results due to its traditional usage as antiseptic and anti leishmananiosis .

Key Word : Perovskia abrotanoides Karel. aerial parts, essential oil composition, North of Iran

Chemical composition and antimicrobial effects of essential oils of Ten Eucalyptus species against Micrococcus loteus and Escherichia coli

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Using natural products for treatment of microbial diseases, instead of synthetic drugs is very important. Medicinal plants are one of the sources for natural compounds with medicinal properties. There are over 700 different species of Eucalyptus in the world, of which at least 500 produce a type of essential oil. The Eucalyptus oils can be used in some medicinal products because of their antimicrobial effects [1-3]. The leaves and oils of many Eucalyptus species are especially used for respiratory ailments such as bronchitis and croup [4-7]. In this research the essential oils of ten Eucalyptus species were obtained by hydro-distillation and examined against Escheichia coli (1330) and Micrococcus loteus (1110). The Eucalyptus species were Eucalyptus gilli, E. microcarpa, E. kingesmillii, E. loxophleba, E. gongylocarpa, E. salubris, E. erythrocorys, E. salmonophloia, E. spathulata and E. flocktoniae. The essential oils were analyzed by capillary GC and GC/MS. For investigation of antimicrobial effects, the disk diffusion method was used after dilution of the oils with DMSO. The results showed the main component of all essential oils were 1,8-cineole and á-pinene. The highest amount of 1,8-cineole (82.1%) was found in the oil of E. kingesmillii and the lowest amount (59.6%) was found in the oil of E. salubris. The results of antimicrobial studies showed that all oils were effective against two bacteria. The diameters of inhibitory zones on Micrococcus loteus were 10 to 49 mm and on Escheichia coli were 10 to 22 mm.

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PP-A37

Comparison of three analytical methods for the extraction of the volatile fraction from stem and leaf of *Helichrysum Aucheri*

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Solid-phase microextraction (SPME) is a modern analytical tool for easy effective extraction of various types of compounds. It is worthwhile to mention that HS-SPME is a non-destructive modern application for trapping/extracting volatiles, fragrances, emissions, etc. It is a fast, easy, convenient and effective technique with good future prospects [1-4].

Solvent-free microwave extraction (SFME) is a new technique which combines microwave heating with dry distillation at atmospheric pressure for the isolation and concentration of the essential oils. In SFME method, there is no need to add any solvent or water if fresh plant material is used. If dry plant material is used, the sample is rehydrated by soaking in water for some time and then draining off the excess water [5].

In this study we investigate the volatile fraction of leaf and stem of *Helichrysum Aucheri* that it wasn't done the subject of investigation previously. The volatile fraction of leaf and stem of *Helichrysum Aucheri* collected from Abasabad Valley of Hamedan, in June 2008, were isolated by solvent free microwave assisted extraction (SFME) and headspace-solid phase microextraction (HS-SPME) and comparison the compositions of the volatile fractions extracted by these methods with Hydrodistillation (HD) method. The volatile compounds which obtained by Hydrodistillation, SFME and SPME were analyzed by means of GC and GC/MS. The percentages of the components have been obtained by their peak areas chromatograph of GC/FID. α-pinene (39.6%), β-caryophyllene (7.3%), 1,8 cineole (19.7%), were the major components in HD method. The main compounds which entered by HD-SPME were α-pinene (10.1%), β-caryophyllene (6.9%), 1,8 cineole (16.2%). Limonene (16.0%), 1,8 cineole (10.0%), z-β-farnesene (7.0%), spathulenol (18.3%) were found to be the main constituents in the SFME method.

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Chemical composition of the essential oil from leaves of *Stachys pubescens* Ten. Growing wild in North-west of Iran.

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The genus *Stachys* consists of 250 species widespread throughout the word. Among the 34 species present in Iran, 13 species are endemic [1]. The compositions of the oils of some *Stachys* species such as, *S. acerosa* [2], *S. aegyptiaca* [3], *S. athorekalyx* [4] and *S. recta* [5] have been reported. *Stachys* species also have several folkloric uses, e.g. the leaf of *S. officinalis* L. Trev. is used as a carminative and to relieve headaches[6]. *S. botenica* L. is used as a tonic, astringent and to relieve headaches, while *S. palustris* L. is used as an antiseptic, to relieve gout and to stop hemorrhage. *S. byzantina* is used as an ulcer healer and antiseptic too. In this study, the essential oil of *Stachys pubescens* Ten. leaves growing wild in North-west of Iran was analyzed by GC and GC-MS methods. The plant material was collected in July 29, 2009 in Ardabil area, North-west Iran at an altitude of 1450 m. The air-dried leaves of plant material (150g) were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. The yield of total volatiles was 0.06% (w/w). A total of 21 compounds representing 93.1% of the oil were identified. The main components of the oil were thymol (35.5%), geraniol (23.7%) and linalool (9.0%).

Key words: Stachys pubescens Ten., Lamiaceae, essential oil composition, Thymol, geraniol.

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Essential oil composition of fresh and dried flowers of Musk rose (*Rosa moschata*) from Iran

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Iran is an origin of Musk rose. This specie is important to extract rose water and rose oil. Musk rose has been used in food as flavoring agent and also in folk medicine [1]. The oil composition of *R. damascena*, R. *brunonii*, *Rosa davurica* and *R. centifolia* were reported previously. In this study the essential oil composition of fresh and dried flowers from two important cultivation regions were studied by GC and GC-MS techniques. Collected flowers were divided into fresh and dry parts. One part was air dried at ambient temperature in the shade. Both parts were steam distilled by using a Clevenger type apparatus for 3 hours. The yield of the oils were 0.12% -0.36%. Retention indices were determined by using retention times of n-alkanes which had been injected after the oil under the same chromatographic conditions. The retention indices for all the components were determined according to the Van Den Dool method using n-alkanes as standard [2]. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra [3]. The main compounds in all of the oils were eugenol (21.1%-38.6%), heneicosane (20.5%- 26.5%), tricosane (5.1%-8.5%) and nonadecene (5.0%-6.6%). Drying process decreased the yield of the oil and also the relative amount of alcoholic monoterpenes which are important in Rose oils.

The stearoptene content consisted high percent in all samples. In dried samples the percentage (60.9-61.2%) was higher than in fresh ones (46.7-50.2%) due to their nonvolatile characters and contained mainly heneicosane, nonadecene and nonadecane. These compounds were found in some Rose oil (*R. damascena*) at level 10-15% and were also presented in the oil of *R. moschata* and give a fixative property to it. In our samples, because of high level of these compounds, the oils were waxy and solidified in refrigerator.

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Composition of the essential oil of *Libanotis transcaucasica* from Iran

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Libanotis transcaucasica Schischk., being a perennial, is distributed in Europe, NW Iran and Caucasia [1]. In Chinese traditional medicine, some *Libanotis* species were reported in ancient literature as having various healing effects such as *Yan Feng* (root of *Libanotis buchtormensis*) and *Fang Feng* (*Libanotis laticalycina*) [2]. In this study the essential oil composition of the plant were studied by GC and GC-MS techniques. Retention indices were determined by using retention times of n-alkanes which had been injected after the oil under the same chromatographic conditions. The retention indices for all the components were determined according to the Van Den Dool method using n-alkanes as standard [3]. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra [4]. Analysis of the essential oils of *Libanotis transcaucasica* Schischk. led to identification of 55 compounds, which represent 84% of the total oil (Table I). The main components of the oil were Germacrene B (20.2%), Isospathulenol (11.0%), Germacrene D (9.2%) and Kessane (5.5%). In another study, the essential oil of *Libanotis buchtormensis* indicates that, Epiglobulol and Germacrene D were the major constituents.

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Chemical Composition and Biological Activity of Essential Oils of *Dionysia taptodes* & *Dionysia diapensifolia* Species

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Dionysia genus (Primulaceae family) contains almost 50 known species of which 30 species are native to Iran [1]. The aerial parts of the plants were collected during flowering stage from Zagros mountains in Iran.The essential oils of air-dried *Dionysia taptodes* and *Dionysia diapensifolia* aerial parts were obtained by hydrodistillation with yields of 0.2 and 0.1% (w/w), respectively. These plants are endemic to Iran. The oils were analyzed for the first time by GC and GC-MS [2,3]. Thirty-four compounds in *D. taptodes* and forty-six compounds in *D. diapensifolia* were identified that included 90.3% and 90.7% of the oil components, respectively. 2-Hydroxy-5-methoxy acetophenone (36.5%), (E)-chalcone (31.2%) and p-hydroxy acetophenone (5.1%) were the major compounds of *D. taptodes* essential oil. The major components of the *D. diapensifolia* oil were (E)-chalcone (43.5%), 1,3-diphenyl, 1,3-propanedion (14.4%) and methyl 4-methoxysalicylate (5.1%). In the DPPH radical scavenging assay, *D. taptodes* oil showed moderate to good activity with IC_{50} value of 9.2 mg/ml while *D. diapensifolia* showed weak antioxidant activity with IC_{50} of 41.4 mg/ml. Cytotoxic activity assessed on 3 human cancer cell lines (Hela, LS180 and Raji), showed that essential oil from *D. Diapensifolia* (IC_{50} values; 0.138 – 0.447 mg/ml) had good cytotoxic activity.

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Composition of essential oil from *Oenothera biennis* L. of Iran

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Evening primrose (*Oenothera* biennis) is a common weed that is native to North America. It is now commonly found in many temperate zone around the world. The edible oil from its seeds has been shown to have several pharmacologic effects. It is best known for its use in the treatment of systemic diseases marked by chronic inflammation, such as atopic dermatitis and rheumatoid arthritis. It is often used for several women's health conditions, including breast pain (mastalgia), menopausal and premenstrual symptoms, cervical ripening, and labor induction or augmentation [1].

Recently has been demonstrated specific anti-tumour activity of an ethanol extract from evening primrose defatted seeds[2].

In this work,the aerial parts of Evening primrose grown in Iran were hydrodistilled for 4 hours, using a Clevenger-type apparatus to yield 0.05% (w/w) of dark yellowish oil. The oil was analyzed by GC and GC-MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [3].

Ten compounds were characterized in the essential oil of Evening primrose, representing 93.06% of the oil, of which n-cety alcohol(65.55%),phytol (8.66%), n-docosane(7.68%) were found to be the major components.

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Chemical Composition from *Thymus Kotschyanus* Cultivated in Iran

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The genus *Thymus* L. (Labiatae) consists of about 215 species of herbaceous perennials and subshrubs. This genus is represented in Iranian flora by 14 species, *four of which are endemic.* Studies indicating *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities (1, 2).

Among them, Thymus kotschyanus known as a member of thyme species is used as traditional medicine among people.

We report on the essential oil composition of Thymus kotschyanus cultivated in Hamedan. Aerial parts of dry plant were steam distilled for three hours using a Clevenger-type system. Essential oils were dried with anhydrous sodium sulfate and kept in amber vials at 4 °C until chromatographic analysis. The oil yield was 0.3% on dry weight basis for yellowish oil.

The oil was analyzed by GC and GC-MS. The components of the essential oils were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples (4).

Thirty nine components were characterized in the essential oil and linalool (24.76%), carvacrol (21.13%) and trans-caryophyllene (8.56%) were found as the major constituents.

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Essential oil composition and in vitro antibacterial activity of essential oils from two Satureja species growing in Iran

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Satureja bachtiarica Bunge. and *S. Khuzistanica* Jamzad. are two endemic species growing wild in Iran (2,3). Aerial part of two species were collected from Isfahan and Lorestan provinces of Iran at full flowering stages in 2009. The essential oil of each air-dried samples were isolated by hydrodistillation and analyzed by combination of GC with GC-FID and GC-MS(1). Analyses resulted in the identification of 17 components corresponding to 98.6% of *S. bachtiarica* essential oil. The oil was mainly consisted of oxygen-containing monoterpenes (72.1%) and sesquiterpenes (20.8%), with a small amount of monoterpene hydrocarbons (4.9%). The major constituents were the terpenoid phenols thymol (28%) and carvacrol (13.2%), caryophyllene oxide (17%), borneol (11.6%) and linalool (9.6%). In the essential oil of *S. khuzistanica*, a total of 19 compounds were characterized comprising 98.3% of composition. In this sample, monoterpenoids (94.1%) with carvacrol (68.8%) and its precursors p-cymene (7.3%) and γ-terpinene (5.5%) were dominant as the principal components. Due to antimicrobial effect of phenolic compounds,The in vitro antimicrobial activities of these oils were studied against two Gram-positive and two Gram-negative bacteria (Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa) (3). The results of the antimicrobial activities of the essential oils according to the disc diffusion method and minimum inhibition concentration (MIC) values indicated that essential oils tested in this study, especially those of *S. khuzistanica*, exhibited significant inhibitory activity against both Gram-negative and Gram-positive bacteria. In conclusion, the essential oils of studied Satureja species have the potential to be considered as alternatives for synthetic food preservatives. they can be taken into account as potential preservatives for food or other related industries.

Keywords: Satureja spp.; Antibacterial activity; Essential oil; Carvacrol; Thymol

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The Chemical Constituents of Essential Oil of *Thymus trautvetteri Klokov & Desj-Shost.* Aerial parts (Lamiaceae).

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Genus of Thymus which belongs to SubClass of Asteridae, order of Lamiales and family of Lamiaceae, this family has 224 Genus and 5600 species distributed throughout the world, especially in Mediterranean area and central Asia. The chemistry of this genus has been studied by many investigators. The Iranian flora comprises 14 species of Thymus, of which some are endemic. The popular Persian name of the most of these species is "Avishan". *Thymus trautvetteri* is one of these species which is distributed in different regions of Iran [1] several species of Thymus have been used in folk medicine, for example as a treatment of bronchitis, asthma, whooping-cough, rheumatism, gout and dysmenorrhea.

Anti-inflammatory, Anti-spasmodic, Antimicrobial, Bronchodilator and expectorant activities of some species of Thymus have previously been reported [2-6]. This investigation describes the constituents of the oil of T.trautvetteri which has not been studied previously.

The essential oil of aerial parts of *Thymus trautvetteri* obtained by hydro distillation and steam distillation methods and was analyzed by GC and GC/MS. Among the 44 identified constituents accounting for 97.4% of the total oil which was collected by hydro distillation, the major components were Thymol (56.8%), p_Cymene (10.9%), gama_Terpinene (8.5%) & Carvacrol (3.7%) and The essence which was collected by steam distillation, 40 compounds were identified which most important ones were Thymol (51.3%), 1,8_ Cineole (12.2%), gama_Terpinene (7.9%) & Carvacrol (4.4%).

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POSTER PRESENTATIONS

SESSION B

Isolation and methods of analysis of essential oils

Detecting traces of methyl eugenol in essential oils

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Methyl eugenol is a naturally occurring flavour and fragrance[1] found in a variety of different food sources, including spices (nutmeg, allspice), herbs (basil and hence pesto, tarragon), bananas, and oranges. It is also found as a component of natural essential oils. Commonly used oils with more than 0.1% of methyl eugenol include calamus, rosewood, elemi, ylang ylang, cymbopogon, star anise, lovage, verbena, nutmeg, basil, pimento, bay leaf, rose and clove[2]. In addition there are other potential sources of exposure to methyl eugenol, including agriculture (insect lures); consumption of wine and as part of the ambient background in air and water[1].

Because high doses of some allyl alkoxybenzenes have induced tumours in rats and mice[4], use is recommended as either restricted[5,6] or, in the case of safrole, prohibited[5]. Many reviewers and researchers[2,3,7] present clear evidence that these restrictions are excessive, especially those who accept that carcinogenesis is a threshold phenomenon[7].

This poster describes suitable gas chromatographic methods for the determination of trace amounts of methyl eugenol in the essential oil of tea tree, *Melaleuca alternifolia*, terpinen-4-ol type, where published "trace" amounts[8] were interpreted as being as high as 0.3-0.9% by one regulator[9]. Peak assignment by GCMS and co-elution with a standard, facilitated the GCFID determination of more than 100 commercial samples on a 60m Alltech 1701 column. Inter-laboratory confirmation was achieved using GCMS-SIM. These determinations indicated that the methyl eugenol content of tea tree oil ranged from <0.01-0.06%, mean 0.02% i.e. 20-fold lower than the regulator's interpretation[9] and one million times lower than the logarithmic scale levels known to cause carcinomas in rats[10]

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Binary mixture thermal studies based on a minor compound extracted from *Syzygium aromaticum* L.

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Eugenyl acetate is a natural derivative extracted from *Syzygium aromaticum* L. Studies have demonstrated that it has some pharmacological properties such as an analgesic, bactericidal and others [1].

However, this oil derivative has a lipophylic characteristic which implies that it has a major water solubility problem. Therefore, this molecule presents a difficult relationship between its risky low water solubility and oral bioavailability. Nonetheless, one attractive option to improve this drug's solubility is producing eutectics [2].

The aim of this study was to investigate the formation of blends using different compositions of eugenyl acetate, and polyethylene glycol 3350, and later differentiated based on their thermal behavior.

The thermal analysis was performed using DSC Q-10. The DSC scans were recorded at 5°Cmin^{-1}. The initial and final temperatures were 10°C and 70°C, respectively.

The binary mixtures for these materials yielded 1-2 endothermic events. The first peak represents the eutectic composition of the binary mixture. The second peak occurs approximately at the same temperature, and it corresponds to an endothermic peak of PEG 3350 which is shifted to a few degrees lower than the PEG 3350 peak probably due to the melting point of PEG being lowered due to the 2nd component acting as an impurity.

Based on this phase diagram, 80% of Eugenyl acetate is established as the eutectic mixture which is characterized by having the lowest melting temperature compared to the individual components, at 21.3°C.

These blends present several advantages such as particle size reduction and the enhancement in their hydrophilic profile, which improves their dissolution behavior [2,3]. This proved to be an interesting fact in order to optimize the therapeutic activity of a formulation for the pharmaceutical industry using natural compounds [4].

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Hydrodistillation versus Steam Distillation

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Hydrodistillation und steam distillation are well known techniques for isolation of essential oils. Differences in oil yield and oil composition can be explained by the different role of water solubility of specific components in the evaporation and separation process [1].

Using the example of caraway and fennel, chamomile and lavender, peppermint and marjoram the apparatus for hydrodistillation of the European Pharmacopeia and the steam distillation apparatus of the company NORMAG were compared (consistent in water amount, distillation time and dry plant amount in both cases) (Fig.1)

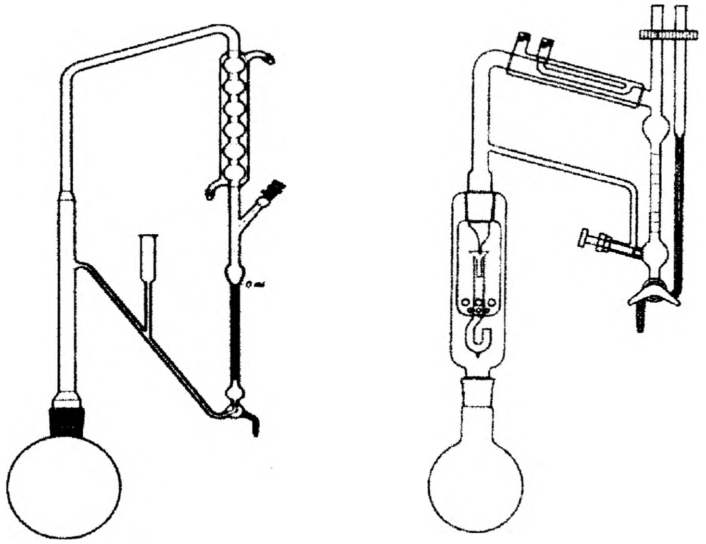

Fig.1: Equipments for hydrodistillation (left) and steam distillation (right)

Generally it can be shown that steam distillation delivers significantly higher essential oil yields than hydrodistillation in all cases (Tab.1). The compositions of the essential oils are partly similar (fennel, peppermint), partly significantly different (caraway, chamomile, lavender, marjoram).

Tab.1: Essential oil contents (mL/100 g) depending on the distillation method

	Caraway	Fennel	Lavender	Chamomile	Peppermint	Marjoram
Hydrodistillation	2.2	7.3	0.6	0.3	1.5	0.8
Steam distillation	3.0	12.0	2.0	1.0	4.0	2.5

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Authentication of bergamot essential oil by Gas-Chromatography-Combustion-Isotope Ratio Mass Spectrometer (GC-C-IRMS)

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Bergamot (*Citrus bergamia* Risso) essential oils belong to essential oils of the genus citrus, very complex mixtures characterized by a volatile fraction which comprises 85-99% of their composition, mainly represented by monoterpene and sesquiterpene hydrocarbons, and their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters [1].

Bergamot essential oils are articles of commerce in many parts of the world, they are particularly requested in perfumery and in food industry.Reconstituted bergamot oils are easy to find on the market and are generally obtained by mixing monoterpenes and distilled oils of different origins, linalyl acetate and citrus oils other than bergamot oil [2]. Besides, the most common practice to reconstitute a bergamot oil, with a composition very similar to a genuine one, is the addition up to 8% of linalool [3] of natural or synthetic origin.

The target of this work is the application of Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometer technique [4] in order to investigate the quality control of bergamot essential oils. An authenticity range was obtained investigating the carbon stable isotope ratio of genuine Italian bergamot essential oils (harvest period 2008-2009), in order to compare the GC-C-IRMS data of several industrial, commercial and foreign bergamot essential oil samples. Moreover, with the aim to test the efficiency and the sensibility of IRMS device, self-adulterated in laboratory bergamot oil samples were analyzed. The data were compared with those achieved by conventional Enantioselective Gaschromatography (Es-GC) and high resolution Gaschromatography (GC-FID). Results of this work indicated that GC-C-IRMS was able not only to detect the presence of adulterants in the samples, but also to discriminate the bergamot oil samples according to their geographic provenance and the nature of the adulterants added.

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Cellulosic-based liquid chromatographic enantiomeric separation for determination of coumarins in *Citrus* essential oils

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Coumarins are found in many natural sources, mainly in plants, in particular in Citrus essential oils. Coumarins have received considerable attention for their chemical, biological and farmacological properties. In recent years it has been given much importance to the study of chirality, because the enantiomers have the same physical and chemical properties but different biological properties. In fact, it is known that the biological activity of enantiomeric compounds can vary widely. Some of the coumarins present in the citrus essential oils have, in their structure, some chiral centers, hence a separation of enantiomers could be a valid tool for discrimination between synthetic and natural products.

To date, the study of chiral coumarins is limited and there are not methods published in the literature pertaining to the enantioseparation of racemic mixture coumarin. As a consequence, the recent introduction of new chiral stationary phases for liquid chromatography contributed to the development of efficient HPLC methods for the separation of enantiomers in order to assess the biological activity of each enantiomer of a chiral compound.

The development of a new HPLC method allowed the enantiomeric separation of chiral coumarins through the use of a column packed with cellulosic-based chiral stationary phase.

With this HPLC separation is possible to separate coumarin's standard obtained from synthetic process that occur in racemic mixture while those isolated from Citrus essential Oil that are enantiopure form.

Conventional and Fast Gas Chromatography Analysis Of Essential Oils Using An Ionic Liquid Stationary Phase.

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GC analysis of volatile fraction of essential oil, both for qualitative and quantitative purpose, is often run both on non polar and on polar columns. Non polar columns are preferred in the identification of unknown compounds achieved with an MS detector by means of the use of linear retention index (LRI). LRI are calculated using as reference series of n-alkanes that often present fluctuating retention behaviour on polar columns. Polar columns, on the other hand, are often used in order to resolve coelutions that can possibly occur on the non polar ones, hence in order to obtain the complete elucidation of the matrix. Recently, a new class of compounds has received increasing attention as stationary phases for gas chromatography: room temperature ionic liquids. Room temperature ionic liquids represent a class of organic non-molecular solvents generally constituted of an organic cation containing N- or P- (i.e., alkyl imidazolium, phosphonium) counterbalanced by an anion of organic or inorganic nature and that are liquid at 20°C. Ionic liquids have been widely employed in several fields of chemistry. Their peculiar properties such as low volatility, high thermal stability, excellent selectivity towards specific chemical classes and moreover good wetting abilities on the inner wall of fused silica capillaries make this class of compounds suitable as stationary phase in GLC. IL as stationary phases in gas chromatography have been used for the analysis of polyaromatic hydrocarbons essential oils, chlorinated pesticides, fatty acid methyl esters [1-2], and flavours and fragrances. IL stationary phases have also been used in multidimensional gas chromatography (MDGC) [3] and in comprehensive two-dimensional gas chromatography. In the present work a new GC capillary column coated with an ionic liquids stationary phase (SLB-IL59) was evaluated in terms of efficiency and in terms of polarity. The results showed for this stationary phase a polarity close to that of the 100% polyethylene glycol, hence the SLB-IL59 was utilized as polar stationary phase in the analysis of essential oils both in conventional and fast gas chromatography.

Extraction of essential oil using ILs

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Essential oils are extensively used in many industrial areas: medicinal products, cosmetics, foods. The relatively simple process of distillation has as drawback the very low yield of product, due to the small amount of oil contained in the plant, and the difficulty of separation from the lignin-cellulosic matrix. The low yield and the high market demand make these substances high value-added products and it could be extremely interesting to improve the process yields with lower costs and increased profits.

To increase hydrodistillation yield the use of ionic liquids has been evaluated. Four different ionic liquids have been chosen among those reported in the literature for dissolution of cellulose[1]. Various concentrations of ionic liquid and different modes of macerations have been attempted to determine the best procedure for yield increasing. This method have permitted to obtain higher amounts of essential oil respect to normal hydrodistillation[2] ranging from about 5 to 22 % without affecting the final composition of the product. The possibility to use small amounts of additive permits to contain costs, increase the economic revenue, especially for very expensive essential oils. The wide range of available ionic liquid allows to find the best additive for each specific plant material, both from the chemical and the environmental point of view.

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Estimation of odour active compounds of marjoram and caraway after decontamination by GC/MS, SPME/GC and GC/O

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Spices are commonly used to enhance the attractiveness of aroma in foodstuffs. Unfortunately, they are spices which are highly contaminated microbiologically. Contaminated spices may cause adverse sensory changes in foodstuffs, reduce their shelf life and have an adverse effect on the consumer's health. For this reason several methods of spices decontamination are used. It is very important, that the method which reduces microflora, at the same time doesn't change the sensory quality of spices.

The aim of the study was to investigate the effect of the applied steam decontamination on contents of odour active compounds in selected leaf spice (marjoram) and fruit spice (caraway) by used GC/MS, SPME/GC, GC/O methods, including chiral analysis.

Volatile aromatic compounds were identified in spices. Quantitative composition of these compounds was determined before and after spice sterilization. The GC/O analysis showed that the primary compounds responsible for the aroma in analyzed spices were hydrate cis-sabinene and linalool in marjoram and carvone in caraway, which received the highest dilution factor values (FD) both before and after decontamination. It was determined that the content of the main odorants may be estimated by a rapid SPME/GC method, for which a high correlation was obtained with distillation. The effect of decontamination on enantiomeric ratios in spices was determined by chiral analysis of essential oils both in distillates and SPME. It was found that steam decontamination caused considerable losses of active compounds in marjoram as well as all the volatiles. No qualitative changes or losses were recorded for volatile aroma compounds in steam-decontaminated whole seeds of caraway. No changes were found in the composition of enantiomers of primary aroma compounds in marjoram and caraway under the influence of steam decontamination. The application of SPME made possible a rapid assessment of the effect of this process in the quality of aroma in spices on base of active odour compounds.

PP-B9

Efficient method of isolation of pure (–)-α- and (+)-β-thujone from *Thuja occidentalis* essential oil

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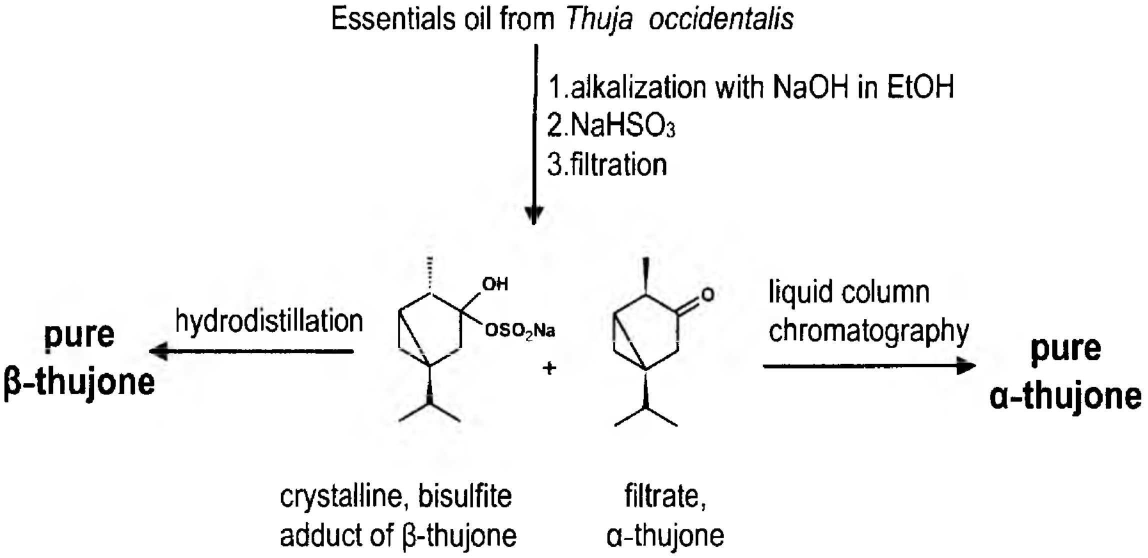
Thuja occidentalis is one of three species of Thuja cultivated in Poland [1]. Leafs of Thuja occidentalis contain relative high amount of essential oil. Major constituents of this essential oil are: α- and β-thujone (80%), fenchone (6%), sabinene (2%). The ratio α- to β-thujone is 9:1. In folk medicine, Thuja occidentalis has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism [2].

The efficient method of isolation and separation of α- and β-thujone from Thuja occidentalis was elaborated (Scheme 1).



Scheme 1.

Essentail oil from Thuja occidentalis was alkalized with sodium hydroxide in ethanol and then saturated solution of sodium bisulfite was added. Only β-thujone gives crystalline adduct with sodium bisulphite [3]. The solid was filtrated and draied in room temperature. The hydrodistillation of this adduct with application of Deryng apparatus gave pure (99,8 % according to GC) (+)-β-thujone. Furthermore, column chromatography (silica gel, hexane:ethyl acetate 100:1 v/v) of filtrate afforded pure (96% according to GC) (–)-α-thujone.

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Comparison of different types of distillation on chemical and sensorical properties of 12 selected essential oils.

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During the last years importance of microwaves for isolation of essential oils from plant-material is significantly developing [1]. Main difference between conventional and microwave heating is gradient of temperature internal and external part of extraction vessel. In traditional heating highest temperature value is observed in external surfaces of extraction vessel but in microwave heating heat transport gradient is located from central parts of extraction/separation bed [2]. For more conversion of microwave energy field into heat, frequently used ferromagnetic substances (conversion magnetic component of electromagnetic radiation) e.g carbonyl iron powder (CIP), iron oxide. We investigated three types of steam-distillations: with typical heating system, focused-microwave assisted and focused-microwave with iron oxide powder. In this technique focused microwave waves heating material in small volume, but absorbing of microwave irradiation by bed, is overall in the same time. Focused microwave was generated and applied by special generator located in microwave oven. We checked 14 essential oils bearing plants: oregano (Origanum vulgare), rosemary (Rosmarinus officinalis), marjoram (Origanum majorana), tarragon (Artemisia dracunculus), bay laurel (Laurus nobilis), allspice (Pimenta dioica), lavender (Lavandula angustifolia), camomile (Matricaria chamomilla), peppermint (Mentha × piperita), winter savory (Satureja montana), summer savory (Satureja hortensis), black pepper (Piper nigrum). All obtained essential oils were submitted into GC-MS analysis. Identification of compounds were accomplished on the basis of retention indices, mass spectrum (NIST 05 library) and real standards. In most cases the composition of essential oil was slightly differ, depending on methods of distillation. In the case of focus microwave distillation with iron oxide presence the time of efficient-yield distillation was 10 min whereas typical heating system required 60 min. Sensory analyses of acquired essential oils gave the inconsiderably distinctions

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Study on the Odor of α-Santalol and Its Side-Chain Modified Derivatives

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Sandalwood (*Santalum album* L.) is a valuable scent material in the fragrance industry. Both α-santalol and β-santalol are major constituents of sandalwood odor, and have distinctive woody odors. Many studies have been reported on sandalwood, and the structure–odor relationships of β-santalol and its related compounds have been investigated in detail [1-3]. Previously, we reported that the odor of sandalwood chips is formed by a combination of santalols and their aldehyde and formate derivatives [4]. In the present study, we report the interesting relationship between the structure and odor of α-santalol and its side-chain modified derivatives.

We compared the odors of the hexene-extracted sandalwood oil and a commercial sandalwood oil to that of the original base scent material (sandalwood chips). The odor of the extracted oil was found to be similar to that of the base material. We isolated the minimum odorant groups necessary to retain the characteristic fragrance of the scent material by precise fractional distillation and chromatography. Each group consisted of several organic compounds with similar structure (i.e., the aldehydes, the formates, and the acetates of α-santalol). We compared the odors of these compounds. The odors of the Z-isomers of the compounds were similar to those of the corresponding saturated compounds, but different from those of the E-isomers. We synthesized the compounds from α-santalol, which was prepared from (+)-3-bromocamphor as starting material by the reported synthetic route with modifications (six steps). We confirmed the above result by sensory evaluation of the synthesized compounds.

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Structure-odor Relationship on Sandalwood Odorants: Synthesis and Olfactory Analysis of β-Santalol Analogues

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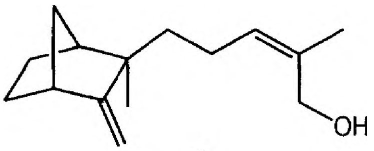
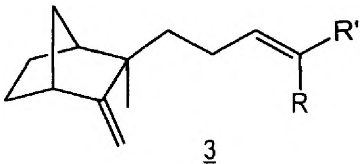
Sandalwood essential oil is a high valued raw material used in perfume formulations obtained from *Santalum album* wood. Its particular fragrance is in part due to β-santalol (1) (~30% of the essential oil) which exhibits powerful sandalwood, milky, urinous tonality. Despite many works, β-santalol synthesis is still too expensive for perfumery purposes[1, 2]. Many β-santalol analogues have been prepared, expanding the knowledge of sandalwood structure-odor relationship (SOR)[3].

Other natural sandalwood odorants have been identified in this precious essential oil such as β-photosantalol (2) (0.05% of the essential oil), which possesses a strong sweaty sandalwood scent. In order to bring new data in the SOR of sandalwood odorants, we synthesized the *Z* and *E* isomers of the β-photosantalol analogue (3) from natural β-santalol, as well as some other analogues (4). Olfactive potency of all products and intermediates was evaluated by GC-O and compared to those of β-santalol using AEDA method. All these data were then treated by computational methods to bring new insights on the sandalwood olfactophores.

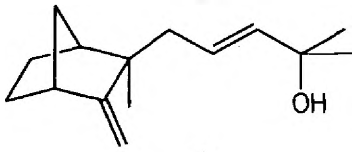


1



3

R=$C(CH_3)_2OH$	R'=H
R=H	R'=$C(CH_3)_2OH$



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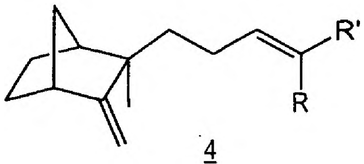

4

R=CH_2OMe	R'=H
R=CH_2SH	R'=H
R=CN	R'=H
R=$CH(CH_3)OH$	R'=H
R=H	R'=$CH(CH_3)OH$
R=CHO	R'=H
R=H	R'=CHO
R=$COCH_3$	R'=H
R=H	R'=$COCH_3$

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The strategy of sample preparation step exemplified by the volatiles of Teucrium marum L.

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To extract the volatiles from plant material many sample preparation techniques were reported and among them, hydrodistillation (HD) was the common procedure for isolation of essential oil. As the emitted volatile fraction plays a fundamental role in the plant's life, there has been a remarkable renewal of interest in headspace sampling techniques (HS) such as Solid Phase Micro-Extraction (SPME).

Four works deals with the volatiles of *T. marum*, three of them reported the occurrence of iridoids compounds (dolichodial, teucrein and dolicholactone) and sesquiterpenes ((E)-β-caryophyllene, caryophyllene epoxide and (E)-β-bisabolene) extracted with organic solvents by maceration then distillation [1], soxhlet [2] and simultaneous distillation-extraction [3]. Only one study described the chemical composition of Sardinian essential oil obtained by hydrodistillation in which isocaryophyllene (20.2%), (E)-β-bisabolene (14.7%), β-sesquiphellandrene (11.2%), α-santalene (10.9%) and dolichodial (9.3%) were reported as main components [4].

The aim of the study was to study the chemical variability of the Corsican *T. marum* by investigating its volatiles components. From the aerial parts of the same plant material, we extracted the essential oil by HD and the volatile fraction by HS-SPME. Essential oil was investigated using GC/RI, GC-MS and Carbon-13 NMR spectroscopy after fractionation on chromatography column. The volatiles sampling from the gaseous phase in equilibrium of *T. marum* was analysed using GC/RI and GC-MS. To our knowledge, it's the first report on the *T. marum* volatiles extracted by HS-SPME.

The analysis of 25 sample oils of *T. marum* allowed the identification of caryophyllene oxide (0.4-20.5 %), (E)-β-caryophyllene (0.9-18.9 %), (E)-α-bergamotene (1.0-18.8 %), (3Z, 6E, 10E)-α-springene (1.1-17.8 %), β-bisabolene (0.8-14.3 %) and β-sesquiphellandrene (0.9-11.5 %) as main components. Corsican oils were close to the sample oil reported in the literature except for dolichodial which was never detected in the 25 Corsican sample oils analyzed. The analysis of the volatile fractions emitted from the same plant material, allowed to identify dolichodial (16.8-83.4%), (E)-α-bergamotene (0.2-21.5%), (E)-β-caryophyllene (0.3-21.5%), (E)-β-bisabolene (0.2-11%) and β-sesquiphellandrene (0.2-8.3%) as major components. The absence of dolichodial in *T. marum* essential oil and its occurrence in the HS-SPME fractions, led us to investigate the hydrolate-extract obtained by Liquid-Liquid Extraction. The hydrolate-extract analysis allowed identifying five iridoids compounds such as dolichodial (23.3 %), dolicholactone (20.0 %), epi-dolichodial (5.0 %), iridomyrmecine (3.7 %) and nepetalactol (1.9%). The absence of dolichodial in the essential oil could be explained by its water solubility.

This work is a good example to demonstrate the need to adapt the sample preparation strategy for the complete characterization of the volatiles of plant material. Moreover, in order to determine novel oil chemotypes or characterize the volatiles of plant material not yet studied, it could be interesting to carry out at least two sample preparation techniques.

Artificial Crushing Finger (ACF) : a versatile device for rapid evaluation of native oils from fresh aromatic plants

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Essential oil plants are aromatic crops used in perfumery, cosmetic and food industry which extracts were obtained by hydrodistillation or steam distillation. During extraction process, essential oil storage vesicles were explosed due to temperature and mechanical factors and consequentatively aromatic compounds could be more easily extracted by water vapors. Flavorists and farmers duplicated this fact when they crush between their fingers a couple of leaves or flowers followed by sniffing of the generated odor in order to set up a rapid evaluation of the aromatic potential of such plants. In the framework of the AROMATIC Program, we develop/adapt instruments to be used for a rapid evaluation of aromatic potential of essential oil plants based on headspace analysis. The so-called Artificial Crushing Finger device (ACF) was based on a modified artificial mouth and has the objective to perform a rapid evaluation of aromatic potential of essential oil plants with only a couple of leaves through a controlled Dynamic HeadSpace (DHS) or Solid Phase Micro extraction (SPME) concentration of volatiles emitted by crushed fresh leaves followed by GC-MS analysis [1]. First developped for analysis of native oils from medieval aromatic plants fresh leaves [2] , the use of ACF was then extended to other aromatic plants leaves and citrus peels. In the present paper, we reported optimization of ACF parameters (especially piston rotation speed, descent speed up and down) applied for crushing of fresh hard leaves, soft leaves and citrus peels. Comparison of volatiles chromatographic profiles of essential oil, obtained by hydrodistillation or cold expression, with the ones obtained with entire fresh leave/peel volatiles concentrated by SPME (PDMS-DVB-CAR) and analyzed by GC-MS stated that ACF could be a powerful tool for a rapid evaluation of native oil content from freshly crushed samples [3].

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An UPLC-MS/MS method for the quantification of furocoumarins. Comparison with classical methods.

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Furocoumarins (heterocyclic oxygenated compounds) are naturally present in natural raw extracts, mainly in citrus spp. Physiological effects [1, 2] of furocoumarins were known and a regulation was edicted, limiting their presence in cosmetic products (level below 1 ppm).

Many studies described the analyses of furocoumarins, most of them using HPLC technique (HPLC-DAD-UV [3]). A study performed on 15 furocoumarins compared three different detection modes: HPLC-DAD-UV and HPLC-MS, HPLC-FLD [4]. A more recent article using the HPLC-MS/MS method [5] described the quantification of bergapten and bergamottin in both essential oils and various commercial samples using two different ionization modes.

We developed a method for a simultaneous quantification of ten furocoumarins. The separation was performed by UPLC, using sub-2µm particles sizes with high linear solvent velocity. Quantification was achieved on a Triple Quadrupole Mass Spectrometer with an electrospray ionization source (ESI). Furocoumarins were analysed in multiple reaction monitoring (MRM) mode. MRM transitions were monitored for each analyte.

Performances of the method (linearity, repeatability, repeatability intraday, reproductibility, limit of quantification and limit of detection) were established and compared with those of the two classical methods: HPLC-DAD-UV and HPLC-FLD. The UPLC-MS/MS method was applied for the quantification of furocoumarins in various natural extracts. Results were compared with those obtained from the two classical HPLC analytical methods (HPLC-DAD-UV, HPLC-FLD). Due to its high sensitivity and specificity, the UPLC-MS/MS method appears to be the most appropriate choice for the simultaneous quantification of furocoumarins in natural extracts.

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Volatile compounds of thyme infusion

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The relative proportions of chemical classes (hydrocarbons, oxides, alcohols/ethers, ketones/ketones and esters) in the essential oil of thyme (*Thymus vulgaris* L., Lamiaceae) and in the volatile fraction of extracts prepared by infusion were examined and showed remarkable differences. The volatile compounds of the infusion were isolated by two different methods, hydrodistillation and solid phase extraction (SPE). The main constituents of the volatile fraction of *T. vulgaris* infusion were (hydrodistillation/SPE): thymol (75.2%/79.3%), carvacrol (5.5%/5.9%), linalool (3.8%/2.9%), borneol (2.3%/2.1%) and *p*-cymene (3.0%/1.1%). For comparison, the major constituents of the essential oil of the leaves were thymol (51.6%), *p*-cymene (16.8%), γ-terpinene (7.3%), carvacrol (3.7%) and linalool (2.1%). Comparison of the total essential oil yield quantified by hydrodistillation of the infusion (0.81% v/w) with the essential oil yield of the leaves (2.18% v/w) revealed that 37.2% of the initial oil could be extracted by infusion. Furthermore investigations of the volatile fraction of infusions of variable mixtures of thyme with Primulae radix and Liquiritiae radix were performed. Both roots are well known saponine containing herbal drugs and often used as an integrant of various tea mixtures in combination with thyme in the treatment of upper respiratory tract infections [1]. Therefore, the qualitative and quantitative effects of these roots on the volatile fraction of the infusion extracts were determined and statistically evaluated.

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Encapsulation of cinnamon oil in β-cyclodextrin

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Inclusion complexation of flavors with β-cyclodextrin has been applied to protect oil volatiles against oxidation, heat and light degradation, evaporation and moisture.

The aim of the present study was to prepare the inclusion complexes of the *Cinnamomum verum* essential oil with β-cyclodextrin, using a co-precipitation method, and to investigate the amount as well as the composition of the surface adsorbed and entrapped oil by GC/MS and compared with the composition of the initial oil.

Inclusion complexes between the *Cinnamomum verum* essential oil and β-cyclodextrin were prepared with the four oil to β-cyclodextrin ratios of 5:95, 10:90, 15:85 and 20:80 (w/w) in order to determine the effect of the ratio on the inclusion efficiency of β-cyclodextrin for encapsulating oil volatiles. The retention of essential oil volatiles reached a maximum of 94.18 % at the oil to β-cyclodextrin ratio of 10:90. Though, the maximum inclusion efficiency of β-cyclodextrin was achieved at the ratio of 15:85, in which the complex powder contained 117.2 mg of oil/g of β-cyclodextrin. Thirty-one flavor compounds were detected in the original oil and 21 compounds with the content higher than 0.10 % were considered in complexation process. The qualitative and quantitative composition of the volatiles in the total oil extracts was similar to the initial oil. However the chromatographic profile of the surface adsorbed oil was different. The thirteen major flavor compounds, found in the commercial *Cinnamomum verum* essential oil, were present in all of the extracts, but in the different proportions between the total and surface oil extracts.

Key Words: Cinnamomum verum oil, β-cyclodextrin, inclusion complex, flavor volatiles.

Average Mass Scan of the Total Ion Chromatogram vs. Percentage Chemical Composition in Multivariate Statistical Comparison of Essential Oils

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The analysis of complex volatile mixtures (essential oils) by GC/MS is a time-consuming process. It involves separation and identification of the components based on their retention times and fragmentation patterns, followed by determination of their relative percentages from integration of their peak areas. Herein we show that multivariate statistical analysis of the relative abundances of the *m/z* values obtained from the average mass scans (AMS) of the essential oils a faster and potentially more reliable method of assessing these complex volatile mixtures. To achieve this, 28 essential oils (24 different species from the following 12 genera: *Artemisia*, *Anthemis*, *Achillea*, *Sonchus*, *Tanacetum*, *Ramonda*, *Foeniculum*, *Telekia*, *Taxus*, *Calamintha*, *Vaccinium*, *Arctostaphylos*) were hydrodistilled using an original Clevenger type apparatus, and analyzed using GC and GC/MS. More than 1000 different compounds (mono- and sesquiterpenes, phenylpropanoids, carotenoid derived compounds, fatty acids and fatty acid esters, fatty acid derived compounds, "green leaf" volatiles, etc.) were successfully identified in the oil samples. The AMS profile and chemical composition of each oil were compared to one another using agglomerative hierarchical cluster analysis and principal component analysis. The results obtained strongly suggest that multivariate statistical analysis of AMS profiles is a promising, time saving and reliable tool for analyzing complex volatile mixtures, in particular essential oils.

α-Linalool as a constituent of sweet basil (*Ocimum basilicum* L.) essential oil: the first report

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Ocimum basilicum L. (Lamiaceae), or sweet basil, is a highly fragrant culinary herb of major importance. Essential oils extracted from this plant species have been extensively studied (chemical composition, pharmacological activity, etc.) [1].

Here in, we present results of detailed chemical analyses (GC, GC-MS) of 4 different *O. basilicum* essential oils. The main constituent of all 4 samples was β-linalool (44.1-79.5%; 3,7-dimethylocta-1,6-dien-3-ol). This acyclic monoterpene alcohol has been reported as the main constituent of a great number of previously analyzed sweet basil essential oils [1]. However, in one of the samples (OB1; commercial oil obtained from the local pharmacy), α-linalool (16.0%; 3,7-dimethylocta-1,7-dien-3-ol), less stable of two linalool isomers, has been also detected. When speaking of its natural occurrence, the latter has been previously reported only in a limited number of studies [2,3]. This is the very first time α-linalool was detected as an *O. basilicum* volatile and a major one. To corroborate the GC-MS identification, OB1 oil was fractioned using column chromatography (MPLC; SiO_2, gradient elution: hexane/diethyl ether (0-100% diethyl ether)). β- And α-linalool, estragol, as well as some other minor OB1 constituents, obtained in pure state, were fully spectrally characterized (^{1}H-NMR, ^{13}C-NMR, IR, MS, UV).

Chemical compositions of the four analyzed and 24 other, randomly chosen corresponding oils (including several different *Ocimum* taxa) were compared using multivariate statistical analyses (agglomerative hierarchical cluster analysis and principal component analysis). Results of the chemical and statistical analyses once again pointed out to the existence of (at least) two different (β-linalool or estragole) *O. basilicum* chemotypes, but did not separate any α-linalool group.

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Title: Some Theoretical Studies on the Structural Property of a Group of Essential Oils of Salvia

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Salvia is a widely distributed annual herbaceous plant. The hydro distillated essential oil of salvia hydrangea was analyzed by GC-MS and 54 different compounds representing 95.9% of the compounds in the oil were identified. And the antibacterial activity of the oil was determined against 30 bacterial. In this work we have some theoretical calculations on the structural property of these antibacterial compounds by using the density functional method at the B3LYP/6-31G (d,p)[1] level of theory was used to calculate the geometry and quantum descriptors of this molecules. The results indicate a strong intramolecular hydrogen bond of the type O-H...N and predominance of phenol-imine tautomeric form. Mulliken populations, eigenvalues, contribution of the atomic orbital to the molecular orbitals of these compounds are reported in this work, and the electronic properties of these compounds were suggested by this method [2, 3].

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Evaluation of Simultaneous Hydrodistilation and Extraction for Isolation of Volatile Oils from *Salix aegyptiaca.L.*

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Volatile components obtained from *Salix aegyptiaca L.* from Iran was fractionated by simultaneous hydrodistilation and extraction (SHDE) with xylene as solvent using modified Clevenger apparatus and with different solvents using the methods of hydrodistilation liquid-liquid extraction ultrasound assisted (HD-LLE-USA) and Soxhlet extraction(SE). Then Salix Iranian oils were analyzed by capillary gas chromatography (GC), using flame ionization and capillary gas chromatography mass spectrometry (GC/MS) detection. The identification of the compounds was based on a comparison of retention indices and mass spectra with those of authentic samples and with NIST MS library. The identification was also confirmed by comparison of the retention indices with data in the literature [1-3]. The percentages of compounds were calculated by the area normalization method, without considering response factors.

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POSTER PRESENTATIONS

SESSION C

Biological activity of essential oils and their components

Inhibition of LPS Induced Inflammatory Cytokines in Murine RAW macrophage-like Cells by Oregano Oil (*Plectranthus amboinicus*)

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A variety of compounds in Oregano oil (*Plectranthus amboinicus*) have Anti-oxidant activity that inhibits production of pro-inflammatory substances such as tumor necrosis factor alpha (TNFα) and interleukin – 6 (IL-6). Compounds with Anti-oxidant activity have been linked to the prevention of cancer and heart disease. Since TNFα and IL-6 play an important role in the inflammatory process due to its release from stimulated macrophages, inhibition of excessive TNFα and IL-6 production provides an indication of the Anti-oxidant activity and anti-inflammatory potential of a compound. In this study, the anti-inflammatory properties of various concentrations of steam-distilled oregano oil (*P. amboinicus*) were tested via its ability to inhibit pro-inflammatory cytokine production in LPS-stimulated RAW cells using ELISA. The results demonstrate the anti-inflammatory properties of Oregano oil (*P. amboinicus*) compared to oil from other oregano species. In addition, we demonstrate the novel finding that 10-4 and 10-5 dilutions of Oregano oil (*P. amboinicus*) inhibited the production of IL-6 by 58.5% and 43.9%, respectively; the same dilutions of Oregano oil (*P. amboinicus*) also inhibited the production of TNFα by 81.1% and 42.1%, respectively.

Biological activities of essential oil of Nepeta glomerata Montbret et Aucher ex Bentham from Lebanon.

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The genus *Nepeta* L. belongs to the family Lamiaceae, subfamily Nepetoideae, tribe Mentheae. It is one of the largest genera of Nepetoideae and comprises about 300 herbaceous perennial, rarely annual species distributed in temperate Europe, Africa and southwest Asia. Several *Nepeta* spp. are used in folk medicine as diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic, febrifuge, emmenagogue, sedative agents and for the antiseptic and astringent properties as topical remedy in children cutaneous eruptions, snakes and scorpion bites [1]. The diversity, species richness and variation as well as chemical properties have led to much research into the genus: nepetalactones, iridoids, and their glucosides, diterpenes, and triterpenes are reported in the literature as major constituents of *Nepeta* species [2]. Publications on *Nepeta* volatile oils clearly demonstrate that chemical polymorphism is characteristic of this species, and the oil composition depends on variety, growing site, climatic conditions, and analysis method. Because of the multiple biological activities of *Nepeta* species and their use in folk medicine, we were interested in the essential oil content, composition and biological activity of members of this genus collected in Lebanon [3]. *Nepeta glomerata* Montbret et Aucher ex Bentham, catmint, has an aromatic, characteristic odour, which bears a certain resemblance to that of both Mint and Pennyroyal. In this communication we report the study of the chemical composition of the essential oil of *N. glomerata* collected in the village Ainnete, Makmel mountain, that is considered as the East's Plateau and Lebanon's water reservoir and has unique natural habitat with rare indigenous Middle Eastern trees and plants. The aim of this work was besides to get a better knowledge on the potential biological activity of *N. glomerata* essential oil. Particularly, in the present study we investigated the susceptibility of bacteria that play a role in respiratory tract and skin infections to the oil with regard to its chemical composition. In addition, we studied anti-inflammatory activity of the oil and its *in vitro* cytotoxic assay on two human cancer cell lines, renal adenocarcinoma (ACHN) and amelanotic melanoma (C32).

GC and GC/MS analysis enabled the identification of 70 compounds representing 89.4% of the total oil. The most abundant compounds were α-pinene (13.4%), spathulenol (8.6%), carvacrol (5.4%) and hexadecanoic acid (4.2%). The oil showed antibacterial, anti-inflammatory and cytotoxic activity. Gram + bacteria were particularly sensitive to the action of the oil when it was tested *in vitro* against eight bacteria species; besides, *N. glomerata* oil effectively inhibited LPS-induced NO production in macrophage cells line RAW 264.7 with IC_{50} of 78.1 µg/ml. The *in vitro* cytotoxic assay on two human cancer cell lines showed that the oil was more active on ACHN cell line (48% of inhibition of proliferation at 100 µg/ml) in comparison to C32 cell line.

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Cytotoxic activity of essential oils, fractions and constituents of *Curcuma longa* and *Curcuma xanthorrhiza*

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Curcuma species have a long history of use in ethnomedicine. Extracts, essential oils and individual constituents (especially less-volatile curcumin and volatile turmerone isomers of *Curcuma longa* and volatile xanthorrhizol of *Curcuma xanthorrhiza*) from different parts of *Curcuma* plants have been proved to possess specific antibacterial and antiviral activity as well as anticancer and non-specific effects on DNA, RNA and protein metabolism *in vitro* [1, 2, 3]. Recently, an inhibitory activity of xanthorrhizol in combination with curcumin have been shown against breast cancer cells [4]

Cytotoxicity of *C. longa* and *C. xanthorrhiza* essential oils and their polar and apolar fractions as well as xanthorrhizol were evaluated on HeLa cells using Cell Proliferation Reagent WST-1 (Roche). The substances of interest were dissolved in DMSO. Cytotoxic effect was measured after 24 hours of incubation of the cell culture on the medium with the studied substance in concentration 1 to 1000 µg/ml. Commercially available curcumin and actinomycin D were used as reference compounds (Sigma Aldrich).

Essential oils and polar fractions of *C. longa* and *C. xanthorrhiza* demonstrated a similar level of cytotoxicity (CC_{50} 10.7±2.3 µg/ml and 12.0±4.0 µg/ml for *C. longa* essential oil and its polar turmerone-rich fraction, respectively; CC_{50} 10.7±2.3 µg/ml and 8.7±3.1 µg/ml for *C. xanthorrhiza* essential oil and its polar fraction containing around 30% of xanthorrhizol, respectively). Apolar fractions of *C. longa* and *C. xanthorrhiza* showed a CC_{50} 30.0±8.7 µg/ml and 23.3±6.4 µg/ml, respectively. Cytotoxic activity (CC_{50}) of individual compounds was 5.7±2.5 µg/ml (26.7±6.4 µM) for pure xanthorrhizol, 5.5±1.8 µg/ml (15.0±5.0 µM) for curcumin and 0.03 µg/ml (0.02 µM) for actinomycin D.

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Chemical composition, olfactory analysis and antibacterial activity of *Thymus vulgaris* L. chemotype "geraniol"

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Thymus vulgaris L. exists in a variety of some chemotypes [1-5], characterized by their different chemical compositions. In particular 6 chemical types of this thyme species are wellknown since many years: thymol, carvacrol, geraniol, linalool, terpinen-4-ol and α-terpineol/4-thujanol [6].

The used essential *T. vulgaris* L. ct "geraniol" oil was cultivated in the south of France and analyzed by GC/FID and GC/MS polar and apolar columns. The following major compounds could be detected: geranyl acetate 41.6%, geraniol 26.4%, β-caryophyllene 5.2% and *trans*-sabinene hydrate 2.6%. Thymol and carvacrol are minor componts with 0.5% and 0.2%.

The oil was olfactorily evaluated by two professional perfumers and two aroma chemists. The odour was characterised as spicy, flowery – soft rosy and balsamic.

The antimicrobial activity of the essential oil was tested against eleven different genera of Gram-positive and Gram-negative bacteria by using serial broth dilution [7] method. It could be verified, that the essential oil posesses high antimicrobial activity against both strains of Gram-positive *Staphylococcus aureus*, and Gram-negative *Escherichia coli*, as well as three strains of *Salmonella abony* but less against two strains of *Pseudomonas aeruginosa* and *Ps. fragi*. The highest activity was verified with *Brocothrix thermosphacta*.

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Aroma-profile and antioxidant activity of 14 cultivars of basil (*Ocimum basilicum* L.) as affected by drying.

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The genus Ocimum (*Lamiaceae*) consists of about 150 species with a large number of varieties containing both terpene and non-terpene constituents in their essential oils. Fresh and dried basil is widely used in the Mediterranean cuisine, for example in tomato-based products, vegetables, salads, pizza, meat, soups and marine foods. Essential oils from 14 basil cultivars available in Poland were analyzed by GC-MS. Basil plants were grown in 2009 under the same field conditions at a Research Station near Wroclaw (Poland): *Dark Opal*, *Balloso Napoletano*, *Largo Dolce per Vasi*, *Clasic*, *Clasic* (Polish cultivar), *Thai Siam*, *Verde a Piccolo Foglie*, *Greco a Palla*, *Red Rubin*, *Alla Canella*, *Genovese*, *Al Limone*, *Palla verde scuro*, *Foglie di Latuga*. Basil seeds (Franchi Sementi S.P.A.) were sown, under greenhouse conditions, on April 14th, in multi-pots filled with peat substrate; seedlings were transplanted to the greenhouse soils at the end of May. Leaves for chemical analysis were harvested twice when basil plants were blooming (middle of July and September). Steam-distillation was conducted on samples of fresh and dried herbs. The Deryng apparatus was used to obtain the essential oils. The antioxidant activity was evaluated using the ABTS method. GC-MS analysis provided information (composition and concentration) of about 50 compounds in each sample. The predominant compounds were: i) linalool (the most abundant one, e.g. 50 % in *Italiano classico*), ii) estragol (34 % in *Balloso Napoletano*) and iii) eugenol (40 % in *Palla verde scuro*). The effects of the drying process on the amount and composition of the essential oils was also studied. The most sensitive cultivar to the drying process was *Palla verde scuro*, which experienced a 30 % loss of volatiles compounds. The antioxidant activity was dependent on the basis cultivar and ranged from 1272 to 148 μM Trolox/g of essential oil in *Greco a Palla* and *Alla Canella*, respectively.

Deterrent and repellent activities of selected monoterpenoids against lesser mealworm , *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae).

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Essential oils, especially their major components, often various monoterpenoids, have been the object of great interest of researchers in recent years as a potential source of ecologically safe botanical insecticides due to their toxic, repellent, antifeedant and/or attractant properties [1, 2]. In these studies fifteen compounds occurring naturally in the plant essential oils (citral, eucalyptol, thymol, (*S*)-carvone, (*R*)-carvone, (+)-α-pinene, (-)-α-pinene, (*L*)-neopinocampheol, (*D*)-neopinocampheol, α-thujone, β-thujone, (+)-fenchone (-)-fenchone, α-terpinene and geraniol) were evaluated for antifeedant activity against adults and 30 day old larvae of the lesser mealworm, *Alphitobius diaperinus* Panzer. Most of investigated compounds were purchased from Aldrich. α-Thujone and β-thujone were isolated in new, organic-solvent free approach from essential oil of *Thuja occidentalis* [3]. Campheols were isolated from essential oil of *Hyssopus officinalis* or obtained from chemical reaction. Purity of all used compounds was above 99%.

To examine the feeding deterrent activity 1% alcohol solutions of compounds in choice and no-choice tests were used. In these bioassays oat flakes were used as test food. One gram of the oat flakes was saturated by dipping in 1mL of the test solution or in acetone alone as control. When the solvent was evaporated the oat flakes were offered to insects. After 3 days of feeding (29 ± 1°C in the dark) the mean weight of food consumed in each test was calculated. Basing on the amount of food consumed feeding deterrence coefficients: relative (choice test), absolute (no-choice test) and total were calculated. Especially active compounds were tested for their effect on the growth and development of larvae *A. diaperinus*.

The antifeedant activity of compounds studied varied with developmental stage. Citral, thymol, eucalyptol and (*D*)-neopinocampheol were the most effective feeding deterrents to adults. Some of them such as citral, eucalyptol, and thymol exhibited repellent activity against imago. All compounds, except for thymol and citral were weak antifeedants to larvae *A. diaperinus*. Geraniol, α-thujone and (*L*)-neopinocampheol showed the attractive properties. Compounds with high deterrence against larvae i.e. thymol and citral had an inhibitory effect on the growth of *A. diaperinus* larvae.

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Microbiological activity of caraway essential oil obtained from different origin.

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Caraway (*Carum carvi* L.) is one of the most important medicinal plant cultivated in Poland on area 8 000 ha. Caraway essential oil performs medium antimicrobial activity, although it inhibits growth of many bacteria and fungi [1,2].

The aim of the study was to evaluate microbiological activity of caraway essential oil obtained from seeds originated from different genotypes and investigate the correlation between this activity and essential oil content and its main components (carvone and limonene) content.

In the experiments done in 2008 and 2009, essential oil of 25 selected caraway genotypes originated from: European botanical gardens (18), two cultivars: 'Rekord' and 'Kończewicki' and our own breeding strains, which grown in Poznań, was tested. Antibacterial activity of caraway oil was analyzed by standard dilution in liquid media according to Cavanagh [3]. The Minimal Inhibitory Concentration (MIC) of essential oil, which inhibited standard bacterial strain (*Staphylococcus aureus* ATCC 6338 P), was investigated. MIC value was recalculated to antibiotic units (AU). One-way analysis of variance and correlation analysis were carried out to determinate variability of MIC and AU. and their correlation to essential oil, carvone and limonene content.

The obtained results showed that microbiological activity of caraway oil of the tested objects was significantly different. The strongest activity performed oil of population from Cluj (MIC=0.16 mg/ml; AU=8650), while the weakest activity was determined for oil of population from Kraków (MIC=1.75 mg/ml; AU=582). A significant negative correlation was observed between MIC and carvone content, however positive correlation was observed between MIC and limonene content. Caraway essential oil exhibited medium antimicrobial activity and carvone can be recognized as a one of the active component.

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Kombucha – new active ingredient using in cosmetic preparations

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Kombucha has been used for years in many countries, especially in Japan, Russia, China and Eastern Europe. It is consumed in the form of a refreshing beverage as a dietary supplement and has strengthening and life-prolonging effect on the human body [1].

Kombucha is the colony of multiple bacteria and yeast, living in symbiosis [2, 3].

Kombuchka (a trademarked name) is a new active ingredient in cosmetic industry, and is obtained by the fermentation of sweet black tea and symbiotic microorganisms [1].

On the basis of previously identified Kombucha composition, we may suppose that it has a beneficial effect on skin.

The most important components, identified in the fermented beverage, include: organic acids, B-vitamins, vitamin C and ions of zinc, copper, iron and manganese [4, 5, 6].

For this reason, in modern cosmetology, Kombucha is more and more often found in exclusive cosmetic preparations, having the function of epidermal keratosis regulator, moisturizing, skin-smoothing and lipo-filling substance.

Kombucha is used in various cosmetic's forms – such as anti-age creams for day and night, face serum, anti-wrinkle gel and concentrates with exfoliating effect, moisturizing lotions and emulsions, deep moisturizing, regenerating and calming mask, furthermore in cosmetics improving the structure and elasticity of the skin.

For this reason it gives the skin a fresh, more radiant and vitalized appearance.

The aim of this paper is to present the properties of Kombucha as cosmetic ingredient, which gives a favourable effect to the skin.

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Activity of essential oils against food spoiling yeast

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Yeasts are recognized as one of the main factors of food spoilage. Their development results in changes in food texture, colour and organoleptic features. *Candida*, *Debaryomyces*, *Kluyveromyces*, *Rhodotorula*, *Trichosporon* and *Yarrowia* are counted to be the most frequent food borne yeast [1]. The use of spices in food preservation is based on their anti-microbial activity. However antibacterial activity of essential oils is well documented [2, 3], their action against yeast has not been extensively searched. The oils seemed to be a good preservative of natural origin directed against yeast.

In the course of the study antifungal activity of eight essential oils against nine yeast species associated with food spoiling microflora was examined. Following commercial essential oils were tested: basil (*Ocinum basilicum* L.), onion (*Allium cepa* L.), garlic (*Allium sativum* L.), lemon (*Citrus limon* L. Burm.), grapefruit (*Citrus paradisi* Macfad.), marjoram (*Origanum majorana* L.), peppermint (*Mentha pipperita* L.) and thyme (*Thymus vulgaris* L.). *Candida rugosa* Ł0002, *Debaryomyces hansenii* Ł0015, *Kluyveromyces marxianus* Ł0025, *Rhodotorula glutinis* Ł0051, *Rhodotorula minuta* Ł0053, *Saccharomyces cerevisiae* Ł0233, *Trichosporon cutaneum* Ł0252, *Yarrowia lipolytica* Ł0264 and *Zygosaccharomyces rouxii* Ł0266 were used. The microorganisms originated from Collection of Pure Cultures of the Institute of Fermentation Technology and Microbiology, Technical University of Lodz, ŁOCK 105. The sensitivity of yeast strains was tested by agar disc diffusion method using paper discs of 6mm in diameter. The results were given as the mean value of six repetitions. The activity of the essential oils was estimated by an impedimetric method using Bactometer M64 (bioMerieux), as described previously [4]. MIC and MFC values were the result of three repetitions. Results were analyzed using a 3-way ANOVA test at a confidence level of $p<0.05$.

All of the tested yeasts expressed the highest sensitivity against thyme oil (inhibition zones 25.5-58.0mm). Generally, the onion oil was the least active and did not affect majority of tested strains. The most susceptible were *D. hansenii* and *S. cerevisiae*, showing inhibition zones from 15.7 to 58.0mm. The estimated MIC values ranged from 0.2 to 24.0µl/ml. The fungistatic activity of essential oils was as follows: thyme > marjoram > peppermint > basil > lemon > grapefruit > onion, garlic oils. The fungicidic activity of the tested oils was slightly different. The most effective were: thyme, marjoram, peppermint and basil oils (MFC 1-24µl/ml). The other tested oils (onion, garlic, lemon and grapefruit) up to the concentration 30µl/ml did not eliminate the yeasts.

The thyme, marjoram and peppermint oils were proved to be the most effective against majority of tested yeast strains. Their application as natural preservatives will be tested in food matrices.

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Tea tree and lemon oils as active agents in facial skin cosmetics

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Essential oils are known for their antimicrobial activity as well as beneficial effects on the mammalian cells. Tea tree and lemon oils are frequently used in cosmetic formulations because of their fragrance and biological action.

In the course of the study a preservative value of tea tree (*Melaleuca alternifolia*) and lemon (*Citrus limon* L. Burm.) oils in facial skin cosmetics was estimated. Moreover, the influence of tea tree and lemon oil preparations on decrease of facial sebum production and intensity of skin hyperpigmentations, respectively, were checked.

Cleansing lotion and tonic with 0.5% tea tree or lemon oils were prepared. The preservative effect of oils against *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida* sp. ŁOCK 0008 and *Aspergillus niger* ATCC 16404 was estimated *in vitro* by bioimpedimetric method [1]. The oil antimicrobial activity *in situ* in cosmetics was evaluated in challenge test according to E.P. 5.0 [2]. Facial sebum production was monitored in 20 patients, aged 18-40 years with moderate seborrhoea on face. Lipid coat was measured twice by sebumeter before and after trial. The study of facial skin hyperpigmentations was performed in 35 healthy females, aged 35-50 years, with the melasma lesions. 25 patients were treated with cosmetics containing lemon oil and 10 patients were set as a control group treated with cream containing 2% hydroquinone.

The antimicrobial activity of tea tree and lemon oils against the tested microorganisms varied. MIC values ranged between 0.02-0.04% for *Asp. niger* to 0.10% for *Ps. aeruginosa*. The Minimal Bactericidal/Fungicidal Concentration (MBC/MFC) of oils was usually slightly higher, from 0.04 to 0.20%. Formulations containing essential oils met Criterion A of the Pharmacopoeia for *S. aureus* (reduction of the inoculum by 3 logarithmic units within 7 days with no increase up to the 28th day) as well as *Candida* sp. and *A. niger* (reduction of the inoculum by 2 logarithmic units within 14 days with no increase up to the 28th day). At the same time, none of the oils affected *P. aeruginosa* within 28 days of incubation. During clinical trials, the sebum level in patients was decreased in 11.8% after four weeks therapy with tea tree oil preparations. A beneficial effect of treatment with lemon oil cosmetics was noted in about half of the patients, however better effects were observed in control group treated with cream containing hydroquinone.

Both the tea tree and lemon oils may serve as active components of facial skin cosmetics, acting as elements of preservative system in formulations. They may also bring a profitable effect in some dermatological problems.

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Geranium macrorrhizum L. (Geraniaceae) Essential Oil: A Potent Agent Against *Bacillus subtilis*

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Geranium macrorrhizum (wild geranium or zdravac, in Serbian, the meaning of which is related to the English term "health"), a common species of the Balkan Peninsula, is the most economically important species of the genus *Geranium* for its aromatic and healing properties. It is highly valued in Serbian tradition and traditional medicine of other Balkan peoples for the treatment of stomach disorders [1–2], as an aphrodisiac [3–4] and emblematic plant, as a symbol for good health [5–7]. Previous phytochemical investigations of this species resulted in the identification of flavonoids [8–11], terpenoids [6,12–16] and tannins [10]. The extracts of *G. macrorrhizum* have been reported to possess a broad spectrum of antimicrobial, hypotensive, spasmolytic, astringent, cardiotonic, antioxidant, capillary and sedative activities [9–10,17–18].

The volatile hydrodistilled compounds from aerial parts and rhizomes of *Geranium macrorrhizum* L. were screened for their antimicrobial activity in disc diffusion and microdilution assays. The assays pointed out to the very high and selective activity of the oils against *Bacillus subtilis* having a minimum inhibitory concentration with the order of value of 1 μg/ml. This provoked us to perform detailed compositional analyses of the oils. GC and GC/MS analyses allowed the identification of 283 constituents. The oils consisted mainly of sesquiterpenoids, the main ones being germacrone (49.7% in the oil from aerial parts) and δ-guaiene (49.2% in rhizome oil). Significant qualitative and quantitative compositional differences in the oils from the two plant parts were observed. Further antimicrobial testing made possible to determine that germacrone, the major constituent of the oil from aerial parts, was not the sole agent responsible for the observed activity.

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Free radical scavenging, antioxidant, antimicrobial and antifungal capacity of extracts from the leaves of *Eremophila longifolia* (F. Muell) (Myoporaceae): an indigenous Australian medicinal plant with high geographic chemovariabiliy

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Hydrodistilled essential oils, as well as total solvent and aqueous extracts from leaves of the Australian Aboriginal medicinal plant *Eremophila longifolia* (Emu Bush) collected at various sites in western New South Wales, Australia were chemically characterised using GC/MS and TLC. Extracts were then assayed for their antimicrobial and antifungal capacities and assessed for free radical scavenging and antioxidant capacities. Antimicrobial/antifungal capacities were investigated using disc diffusion, broth dilution and TLC bioautography to identify separated individual active components. Methods for the determination of antioxidant and free radical scavenging consisted of abrogation of AAPH induced haemolysis, DPPH reducing ability (also in conjunction with TLC to identify individual separated active components), a beta-carotene/linoleate system, DMPD scavenging and ferric iron reducing ability. Chemical characterisation was consistent with the geographical chemotypic distribution already identified elsewhere [1, 2], with additional variability revealed. Essential oil composition exhibits a broad range of cyclic aliphatic and aromatic monoterpenes; including alcohols, ketones and alkenes; with overall oil yield ranging from very low (<0.1% of wet weight) to extremely high (>7% of wet weight). Antimicrobial/antifungal assays of variously prepared extracts of NSW chemotypes revealed moderate to high activity, the variation of which in most cases correlated with the cognate variation in antioxidant and free radical scavenging ability. Some extracts had normalised radical scavenging activity comparable to that of green tea extracts used as positive control. Further investigations, such as DNA fingerprinting, anti-inflammatory activity and cultivar chemotype requirements are in progress.

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Phytochemistry and Pharmacology of Volatile Components of *Plectranthus graveolens* R. Br.

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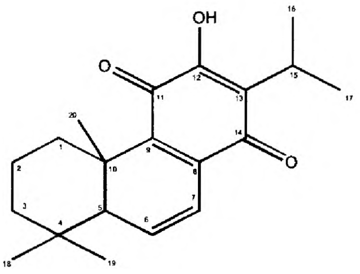
The chemical composition and pharmacological activity of the essential oil of *Plectranthus graveolens* R. Br. (family Lamiaceae) was determined. The plant materials (leaves and stems) were steam distillated with cohabation and GCMS profile of the red oil showed that the volatile components are composed of mono-, sesqui- and diterpenes. Bioactivity-guided fractionation was performed on normal phase column chromatography and size exclusion chromatography. Three abietane diterpenes were isolated: 12-hydroxy-6,8,12-abietatriene-11,14-dione (syn. 6,7-dehydroroyleanone) (**1**); 8,11,13-abietatrien-12-ol (**2**); and 7,13-abietadiene (**3**). Compound **1** was isolated as a red orange crystal and was identified as the compound responsible for the red colour of the oil. Compounds **2** and **3** are known diterpenes but have not been previously reported in this *Plectranthus* species, with compound **2** being one of the major components of the oil.



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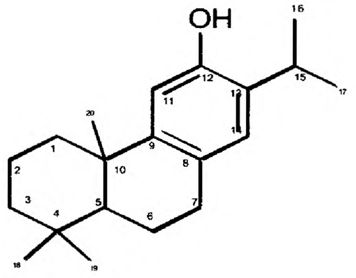

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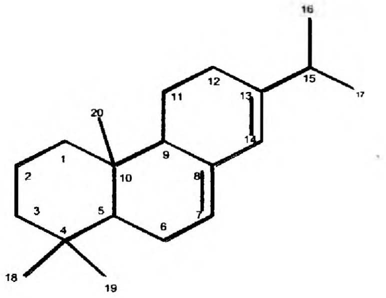

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The ORAC assay was used to measure the antioxidant scavenging activity of the essential oil, fractions and the isolated compounds against peroxyl radicals induced by 2,2′-azobis (2-amidinopropane)dihydrochloride (AAPH). The ORAC value of the crude oil was 230 µmolTE/g, while higher activity was found in some fractions compared to the crude oil. Fraction 8 showed the highest acitivity with 2418 µmol TE/g, which was attributed to its high phenol and p-mentha-1,5-dien-8-ol content. Compound **1** showed a relatively high antioxidant activity at 2244 µmol TE/g. Compounds **2** and **3** gave ORAC values of 808 and 222 µmol TE/g, respectively. The measurement of cell proliferation (P388 mouse lymphoblast cell line) was obtained by using the adenosine triphosphate luminescent (ATPLite™) assay. The crude oil gave an IC_{50} = 202.95 µg/mL. Compounds **1**, **2** and **3** showed remarkable cytotoxic effect with IC_{50} values of 10.75 µg/mL, 4.50 µg/mL and 9.62 µg/mL, respectively.

Preliminary Investigation into the Phytochemistry and Pharmacology of Volatile Oil of *Plectranthus* sp. 'Hann Tableland'

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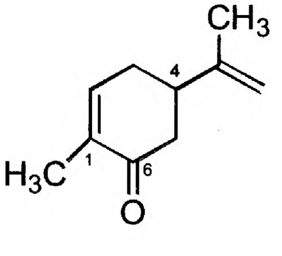
The chemical composition of the steam-distilled essential oil of the botanically undescribed *Plectranthus* sp. 'Hann Tableland' was investigated. The GCMS profile showed that the major volatile components are the monoterpenes δ-3-carene (42.34%), limonene (15.80%), *p*-cymene (8.86%), α-pinene (7.44%) and α-phellandrene (5.72%). The cytotoxicity of the crude oil against the P388 mouse lymphoblast cell line showed IC_{50} = 97 μg/mL and the oxygen radical absorption capacity (ORAC) value was 1111 μmole TE/g oil. The oil was fractionated by C18 SPE chromatography and fractions D and E showed high ORAC values (2368 and 1601 μmole TE/g, respectively), which was attributed to the carvacrol content. Fraction F was the most cytotoxic and had IC_{50} = 29 μg/mL, which was attributed to its intermedeol (11-selinen-4-ol) content.

Fractions C and D were further fractionated by reversed phase preparative HPLC and yielded 6 components: carvone (**1**); carvacrol (**2**); 3,6,6-trimethyl-2,4-cycloheptadien-1-one (**3**); 3-caren-2-one (**4**); *m*-cymen-8-ol (**5**); and *m*-mentha-4,6-dien-8-ol (**6**). Compounds **3** and **5** are known compounds but have not been reported in this genus before. Compound **6** is a novel compound and it is interesting to note that similar to **5**, these two compounds could have originated from a different biosynthetic scheme than the *p*-menthane cascade.



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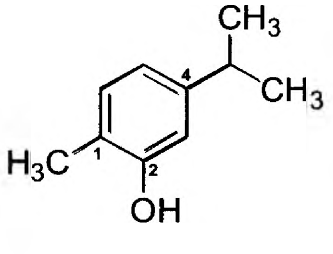

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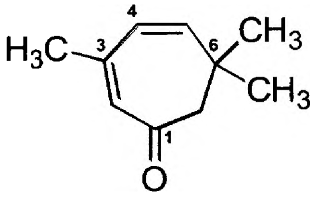

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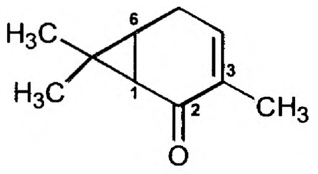

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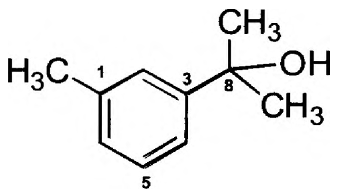

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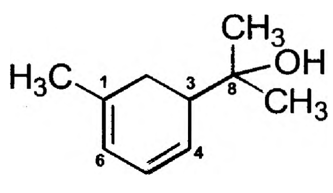

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Inhaling essential oils of two Santalum species affects physiological parameters in humans

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Consistent with its indications in traditional medicine as well as aromatherapy, East Indian sandalwood odour acts as a sedative in animal[1] and human[2] studies. In the present study, the physiological effects provoked by inhalation of the essential oils of East Indian sandalwood (*Santalum album L.*) and Western Australian sandalwood (*Santalum spicatum R.Br.*) in 24 humans exposed to experimental stress were investigated. During the individual testing sessions, several saliva samples were collected, blood pressure was regularly measured and parameters of the autonomic nervous system (heart rate, skin conductance reactivity) were continuously monitored. Salivary cortisol as an endocrine stress indicator was determined by TR-FIA. Statistical analyses evidenced that both essential oils elicited a considerable physiological deactivation concerning systolic blood pressure: East Indian sandalwood oil significantly reduced the peaking of blood pressure during testing phase, whereas Western Australian sandalwood oil did not affect the blood pressure during testing phase but produced a faster and major decrease of blood pressure during recreation phase compared to control. This finding corresponds with a distinct reduction of salivary cortisol levels during recreation in the Western Australian sandalwood oil group compared to the other experimental groups. In conclusion, the results point to the fact that the essential oils of the *Santalum* species can alleviate the physiological reactions to psychological stress and facilitates recovery after exposition to stress.

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Composition and Antimicrobial Effect of Essential Oils Isolated from Different Lavender Species

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The antimicrobial effect of essential oils isolated from different plants is well-known [1-3] and its investigation is very important because, in contrast to antibiotics, resistance rarely occurs due to the large number of components in each essential oil. The aims of our research include the phytochemical characterization of the essential oils extracted from different lavender species (*Lavandula vera* L., *L. intermedia* L., *L. pyrenaica* DC., *L. stoechas* ssp. *stoechas*) that are important from therapeutic and economic aspects, the optimization of direct bioautography for selected *Pseudomonas* strains and the microbiological investigation of the effect of essential oils using broth dilution method.

Lavender species were collected from the Botanical Garden of the University of Pécs (Hungary) in 2009, and essential oils were isolated by steam distillation. One essential oil was obtained from the Lavender Garden of Tihany (Hungary). The chemical and percentage composition of essential oils were analysed by GC and GC-MS.

The main component of the essential oils was linalool in the majority of lavender species, while L-fencon and camphor were identified in the largest amount in *L. stoechas* ssp. *stoechas*.

Various methods can be used for evaluating the antimicrobial effect, but often it is difficult to compare their results. In the present study direct bioautography and broth dilution methods were applied. Direct bioautography was optimized by choosing the appropriate circumstances (incubation time, temperature, agar medium, density of bacterial suspension). However, detection with MTT was not successful in the case of *Pseudomonas* strains. Broth dilution is one of the most frequently used methods, its advantages include that it is easy to perform and provides information about MIC (minimal inhibitory concentration) as well. Because of the lipohilic character of essential oils, emulsions containing 0.2% polisorbate 80 were prepared from the samples.

In each of the eight investigated essentil oils an inhibitory effect was detected and MIC value was in the range 12.5-50 µl/ml.

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The Chemical Characterisation of some Salvia Essential Oils and their Inhibitory Effects on Acetylcholinesterase and Butylcholinesterase

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The genus *Salvia* L. (Lamiaceae) represents 95 species, 48 of which are endemic in Turkey. *Salvia* species are used for their culinary, horticultural and medicinal properties since centuries. They are generally known as 'adaçayı' in Turkey and consumed as a herbal tea or in other preparations also for the treatment of various diseases and complaints in folk medicines.

In our continuing research on *Salvia* species, we have investigated *S. ballsiana* (Rech. fil.) Hedge (endemic), *S. cyanescens* Boiss. & Bal. (endemic), *S. pachystachys* Trautv., *S. pseudeuphratica* Rech. fil (endemic) and *S. russellii* Benth. essential oils and their inhibitory effect on acetylcholinesterase (AChE) and butylcholinesterase (BuChE) activity for the first time. Essential oils were obtained from the aerial parts by hydrodistillation and were subsequently analyzed both by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems, simultaneously. Enzyme activities was measured with colorimetric Ellman method [1].

The major components were identified as follows: caryophyllene oxide (34.1%), β-caryophyllene (8.2%) and α-pinene (7.5%) in *S. ballsiana*; spathulenol (23.2%), *p*-cymene (10.3%), 1,8-cineole (9.1%), α-pinene (6.4%) and β-pinene (6.2%) in *S. cyanescens*; β-pinene (24.0%), α-pinene (12.2%), spathulenol (10.4%), viridiflorol (7.7%) and 1,8-cineole (6.5%) in *S. pachystachys*; camphor (48.3%) and 1,8-cineole (17.4%) in *S. pseudeuphratica*; β-pinene (20.4%), 1,8-cineole (9.5%), α-copaene (8.7%), valeranone (8.7%) and α-gurjunene (4.8%) in *S. russellii* essential oils, respectively. In the enzyme inhibition assays, the % inhibitory effect of essential oils at 80 μg/ml concentration of *S. ballsiana, S. pseudeuphratica, S. russellii* on AChE activity was higher than 40%, respectively. Essential oils of *S. pseudeuphratica* and *S. cyanescens* showed the highest inhibitory effect on BuChE activity (49.18±1.15 and 41.87±4.14) at the same test concentrations, respectively. Eserin was used for comparison as a reference substance with 89.04±2.55% inhibition in the assays.

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Antimicrobial and antioxidant evaluation of *Vitex agnus castus* essential oils

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Vitex agnus castus L. of Verbenaceae is a shrub which is widely distributed in the Middle East and Southern Europe. It is used today in the treatment of climacteric complaints such as menstrual disorders, spasmodic dysmenorrhea etc. also many traditional applications are recorded. In this present study, two samples of *V. agnus castus* collected from different localities in Turkey were investigated for their essential oil compositions, antioxidant and antimicrobial activities. The essential oils were isolated from aerial parts by hydrodistillation which were analyzed by GC and GC/MS, simultaneously. Major compounds were identified as sabinene (20.8%) and 1,8-cineole (19.5%) for the İzmir sample; sabinene (18.6%) and β-caryophyllene (10.5%) for the Antalya sample. The essential oils were screened for their antimicrobial and radical scavenging activities by using a microdilution assay against a panel of human pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Salmonella typhimurium*, *Candida parapsilosis* and for the antioxidant activity with DPPH radical, respectively. İzmir sample showed weak inhibitory activity against *C. parapsilosis* and *P. aeruginosa* while Antalya sample showed weak inhibitory activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *C. parapsilosis* compared with antimicrobial standards. Both samples showed weak antioxidant activity compared to ascorbic acid. To the best of our knowledge, this is the first comparative antimicrobial and antioxidant evaluation of the aerial parts of *V. agnus castus* growing in Turkey.

The effects of *Nigella sativa* L. and its contituents against plant pathogenic fungi

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The genus *Nigella* (Ranunculaceae) is represented by various species of Mediterranean and western Asian origin. However, the most popular and known species *N. sativa* is cultivated in Afyon, Burdur, Isparta and Denizli provinces since centuries in Turkey. The plant is known as black cumin and the spicy seeds are used commonly in food and bakery. The medicinal usage is dating back to the ancient Egyptians, Greeks and Romans. In his Canon of Medicine Avicenna states the black seed acts as an expectorant, it stimulates the body's energy and helps recovery from fatigue and dispiritedness among many other uses. *N. sativa* and its preparations are extensively used in traditional medicine, for healing various respiratory and gastro-intestinal, microbial, immune and dermal diseases.

The major constituent of the essential oil obtained by hydrodistillation was found as thymoquinone. Thus, thymoquinone was used in the assays with the oil comparatively. Antifungal activity of the oil was observed against the strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* using direct bioautography on silica gel TLC. *N. sativa* essential oil showed non-selective activity against all three *Colletotrichum* species at 80 µg. The identification of active antifungal compounds was confirmed on the TLC plate. 1D-TLC bioautography revealed that R_f value of 0.59 corresponded with the R_f value of the thymoquinone standard in *n*-hexane:diethyl ether (8:2, v/v) system. Thymoquinone was subsequently evaluated using a 96-well microbioassay system against *C. acutatum, C. fragariae, C. gloeosporioides*, *Botrytis cinerea*, *Phomopsis obscurans*, *P. viticola* and *Fusarium oxysporum.* Secondary screening using this micro-dilution broth assay showed that thymoquinone possessed selective antifungal activity at 30 µM and inhibited the growth of *P. viticola* by 60% after 144 h exposure. However, inhibition of *Colletotrichum, Botrytis*, *Phomopsis* and *Fusarium* species was not observed at other tested concentrations when compared with standard antifungals.

Chemical Composition and Antimicrobial Activity of Essential oils of Roots from *Centaurea virgata* Grown in Kakhk area in Iran

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Centaurea genus is one of the most important plants in Iran, some of them are endemic. In this study root of *Centaurea virgata* was collected from Kakhk in Khorasan-e-Razavi in Iran, chemical constituent of essential oils of roots of *Centaurea virgata* determined. Air-dried roots (180gr) were subject to hydrodistillation in a Clevenger – type apparatus until there was no significant increase in the volume of the oil collected (3h). The yield of the yellow oil was 0.7% (w/w). The essential oil was analyzed by GC and GC/MS.

Indentification of the component was based of GC retention indices computer matching with wiley GC-MS library, and by comparison of the fragmentation patterns of the mass spectra those reported in the literature. 25 components were identified constituting more than 95.07% of the oil. δ-3-Carene (5.85%), Camphor (42.49%), β-Caryophyllene (6.81%),Viridiflorel (5.02%), δ-Cadinene (4.15%),Borneole(6.90%) and α-Humulene (5.20%) were major components that in roots of *Centaurea virgata* identified.

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

POSTER PRESENTATIONS

SESSION D

Synthesis and biotransformations of isoprenoids

R-(-)-Carvone and *S*-(+)-carvone biotransformation and glycosylation capacity of *Anethum graveolens* hairy roots: effect on morphology, growth and volatile components

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The biotransformation capacity of *Anethum graveolens* hairy roots cultures was studied by evaluating the influence of the addition of *S*-(+)-carvone or *R*-(-)-carvone (25mg/l) in the morphology, growth and volatiles production. Hairy root cultures were maintained for 7 weeks in SH medium [1], at 24°C and 80 r.p.m. in darkness. Substrates were added 15 days after inoculation. Root morphology was studied by SEM, according to [2]. Growth was measured by the dissimilation method [3] and by fresh and dry weight determination. The volatiles were isolated by distillation-extraction and analyzed by GC and GC/MS. Glycosidic bound volatiles were evaluated by the Baerheim Svendsen and Merkx method [4].

Scanning electron microscopy observations showed that the root morphology was not influenced by the substrate addition in spite of the phenolization of some of the monoterpene-added hairy roots.

Dissimilation in the *R*-(-)-carvone added cultures was higher than that in the control and in the *S*-(+)-carvone added cultures.

The constitutive volatile oils were composed, in more than 50%, by falcarinol (1-59%), apiole (2-37%), palmitic acid (1-8%), linoleic acid (1-2%), myristicin (1-8%) and *n*-octanal (2-20%). The addition of the monoterpene substrates induced the production of nine new compounds: *cis*-dihydrocarvone, dihydrocarveol, *trans*-carveol, *cis*-carveol, dihydrocarveol acetate, *iso*-dihydrocarveol acetate, *trans*-carvyl acetate, *neo-iso*-dihydrocarveol acetate and *cis*-carvyl acetate. However, the relative amount of the new volatiles differed with the monoterpene enantiomer added. Dihydrocarveol acetate was the main biotransformation product (21%) in the *R*-(-)-carvone added cultures, whereas *cis*-carvyl acetate was dominant (11%) in *S*-(+)-carvone added cultures. The relative amounts of the substrates and some of its biotransformation products showed a time-course decrease.

The analysis of the extracted volatiles, after the addition of β-glycosidase to the decoction water, showed that part of both substrates was also glycosylated.

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Biotransformation of (-)- and (+)-Menthone by the Larvae of Common Cutworm (*Spodoptera litura*)

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The use of biological transformation as a biocatalyst processes the advantage of being able to proceed under mild condition and with high regio- and enantioselectivility. In the present study, the biotransformation of terpenoid was attempted by the larvae of common cutworm (*Spodoptera litura*). The reasons for using the larvae of *S. litura* as biological catalyst are as followed: lepidoptera larvae feed on plants contained terpenoids as their diet and, therefore possess a high level of enzymatic activity against terpenoids; the worm consumes a large amount of plants, making it possible to obtain more metabolites; and the worm is easy to rear on a laboratory scale. Previously, we reported biotransformation of monoterpenoids by the larvae of common cutworm (*Spodoptera litura*) [1-5]. This study demonstrated biotransformation of metabolism of (-)-menthone (**1**) and (+)-menthone (**2**) in *Spodoptera litura* larvae has been investigated. Compound **1** was transformed to (1*R*,4*S*)-(-)-7-hydroxymenthone (**1-1**), (1*R*,3*S*,4*S*)-(+)-7-hydroxyneomenthol (**1-2**) and (1R,4*S*,8*R*)-(-)-*p*-menth-3-one-9-oic acid (**1-3**). Similarity, compound **2** was transformed to (1*S*,4*R*)-(+)-7-hydroxymenthone (**2-1**), (1*S*,3*R*,4*R*)-(-)-7-hydroxyneomenthol (**2-2**) and (1*S*,4*R*,8*S*)-(+)-*p*-menth-3-one-9-oic acid (**2-3**). In conclusion, C-9 position of (-)- and (+)-menthone were carboxylated by *S. litura*, which is characteristically metabolic pathway compared with any other biocatalysis.

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Metabolism of (-)-*cis*- and (-)-*trans*-Rose oxide by Human Liver Microsomal P450s

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We reported that a number of monoterpene cyclic ethers such as 1,8-cineole and 1,4-cineole are metabolized by human liver microsomal P450s [1,2]. In this study, the *in vitro* metabolism of (-)-*cis*- and (-)-*trans*-rose oixde was investigated using human liver microsomes and recombinant cytochrome P450 (P450 or CYP) isozymes. Both isomers of rose oxide were incubated with human liver microsomes, and the formation of respective 9-oxidized metabolite was determined by using gas chromatography-mas spectrometry (GC-MS). Of 11 different recombinant human P450 isozymes used, CYP2B6 and CYP2C19 were the primary enzymes catalyzing the metabolism of (-)-*cis*- and (-)-*trans*-rose oxide. CYP1A2 also efficiently oxidized (-)-*cis*-rose oxide at the 9-position but did not (-)-*trans*-rose oxide. α-Naphthoflavone (a selective CYP1A2 inhibitor), thioTEPA (a CYP2B6 inhibitor) and anti-CYP2B6 antibody inhibited (-)-*cis*-rose oxide 9-hydroxylation catalyzed by human liver microsomes. On the other hand, the metabolism of (-)-*trans*-rose oxide was suppressed by thioTEPA and anti-CYP2B6 at significant level in human liver microsomes. However, omeprazole (a CYP2C19 inhibitor) had no significant effects on the metabolism of both isomers of rose oxide. Using microsomal preparations from 9 different human liver samples, (-)-9-hydroxy-*cis*- and (-)-9-hydroxy-*trans*-rose oxide formations correlated with (*S*)-mephenytoin *N*-demethylase activity (CYP2B6 marker activity). These results suggest that CYP2B6 plays important roles on the metabolism of (-)-*cis*- and (-)-*trans*-rose oxide in human liver microsomes. In addition, CYP1A2 may be expected to play important roles for some of the (-)-*cis*-rose oxide 9-hydroxylation in human liver, since CYP1A2 mean contents are much higher than those of CYP2B6

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Biotransformation of 4-*tert*-butylcyclohexanone by fungi.

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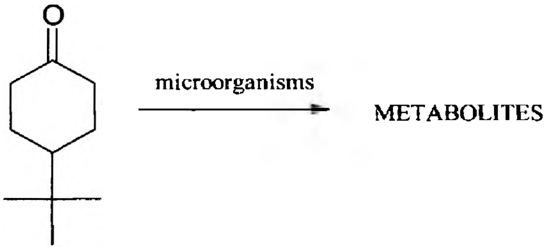
Green chemistry is a field of science, which has developed very dynamically over the last few years. One of its major techniques is the application of biocatalysts, namely enzymes or whole cells (mostly microbial ones), to obtain desired products of various interests [1]. Fungi can be considered as a promising source of new biocatalysts, mainly for chiral reactions [2]. Biotransformation is an effective tool for the structural modification of bioactive, natural and synthetic compounds with various [3].This method is useful in bioorganic chemistry due to its versatility and simplicity.

Searching for the new compounds with potential biological activity we carried out biotransformation of purchased 4-*tert*-buthylcyclohexanone by the following microorganisms: *Fusarium oxysporum*, *Fusarium culmorum* and *Aspergillus niger*.



Microorganisms were cultivated rotatory (160 rpm) at 28°C for 24-48 hours in the 250 ml Erlenmeyer flask. After the full growth of microorganisms the substrate was added (50 mg) and incubated for 7 days under the same conditions. The progress of biotransformation was monitored by TLC. After filtration of the cultured broth the aqueous layer was extracted with ethyl acetate. The concentrated ethyl acetate extract was evaluated by GC/MS and then applied to silica gel chromatography column for isolation of the metabolites. The structures of isolated metabolites were established by ^{1}H, ^{13}C NMR and IR spectroscopy.

Biotransformation and structure details of new compounds will be presented.

Acknowledgements: This work was supported by the project "Biotransformations for pharmaceutical and cosmetics industry" No. POIG.01.03.01-00-158/09-01 part-financed by the European Union within the European Regional Development Fund.

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Synthesis of new bicyclic lactam from (+)-3-carene via Beckmann rearrangement

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Lactams are compounds with many applications. Some of them are biologically active. Pennicillins and cephalosporins are classes of antibiotics with *β*-lactam ring in their structure. [1]

Pyrrolid-2-one derivatives have procognitive properties. They are used in treatment of cognitive dysfunctions associated with ageing, head trauma or neurodegenerative diseases. [2] They are also used for scopolamine – induced amnesia [3] and neuropathic pain. [4]

Synthesized bicyclic lactam is a piperid-2-one derivative. These kinds of compounds are suspected to inhibit apoptotic processes. [5] It is proven that some piperid-2-ones influence serotonin and dopamine release by interacting with $5HT_{1A}$ serotonin receptor. [6] Mechanism of piperid-2-ones action depends on *N*-substituted ring's moiety.

Research in our laboratory is directed towards chemical transformations of (+)-3-carene leading to compounds with potential industrial and pharmaceutical applications. (+)-3-carene is a bicyclic hydrocarbon derived from common in Poland Scotch Pine (*Pinus sylvestris*). We used it as a starting material in seven-step synthesis of new bicyclic piperid-2-one derivative in form of racemate.

Acknowledgements: This work was supported by the project "Biotransformations for pharmaceutical and cosmetics industry" No. POIG.01.03.01-00-158/09-01 part-financed by the European Union within the European Regional Development Fund.

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Synthesis and odor characteristics of racemic and optically active oxy-derivatives of *gem*-dimethylcyclohexene.

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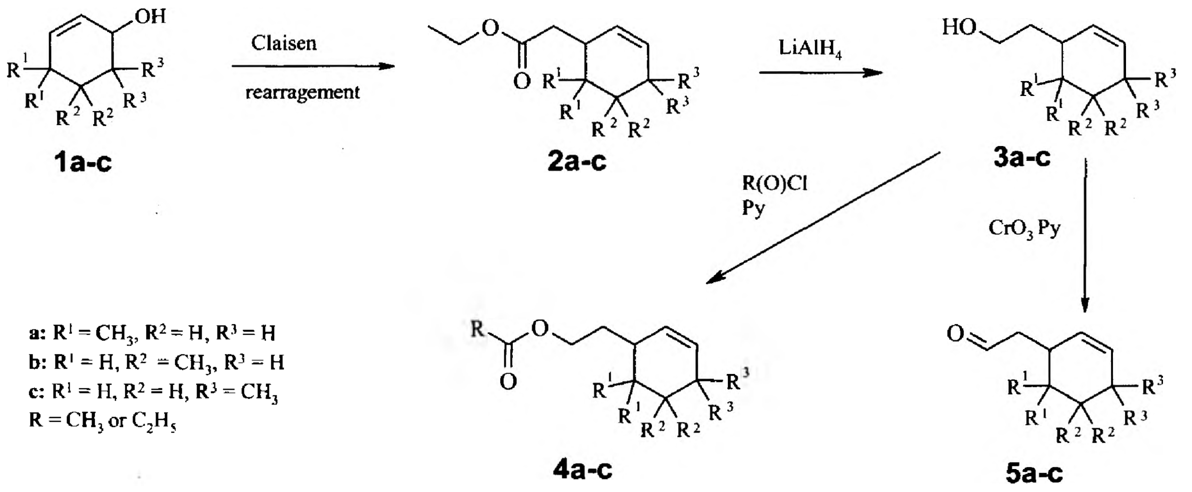
Many fragrances compositions are based on natural and synthetic compounds with *gem*-dimethylcyclohexene ring. Their isolation from natural sources e.g.: β-damascone from rose oil and limonene from citrus peels oils has been the main topic of many perfumery reaserches. Up to now many structural analogs or homologs of natural compounds (methylionone, isomethylionone, dynascone) were obtained. [1-4].

In the course of studies on synthesis of biologically active bicyclic lactones with alkylsubstituted cyclohexene ring, we have observed that many oxy–derivatives with *gem*-dimethylcyclohexene system possess interesting and valuable odors [5,6]. This observation pushed us to synthesis of odoriferous oxy–derivatives of *gem*-dimethylcyclohexene. Recently we published the synthesis and odor characteristics of oxygene conteining derivates of trimethylcyclohexene [7]. Here we present the synthesis of ethyl esters (**2a-c**), alcohols (**3a-c**), aldehydes (**5a-c**), acetates and propionates (**4a-c**) (Scheme 1.).



a: $R^1 = CH_3$, $R^2 = H$, $R^3 = H$
b: $R^1 = H$, $R^2 = CH_3$, $R^3 = H$
c: $R^1 = H$, $R^2 = H$, $R^3 = CH_3$
$R = CH_3$ or C_2H_5

Scheme 1.

The odoriferous characteristics of compounds obtained are also presented.

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The plant-mediated biotransformation of α- and γ-terpinene

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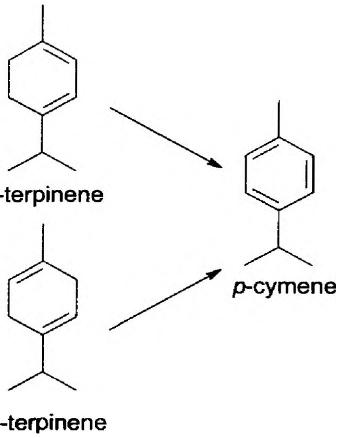
p-Cymene (1-isopropyl-4-methylbenzene) is an important intermediate used in industrial fine chemicals syntheses for fragrances, flavourings, herbicides, pharmaceuticals [1, 2, 3]. At present approximately 4000 tons of this compound are produced annually [2]. Currently, conventional preparation of *p*-cymene is based on the Friedel–Crafts alkylation of petroleum derivatives: benzene with methyl and isopropyl halides using $AlCl_3$ as an acid catalyst, or toluene with propene or 2-propanol over hydrochloric acid containing $AlCl_3$, BF_3 or H_2SO_4 [1, 3]. It is worth noting that the alkylation processes produce a mixture of the three different isomers of cymene, with the subsequent need for costly separation processes. In addition, the use of these homogeneous acids gives rise to many problems regarding handling, safety, corrosion and treatment of the disposed catalyst [3].

This information encouraged us to undertake research into transformations of α- and γ-terpinene by means of enzymatic system of comminuted plant parts. The method used by us has some advantages compared to other methods, for example a very low cost, the availability of the material and the easiness of reaction work-up. There is no information in literature about using plant biocatalysts in transformation of α- and γ-terpinene.

In our research we used five biocatalysts: four vegetables (*Petroselinum crispum* L. - parsley, *Daucus carota* L. - carrot, *Apium graveolens* L. - celeriac, *Solanum tuberosum* L. - potato, *Beta vulgaris* L. - beet) and one fruit (*Malus pumila* L. - apple). All biocatalysts were able to aromatize ring of α- and γ-terpinene, which caused transformation of both substrates to *p*-cymene.



The best results for both substrates were obtained by means of enzymatic system of comminuted apple. *p*-Cymene was obtained with yield 30% and 31% respectively after 48h transformation.

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Bioconversion of cyclic terpenoid ketones by fungal strains

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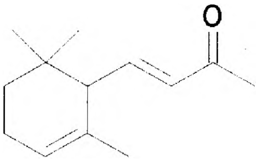
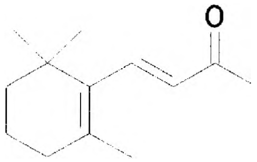
The C13-norisoprenoids are widespread in nature. They are found in a variety of essential oils. As a value aroma components with attractive sensory properties they are used mainly in perfumery industries but they also possess noteworthy biological and therapeutic activities. Interesting representatives of this group are compounds known as rose ketones: ionones and damascones. β-Ionone is an important precursor in the synthesis of vitamin A whereas α-ionone is in high demand in the fragrance industry because of their violet and woody-fruity odor [1,2,3]. The damascones have been identified as components of tea aroma. They exhibit fruity, rose like odors that are desired in various flavor and fragrance compositions [1,4].

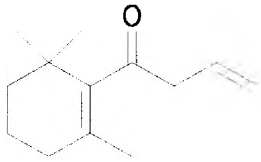
Microbial transformation of norisoprenoids are a good way for synthesis of many new bioactive compounds with isoprenoid structure. Recently we have published the synthesis of oxyderivatives of farnesol by fungal strains [5]. Here we report the biotransformation of α-ionone (**1**), β-ionone (**2**) and damascone (**3**) by means of three strains of microorganisms, which were selected in the screening procedure. The substrates was transformed by *Penicillium chermesinum 113, Absidia cylindrospora 336* and *Aspergillus ochraceus 456* into oxyderivatives. Biotransformations of ionones and damascones have been subject to an studies of influences the structure on the process of bioconversion and fragrance of obtained oxyderivatives.



α-ionone (**1**)



β-ionone (**2**)



β-damascone (**3**)

Acknowledgements: This work was supported by the project "Biotransformations for pharmaceutical and cosmetics industry" No. POIG.01.03.01-00-158/09-01 part-financed by the European Union within the European Regional Development Fund.

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PP-D9

Biotransformation of Myrcene by Two Species of *Pseudomonas*

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Dihydrolinalool and terpineol are sources of fragrances that provide a unique volatile terpenoid alcohol of low toxicity and thus are widely used in the perfumery industry and have applications as well in folk medicine and in aromatherapy. They are important chemical constituents of the essential oils of many plants. Previous studies have concerned the biotransformation of limonene by *Pseudomonas putida*. The objective of this research was to study the pathways involved during biotransformation of myrcene by *Pseudomonas aeruginosa* and *P. putida*. The culture preparation was done using such variables as different microbial methods and incubation periods to obtain maximum cells of *P. aeruginosa* and *P. putida* for myrcene biotransformation. It was found that myrcene was converted to dihydrolinalool and 2,6-dimethyldecane in high percentages by *P. aeruginosa*, and dihydrolinalool, *cis*-β-dihydroterpineol, linalool, and *cis*-ocimene-8-oxo were obtained in high percentages by *P. putida*. The biotransformation products were identified by theoretical study (TS), Fourier-transform infrared spectroscopy (FT-IR), ultraviolet visible (UV), gas chromatography (GC), nuclear magnetic resonance (NMR), and gas chromatography/mass spectroscopy (GC-MS). Comparison between *P. aeruginosa* and *P. putida* of different incubations was showed in *P. aeruginosa* 2,6-dimethyldecane (90.0%), dihydrolinalool (79.5%), and *cis*-β-dihydroterpineol (67.6%); linalool (25.8%), dihydrolinalool (16.7%), and *cis*-ocimene-8-oxo (61.6%) were the main compounds comprising *P. putida*[1].

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Application of menthol for the enzymatic resolution of α-halopropionates

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Transesterification of methyl α-halopropionates is an interesting reaction when racemic alcohol is used for the resolution of racemic ester. A perfect process could produce a mixture of products, which would give four optically pure compounds after few relatively easy physical separation steps. Every enantiomerically pure product of this reaction could be used as a synthon for the asymmetric synthesis of valuable organic compounds: α-halopropionates are the substrates for the synthesis of chemicals used for crop protection and in pharmacy, whereas chiral alcohols are used in perfumery and for flavouring foods.

We used *Candida rugosa* lipase for the enzymatic transesterification of methyl α-chloro-, and α-bromopropionates with racemic (±)-menthol in organic solvents. The enzyme transformed α-chloropropionate with 100% yield in hexane, and was selective regarding the alcohol: only traces of (+)-menthol formed the ester, and the product formed from (-)-menthol had 31% de. More polar solvent, MTBE, was also suitable for the complete transesterification of the methyl chloroester, but only a slight preference (5% de) for the S enantiomer of the substrate was recorded.

The use of a single isomer, (-)-menthol, caused incomplete transesterification: 95% in hexane, and 63% in MTBE with poor diastereoselectivity.

When bromopropionate is used as the substrate, only 41% conversion was achieved in hexane, but only when pure (-)-menthol was used. The selectivity was twice as high comparing to the chloro derivative. Additionally, we observed opposite absolute configuration at the asymmetric carbon attached to the halogen. Surprisingly, racemic menthol did not undergo transesterification. We noticed a stronger solvent effect with bromo ester: reaction took place only in hexane.

In all cases *Candida rugosa* lipase strongly favoured apolar solvents, as the enzyme action was not observed in tetrahydrofuran nor acetone.

Organization of a Proficiency Testing Scheme on Aromatic Products

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Founded in 1970, Bipea is a non profit organization which assists laboratories in the evaluation of their performances. Bipea is an accredited organizer of proficiency testing schemes based in France. Today, Bipea offers 59 different Proficiency Testing Scheme in the following areas:

- Raw materials (cereals, oilseeds and animal feed)
- Food industries (beverages, honeys, oils)
- Environment (waters, soils) and contaminants (pesticides, mycotoxins, GMOs).

More than 1130 laboratories trought out 60 countries participate on regular basis in our PTS. In 2010, Bipea has launched the first Proficiency Testing Scheme dedicated to the analysis of aromatic products. The aim of this PTS is to set up a confidential frame where testing laboratories can compare anonymously their test results on several aromatic products. When joining a Bipea PTS a laboratory determine regularly its bias in regard to the consensus value; thus giving an indication of its tendency to under-or over-estimate test results. It enables to set up accuracy monitoring chart for each given criterion and check for its performance in the long run. Proficiency Testing schemes are a useful quality management tool for the laboratory managers.

This experimental PTS "Aromatic products" was carefully elaborated with the support of a group of experts from the SNIAA (French Association of Flavours). Four trials took place from January to April 2010. The matrixes chosen were: an essential oil of lavender, a liquid aroma of Tonka Bean, a solid aroma and a vanilla extract with coumarin.

With this PTS, the laboratories have the opportunity to compare themselves on more than 12 parameters including Relative density, Refractive index and specific compounds. A specialized committee chaired by Doctor Hughes Brevard from Robertet with the assistance of Doctor Christine Schippa from Mane was in charge of the validation of the in house statistical treatment (according to ISO 13528 Standards) and responsible for the evolution of the PTS for the next year.

For the launching year, the contribution to this PTS has been satisfied with a participation of 13 laboratories from 4 different countries. The beginning of the new PTS series is schedule for September 2010.

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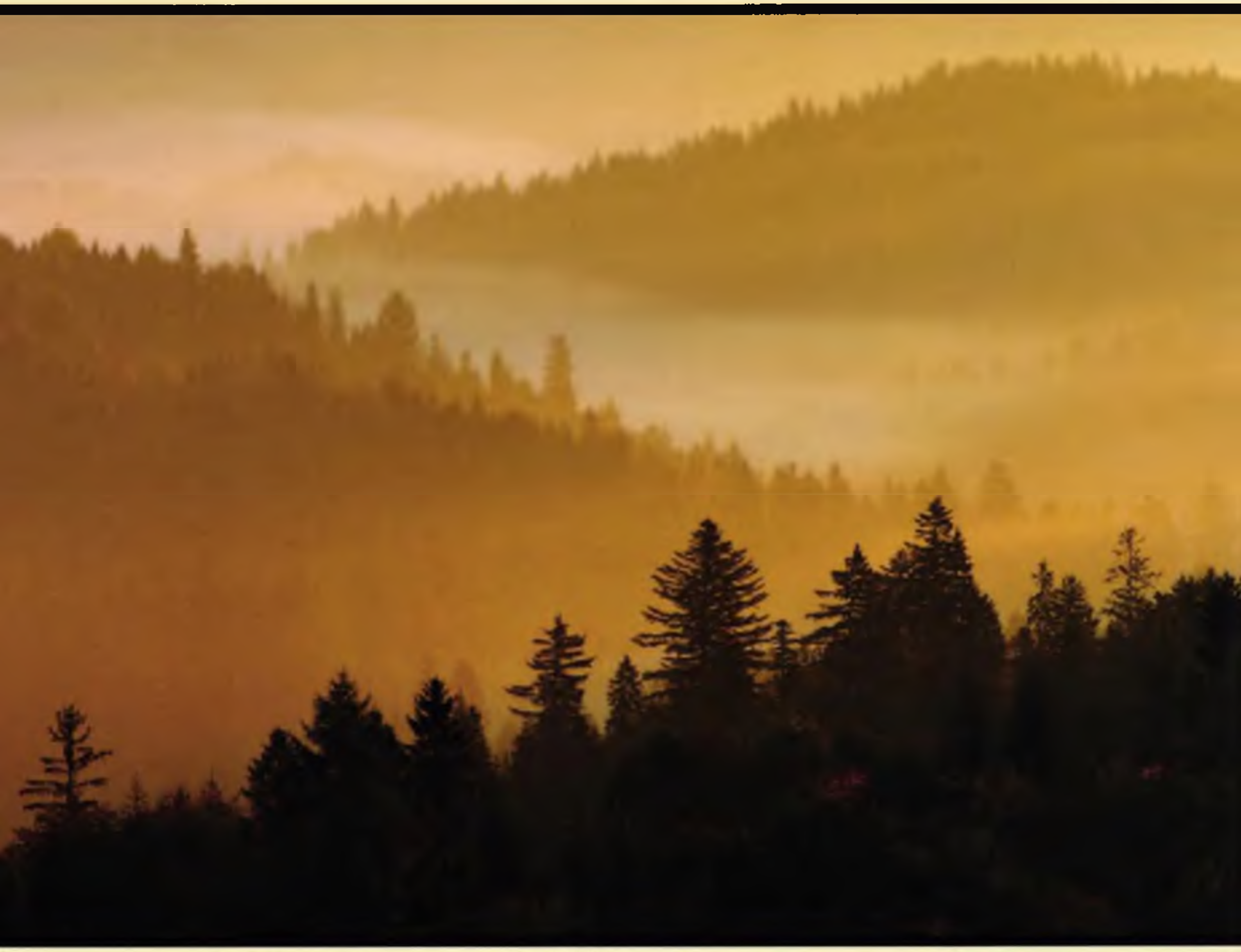

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