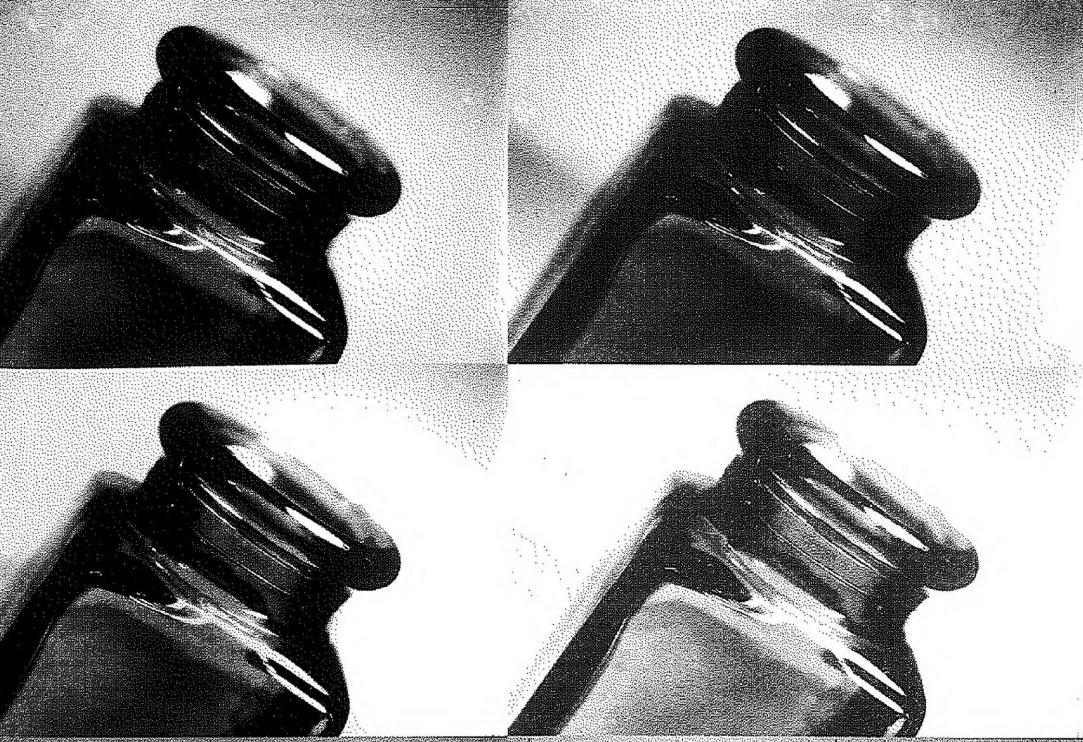

8-12 September 2013

Budapest, Hungary

Book of abstracts

44th ISEO

International Symposium on Essential Oils

44TH INTERNATIONAL SYMPOSIUM ON
ESSENTIAL OILS 2013

PROGRAMME
&
BOOK OF ABSTRACTS

DANUBIUS HOTEL FLAMENCO
BUDAPEST, HUNGARY
8-12 SEPTEMBER 2013

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GENERAL INFORMATION

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Date and Venue
The Congress is held at the Danubius Hotel Flamenco between 8 - 12 September 2013.

Congress and Exhibition Venue
Danubius Hotel Flamenco
Address: H-1113 Budapest, Tas vezér utca 3-7.
Website: www.danubiushotels.hu
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Foreign Exchange, Banking Facilities
The official national Hungarian currency is the Hungarian Forint (HUF). All the major credit cards are accepted in Hungary in places displaying the emblem at the entrance. Though Hungary is a member of the European Union, only a few shops and restaurants accept Euros (EUR) for payment. Currency exchange booths are available in Budapest at the airport terminals, railway stations, travel agencies, banks and various places in the city. The exchange rates applied may vary. Traveller's cheques and convertible currency may be exchanged at these facilities. Major credit cards are usually accepted in most hotels, restaurants and certain shops in the city. Obtaining cash against ATM or credit cards is very easy from the ATM cash machines that can be found at almost every office, hotel or on the street.

Liability and Insurance
The organisers cannot accept liability for personal accidents, loss of belongings or damage to private property of participants and accompanying persons that may occur during the Congress. Participants are advised to make their own arrangements to obtain health, travel and property insurance before their departure to the SIL2013.

Public transport in Budapest
Public transport in Budapest is provided by Budapest Transport Ltd. (known to all Hungarians simply as BKV). Budapest has an efficient public transport network. In general the buses, trams and trolleybuses operate between 4.30 AM and 11 PM. All night bus service operate on the major thoroughfares in the city (night bus timetables are posted at stops and in most metro stations). The three metro lines intersect at Deák Square in the centre of the town. Metros run at 2-15 minutes intervals from about 4.30 AM to 11.15 PM. There are also five suburban railway lines (HÉV) serving the outskirts of the city.

Shopping in Budapest (opening hours)
Food shops are open from 7 AM - 6 PM Mon - Fri, 8 AM - 1 PM Sat (but there are several larger stores open on Sunday morning and a number of small 24-hour shops). Other shops are open between 10 AM-6 PM Mon - Fri, 10 AM - 1 PM Sat. Most shopping centres are open 10 AM - 8 PM even on Sunday. Tesco is open 24 hours a day, seven days a week.

Office hours: generally from 8 AM - 4 PM Mon - Fri.
Post offices: Mon - Fri: 8 AM - 6 PM, Sat: 8 AM - 1 PM
Banks: Mon - Thu: 8 AM - 3 PM, Fri: 8 AM - 1 PM.

Taxis in Budapest
Budapest taxis have yellow number plates and a taxi sign in yellow. Any vehicle without these features is operating illegally. It's a good idea to avoid drivers who volunteer their services but don't have a registered taxi sticker on their car. This can prevent unpleasant surprises when it comes time to pay. All cars must have a taximeter installed, and these also print out a receipt. Taxi drivers are required to give an invoice on request. Tipping: in general 10% of the fare is acceptable. It is worth calling a taxi by phone because most of the taxi companies charge lower rates in this case than in the case of hailing a taxi in the street.

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The international code for Hungary is 36, the area code for Budapest is 1. To call a number within Hungary, first dial 06. Budapest telephone numbers have seven digits, all other areas have six digits (in addition to the area codes). To make an international call from Hungary, first dial 00, then the country code followed by the area code and the subscriber's telephone number. Public telephones accept either coins (20, 50, and 100 HUF) or phone cards (available from tobacconists, newsagents, post offices, and petrol stations). To call a (Hungarian) mobile phone, from a public telephone first dial 06, followed by the subscriber's seven-digit number starting with 20-, 30- or 70-.

Important phone numbers
(English is usually spoken at the emergency numbers listed below. In case English is not spoken, dial 112)

Ambulance: 104
Fire brigade: 105
Police: 107
Central help number: 112
General enquiries: 197
Inland enquiries: 198
International enquiries: 199
Hungarian Automobile Club help number: 188

Time
Hungary is in the Central European Time Zone. In the summer months clocks are set at GMT + 2 hours.

Weather
The weather in Hungary in September is usually sunny and warm. Temperatures are usually in the range between 23 and 26 °C during the day.

Registration and Information Desk
The registration desk is located on the entrance level of the Congress Venue.

Opening hours:

19:00 – 21:00 Sunday, 8 September, 2013
08:00 – 20:00 from Monday, 9 September – till Wednesday, 11 August

Congress delegates and their companions will receive their badges, Congress materials, social event tickets at the desk.

Badges
All participants and accompanying persons will receive a personal badge upon registration. You are kindly requested to wear your name badge when attending the meeting or social events. Only participants who are wearing their name badge will be admitted to the lecture halls.

Please note that accompanying persons will be not be admitted to the technical sessions.

Delegate and PhD registration fees include:

- Admission to all scientific sessions
- Admission to the exhibition
- Printed material of the symposium with conference bag
- Welcome reception
- Coffee breaks
- Lunches

Accompanying persons' registration fee includes:

- Welcome reception
- Lunches
- Budapest excursion (half-day on 9 September)

Admission to the technical sessions is not included in this registration category.

On-site Registration

	for delegates	for students	for accompanying person
on-site fees	400€	300€	150€

Programme Changes
The organisers cannot assume liability for any changes in the programme due to external or unforeseen circumstances. Please note, that in case an oral presentation is off, the following presentations will start at the planned start time.

Message
Personal message can be placed on the message board located at the info desk.

Catering Services during ISEO 2013

- Welcome reception - Danubius Hotel Flamenco, Congress venue, Sunday, 8 September (including in the registration fees)
- Lunches -Danubius Hotel Flamenco, Congress venue, Monday-Tuesday, 9-10 September (included in the registration fees)
- Coffee breaks -Danubius Hotel Flamenco, Congress venue, lobby (included in the registration fees)
- Symposium dinner - Szekércsárda, Tuesday, 10 September (NOT included in the registration fees, price: 40 EUR)

Advice for your departure

Airport Minibus

Participants leaving from Budapest International Airport are advised to use the Airport Minibus shuttle service (www.airportshuttle.hu), which takes one from any address in Budapest for a fee of 3200 HUF/person (cca. 11 EUR).

You may order your Airport Minibus at the Hotel's reception desk (entrance level) at least one day before your departure. Telephone number: (36-1) 296-8555.

Taking a taxi - the tariffs of the taxi companies may differ but share a fixed common tariff ceiling. If you take a taxi, you can find the tariff chart on both dashboard and on the right rear window.

You get a price reduction if you order your taxi by phone, so it is better to order a taxi from the Hotel Reception or from a restaurant, rather than getting in a car in front of your Hotel.

Phone numbers of some taxi companies: Fotaxi 222-2222, City Taxi 211- 1111, Radio Taxi 377- 7777, Taxi 2000 200-0000. During the Congress the congress secretariat has entered a contract with City Taxi.

Cancellation Policy

Registration fee

The cancellation policy for the conference is:

- Full refund before 31 July 2013
- No refund was possible for cancellations after 1 August 2013

Cancellation of registration must be made in writing, which will be confirmed by the Conference Secretariat. All refunds will be paid only after the conclusion of the Congress. As alternative to cancellation, please consider a transfer of your registration to another person.

SCIENTIFIC PROGRAM

SCIENTIFIC PROGRAM

8th September

19.00-21.00	**Cocktail reception**		

9th September

9.30-10.20	**Opening ceremony**	Chair: **Zámbori-Németh, É.**	
10.20-10.50	**Coffee break**		
10.50-12.15	**Session 1.**	Chair: Buchbauer, G.	
10.50-11.35	**Plenary lecture**	**Kaiser, R.:** "Scent of the Vanishing Flora" – Highlights from 500 investigated endangered species	25
11.35-11.55	**Oral lecture**	**Sadgrove, N.:** New directions in Australian essential oil research: chemistry, chemotypology and chemotaxonomy; the three C essentials	33
11.55-12.15	**Oral lecture**	**Bizzo, H.:** Scents from Brazilian Cerrado: Chemical composition of the essential oil from *Pseudobrickellia brasiliensis (Asteraceae)*	34
12.15-13.30	**Lunch**		
13.30-15.35	**Session 2.**	Chair: Kubeczka, K-H.	
13.30-14.15	**Plenary lecture**	**Zámbori-Németh, É.:** Biological background of variability of essential oil compounds	26
14.15-14.35	**Oral lecture**	**Sparinska, A.:** Variability of volatile compounds in petals and hydrosols of *Rosa rugosa* and its hybrids	35
14.35-14.55	**Oral lecture**	**Belhassen, E.:** The secret of vetiver scent: determination of character impact constituents	36
14.55-15.15	**Oral lecture**	**Ludwiczuk, A.:** Chemical relationships between liverworts of the *Lejeuneaceae* family	37
15.15-15.35	**Oral lecture**	**Coulerie, P.:** New Caledonian liverworts: A new source of original and bioactive compounds	39
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16.05-17.30	**Poster session 1.**	Even numbers (Moderator: Baser, K.H.)	
18.00-20.00	**Meeting of Scientific Committee**		

10th September

9.30-10.55	**Session 3.**	Chair: Baser, K.H.	
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10.35-10.55	**Oral lecture**	**de March, C.:** The human OR1G1 olfactory receptor is differentially activated by various sandalwood odorants. A joint approach combining in silico and in vitro experiments	41
10.55-11.25	**Coffee break**		
11.25-12.25	**Session 3.** (ctd)	Chair: Baldovini, N.	
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11.45-12.05	**Oral lecture**	**Kuraya, E.:** Improving the efficiency of essential oil extraction from shell ginger (*Alpinia zerumbet*) with an underwater shock-wave pretreatment	43
12.05-12.25	**Oral lecture**	**Horváth, Gy.:** Application of TLC-bioautography for detecting the antibacterial activity of essential oils	45
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13.45-15.10	**Session 4.**	Chair: Asakawa, Y.	
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14.50-15.10	**Oral lecture**	**Sciarrone, D.:** On-line LC-MDGC-prep system for the isolation of high amounts of pure components	47
15.10-15.30	**Oral lecture**	**Pluhár, Zs.:** Seasonal variation of the essential oil at different *Thymus vulgaris* chemotypes	48
15.30-16.00	**Coffee break**		
16.00-17.30	**Poster session 2.**	Odd numbers (Moderator: Franz, Ch.)	
19.15-	Departure for Symposium dinner (40 EUR) at Szekércsárda (departure from the hotel by bus at 19.30)		

11th September

9.30-10.55	**Session 5.**	**Chair: Bernath, J.**	
9.30-10.15	**Plenary lecture**	**Novak, J.: The place of molecular biology in essential oil research**	29
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11.25-12.25	**Session 5. (ctd.)**	**Chair:** Novak, J.	
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12.05-12.25	**Oral lecture**	**Kirbaslar, S.I.:** Analysis of lemon oil obtained by supercritical CO_2 extraction, cold pressing and hydrodistillation methods	55
12.25-12.45	**Closing of the Symposium**		

Poster presentations

Abbrevations:

- BV: Biological variability of plant volatiles
- BA: Biological activities of essential oils and their constituents
- AN: Recent approaches in essential oil analysis

All posters are on show during the whole symposium.

Discussion of posters: 9th September 16.05h even numbers
10th September 16.00h odd numbers

ABSTRACTS - PLENARY LECTURES

PL-1
"SCENT OF THE VANISHING FLORA" – HIGHLIGHTS FROM 500 INVESTIGATED ENDANGERED SPECIES

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Keywords: Endangered plants, new compounds, olfactory concepts

As a part of our broad and ongoing search for new scent molecules and scent concepts in nature during the past 25 years, we have encountered an astounding number of interestingly scented species which, today, have to be considered as endangered. In our appreciation of these wondrous plants and in the hope to sensitize people for the many reasons making conservation activities so important, we decided in 2001 to focus even more on highly endangered scented species worldwide and to compile their scent compositions as well as complimentary information in a book entitled "Scent of the Vanishing Flora" which has been recently published as a contribution to the 2010 UN International Year of Biodiversity. The purpose of this lecture is to give with a series of interesting examples an impression on the 500 endangered species investigated within this project from which 267 representatives are summarized in the book cited above.

The lecture starts with some species which have already vanished from nature as *Franklinia alatamaha* (Theaceae) and *Paphiopedilum vietnamense* (Orchidaceae). Among others, also the olfactory concepts and new compounds found in the flower scents of *Gardenia brighamii* (Rubiaceae, endemic to Kauai, critically endangered), *Portlandia albiflora* (Rubiaceae, endemic to St. Andrews on Jamaica, critically endangered), several *Kefersteinia* species (Orchidaceae, Neotropics, all extremely rare and endangered,), *Santalum album* (Santalaceae, India, vulnerable) and *Lilium candidum* (Liliaceae, East Mediterranean, endangered) are discussed. Finally, the structurally and olfactorily interesting compounds of the needle scent of *Abies nebrodensis* (Pinaceae, endemic to Sicily, critically endangered) as well as the wood scents of *Widdringtonia cedarbergensis* (Cupressaceae, endemic to the Cederberg Mountains, South Africa, critically endangered) and *Vouacapoua americana* (Caesalpiniaceae, Amazonia, critically endangered) will be presented. As an olfactory illustration the audience will be able to evaluate 11 of the discussed scents as close-to-nature reconstitutions.

PL-2
BIOLOGICAL BACKGROUND OF VARIABILITY OF ESSENTIAL OIL COMPOUNDS

Németh ZÉ

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Keywords: quality, biodiversity, chemotaxonomy, biosynthesis, genotype, phenotype

The quality of essential oil from the same species may be numerous. Quality requirements depend on the purpose of use. Standards like PhEur, ISO and internal ones have distinct priorities.

Although processing technologies may have enormous effects on the oil quality, background of the natural variability starts from the plant itself.

It is well known that intraspecific units may often produce diverse pattern of volatile components. Phenotypic appearance of the genetic background depends further on the current ontogenetic stage and examined plant organ. Besides, these biological factors are always manifested in well defined environments and influenced by external effects. Species like fennel (*Foeniculum vulgare* Mill.) or peppermint (*Mentha piperita* L.) are good examples for this phenomenon.

Formerly, chemotaxonomic studies have been restricted to the detection and description of chemical variability. While some species exhibit an extraordinary wide spectrum of oil composition (e.g. thyme – *Thymus* spp. or yarrow – *Achillea* spp.) origin of many quality problems in the practice, others, (e.g. lavander – *Lavandula angustifolia* Mill., caraway- *Carum carvi* L.) show only a slight variation. In the last decades, more and more information accumulates on the molecular mechanisms of volatile biosynthesis and accumulation in plants. Recently, special attention has been devoted to enzymatic systems and their transcriptional regulation producing the wide spectrum of individual volatile components. Metabolomic processes seem to be able to explain now many of the long known chemotaxonomic features however it is also obvious, that each result arises multiple further questions.

Molecular genetic and physiologic studies are indispensable tool in understanding the background of biodiversity in volatile formation, however, practical realisation can not go without in vivo trials.

Literature is still full of reports on chemical diversity concerning essential oil components in different aromatic species, however, a small proportion of them may really contribute to our understanding of biological background. The lecture presents good and bad examples of them.

PL-4

NEW BIOLOGICALLY ACTIVE TERPENOIDS OBTAINED FROM TURPENTINE

Lochyński S

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Applications of naturally occurring (+)-3-carene for synthesis of biologically active compounds are presented. This bicyclic chiral monoterpene hydrocarbon is an inexpensive, little explored and readily available natural component of turpentine obtained from some species of pine (in Poland from *Pinus sylvestris* L.)

Investigations were focused on three classes of biologically active compounds: (i) odoriferous substances as components in perfume and food industry, (ii) analogues of pyrethroids, juvenoids and feeding deterrents as potent insecticides and (iii) derivatives possessing pharmacological activity as potent local anesthetic, cardiovascular and neuromodulatory drugs. Oxoderivatives with preserved bicyclo[4.1.0]heptane skeleton, as well as *gem*-dimethylbicyclo[3.1.0]hexane and *gem*-dimethylcyclohexane system were substrates in designed and elaborated synthetic methods for these analogues. Finally, many new compounds were synthesized, using novel stereocontrolled methods of synthesis and resolution of isomeric mixtures.

All obtained compounds were subjected to biological tests (olfactory, insecticidal and pharmacological). In the group of odoriferous compounds, derivatives with preserved carane skeleton, 6,6-dimethylbicyclo[3.10]hexane moiety and cyclic analogues of dihydroneryl and dihydrogeranyl system possessed very interesting and valuable odour characteristics. Analogues of acyclic juvenoids were morphogenetically active against pest of cotton (*Disdercus cingulatus*). New spirolactones with carane backbone exhibited good feeding deterrent activity against storage pest insect, khapra beetle (*Throgoderma granarium* Ev. lervea and adults).

Pharmacologically active compounds were the most interesting group. Among all synthesized analogues the aminohydroxyiminocarane derivative proved to be most valuable. Hydrochloride salt of (*R,S*)- mixture possessed the strongest local anaesthetic activity in corneal infiltration anaesthesia tests. It is very promising that this salt does not evoke any toxicodermal effects and does not induce any allergic reaction in response to topical application. Hydrolytic kinetic resolution (HKR) process led to separate (*R*)- and (*S*)-diastereoisomer with high enantiopurity (over 98% *ee*). Interdisciplinary investigations including comparative pharmacological tests, X-ray crystallography and phospholipids bilayer study by molecular dynamic stimulation were carried out.

In summary, (+)-3-carene proved to be valuable and uncommonly useful chiral substrate in synthesis of structurally various compounds with broad spectrum of biological activity. General accessibility of this hydrocarbon, component of many kinds of turpentines including the very cheap sulphate turpentine (0.5 $ per gallon as a waste during cellulose production) creates large technological perspectives for this natural product.

PL-3
ESSENTIAL OILS: TOXICOLOGY AND CHEMICAL SAFETY ASSESSMENT (CSA)

Bursch W

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A major task of toxicology is to recognize the risk imposed on environmental and human health by industrial chemicals, medicinal products, food additives and contaminants, cosmetics etc. (risk characterization) and thereby, to provide a rational basis for therapy or health protection (risk management). To ensure a high degree of reliability and adequacy of procedures and data applied for chemical safety assessment (CSA), a large body of internationally recognized guidelines has been developed, the details of which may differ depending on the legal framework of the respective political entities such as the European Union. In principle, a toxicological risk characterization links the following information:

1. Exposure assessment. Essential oils are plant constituents, in general complex mixtures of lipophilic substances such as terpenes and phenylpropanoids. Being constituents of parfums, cosmetics, cold remedies, food flavourings and much else, essential oils are pervasive in everday life. Depending on the use pattern, intake can ensue inhalatory, dermal or orally; exposure of environment and consumer covers a wide concentration/dose range.
2. Hazard assessment. Identification of type of damage and establishing a dose-response relationship. Type and severeness of toxic effects of essential oils - like any other chemical substance– depends on (physico)chemical properties, route of exposure and dose. Hazards caused by essential oils comprise a broad spectrum incl. local irritation, allergenic effects, photoxicity. Based upon their lipophilicity, essential oils are readily absorbed by intact skin and mucosa and therefore, may also provoke systemic toxicity, for instance in nervous system, liver, reproductive organs.
3. Extrapolation of risk, i.e. the likelihood that an adverse effect will become manifest in humans (worker, consumer) or environment under reasonably foreseeable conditions of use of a given chemical or mixture of substances; the risk is being quantified by comparing a given exposure level (dose) with effect criteria.

Risk characterization as performed at scientific level may be subjected to a risk-benefit analysis at socio-economic-level, i.e. establish an acceptable risk according to ethical and social standards. Risk characterization also serves to establish adequate measures for health protection, ranging from warning notices, maximum permissible values to prohibition of use or production of a given substance (risk management). In the context of the present lecture, principles of chemical safety assessment of essential oils will be discussed.

PL-5
THE PLACE OF MOLECULAR BIOLOGY IN ESSENTIAL OIL RESEARCH

Novak J

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Keywords: *molecular biology, phylogeny, taxonomy, DNA barcoding, genetic regulation, population biology, genetically modified organisms*

Molecular biology is the study of biology at a molecular level, i.e. the understanding of the interactions between the various systems of a cell, including the interactions between DNA, RNA and protein biosynthesis as well as learning how these interactions are regulated. As in any scientific areas, a set of specific tools/methods have been developed. Especially the invention of PCR and DNA sequencing have been milestones that fueled the speed of development in this area. These methods where in succession also used in areas, where DNA information was helpful to study the relatedness between individuals like in systematics ('molecular systematics'/'molecular phylogeny') or population biology ('molecular population biology').

Molecular systematics is giving us decision support on taxonomically difficult questions (1). Turning this approach around, DNA can also be used like a barcode to identify plants ('DNA-barcoding' (2)) where to a certain degree such an identification is also possible in processed materials and end-products (3). In population biology molecular data has become a valuable tool in plant breeding and can be used in analogy to the DNA barcoding approach at the species level to identify the origin of plant material (4). In the future molecular population biology may be useful to guarantee sustainable wild collection by determining the 'genetic health' of populations avoiding their over-exploitation.

Understanding the formation and accumulation of essential oils in the plant is of importance to improve their quality and yield. Molecular biology has contributed essentially to elucidate the genetic background of biosynthesis and regulation, although only in a few model essential oil plants like mint (5) or sage (6). The information about the genetic background can be subsequently used to improve plants by genetic engineering (7).

Molecular biology has become very important in many sectors like medicine, forensics and food science. Therefore methods of molecular biology are tremendously fast evolving leading to revolutionary applications within short time periods that will also influence essential oil research and production.

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ABSTRACTS - ORAL LECTURES

O-1

NEW DIRECTIONS IN AUSTRALIAN ESSENTIAL OIL RESEARCH: CHEMISTRY, CHEMOTYPOLOGY AND CHEMOTAXONOMY; THE THREE C ESSENTIALS

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Keywords: Astralia, essential oil, chemotaxonomy, Eremophila, Phebalium

We have initiated several investigations of essential oils from a variety of native Australian plant species aimed at examining the extent and aetiology of essential oil chemovariation. Chemical fingerprints thus derived have been used to complement taxonomic studies aimed at re-evaluating existing species classifications. For example, using relative fluorescence in flow cytometry, significant observed chemotypic variation of *Eremophila longifolia* has been shown to be consistent with two separate ploidy types; potentially warranting revision of the species. In another example, using NMR and GC-MS to identify some unusual and uncommon essential oil compounds in species of the genus *Phebalium*, we have uncovered a number of taxonomic discrepancies, hitherto suspected but unconfirmed. In particular, dihydrotagetone (2,6-dimethyloct-7-en-4-one) is an omnipresent characteristic of the *Phebalium glandulosum* subspecies complex; hence other species expressing dihydrotagetone were further examined for morphological characteristics consistent with the *P. glandulosum* group. Another unusual essential oil ketone, the sesquiterpene squamulosone, was found to be an important complement to taxonomic studies aimed at revising the *P. squamulosum* subspecies complex. Furthermore, investigation of sesquiterpenoid essential oils from *Prostanthera* species has uncovered a number of unusual and novel compounds that may also complement future taxonomic studies. Other species, such as those belonging to the genus *Zieria* and *Geijera*, have also recently been investigated and we shall report on the taxonomic implications of our results and the overall contribution of chemical analysis to a contemporary understanding of the Australian taxa.

O-2

SCENTS FROM BRAZILIAN CERRADO: CHEMICAL COMPOSITION OF THE ESSENTIAL OIL FROM *PSEUDOBRICKELLIA BRASILIENSIS* (ASTERACEAE)

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Keywords: medicinal plant, terpinen-4-ol, biodiversity, Asteraceae, arnica

Cerrado (savannah) is the second largest Brazilian biome, but the first in number of endemic species and the most threaten by anthropic pressure. A small fraction of the 12.000 known botanical species were chemically investigated. Cerrado is a very promising source for flavour and fragrance applications. Considering this, Embrapa has started a research project to study the aromatic species from this biome in order to propose sustainable alternatives for their commercial use. *Pseudobrickellia brasiliensis* (Spreng.) R.M. King & H.Rob. (Asteraceae) is a shrub 1-1.5 m tall, largely distributed in Central Brazil at Cerrado vegetation (1). It is commonly known as "arnica-do-mato" and used in traditional medicine to treat pain and as antiinflammatory. In this study the chemical composition of the essential oil from the leaves was investigated.

Leaf samples were collected at Araçuai, Minas Gerais State, and voucher specimen deposited at the herbarium of Embrapa Genetic Resources and Biotechnology (CEN) at number JBP339. Dried plant (aerial parts) was extracted in a Clevenger type apparatus for 2 hours. The oil was analyzed by gas chromatography and mass spectrometry using an Agilent 7890A gas chromatograph equipped with a FID and an Agilent 5973N MSD. A DB-5 capillary column (30m X 0.25mm X 0.25 μm) was used, with either hydrogen (for FID) or helium (for MS) as carrier gas, at 1.0mL/min. Column temperature was programmed from 60°C to 240°C at 3°C/min. Mass detector was operated in electronic ionization mode at 70eV. Identification of compounds was achieved by comparison of both mass spectra and retention indices with spectral libraries and literature data (2,3).

Thirty-five out of 39 compounds were identified in the oil. Terpinen-4-ol (38.6%), gamma-terpinene (19.5%), alpha-terpinene (7.8%) and alpha-terpineol were the major components. A previous investigation of the same species pointed out alpha-pinene (32.6%), beta-pinene, alpha-thujene and sabinene as major compounds in plants from the same geographical area (4). Further studies on volatiles variability are needed to evaluate the possibility of occurrence of chemotypes.

Acknowledgements: CNPq, Embrapa

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O-3

VARIABILITY OF VOLATILE COMPOUNDS IN PETALS AND HYDROSOLS OF *ROSA RUGOSA* AND ITS HYBRIDS

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Keywords: *Rosa rugosa, volatile aroma compounds*

In the climatic conditions of the Baltic countries and North Europe Rugosa hybrids are the most winter hardy groups of roses, therefore they are bred for use in landscape architecture for public places. Unfortunately it has spread out of gardens and made huge populations on the Baltic coast and roadsides. Proper uses of this plant could provide sustainable management solutions instead of costly and complicated destruction of rose communities. This study is aiming at identifying new qualities of Latvia bred Rugosa hybrids by focusing on testing, if flowers of Rugosa hybrids would be suitable for production of rose water and, furthermore, rose oil.

Volatiles of rose petals and rose water from 10 cultivars were extracted using solid phase microextraction (SPME) with subsequent separation by gas chromatography and identification by comparison of their mass spectra with mass spectral libraries (Nist98). Fifty volatile aroma compounds were identified in petals. Among those, phenylethylalcohol, ß-citronelol and nerol were predominant, but the composition of compounds varied among varieties. Species Rosa rugosa and cv. 'Plena' showed the highest total level of volatiles and contained 26% and 31% ß-citronelol, respectively, and 17-19% of phenylethyl alcohol. Cv. 'Raita' produced a smaller total amount of volatiles, but a higher proportion of ß-citronelol (ca. 44%) and phenylethyl alcohol (ca. 30%). Main volatile compound were detected in rose water in the same proportions, but their concentration was twice as high as in petals.

In summary, cultivar 'Raita' is recommended for use as a decorative plant, as well as a source of volatile oil or rose water. Wild populations of *R. rugosa* are suitable for production of rose water and nutrients.

O-4

THE SECRET OF VETIVER SCENT: DETERMINATION OF CHARACTER IMPACT CONSTITUENTS

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Keywords: Vetiver, gas chromatography-olfactometry, odor impact coumpounds

The vetiver essential oil is one of the most important natural woody ingredient of the perfumer's palette. It is then surprising that, as quoted in the recent litterature "no synthetic vetiver odorant is commercially available. There is not even a consensus about which constituent contribute to its distinct and characteristic suave and sweet woody-earthy odor..." (1). This is due in part to the chemical complexity of this essential oil which is still not completely characterised.

In the course of our studies on the identification of odor active constituents of natural raw materials, we analysed Haitian vetiver oil and its derivative (vetiveryl acetate) by means of two-dimensional GC-MS (2) and GC-Olfactometry (GC-O). Most of the odorant compounds determined as characteristic of vetiver are sesquiterpenic ketones (Figure 1) but many other molecules also bring an important contribution. In addition, while khusimone was so far pointed out as the main odour active constituent (3,4,5), we identified several even more important contributors to the typical vetiver scent.

In order to ensure the correct identification of odorant molecules, we undertook a synthetic work mainly focused on derivatives of the zizaane family.

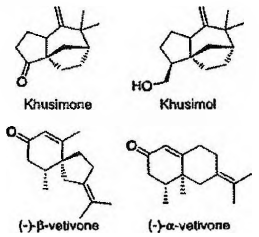

Figure 1: Constituents of vetiver oil previously described as olfactorily important by Maurer (3), Weyerstahl (4), and Mookherjee (6).

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O-5
CHEMICAL RELATIONSHIPS BETWEEN LIVERWORTS OF THE *LEJEUNEACEAE* FAMILY

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Keywords: chemotaxonomy, volatiles, terpenoids, PCA and CA

The Lejeuneaceae is the largest family of the liverworts, with more than thousand species. Most of the liverworts belonging to this family are epiphytes and are abundant in tropics and subtropics [1]. Due to the high degree of morphological homoplasty within Lejeuneaceae, the division of this family into natural subunits is considered to be notoriously difficult, and many conflicting classifications have been proposed. At present the division of Lejeuneaceae into two subfamilies: Ptychanthoideae and Lejeuneoideae is widely adopted. However, recent morphological and molecular phylogenetic analyses have resolved a monophyletic Lejeuneoideae and paraphyletic Ptychanthoideae indicating problems with this division [1,2].

Our previous studies showed the possibility of use the chemistry of liverworts to resolve the taxonomic problems, especially at genus and species level [3-5]. The aim of the present work was to find the chemical relationships between liverwort species belonging to Lejeuneaceae family on the basis of sesqui- and diterpenoids composition. GC-MS analysis of the 31 liverworts specimens belonging to the Lejeuneaceae family indicated variability of the chemical composition of terpenoids depending on liverwort species. Each of the analyzed liverworts is characterized by the presence of own peculiar compounds, however, there are some characteristic components, which can be chemical markers of this liverwort family. These are punguisane and monocyclofarnesane type sesquiterpenoids, fusicoccane type diterpenoids, and also sesquiterpene hydrocarbon, isolepidozene. Chemical data also showed the differences among liverworts classified in different subfamilies within the Lejeuneaceae. All of the mentioned characteristic components are present in liverworts belonging to the Ptychanthoideae subfamily. On the other hand, among all characteristic components only β-pinguisene was detected in *Myriocolea irrorata*, *Myriocoleopsis gymnocolea* and *Cololejeunea stotleriana* classified in Lejeuneoideae subfamily. Neither isolepidozene nor pinguisanes, fusicoccanes and monocyclofarnesanes was found in *Lejeunea topoensis*. The existence of the chemical polymorphism between both subfamilies is also evident form PCA and CA analyses.

In conclusion, the composition of volatiles present in liverworts can be use to resolve some taxonomical problems also at family level. Results from the chemical investigations can help to understand relationships among the taxa within one family, but especially can be used as additional support for molecular studies.

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O-6

NEW CALEDONIAN LIVERWORTS: A NEW SOURCE OF ORIGINAL AND BIOACTIVE COMPOUNDS

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Keywords: liverworts, New Caledonia, Frullania, Lejeuneaceae, volatiles compounds

New Caledonia is a sub-tropical island in the South Pacific with a flora characterized by a high rate of endemism (1). Considering the liverworts, it is estimated that 45 to 50% of the 460 of native species described are endemic to this island (2). Despite the great interest of the liverworts for the research of new active compounds, none of the New Caledonian liverworts has been investigated for its chemistry yet (3). Here we present some of the most interesting results obtained from the GC-MS analysis of the ether extracts of 90 Caledonian species.

5 *Frullania* species were analysed: *F. bella**; *F. falsicornuta**; *F. mammillosa**; *F. multilacera*; *F. scalaris** only contain few monoterpenes. One non identified compound (M=136) was observed in *F. bella*, *F. mammillosa* and *F. scalaris*. Limonene and terpinolene were also detected as minor compounds in *F. bella* while *F. falsicornuta* contained α-Pinene, Δ^3-carene and and 1-octen-3-yl acetate. Both four *Frullania* species contained a large amount of non oxygenated sesquiterpenoids including one non identified compound (M=204), Africa-3(15)-ene, β-caryophyllene, α-humulene, β-longipinene, 4,5-*diepi*-aristolochene, isolepidozene (a major compound of *F. bella* and *F. mamillosa*), eremophilene (a major compound of *F. falsicornuta*) and hinesene (a major compound of *F. multilacera*). Interestingly 3 *Frullania* species (*F. bella*, *F. mamillosa*, *F. scalaris*) were characterized by the abundance of oxygenated sesquiterpenoids while only traces of such derivatives were detected in *F. falsicornuta* and *F. multilacera*. Cyclocolorenone was found only in *F. Falsicornuta*. Eudesm-3-en-6,7-oxide was a major compound of *F. bella* and was found only in this species. The dihydrofuranollide and a non identified compound with M=232 were detected as major compounds in *F. mamillosa* and *F. scalaris*.

15 Lejeuneaceae species has been analysed by GC-MS and are curently studied for botanical identification. Among this familly which is represented by 141 species in New Caledonia (2), we particularly focused on the epiphyllous specimens which often express a very strong odour. Thus, the characteristic smell of a *Leptoleunea sp* is due to the presence of 4-ethylphenol and 1-acetoxy-4-ethylbenzene. In *Colura leratii* ether extract we identified frullanolide as a major compound. To the most of our knowledge this is the first time that a frullanolide derivative is described from the Lejeuneaceae.

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O-7

ANTIMICROBIAL EFFICACIES OF COMMERCIAL ESSENTIAL OILS: SINGLE COMPOUND TO INTERACTIVE PROFILES

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Keywords: antimicrobial, interaction, Lavender, Myrrh, frankincense, efflux inhibition

Essential oils have been used to combat infectious diseases since antiquity. With reference to specific diseases, some essential oils will be highlighted and noteworthy activities presented. The MIC of essential oils such as *Laurus nobilis* (bay leaf), *Cinnamomum zeylanicum* (cinnamon), *Pogostemon patchouli* (patchouli), *Santalum album* (sandalwood) and *Andropogon muricatus* (Vetiver) showed some of the most noteworthy antimicrobial activity, with efficacies as low as 0.1 mg/ml against *Brevibacterium laterosporus*, a micro-organism associated with foot odour. The oils were also assayed in combination and the fractional inhibitory concentration (ΣFIC) calculated. Isobolograms were used to graphically demonstrate efficacies of combinations in varied ratios. Combination studies will be emphasised, as this forms the basis of aromatherapeutic, complimentary and traditional healing practises. The *in vitro* interactive efficacies of the combination of frankincense spp. (*Boswellia rivae, B. neglecta, B. papyrifera*) with two essential oil samples of myrrh (*Commiphora guidotti* and *C. myrrha*) will be presented, highlighting synergistic and additive interactions. Particular attention will be given to *Lavandula angustifolia* (Lavender). Synergistic combinations were evident when *Lavandula angustifolia* was combined with *Daucus carota* (ΣFIC 0.50 and 0.50); *Juniperus virginiana* (ΣFIC 0.50 and 0.50); *Cinnamomum zeylanicum* (ΣFIC 0.40 and 0.50) and *Citrus sinensis* (ΣFIC 0.42 and 0.38) when tested against *Candida albicans* and *Staphylococcus aureus*, respectively. Studies on essential oil constituents not only demonstrate efficacy, but selected compounds (menthone and carvacrol) also show the ability to inhibit *Staphylococcal* efflux activity. These examples detailing antimicrobial efficacy and pharmacological interactions, whether synergistic, antagonistic or on an additive level, play an important role in the understanding of the anti-infective properties of essential oils.

O-8

THE HUMAN OR1G1 OLFACTORY RECEPTOR IS DIFFERENTIALLY ACTIVATED BY VARIOUS SANDALWOOD ODORANTS. A JOINT APPROACH COMBINING IN SILICO AND IN VITRO EXPERIMENTS

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Keywords: Molecular dynamics, olphactophore ,olfactory receptor, sandalwood

With its characteristic woody fragrance and its fixative properties, sandalwood is one of the most precious natural raw materials used in perfumery. The main odorant components of natural sandalwood are (+)-(Z)-α-santalol and especially (−)-(Z)-β-santalol which shows the most typical and characteristic sandalwood odor. Today, a large number of synthetic substitutes of β-santalol are available for the perfumers in search of affordable sandalwood odorants, and many of them have a molecular structure completely different from that of β-santalol.

Despite many structure-odor relationships studies on sandalwood, the action mode of sandalwood odorant molecules remains to be addressed. In fact, olfactory receptors involved in their perception have not yet been identified.

In our study, the interaction of a human olfactory receptor, hOR1G1, with sandalwood odorants has been investigated. By means of cellular biology and functional tests experiments, we provide an additional insight to our atomic model of OR1G1 and our olfactophore approach performed on various odorants. The studied odorants cover a wide range of structures and sandalwood intensities. We experimentally show that β-santalol is a strong agonist of hOR1G1, contrarily to α-santalol and other closely related compounds. An atomic-scale model is then inferred through the use of both a sandalwood pharmacophore and the prediction of the position of these compounds within the cavity of the receptor.

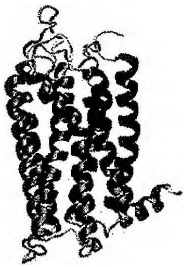

Figure 1. hOR1G1 structure obtained by homology modeling

O-9
AROMA PROFILE OF STAR ANISE AND THE STRUCTURE-ODOR RELATIONSHIP OF ANETHOLE

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Keywords: star anise, anethole, aroma profile, structure-odor relationship

The reception mechanism behind odor recognition is gradually becoming better understood. For example, some eugenol derivatives play an important role in the interaction with receptors [1]. We have proposed that the structural similarity of the constituents is important for the aroma profile of materials [2]. In this paper, we report the aroma profiles of star anise and the structure-odor relationship of anethole. GC-MS analysis showed that star anise contains many components that have structures similar to (*E*)-anethole. The results indicate that anethole is the key compound in the odor of star anise and the similarity of the structures is important in the aroma characteristics. We examined the effect of odorant structure on the aroma characteristics of (*E*)-anethole, focusing on the methoxy and 1-propenyl substituents. Altering the 1-propenyl group changed the odors of all the anethole derivatives. Replacing the methoxy group with a hydrogen atom resulted in compounds with a similar fatty odor. This shows that the methoxy group is important for the odor of anethole. We then synthesized anethole derivatives where the methoxy group was replaced with a methyl group. In both systems (methoxy- and methyl-substituted anethole derivatives), altering the 1-propenyl group changed the odors of all the derivatives. The results indicate that the structural characteristics of anethole are closely related to its odor expression.

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O-10

IMPROVING THE EFFICIENCY OF ESSENTIAL OIL EXTRACTION FROM SHELL GINGER (*ALPINIA ZERUMBET*) WITH AN UNDERWATER SHOCK-WAVE PRETREATMENT

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Keywords: Alpinia zerumbet, underwater shock wave, antioxidant activity, melanogenesis

Alpinia zerumbet (Pers.) Burtt et Smith (shell ginger) is an aromatic plant distributed widely in tropical and sub-tropical regions around the world. In Japan, it is distributed from southern Kyushu to Okinawa Prefecture. Shell ginger, known as "sannin" in the local Okinawa dialect or "getto" in Japanese, is used to flavor and wrap "mochi" rice cakes and its leaves are sold as herbal tea. Its essential oil has antioxidant activities [1,2] and it is very expensive because only low concentrations can be detected in the leaves and stems.

Underwater shock waves consist of instantaneous high pressure that reaches the entire cell, and they selectively destroy the cell walls of leaves and stems via spalling destruction. As a preprocessing step, the application of an underwater shock-wave treatment to the leaves and stems of shell ginger was expected result in more effective extraction of essential oil by subsequent steam distillation [3]. In this study, we prepared extracts from shell ginger materials that were untreated or subjected to an underwater shock-wave pretreatment, and compared their compositions. Then, we evaluated the bioactivity of the extracts to determine whether the pre-treatment affected their functionality.

The leaves and stems of shell ginger were dried at 45°C to a moisture content of 10–20%, and then the materials were subjected to the shock-wave pretreatment or left untreated before extraction done by hydrodistillation. The shock-wave preprocessing treatment resulted in a 1.5-times higher essential oil yield compared with that obtained from untreated material. The essential oil was analyzed by GC-MS and the components were identified from MS libraries. We identified 35 predominant compounds, which represented 94.3% of the total components detected. The main compounds detected were α-pinene (12.2%), camphene (14.5%), β-pinene (5.5%), camphor (2.5%), myrcene (3.2%), p-cymene (18.3%), limonene (14.0%), 1,8-cineole (4.9%), α-phellandrene (3.6%), and β-thujene (4.8%). The water extracts were analyzed by HS-GC-MS, and 27 compounds were identified. The predominant components were camphene (1.8%), 1,8-cineole (39.2%), γ-terpinene (2.2%), o-cymene (5.7%), linalool (12.1%), camphor (18.8%), terpinen-4-ol (4.0%), α-terpineol (1.8%), and borneol (2.3%). The shock-wave pretreatment resulted in a 3.7-times increase in the content of the most abundant compound in the water extract (1,8-cineol), compared with that obtained from untreated shell ginger.

The water extracts were evaluated to determine their antioxidant activity and their inhibitory effect on melanogenesis in B16 melanoma cells. The total antioxidant activity of the water extracts was measured by the 1,1-diphenyl-2-picrylhydrazyl method. The water extract prepared from untreated shell ginger showed no antioxidant activity; however, that prepared from pre-treated shell ginger showed free radical scavenging ability. The inhibitory effect on melanogenesis in B16 melanoma cells was 1.4-times higher in the extract from pretreated shell ginger, compared with that from untreated material. These findings indicate that destruction of the cell wall by the shock-wave treatment resulted in more effective extraction of the bioactive components.

These results suggest that essential oil can be extracted more effectively by using an underwater shock-wave preprocessing treatment. This treatment can also improve the functionality of the extracted oil, as determined by its antioxidant activity and its ability to suppress melanogenesis.

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O-11
APPLICATION OF TLC-BIOAUTOGRAPHY FOR DETECTING THE ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS

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Keywords: TLC-bioautography, gas chromatography, overpressured liquid chromatography (OPLC), essential oil, antibacterial activity

Antibiotics or disinfectants applied for prevention and treatment in agriculture, veterinary and human medicine can cause selective pressure leading to the spread of resistant mutants. As resistance develops more commonly, the need for alternative treatment increases. However, despite a push for new antibiotic therapies, there has been a continuous decline in the number of newly approved drugs (1). Antibiotic resistance, therefore, poses a significant problem.

The aim of the present study was the chemical characterization of some medically relevant essential oils (cinnamon bark, sweet fennel, rosemary, spearmint and clary sage) and the investigation of the antibacterial effect of these oils and their components by the use of a direct bioautographic method. Thin layer chromatography and overpressured liquid chromatography (OPLC) were combined with biological detection in this process. The essential oils were obtained from a Hungarian drug store chain (Herbaria, Hungary). The chemical composition of the oils was determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS). *Trans*-cinnamic aldehyde was the main component of the essential oil of cinnamon bark, *trans*-anethole of sweet fennel oil, and 1,8-cineole, carvone and linalyl acetate of rosemary, spearmint and clary sage oils, respectively. The antibacterial activity of these oils, their separated components as well as of their pure relevant components (*trans*-cinnamic aldehyde, eugenol, *trans*-anethole, 1,8-cineole, borneol, carvone, linalool, linalyl acetate and sclareol) were observed against *Staphylococcus aureus, S. epidermidis*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli, Micrococcus luteus* and *Bacillus subtilis*.

On the whole, the antibacterial activity of the essential oils could be related to their main components, but their minor constituents as well may be involved in this process. *Trans*-cinnamic aldehyde, eugenol, *trans*-anethole, carvone and sclareol were the most active compounds in TLC/OPLC-bioautography. This method is more cost-effective and provides more reliable results in comparison with conventional microbiological methods, e.g., disc-diffusion technique.

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O-12

EFFECT OF DIFFERENT HABITAT AND HARVEST TIME ON THE ESSENTIAL OIL COMPOSITION OF GROUND - IVY (*GLECHOMA HEDERACEA* L.)

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Keywords: Lamiaceae, sesquiterpenes, germacrene D

Ground-ivy is a perennial medicinal plant belongs to the *Lamiaceae* family, *Nepetoideae* subfamily and *Menthea* tribe, it occurs as a common weed also in Hungary. Its essential oil composition has already been evaluated in several European wild growing populations (1,2,3), however, in Hungary only the non volatile phenolic compounds have been analysed. Therefore our aim was to evaluate the chemical variability concerning essential oil composition of the Hungarian wild growing and cultivated populations.

In our investigations four wild growing *Glechoma* populations were analysed; as natural habitats two botanical gardens were also involved. Vegetative propagation was used to make cultivated populations as well in the experimental field of Department of Medicinal and Aromatic Plants, in Soroksár. The wild growing and the cultivated plants were also compared to each other based on their essential oil composition. The stems were cut in full flowering period (in April), and during the vegetative phase (in August) in each case. After the natural drying the drug was hydrodistilled (according to the European Pharmacopoeia) and the oil composition was analysed by GC-MS in three replications. From the first results approximately 20 compontents have been identified in the dried flowering shoots, 38 in the vegetative samples and 48 in the cultivated populations (also vegetative samples). Our results were in accordance with the literature data (1,2,3): in each sample sesquiterpenes were dominant representing more than 70 % of the identified essential oil compounds. The main essential oil compound was germacrene D. Notable minor components were germacrene B, (E,E)-α-farnesene, β-elemene and germacrene A. The different wild growing populations were characterised by different essential oil composition; the time of sample collection (in full flowering period - April, and in the vegetative cycle phase – in August) and the cultivation also had significant effects on the essential oil composition.

Further studies are necessary to make a firm conclusion about possible connection between the essential oil composition and the natural habitats of ground ivy.

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O-13

ON-LINE LC-MDGC-PREP SYSTEM FOR THE ISOLATION OF HIGH AMOUNTS OF PURE COMPONENTS

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Keywords: component isolation, MDGC, Prep-GC

Natural samples are often characterized by the presence of a high number of compounds, belonging to different chemical classes; hence, the correct structural elucidation of a single component is often a very hard task. Usually, wide-bore columns (0.53 mm I.D.) are used, providing an enhanced sample capacity, even if a lower resolution will result in skewed and coeluted peasks. For these reasons, the collection of pure components requires the injection of lower amounts of sample on the wide-bore columns; as a consequence, the collection of highly pure components, at the milligram level, requires an excessive total collection time. Heart-cutting multidimensional gas chromatography (MDGC) is a prime choice, if the complete separation of target compounds, contained in complex samples, is desired. Recently, MDGC systems equipped with Deans switches, have been successfully used in prep GC applications [1–2] for the collection, in a short time, of pure components ranging from 10 to 30% level, collected at the milligram level and characterized by means NMR, vapor-phase IR and MS.

The collection of compounds at concentrations <10%, is difficult to perform because of the limited injection volume into a conventional GC liner resulting in hundred of analysis to collect a lower amount of pure sample. To improve the capability of the system, an on-line LC-MDGC-Prep system was developed to enable the injection of higher sample volumes, the reduction of the total collection times, while maintaining high levels of purity. The system consists of an NP-LC pre-separation step, followed by the transfer of the isolated fraction(s), to a large volume injector positioned in the first GC instrument, by means of an LC-GC syringe-based interface. An SLB-5ms - Supelcowax 10 - SLB-IL59 ionic liquid stationary phase (0.53 I.D.) combination was used in the three GC dimensions, in order to provide three distinct selectivities. A preparative station, connected at the 3rd GC column outlet, allowed the recondensation of pure components in a tube.

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O-14
SEASONAL VARIATION OF THE ESSENTIAL OIL AT DIFFERENT *THYMUS VULGARIS* CHEMOTYPES

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Keywords: *chemotype, thymol, linalool, geraniol, α-terpineol*

Thyme (*Thymus vulgaris* L.) is widely cultivated in almost all of the countries with moderate climate. An increase in utilization and consumption of the herb and the essential oil is predicted in the case of certain chemotypes (thymol-, carvacrol-, and linalool-types), where standardized composition of the essential oils and uniform organoleptic properties are expected by pharmaceutical, perfumery or food industries.

In our studies, seasonal changes of different chemotypes have been investigated, in order to determine the effect of the growing year and the weather conditions of the harvest periods on the quality of essential oil obtained. Samples were collected from 10 clones belonging to 5 chemotypes in spring (May) and autumn (September) periods of 2011 and 2012 in our Experimental Station, Budapest, Hungary.

In general, the amount of the essential oil in *Thymi herba* was higher in spring than in autumn and it was also affected by the weather conditions of the growing year. There were significant differences among clones, where mostly the phenolic chemotypes represented the lower and more variable essential oil levels (0.676-2.568 ml/100g) than those of the geraniol (1.110-3.142 ml/100g), linalool (1.028-3.039 ml/100g) and α-terpineol (2.046-4.068 ml/100g) containing ones.

Chemotype patterns were verified by cluster analysis, however, certain compounds were affected by seasonal changes. Weather conditions caused significant seasonal variation on essential oil composition in the case of phenolic (thymol, carvacrol) and in geraniol chemotypes. In spring cut samples of thymol chemotypes, the level of thymol was higher (63-88 %) than in autumn (35-51%), while percentages of the respective biosynthetic intermediates, p-cymene and γ-terpinene have changed in inverse ratio. As a result of the seasonal effects, geranyl acetate appeared in higher % in spring harvest (21-34 %), while geraniol ratio was more considerable in September (64-75 %) than in May (22-57 %) at the same chemotype. Concerning α-terpineol (13-22 %) chemotype, proportion of its derivative, α-terpinyl acetate was more significant in our clones with quite constant levels (69-80 %), irrespective of the seasonal effects. Only moderate changes have been observed in the case of linalool chemotype as well, where the proportion (77-86 %) of the main compound has not affected by the environmental conditions.

O-15

THE APPLICATION OF CHEMOMETRIC MODELLING IN OPTIMISING ANTIMICROBIAL ESSENTIAL OIL FORMULATIONS

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The antimicrobial activity of essential oils is not a novel concept and a plethora of scientific evidence has been published to confirm (and re-confirm) the antimicrobial activity of essential oils. Some studies have followed a reductionist approach to identify the essential oil component responsible for the antimicrobial activity of the crude essential oil. The hunt for the "needle in the haystack" is a laborious approach and is fast becoming out-dated. Furthermore, traditional healers and aromatherapists administer complex mixtures and oil blends and do not administer individual compounds.

Using an un-targeted chemometric approach we recorded the antimicrobial activity and essential oil composition of commercially available essential oils (N=158). The antimicrobial activity was determined against three Gram-positive, two Gram-negative organisms as well as two yeasts. The essential oil composition was determined by GC-MS and resulting chromatograms were exported to MarkerLynx™ for peak selection and alignment. The OPLS-DA models were constructed and used to filter out putative retention time mass (RTM) pairs associated with samples exhibiting superior antimicrobial activity.

Using various *in vitro* models to confirm synergy (e.g. FIC index / isobolograms) the bioactivity of various compound combinations as predicted in the model were evaluated. Using Design of Experiments the combination of the bioactive molecules were optimised.

The proposed method of using chemometric analysis of GC-MS data and overlaying bioactivity results confirms the advantages of a metabolomic approach which could assist the formulation scientist to produce essential oil blends exhibiting pronounced antimicrobial activity.

O-16

FLORAL SCENT AND MATING SYSTEM TRANSITIONS IN THE YELLOW EVENING PRIMROSE, *OENOTHERA FLAVA (ONAGRACEAE)*

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How and why angiosperms self-pollinate is one of the oldest questions in evolutionary biology. Angiosperm mating systems range from obligate xenogamy to obligate autogamy, spanned by a continuum of mixed mating. My work has focused on how mixed mating angiosperms use floral characteristics, including floral scent, to balance autogamy and xenogamy through communication with pollinators. To do this, I work with a mixed mating species with extensive floral variation.

Oenothera flava (Onagraceae) is a hawkmoth-pollinated plant found in western North America. It produces yellow flowers that open in the evening and wilt by the following morning. *Oenothera flava* subsp. *taraxacoides* flowers are the deepest in North America (~25cm floral tubes), and petals emit large quantities of volatiles, predominantly nitrogenous aldoximes. Positive herkogamy at dusk disappears by dawn, when they self-pollinate. This subspecies grows in disjunct "sky-island" populations and exhibits variation suggesting repeated evolution toward xenogamy. *Oenothera flava* subsp. *flava* is broadly distributed in xeric habitats, and has weakly scented, short-tubed (~6cm) flowers that self-pollinate early.

The floral scent in each subspecies is dominated by two aldoximes. Aldoximes are common to hawkmoth-pollinated flowers, but are better known as intermediates in the glucosinolate and cyanogenic glucoside defense pathways. In these pathways, they result from the oxidation of an amino acid by a cytochrome P450. Total RNA sequencing from the petals of each subspecies yielded 13,000 contiguous sequences, including a candidate gene significantly similar to an aldoxime-producing Poplar Cyp79. Transient expression in *Nicotiana benthamiana* demonstrated this candidate's sufficiency for production of the floral aldoximes. Lastly, a known pollinator, *Hyles lineata*, showed increased visitation to artificial flowers supplemented with one aldoxime.

These experiments investigate the molecular and behavioral strategies used by a versatile plant species to generate adaptive variation in floral phenotype, thereby maintaining populations of showy flowers on sky islands and smaller, weakly scented, self-pollinating flowers in xeric habitats.

O-17
ESSENTIAL OILS AND SOLVENT EXTRACTS FROM LIVERWORTS: MONO- AND SESQUITERPENOIDS, ACETOGENINS AND AROMATIC COMPOUNDS POSSESSING CHARACTERISTIC SCENTS

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Keywords: liverworts, scents, nerol, bicyclohumulenone, ectocarpene, sex pheromones

Marchantiophyta (liverworts) contain appearing cellular oil bodies. When their fresh samples are crushed, very characteristic fragrant or unpleasant odor emits which correspond to the constituents of oil bodies. The present paper summarizes characteristic scent components of essential oils and solvent extracts from several liverworts. Most of the liverworts contain 1-octen-3-ol and/or its acetate which contribute to their mushroom like odor. *Conocephalum conicum* has several chemo-types one of which elaborates strong mushroom like odor, very similar to that of *Tricholoma matsutake*, the most expensive Japanese edible mushroom. This is due to the presence of methyl cinnamate and 1-octen-3-yl acetate and/or 1-octen-3-ol in its essential oils or ether extract. The ether extract of the Greek *Fossombronia angulosa* emits ocean smell which is due to the presence of marine algal sex pheromones, dictyotene (**1**), (*E*)-ectocarpene (**2**), (*Z*)-multifidene (**3**) and dictyopterene (**4**). Dictyotene and ectocarpene have been found in the Tahitian *Chandonanthus hirtellus*. The essential oils of *Plagiochila sciophila* and *Frullania tamarisci* contain bicylohumulenone (**5**) and tamariscol (**6**), respectively, which are responsible for the strong sweet mossy odor and their total synthesis has already been accomplished. The Malaysian *Cheilolejeunea imbricata* contains milky smell which is based on (*R*)-dodec-2-en-1,5-olide (**7**) and (*R*)-tetradec-2-en-1,5-olide (**8**). Unpleasant odor from the ether extract of the Tahitian *Cyathodium foetidissimum* is due to skatole. The New Zealand *Chiloscyphus pallidus* and the Taiwanese *Heteroscyphus coalitus* emit strong sting bug smell which is attributable to the mixture of (*E*)-dec-2-enal (**9**) and (*Z*)-dec-2-enal (**10**) as the major components and (*E*)- (**11**) and (*Z*)-pent-2-enal (**12**). The surfer smell of the ether extract of the Mediterranean *Corsinia coriandrina* is due to the mixture of (*E*)-(**13**) and (*Z*)-coriandrin (**14**) and two ethyliminodithiocarbonates, (*E*)- (**15**) and (*Z*)-*O*-methyltridentatol (**16**). The ether extract of Japanese epiphytic tiny *Leptolejeunea elliptica* elaborates a very powerful, sweet mold-like odor which is ascribed to the mixture of 4-ethylphenol (**17**), 4-ethylanisol (**18**) and 4-ethyl-acetoxybenzene (**19**). The essential oil from *Wiesnerella denudata* shows sweet mossy odor that is due to the high amount of nerol, neryl acetate and γ-terpinene. The pleasant odor of *Takakia lepidozioides* is owing to a large amount of coumarin. The ether extract of *Plagiochila ovalifolia* has a strong turpentine odor; the ether extract contains α- and β-pinenes, camphene and linalool whereas its essential oil contains 1-octen-3-yl acetate and *trans*-nerolidol as the major components. The French *Targionia hypophylla* emits intense and fragrant scent which is due to a mixture of *cis*- and *trans*-pinocarveyl acetates isolated from its methanol extract. *Isotachis* species are very characteristic since they produce benzyl and β-phenethyl cinnamates as the major components in the essential oil. In the case of liverwort constituents the most interesting phenomenon from a

chemical point of is that most of their sesqui- and diterpenoids are enantiomers of those found in higher plants. Moreover, some different species belonging to the same genus produce normal and its enantiomeric sesquiterpenoids. Liverworts are very tiny plant; however, they are rich sources of fragrant components (1-4).

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O-18
THE CHEMISTRY OF THE VOLATILES OF ANNONACEAE FROM VIETNAM

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Keywords: Annonaceae, diversity, sesquiterpenes, monoterpenes

Our findings into the chemical volatile constituents of some Annonacea of Vietnamese flora have been recently published (1, 2, 3). In the present investigation we studied new essential oil contents and compositions of ten individual plants from Annonaceae family cultivated in Vietnam. The air-dried plants were hydrodistilled (PhVn) and the oils analysed by GC and GC-MS. The components were identified by MS libraries and their LRIs. The essential oils content varied between 0.10% and 0.15% (v/w) on a dry weight basis. The main compounds of the leaf oil of *Friesodielsia filipes* (Hook. f & Thomson) Steenis were α-pinene (19.1%), β-caryophyllene (15.7%), Caryophyllene oxide (9.6%) and β-pinene (8.3%) while benzyl benzoate (34.5%) and α-pinene (30.6%) were identified in the stem. Also, α-humulene (20.4%), bicyclogermacrene (15.4%), β-caryophyllene (14.5%) and bicycloelemene (13.9%) dominated the leaf oil of *Meiogyne virgata* (Blume) Miq. And we identified α-humulene (13.3%), benzyl benzoate (10.3%) and bicyclogermacrene (9.2%) as the major compounds in the stem oil. However, β-cubebene (8.4%) and β-caryophyllene (10.2%) in the leaf; as well as eugenol (42.9%) and limonene (24.1%) in the stem were the main oil constituents of *Polyalthia sessiliflora* (Ast) Ban. The main compounds identified in the leaf oil of *Pseuduvaria indochinensis* Merr. were α-copaene (26.5%), α-pinene (7.6%) and 1,8-cineole (5.7%) while methyl eugenol (20.0%) and limonene (19.2%) occurred in the stem oil. We have identified germacrene D (27.8%), *cis-p*-mentha-2,4(8)-diene (21.6%) and bicyclogermacrene (15.3%) as the main compounds of the leaf oil of *Orophea hirsuta* King,, while limonene (15.4%), β-caryophyllene (12.6%), α-thujene (8.8%) and α-pinene (7.6%) were of significant quantities in the stem.

α-Pinene (50.0%) was the most singly abundant constituent of the leaf oil of *Goniothalamus macrocalyx* Ban., while the stem had α-cadinol (14.5%), β-caryophyllene (10.3%) and octadecanoic acid (8.2%) as major compounds. Benzoic acid (18.4%), β-caryophyllene (12.4%) and α-pinene (10.3%) were present in leaf of *Goniothalamus albiflorus* Ban., while limonene (21.2%), β-caryophyllene (12.8%) and α-phellandrene (9.3%) were identified in stem. The leaf oil of *Goniothalamus tamirensis* Pierre ex Fin. & Gagnep., was characterized by abundance of α-pinene (33.4%), viridiflorol (18.5%) and β-caryophyllene (12.4%), while γ-gurjunene (11.2%), β-caryophyllene (10.9%) and δ-cadinene (10.3%) predominates in the stem oil.

The quantitatively significant constituents of *Alphonsea philastreana* (Pierre) Pierre ex Fin. et Gagnep. and *Alphonsea gaudichaudiana* (Baill.) Fin. et Gagnep. were (*E*)-β-ocimene (6.9 % and 8.5%), bicycloelemene (8.9% and 6.3%), β-caryophyllene (5.1 % and 5.9%), α-humulene (5.8% and 4.6%),

bicyclogermacrene (9.3% and 6.3%), guaiol (9.0% and 5.2%) and α-eudesmol (8.3% and 5.5%) respectively.

The chemotaxonomy implications of the present results as well as the biological activities of the oils would be discussed.

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O-19

ANALYSIS OF LEMON OIL OBTAINED BY SUPERCRITICAL CO_2 EXTRACTION, COLD PRESSING AND HYDRODISTILLATION METHODS

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Keywords: Citrus limon (L.) Burm. f., lemon peel oil, GC/MS, supercritical CO_2 extraction, cold pressing, hydrodistillation

Lemon is one of the most important *Citrus* fruit for Turkey, because of its great amount of production and export (1). Lemon is used for fresh fruit market, pectin, juice and essential oil. Fruit size and peel color are important fruit characteristics for fresh market, while, for processing, soluble solids, juice, pectin and essential oil content are important. Recently, Kirbaslar at al. reviewed a lot of cold-pressed lemon peel oil studies in the literature. Although most of these studies concerned commercial oils, some studies were carried out on essential oils obtained laboratory-extracted from the peel *C. limon* species (2-3). The lemon plants used in the present study were of the variety Kıbrıs (*Citrus limon*) planted at Batı Akdeniz Tarımsal Araştırma Enstitüsü "BATEM" located at Antalya, South Turkey. The samples of cloned Kıbrıs lemon KM-2 fruits (Lemon code: BATEM302) were taken from healthy adult trees of the germplasm collection held in 28 November 2010. Identification of individual constituents was based on comparison of their GC Retention indices (RI) on the apolar column determined relative to the retention time of a homolog series of n-alkanes (C_8-C_{18}) with linear interpolation, with those of authentic compounds and computer matching with the MS database [National Institute of Standards and Technology (NIST) and Wiley libraries] commercial spectral libraries of the GC/MS, as well as by the comparison of the fragmentation pattern in the mass spectra with those reported by Adams(4). The lemon oil yield was found 4.34 % by the Clevenger type hydrodistillation apparatus. While 44 compounds were identified in the supercritical CO2 extraction (SFE) and cold-pressing (CP) oils, 41 components were detected in the hydrodistillation (HD) oil. The analyses of the oil exposed a total of 44 constituents: 15 monoterpenes, 9 sesquiterpenes, 7 aldehydes, 7 alcohols, 3 esters, and 3 oxides. The total monoterpene hydrocarbons were present in larger amounts in the HD (93.48 %) oil than in the SFE (92.95 %) and CP (92.87 %) oils. Sesquiterpene hydrocarbon fraction represents about 2.58 % and 2.02 % of the oil of CP and HD while it is higher in the oil of SFE (2.68 %). An important observation of the oil compositions revealed that higher amounts of total oxygenated compounds, (4.48%) are present in the oil extracted by SFE in comparison with the oil extracted by CP (4.39%) and HD (4.12%). The total compounds of this studied CP lemon oil were similar to that reported by Kirbaslar at al. for CP lemon peel oil components, except for slightly variations in some components (3).

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ABSTRACTS -
POSTER PRESENTATIONS

BA 1

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF LAVENDER (*LAVANDULA ANGUSTIFOLIA*) ESSENTIAL OILS FROM LEAVES

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Keywords: lavender, essential oils, antibacterial activity

Lavender is a valuable resource for a wide therapeutic biological activity. In a series of publications, new varieties of lavender are still generating interest in research, resulting in the possibility of using this herb, such as an antibacterial, antifungal, antioxidant, anti-inflammatory remedy as well as acting as a natural antidiabetic and sedative agent (1-4). The aim of the study was comparing the chemical composition of the essential oils from two varieties of lavender (*Lavandula angustifolia* L.) and its antibacterial activity. The material consisted of leaves of two varieties of lavender: 'Blue River' and 'Ellegance Purple'. Plants were derived from experimental cultivation carried out by the Horticulture Department West Pomeranian University of Technology in Szczecin from the set in July 2012. The dried material parts (20 g) were submitted to hydrodistillation for 3 hours using Deryng's type apparatus, according to the Polish Pharmacopoeia. The chemical composition of essential oils was determined by gas chromatography coupled to mass spectrometry (GC/MS) using an Agilent apparatus model 6890 with a chromatographic column HP-5MS. The qualitative analysis was performed based on MS spectra by comparing them with the spectra of the NIST library. The identity of the compounds was confirmed by retention indices with literature data. Quantitative composition was determined by assuming that the sum of the individual compounds is 100%.

Essential oil content in the leaves of lavender varied from 0.45% for the 'Blue River', to 0.60% for the 'Ellegance Purple'. In the oils 42 compounds have been identified, of which the main are: borneol (12.4–13.8%) caryophyllene oxide (8.0–8.7%), epi-bicyclosesquiphellandrene (6.8–8.3%), eucalyptol (3.7–6.2%), linalool (4.9–5.8%), geranyl acetate (4.0–4.1%) and β-pinene (3.5–3.6%). Most of the compounds identified in the oils were belonging to the monoterpenoid group (48.9–49.1%). Monoterpenes accounted from 10.8% of the identified compounds in the 'Ellegance Purple', to 14.0% in the 'Blue River'. Besides monoterpenes and monoterpenoids sesquiterpenes (11.7–14.5%) and sesquiterpenoids (8.0–8.7%) have been also determined.

The oils were assayed against two bacteria, *Staphylococcus aureus* (reference strain and strain isolates from skin MRSA/ORSA), and *Pseudmonas aeruginosa*. Antibacterial activities of the essential oils from leaves were evaluated using disc-diffusion method. Results, suggest potential antibacterial activity of the essential oils of *L. angustifolia*, which may find those application in future research for the pharmaceutical, cosmetic, and food industry.

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BA 2
ANETHUM GRAVEOLENS L. "DILL": A FRAGRANT SPICE USED IN ARAB TRADITIONAL MEDICINE

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Spices and most aromatic herbs are used in the ancient systems of medicine for the treatment of many human afflictions. *Anethum graveolens L.* (Dill, Shibat) [Family: Apiaceae] has long been used in Arab Traditional Medicine for the prevention and treatment of gastrointestinal disorders. The main chemical constituents that have been reported include coumarins; aesculetin in fruit and 4-methyl-aesculetin in fruit essential oil. The present study was carried out to evaluate the gastric antiulcer property of dill in Wistar albino rats. An aqueous suspension of dill was used in two doses, 250 and 500 mg/kg body weight, orally. Gastric acid secretion studies were undertaken using pylorus ligated (Shay) rats. Gastric lesions were induced by noxious chemicals including ethanol, strong alkalis, indomethacin and hypothermic restraint-induced stress. The levels of gastric wall mucus (GWM), NP-SH and MDA were measured in the glandular stomach of rats following ethanol challenge. The gastric tissue was examined histologically.

In pylorus-ligated Shay rats, the dill suspension significantly reduced the basal gastric acid secretion, titratable acid and ruminal ulceration. The suspension significantly attenuated necrotizing agents, indomethacin and cold plus restraint stress induced gastric mucosal injury. The cytoprotective and antiulcer effect was confirmed histologically. Furthermore, the dill suspension replenished the ethanol-induced MDA concentration of the rat's stomach. The results obtained support the use of dill in various gastric ailments including stomach ulcers. Dill contains essential oils and other bio-active phytochemicals which might contribute to its antioxidant, anti-secretory and cytoprotective properties and validates its use in traditional medicine for gastric disorders.

BA 3
FUNGICIDAL ACTIVITY OF THE ESSENTIAL OIL FROM *SATUREJA MONTANA* L.

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Keywords: Fusarium graminearum, Segurelha, fumigation

The genus Satureja is characterized by shrub-like aromatic plants that inhabit sunny, arid, and rocky regions. The Satureja montana. The *Satureja montana* L. species has been used in folk medicine to treat digestive disorders, as an expectorant and antidiuretic agent (1). The use of essential oils is a natural alternative in the search for new substances for pest control because studies show that intensive and indiscriminate use of synthetic substances favor the rise of resistant pests and cause negative effects on the environment and organisms that are exposed to these products (2).

This study sought to assess the fungicidal potential of the essential oil from *Satureja montana* L. against the plant pathogen *Fusarium graminearum*. The essential oil was extracted by hydrodistillation in a modified Clevenger apparatus (3). The qualitative and quantitative analyses of the essential oil were achieved by gas chromatography coupled to mass spectrometry and to a flame ionization detector. To perform the assay, the fumigation test was employed. The essential oil was diluted in ethyl ether to yield concentrations of 50, 100, 125, 250, 500, 750 and 1000 mg L^{-1}. Two parallel plates were prepared: a relative control and an absolute control. The BDA culture medium was used for the test (2). The readings were taken by measuring the diameter of colonies twelve days after initiating the experiment. The principal compounds found in the essential oil from *Satureja montana* L. were thymol (32%), carvacrol (14.35%), *p*-cymene (12.12%) and linalool (8.99%). The effect of the essential oil on the growth of mycelial growth of the phytopathogenic fungus was 0% in the relative and absolute controls; 29.85% for the 50-mgL^{-1} concentration; 97.71% for the 1000 mgL^{-1} concentration, and 100% at concentrations of 125, 250, 500, 750 and 1000 mgL^{-1}. The essential oil from *Satureja montana* L. had an effect on the pathogen at all the concentrations studied, and total inhibition was achieved at the concentration of 125 mg L^{-1}.

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BA 4

ANTI-INFLAMMATORY ACTIVITY AND CYTOTOXICIY OF *MYRTUS COMMUNIS* L. ESSENTIAL OIL FROM ALGERIA

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Keywords: Myrtus communis, Algeria, chemical composition, anti-inflammatory activity, cytotoxicity

In Algeria *Myrtus communis* L. is distributed throughout the Tell Atlas and the coastal regions of Algiers and Constantine, where it is known as «*rihan*» or «*mersin*». The leaves are appreciated for the treatment of respiratory disorders such as bronchitis, sinusitis, otitis, diarrhea and hemorrhoids.

As part of our ongoing work, on the valorization of Algerian myrtle, we reported in this study the anti-inflammatory activity and cytotoxicity of two oil samples (A and B) isolated from the aerial parts of *M. communis* L.

The chemical composition was investigated by GC (FID), in combination with retention indices (GC-FID), GC-MS and by ^{13}C NMR spectroscopy. Sample A and sample B differed mainly in the relative amounts of α-pinene (50.8 *vs* 33.6%), 1,8-cineole (21.9 *vs* 13.3%), linalool (14.8 *vs* 2.7%) and linalyl acetate (9.5 *vs* 0.5%), respectively.

The anti-inflammatory potential of the oils was evaluated using the *in vitro* model of lipopolysaccharide (LPS)-stimutaled macrophages cell line Raw 264.7. The production of NO was measured using a colorimetric reaction with the Griess reagent and assessment of cell viability was made through a colorimetric assay, using MTT.

Both oils were able to significantly inhibit the production of NO without affecting cell viability in concentrations up to 0.64 µL/mL. Nevertheless, our results suggested that sample B possess slightly higher anti-inflammatory activity than sample A. These promising results highlight the potential of myrtle essential oil as anti-inflammatory drug, thus justifying further *in vivo* assays to confirm the effectiveness of the *in vitro* results.

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BA 5
SEASONAL VARIATION OF CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL OF FIVE SALVIA SPECIES FROM BAJA CALIFORNIA MÉXICO: *SALVIA APIANA, S. PACHYPHYLLA, S. MELLIFERA, S. MUNZII* AND *S. CLEVELANDII*

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Keywords: essential oil, seasonal variation, chemical composition, antioxidant activity

Biological activities are correlated to the presence of chemical compounds, particularly secondary metabolites. The presence of these compounds may assist in predicting some traditional uses of medicinal plants (1). The season and even the number of hours plants receive sunlight may influence the phytochemistry of the plant, since some compounds may be accumulated at a particular period to respond to environmental changes (2).

In this work, the composition and antioxidant activities of five *Salvia* species from Baja California México essential oil were investigated. The essential oils were analyzed by GC–MS, and obtained by hydrodistillation using a Clevenger-type apparatus. All the plants were collected throughout the 2012-growing season. The yields of essential oils ranged from 0.20 % to 2.00 %, with the maximum amount in winter for *Salvia pachyphylla*. The yields were based on dry materials of plant sample.

Qualitative and quantitative differences in compositions and in antioxidant activities of the aerial parts essential oils were observed during the season. Mostly quantitative, rather than qualitative variation was observed in the oil composition of each species. The fluctuations in the major constituents of S. *pachyphylla* oil were 3-carene (22-26%), eucalyptol (12-21%), caryophyllene (10-18%). Levels of camphor (17-35%), eucalyptol (10-24%), limonene (13-21%), and caryophyllene (5-20%) fluctuated seasonally in S. *munzii*. In S. *clevelandii* the most important changes occurred for phenylethyacetate (19-43%), camphor (14-21%), δ-cadinene (8-13%), linalool (9-19%) and eucalyptol (5-18%). S. *mellifera* showed levels of camphor (14-21%) and eucalyptol (22-26%). Finally eucalyptol (24-29%) and camphor (42-45%) were detected as the major components for S. *apiana*.

The antioxidant activities were determined by using complementary tests, namely, DPPH radical-scavenging and bleaching β-carotene in linoleic acid *in vitro* assays. *Salvia munzii* showed in summer the highest antioxidant activity in both DPPH (IC50 = 1.2 mg/ml) and β-carotene-linoleic acid (inhibition percentage: 93 %).

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BA 6

SUPPRESSIVENESS OF SELECTED ESSENTIAL OILS ON THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* IN SOIL

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Keywords: root-knot nematodes, control, sustainability, essential oils

Root-knot nematodes, *Meloidogyne* species, are strongly destructive and extremely difficult to manage due to their poliphagy and worldwide spread (1). Control of these parasites has been prevalently based on chemical treatments, but environmental concerns are imposing the adoption of safer management strategies. Plant-derived compounds can represent a concrete alternative to chemicals, as nematicidal secundary metabolites are present in many plant families (2). Plant essential oils and their constituents have a great potential for nematode control since they can be developed for the use as nematicides themselves or can be used as model compounds for the development of more active derivatives (3, 4). Soil drench treatments with 50, 100 and 200 ml kg^{-1} soil solutions of the essential oils from five different species, i.e. *Schinus molle, Cinnamomum camphora, Eugenia caryophillata, Cinnamomum zeylanicum* and *Citrus aurantium* were comparatively tested against the root-knot nematode *Meloidogyne incognita* on tomato in pot. Plant growth parameters, gall infestation and nematode multiplication on tomato roots were evaluated after a two-month permanence of plants in a greenhouse at 25 °C. All the treatments with the five essential oils but the 50 and 100 and 200 ml kg^{-1} soil rates of *C. zeylanicum* significantly suppressed the number of eggs and juveniles of *M. incognita* on tomato roots compared to the non-treated control. Effect of treatments was less evident on gall formation on tomato roots. Essential oils of *S. molle* and *E. caryophillata* were overall more suppressive than the other three oils and generally as nematotoxic as fenamiphos chemical control. Tomato top and root biomass was not affected by all the tested treatments. Soil treatments with essential oils formulations were confirmed to be an effective tool for a sustainable management of root-knot nematode infestations.

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BA 7

ESSENTIAL OIL FROM *LIPPIA ORIGANOIDES* KUNTH.: CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY

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Keywords: Secondary metabolites, Escherichia coli, Staphylococcus aureus, Verbenaceae

Many studies have demonstrated the antimicrobial efficacy of essential oils, indicating the possibility of their use in the control of food-borne pathogens, thereby contributing to a reduction in the future use of antibiotics [1]. *Lippia origanoides* Kunth. (Verbenaceae) is a species native to northeastern Brazil. An ethnobotanical survey of some Brazilian medicinal plants showed that this plant, popularly known as guinea fowl rosemary, is used in the treatment of various diseases, such as gastrointestinal and respiratory infections (2). The present study sought to chemically characterize and evaluate the antibacterial activity of the essential oil of *L. origanoides*. The plant material was collected in the Medicinal Plants Garden of the Federal University of Lavras (Lavras, Minas Gerais, Brazil) and the essential oil was obtained by leaf hydrodistillation [3]. The chemical characterization of the essential oil was determined as reported in [4]. Evaluation of the antibacterial activity against *Escherichia coli* ATCC 11229 and *Staphylococcus aureus* ATCC 6538 was accomplished using the cavity diffusion in agar technique with concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, and 3.9 μLmL^{-1} of the essential oil in DMSO [5]. The sensitivity of the bacteria toward the different concentrations of the essential oil was evaluated from the inhibition diameters. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the essential oil for which an inhibition halo was observed. GC analysis of the essential oil of *L. origanoides* revealed carvacrol, *p*-cymene, γ-terpinene and thymol, as the major constituents. The MIC values of the essential oil were 7.81 and 15.62 μLmL^{-1} for *Escherichia coli* and *Staphylococcus aureus*, respectively.

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BA 8

INFLUENCE OF THE ESSENTIAL OIL FROM *CHENOPODIUM AMBROSIOIDES* L. ON THE FEEDING BEHAVIOUR OF *DIABROTICA SPECIOSA*

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Keywords: natural products, insect, feeding behaviour

Monocultures occupy vast areas of the planet and are often attacked by herbivorous insects, whose populations cause economic damage. The main method of control of these pests is to use synthetic products, which results in general environmental contamination, the reduction of natural enemies, and the poisoning of the farmer and the consumer (1). The knowledge of the damage caused by the indiscriminate use of synthetic products, associated with consumer concerns about the quality of food has motivated studies related to new pest control techniques. The aim of this study was to chemically characterize the essential oil of *Chenopodium ambrosioides* L. and evaluate its effects on the feeding behaviour of *Diabrotica speciosa* (Coleoptera: Chrysomelidae) in beans. The extraction of the oil was performed over a 2h period by hydrodistillation using a modified Clevenger apparatus, and it was subsequently analyzed by GC-FID and GC-MS as in (2). A completely randomized design in a 2 x 5 factorial scheme with 4 replicates was adopted for feeding behaviour evaluation. The bean plants were sprayed with solutions of the essential oil dissolved in Tween at concentrations of 0 (water + Tween), 0.5, 1.0, 1.5 and 2.0% and then provided to the insects for choice (3). Seven days after the application, the percentage of leaves with injury, leaf area consumed, and preference index with regard to consumption was evaluated (4). The essential oil contained α-terpinene (41%), p-cymene (22%) and trans-ascaridol (12%) as the main constituents. No significant difference between the concentrations used was observed with regard to the percentages of leaf injuries caused by *Diabrotica speciosa* and leaf area consumed. However, on the basis of the preference index, it was concluded that the essential oil of *C. ambrosioides* L. presented antifeeding activity.

Acknowledgements: CNPq, CAPES and FAPEMIG. To FCT PEst-OE/EQB/LA0023/2011.

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BA 9

THE EFFECT OF THYME EXTRACT AND AGE ON THE ADHERENT MUCIN LAYER DYNAMICS OF THE INTESTINE, BLOOD CHEMISTRY CHANGES AND PERFORMANCE IN CHICKENS

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Keywords: thyme essential oil, intestinal mucin adherent layer, biochemistry, broiler

Essential oils are mixtures of volatile natural complex compounds characterized by a strong odour formed by aromatic plants as secondary metabolites. There is evidence that essential oils possess broad-range antibacterial, anti- inflammatory, anti-oxidant and glucose - lowering properties. Furthermore, they also function as growth promotors for animals (1).

The epithelium of the intestinal tract is covered by a layer of mucus composed by mucin glycoproteins. Expression of mucins can be regulated by inflammatory cytokines, microbial products, cholinergic stimuli and by diet constituents (2).

The experiment was conducted to evaluate the effect of thyme dietary supplementation and age on mucin dynamics and performance in chickens. One-day-old chickens ISA Brown were randomly divided into three groups at day of hatching. The chicks of group 1 (C1) were fed a commercial diet. Group 2 (C2) was fed the commercial diet supplemented with sunflower oil. Group 3 (E) was fed the commercial diet supplemented with 0.05% thymi aetheroleum of thyme *Thymus vulgaris* L. leaves (*p*-cimene 48 %, thymol 24%) (Calendula, a. s. Stara Lubovna, Slovakia) which was dissolved in sunflower oil, sunflower oil content in diet was 1%. Duration of trial was for 11 weeks. At day 2, 3, 4, 8 and 22 post - hatching 10 chicks were chosen from C1 group.

At the end of experiment, chickens were sacrified by overdose of anaesthetics and intestinal sections were collected and processed for mucin determination using Alcian blue (3). Blood biochemical parameters were determined by the commercial diagnostic kits (RANDOX lab., Ardmore, UK). The density of the mucin adherent layer in the duodenum was higher in the C1 than in the E group and the caecal mucin layer was significantly thicker in the C2 than in the E group. There were no significant differences in body weight and relative weight of internal organs between groups. Plasma total protein level was significantly higher in the E group than in the C1 group. AST activity was significanlty higher in the C1 group than in the C2 group. ALP activity was higher in the C2 than in C1 and E groups. Phosphorus level was higher in the C2 and E groups than in the C1 group. Calcium level was decreased in the E group than in the C1 group.

The density of the mucin adherent layer has increased with age. The duodenal layer was higher on day 22 than on day 8 post - hatch. The jejunal, ileal and caecal mucin adherent layer was significantly higher on day 8 than on day 2,3, 4 and 22 post-hatch.

The results indicate that thickness of adherent mucin layer can be affected by feeding diet supplemented with thyme extract, day posthatch and intestinal region of chickens.

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BA 10
ANTIFUNGAL ACTIVITY OF *SATUREJA MONTANA* L. ESSENTIAL OIL

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Keywords: Satureja montana L., essential oil, antifungal activity

Satureja montana L. (winter savory) is a perennial shrub used as spice and in traditional medicine for its digestive, sedative or bactericidal properties and more recently because of the antioxidant, herbicidal and fungicidal activities of its essential oil (1- 3).

The antifungal activity of the essential oil of a wild population of Satureja montana L. growing in Spain was evaluated in vitro against three species from Kingdom Chromista: Phytophthora citrophthora (R.E. & E.H. Sm.) Leonian, P. palmivora (E.J. Butler) E.J. Butler and Pythium litorale Nechw., and four from Kingdom Fungi: Verticillium dahliae Kleb., Rhizoctonia solani Kühn, Penicillium hirsutum Dierckx, Colletotrichum gloeosporioides (Penz.) Penz & Sacc., all of them provided from the collection of Grupo de Investigación en Hongos Fitopatógenos, Universidad Politécnica, Valencia in Spain. Grover and Moore method (4) was used to study the inhibitory effect on the mycelial radial growth. The fungitoxicity was recorded in terms of percent colony inhibition in comparison with control.

S. montana essential oil completely inhibited mycelial growth of all isolates to the highest dose (1 µL/mL) (Table 1). *R. solani* was the most resistant isolate to the essential oil treatment, since none of the applied concentrations showed antifungal effect. *P. citrophthora*, *Py. litorale* and *C. gloeosporioides* also showed no inhibitory effect at the two lower concentrations, whereas at 0.1 µL/mL the percent of inhibitions were 87.95, 72.1 and 29.62%, respectively. A dose-dependent reduction was observed in fungal mycelial growth.

Table 1. Mycelial inhibition effect of S. montana essential oils

Fungi	0.001 µL/mL	0.01 µL/mL	0.1 µL/mL	1 µL/mL
	Inhib. (%)			
P. citrophthora	0,00 ± 0,00 a	0,00 ± 0,00 a	87,95 ± 0,82 b	100,00 ± 0,00 c
P. palmivora	2,09 ± 0,92 a	3,02 ± 1,19 a	65,56 ± 1,57 b	100,00 ± 0,00 c
Py. litorale	0,00 ± 0,00 a	0,00 ± 0,00 a	72,10 ± 7,58 b	100,00 ± 0,00 c
C. gloeosporioides	0,00 ± 0,00 a	0,00 ± 0,00 a	29,62 ± 1,64 b	100,00 ± 0,00 c
P. hirsutum	1,23 ± 0,76 a	8,90 ± 1,06 b	17,36 ± 2,56 c	100,00 ± 0,00 d
R. solani	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	100,00 ± 0,00
V. dahliae	1,13 ± 0,28 a	4,93 ± 1,57 b	71,83 ± 1,11 c	100,00 ± 0,00 d

Values are means ± standard error of five replicates. Different letters in the same column indicate significant differences with a confidence level of 95% by Fisher's multiple-range test "Least Significant Difference" (LSD)

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BA 11
DIRECT BIOAUTOGRAPHIC DETECTION OF ANTIBACTERIAL COMPONENTS OF SOME ESSENTIAL OILS

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Keywords: rosemary, clary sage, spearmint, sweet fennel, direct bioautography, plant pathogen bacteria, antibacterial effect

The growing resistance of pathogens against the majority of presently known and used antibiotics means an increasing risk in human and animal health as well as in agricultural. Therefore there is a rising demand to the discovery of new, efficient antimicrobial compounds. To search new perspective antimicrobials the plant kingdom is offered as an untapped reservoir containing the most diverse substances. Essential oils, produced by plants, are complex mixtures of hundreds of individual volatile compounds often having characteristic smell and biological activity.

Searching for bioactive natural products requires appropriate bioassays, focused on the desired activity. Direct bioautography, a combination of different versions of layer liquid chromatography and bioassay, is an ideal technical solution for the chemical and biological screening of drug ingredients, because of high-throughput, low cost and easy maintenance. In the course of the biological detection the developed adsorbent layer is dipped into cell suspension and afterwards the bioautogram is visualized. Visualization can be carried out using yellow tetrazolium salts, reduced by living cells to bluish formazan (antibiotics generate white spots) or by the use of the emitted light of bioluminescent bacteria as signal (the lack of light, the black spots indicate the antibacterial activity).

Rosemary (*Rosmarinus officinalis* L.), clary sage (*Salvia sclarea* L.), spearmint (*Mentha spicata* L.), and sweet fennel (*Foeniculum vulgare* Mill.) essential oil components were investigated in the bioassay against the test organisms Gram negative pepper pathogen *Xanthomonas vesicatoria*, luminescence gene-tagged *Arabidopsis* pathogen *Pseudomonas maculicola*, luminescent marine *Vibrio fischeri* bacteria and Gram positive soil bacterium *Bacillus subtilis*.

The main components of the essential oils were active against *P. maculicola* and *V. fischeri*. The *X. vesicatoria* was strongly inhibited by the main components of spearmint and Salvia essential oils, but in the presence of rosemary and sweet fennel components we observed only week antibacterial activity. *B. subtilis* was the less sensitive bacterium, only the sclareol, one of the main components of clary sage showed clear inhibiting/killing effect.

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BA 12

CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF *AARONSOHNIA PUBESCENS* (DESF.) K.BREMER & HUMPHRIES (ASTERACEAE) ESSENTIAL OIL FROM ALGERIA

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Keywords: Aaronsohnia pubescens (Desf.) K.Bremer & Humphries, essential oil, antifungal, chemical composition

The antifungal activity of essential oils has been recognized and researched for a long time (1). In the present study, the chemical composition and the antifungal properties of the essential oil obtained from the aerial parts of *Aaronsohnia pubescens* (Desf.) K.Bremer & Humphries (syn. *Matricaria pubescens*, Asteraceae), a small annual plant endemic to North-Western Africa (2), was investigated.

The hydrodistilled essential oils of leaves and stems of *A. pubescens* were analyzed by means of GC–MS and GC-FID. 31 volatile compounds could be identified representing 94.1% of the total essential oil composition. The major compounds in the essential oil of dried leaves of *A. pubescens* were determined to be monoterpene hydrocarbons limonene (59.7%), myrcene (15.8%) and α-pinene (6.2%).

Additionally, antifungal activity of *A. pubescens* essential oil was investigated using both disc diffusion and broth dilution methods against phytopathogenic fungi, i.e. *Aspergillus niger, A. flavus, F. oxysporum albedinis, Penicillium purpurogenum, P. jensinii,* and *P. expansum*. The essential oil exhibited great antifungal potential as a mycelial growth inhibitor against the fungi tested, with minimum inhibitory concentrations ranging from 0.09 to 0.1 % (v/v) and inhibition zone diameters between 16 and 37 mm. In conclusion, the essential oil of *A. pubescens* could be used as an alternative fungicide on crops.

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BA 13

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *CALYPTRANTHES RESTINGAE* SOBRAL AND THEIR ANTINOCICEPTIVE PROPERTIES IN RODENTS

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Keywords: Calyptranthes, calyptranthone, β-triketone, antinociceptive properties, essential oil

Myrtaceae is one of the largest families of the Brazilian flora, with approximately 23 genera and 1000 species. This is one of the dominant groups of woody plants in Brazilian biomes, especially in the Atlantic Forest, where more than 50 species may occur syntopically [1]. From a pharmacological perspective, the essential oils of myrtles are widely used in the production of drugs [2,3]. Previous studies have shown that some terpenoid oil constituents possess antinociceptive and anesthetic activities in animal experiments [4,5]. Recently, the antinociceptive and hypothermic effects of the essentials oils of *Eugenia uniflora* [6] and *E. candolleana* DC were confirmed in rodents.

The Brazilian state of Sergipe has a variety of ecosystems, ranging from the semi-arid, caatinga scrublands, to rainforests, mangroves and coastal restings, most of which have been poorly studied. These areas may yet yield new plant species, which may even be known to the local populations, but demand research into their chemical and pharmacological potential. The degradation of local ecosystems is an additional incentive to research.

This study reports on the chemical composition of the essential oil obtained from fresh leaves of *Calyptranthes restingae* Sobral (Myrtaceae), collected from adult plants in a thicket of restinga located near the River Pomonga (satellite positioning: S 10.47.325 / W 36.58.414), in the municipality of Santo Amaro Brotas, Sergipe, Brazil and its antinociceptive properties in rodents.

The GC-FID and GC–MS analysis revealed a total of 14 components, but only 5 were identified, accounting for 98.47% of the oil composition: *E*-caryophyllene (2.40%), calyptrantone (81.03%), α-caryophyllene (1.52%), β-selinene (8.54%), α-selinene (5.01%). The structure of the majority compound was elucidated by ^{13}C-RMN and ^{1}H-RMN. This is the first time that a β-triketone has been identified in the essential oil *Calyptranthes genus* and that calyptrantone has been recorded in a natural product.

The results of the pharmacological activity tests support the conclusion that the essential oil of *C. restingae* Sobral possesses antinociceptive and anti-inflammatory properties, probably via opioid

receptors or mediated by the inhibition of the synthesis of inflammatory mediators, such as prostaglandin. Further studies currently in progress will enable us to understand the precise mechanisms.

Acknowledgments: CNPq, CAPES, FAPITEC-SE

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BA 14

BIOLOGICAL ACTIVITY OF *CITRUS MEDICA* L. (CITRON) PEEL ESSENTIAL OIL IN DIFFERENT MATURATION STAGES

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Keywords: *Citrus, Citrus medica, Rutaceae, antifungal, essential oil*

Studies on natural products have highlighted essential oils as alternative control of pathogenic fungi. These oils have been considered more viable to be used because they do not generate toxic residues on the treated (1) Essential oils are complex mixtures of several compounds at distinct concentrations (2). The synthesis and composition of essential oils in many herbs are influenced by genotype, stage of plant development and environmental conditions (3).

The aim of this study was to characterize chemically (GC/MS) the essential oils from citron peels (mature and green) and to compare their fungiotoxicity using in vitro bioassay observing the inhibition of mycelia growth of the *Fusarium oxysporum, Alternaria alternata* and *Colletotrichum musae* pathogen. For biological tests, the vitro fumigation method was used with the following essential oil concentrations: (125, 250, 500, 1000 and 2000 μg/mL). In parallel, two control plates were made, one with only ethyl ether (relative control) and other with untreated (absolute control). The readings were performed seven days after the beginning of the experiment by measuring the opposite diameter to the mycelia (4).

The analysis of the essential oils from citron peels, mature and green by GC/MS displayed limonene (39%; 31%) as the major component followed by γ-terpinene (32%; 13%), p-cymene (9%; 7%), geranial (5%; 18%) and neral (3%; 13%).The essential oil of citron peels, mature and green had a total inhibition of mycelia growth in the concentration 2000 μg/mL for the three pathogens studied. The essential oil extracted from the peels of citron, mature and green showed dose-dependent antifungal activity on phytopathogens A. *alternata*, C. *musae* and F. *oxysporum*. These studies will help the researchers to find some alternative methods for controlling pathogenic fungi using effective sources easily accessible.

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BA 15

ANTIFUNGAL ACTIVITY AND MOLECULAR DOCKING OF MAIN PHENYLPROPANOIDS OF ESSENTIAL OILS OF *PIPER* AGAINST *FUSARIUM SOLANI* F. SP *PIPERIS*, THE CAUSAL AGENT OF FUSARIOSIS IN BLACK PEPPER

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Keywords: Fusarium solani f sp. piperis, antifungal activity, dillapiole, apiole, eugenol, methyleugenol, molecular docking, β-glucosidase inhibition

In Brazil, black pepper (*Piper nigrum* L.) is very susceptible to the Fusariosis, ie to the attack of *Fusarium solani* f. sp. *piperis* Alb., the fungus that causes the root rot and large losses for this culture. In this work, the antifungal activity of *Piper* oils rich in some phenylpropanoids was evaluated. The oils were hydrodistilled and analyzed by GC and GC-MS. The oils were tested at concentration of 5 mg/mL on mycelium growth of *F. solani* f. sp. *piperis*, by agar dilution method and expressed as the percentage inhibition against the mycelia growth diameter (1). The main constituent of the oil of *Piper aduncum* L. was dillapiole (92.0%), that allowed a mycelium growth equivalent to the control ($p > 0.05$), with inhibition of 21.3%. The oil of *P. krukoffii* Yuncker was dominated by apiole (80.0%) and showed an inhibition of mycelium growth of 31.4%. The oil of *P. divaricatum* Meyer, where methyleugenol (75.2%) and eugenol (7.9%) were the main constituents, showed the higher inhibition effect, about 90% of the mycelium growth and with an IC_{50} value of 0.7 mg/mL, determinated by nonlinear regression. The mechanism for the inhibition of these phenylpropanoids on the enzyme β-glucosidase of *Fusarium solani* f. sp. *piperis* was investigated using molecular docking [2]. Their structures were docked into the model of active site of Fsb-Glcin complex with the natural substrate. The results showed that docking simulations between eugenol and methyleugenol and β-glucosidase were successful in producing lowest affinity energy (-65.07 and -68.79 $kJ.mol^{-1}$, respectively). The minimum distance between the structures of eugenol and methyleugenol and the residues of β-glucosidase was lower than 3 Å, which shows one strong interaction between the hydrogen atoms of hydroxyl and methoxyl groups and these catalytic residues. On the other hand, apiol and dilapiol showed more high affinity energy when compared with the natural substrate. The results of docking simulations presented a significant correlation between estimated affinity energy and the antifungal index, which was determinate *in vitro* ($R^2 = 0.947$).

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BA 16
CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF FIVE NATIVE BRAZILIAN *CROTON* L. SPECIES (EUPHORBIACEAE)

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Keywords: Croton, Euphorbiaceae, germacrene D, bicyclogermacrene, 13-ß-podocarp-7-en-3-ß-ol

Croton L. is a pantropical genus with its main diversity centre located in the Neotropics. In Brazil there are about 300 species, of which 36 are located in São Paulo state, represented in all vegetation types, with the exception of mangroves (1). Some species are known to posses antiinflammatory, antiulcerogenic, antidiabetic and antibacterial activities. These activities can be accounted by the presence of a variety of diterpenoids with different skeletons, besides the presence of alkaloids and essential oils. *Croton* volatile oils are composed mostly of terpenoids and phenylpropanoids, however in some species only terpenoids are found (2). In this study we analysed the essential oil composition and antimicrobial activity of five *Croton* species occurring in São Paulo-Brazil: *C. pallidulus* Baill., *C. alchorneicarpus* Croizat, *C. organensis* Baill., *C. dichrous* Müll. Arg., and *C. tricolor* Klotszch ex Baill. The essential oils were obtained from the dried leaves by hydro-distillation in a Clevenger type apparatus for 4 h with yields varying between 0.19 and 0.88%. The component identification was performed by GC-MS through the comparison of their mass spectra with libraries and their Kóvats indices (1). The essential oil from all analysed species contained only terpenoids where Germacrene D was one of the major components in three species (13% in *C. pallidulus*, 11% in *C. alchorneicarpus* and 14% in *C. dichrous*) and bicyclegermacrene was also important in two species (11% in *C. pallidulus* and 20% in *C. tricolor*). The *C. organensis* oil showed the most varied composition, 63 components, with no major predominance of one single compound, presenting also diterpenes in considerable amounts, such as 13-ß-podocarp-7-en-3-ß-ol (9%). The antimicrobial activity was evaluated using the microdilution method in microplates against *Aspergillus niger* (ATCC 6404), *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538) after reading the absorbance at ⊙=630 nm, after 24 h of incubation (3). From all the tested oils, none presented a considerable activity against *E. Coli*, while the *C. pallidulus* oil presented the highest activity against S. Aureus (56% inhibition) and *C. organensis* was the most active against *P. Aeruginosa* (57% inhibition). The *C. tricolor* oil presented showed the best antifungal activity for both *A. Niger* and *C. albicans*, respectively 65 and 71% inhibition. The distinct oil composition found in the 5 *Croton* species afforded a differential antimicrobial activity against the tested microorganisms.

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BA 17
SPORICIDAL EFFECT OF VOLATILE COMPONENTS OF ESSENTIAL OILS ON BOOK CONTAMINANT FUNGI

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Keywords: citral, preservation, moulds, paper, vapour phase

Preservation of literary and art national heritage on paper against contaminant moulds like *Aspergillus* sp. has been of great importance through ages. Moreover these microorganisms present health risks for people working in contaminated areas of libraries or archives (1). Long-term protection methods gentle to document materials and to humans working with them are still missing (2).

Based on our previous research, we tested sporicidal activity of dominant volatile components of the most efficient essential oils (EOs) against book contaminant moulds. We used 7 substances, namely (R)-(+)-limonene, α-pinene, citral, linalool, linalyl acetate, L-menthol, L-menthone, separately or in combinations. *Aspergillus brasiliensis* and *Penicillium aurantiogriseum* were chosen as the most resistant and the most prevalent species, respectively.

The screening tests were done in saturated vapours using a method SOP-NRL/DS-2 developed by The National Reference Laboratory for Disinfection and Sterilisation of The National Institute of Public Health modified for mould cultivation on paper carriers. The spores inoculated to paper as a carrier were exposed to vapours in glass dessiccators for 1, 5 and 7 days. The spores were germinated on agar plates representing the best growing conditions, and in empty Petri dishes in boxes with 98% relative humidity. The substances with the strongest sporicidal effect on both moulds were chosen for tests in a reducted concentration of vapours. The spores on paper carriers were placed for 1-5 weeks in stainless steel boxes (40 l) flushed by saturated vapours of components of EOs diluted by hepa-filtrated air in ratios ranging from 1:4 to 1:9. The rate of gas flow through the box was 150-250 ml/min, the relative humidity was 75% and the temperature was 21°C. Evaluation of sporicidal effect was based on a visual rating scale concerning the germination of spores after exposition to vapours and the rate of subsequent growth.

Comparison of the growth curves of moulds with negative control showed that citral and linalylacetate is the most effective combination, followed by citral and α-pinen. Citral on its own has the strongest sporicidal activity, as confirmed by many studies (3), but its practical aplication in archives or libraries could be problematic due to possible skin irritation in allergic persons. Quenching of citral by terpenes or alcohols can minimize this negative property (4). Minimal sporicidal concentration of vapours will be stated as well as minimal time of exposition of spores to vapours necessary for sporicidal effect.

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BA 18

CHEMICAL COMPOSITION AND *IN VITRO* BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL FROM THE LEAVES OF *PACHIRA GLABRA* PASQ. FROM NIGERIA

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Keywords: Pachira glabra, Bombacaceae, essential oil composition, limonene, β-caryophyllene, biological activities

The chemical composition of essential oil obtained by hydrodistillation from the leaves of *Pachira glabra* has been studied by GC and GC–MS. The air-dried plants were hydrodistilled (PhBr). The components were identified by using MS libraries and based on their linear retention indicies (LRIs). 32 constituents representing 98.4% of oil were identified. The major constituents of the oil were limonene (23.2%), β-caryophyllene (14.5%), phtyol (8.5%) and β-bisabolene (6.3%). The *in vitro* biological activities of P. *glabra* essential oil were assayed using different methods. The results showed that the oil had promising antibacterial activity against the microorganisms. In the antioxidant assay, the oil exhibited significant activity in the DPPH test, and against nitric oxide and superoxide anion radicals. The insecticidal and larvicidal activities of the oil (LC_{50}) to adults *Sitophilus oryzae* and *Anopheles gambiae* larvae were 32.23 and 43.19 mg/mL, respectively.

BA 19

IN VITRO BIOLOGICAL ACTIVITIES AND ESSENTIAL OIL COMPOSITION OF *BLIGHIA SAPIDA* K.D. KOENIG FROM NIGERIA

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Keywords: Blighia sapida, Sapidaceae, essential oil composition, biological activities

The water distilled essential oil (PhBr) from the leaves of *Blighia sapida* K.D. Koenig was analyzed by GC and GC-MS. The components were identified by using MS libraries and based on their linear retention indices (LRIs). The major components of the oil were 6,10,14-trimethyl-2-pentadecanone (12.8%), geranyl acetone (12.0%), phytol (10.8%) and α-ionone (6.1%). The *in vitro* antimicrobial activity of the oil was tested against 12 local isolates using agar-disc diffusion and broth microdilution methods. The results showed that the oil exhibited moderate to high antimicrobial activity. The insecticidal and larvicidal activities of the oil (LC_{50}) against the adult of *Sitophilus oryzae* and fourth-instar larvae of *Anopheles gam*biae were 23.28 and 18.61 mg/mL, respectively.

BA 20
ANTIFUNGUS ACTIVITY OF THE ESSENTIAL OIL FROM *LYCHNOPHORA PINASTER MART.*

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Keywords: Lychnophora pinaster Mart., arnica, fungus, essential oil

The contamination and spoilage caused by fungi are more common than those caused by any other group of microorganisms. It is important not only from the sensory standpoint, but also because of the danger that the production of mycotoxins brings to the consume (1). Currently there is a serious concern by major importing countries regarding the control of contamination by toxigenic fungi and, consequently, the presence of mycotoxins (2). Essential oils are becoming increasingly important in the pesticide industry, since they have insecticidal and fungicidal activities and offer numerous advantages when compared to the use of synthetic products because they are derived from renewable resources and are rapidly degraded, leaving no residue in food and the environment (3). *Lychnophora pinaster* Mart., popularly known as mining arnica, is a plant belonging to the Asteraceae family, and is commonly used because of its analgesic, anti-inflammatory, antiprotozoal, antibacterial and antifungal activities (4). The objective of this study was to evaluate the antifungal activity of the essential oil from *L. pinaster* against *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus carbonarius*. The evaluation of the inhibitory effect of the essential oil from *L. pinaster* was performed using the disk diffusion test, in which 10 μL of essential oil diluted in dimethylsulfoxide was placed on filter paper disks, 6 mm in diameter. The concentrations tested were 500, 250, 125, 62.5, 31.25, 15.62, 7.81, and 3.9 μL mL^{-1}. Measurements of the inhibition zones were performed after 72 hours, and the minimum inhibitory concentration (MIC) was determined. Statistical analysis was performed using a randomized block design with three replications. Data were subjected to analysis of variance, and the means were compared by the Tukey test ($\alpha = 0.05$). The essential oil of *L. pinaster* inhibited the growth of *Aspergillus flavus* and *Aspergillus niger* at a concentration of 31.25 μL mL^{-1}. The MIC for *Aspergillus carbonarius* was 62.50 μL mL^{-1}.

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BA 21

CHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL FROM *BACCHARIS DRACUNCULIFOLIA*

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Keywords: *volatile oil, chemical composition, alecrim-do-campo, antibacterial activity*

Essential oils have a multitude of applications such as antimicrobial, antifungal, and antioxidant agents and as natural insecticides (1). They can be used in association with or for replacement of synthetic additives. Such use adds value and increases consumer acceptance of products to which they are added (2). Additionally, they have applicability in various commodities such as food, cosmetics, and medicines, among others. Given the above, the present study sought to analyze the chemical composition of the essential oil extracted from the leaves of *Baccharis dracunculifolia* (commonly known in Brasil as alecrim-do-campo) and to determine its antibacterial activity. The essential oils were extracted by hydrodistillation in a modified Clevenger apparatus and the chemical composition was determined as reported in (3). The antibacterial activity and sensitivity of *Salmonella cholerasuis, Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli* bacteria to the essential oil was determined by agar cavity diffusion, using the concentrations 3.90;7.81; 15.62; 31.25; 62.5; 125; 250 and 500 $\mu L\ mL^{-1}$ (4). The major constituents present in the essential oil were limonene (31%), *trans*-nerolidol (22%) and β-pinene (15%). The essential oil did not inhibit the growth of *Staphylococcus aureus* or *Escherichia coli*. The MICs *for Listeria monocytogenes* and *Salmonella cholerasuis* were 500 and 3.9 $\mu L\ mL^{-1}$, respectively.

Acknowledgements: *CNPq, CAPES, Fapemig. To FCT PEst-OE/EQB/LA0023/2011.*

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BA 22

EFFECT OF *SALVIA OFFICINALIS* AS FEED SUPPLEMENT ON ANTIOXIDANT STATUS, SMALL INTESTINE INTEGRITY AND BLOOD PHAGOCYTIC ACTIVITY IN LAYING HENS

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Keywords: sage, essential oil, trans-epithelial electrical resistance, poultry

Essential oils (EO) are able to scavenge free radicals, thus playing an important role in disease prevention such as brain dysfunction, cancer and heart diseases and immune system decline.

In this study, the effects of *S. officinalis* EO on glutathionperoxidase (GPx) activity in blood, liver, duodenum tissue and kidney, on malondialdehyd (MDA) concentration in plasma, liver, duodenum tissue and kidney as well as on total antioxidant status (TAS) in plasma were investigated. Intestinal integrity was tested by measuring the trans-epithelial electrical resistance (TEER). Forty animals were divided into five groups. The first group was fed a nutritionally balanced basal diet (BD). The other four groups contained BD supplemented with 0.01%, 0.025%, 0.05% and 0.1% concentration of *S.officinalis* EO. Essential oil in 0.01% concentration significantly ($P<0.05$) increased GPx activity in liver, duodenum tissue and kidney and significantly ($P<0.05$) decreased MDA concentration in plasma and liver. The 0.025% concentration of EO significantly ($P<0.05$) increased the GPx activity in liver and TAS in plasma and 0.1% concentration significantly ($P<0.05$) increased MDA concentration in kidney. From these results, it is evident that the lowest concentration of *S.officinalis* EO positively influenced the antioxidant status of laying hens; however, the highest concentration was not effective to avoid the oxidative processes. We detected a significant enhancement ($P<0.05$) of blood phagocytic activity in laying hens fed the BD supplemented with 0.01%, 0.025% and 0.05% concentration of *S.officinalis* EO, what showed that these concentrations were able to modulate the neutrophil immune function. The TEER values significantly ($P<0.05$) increased at 6 min, 9 min and 12 min of intestine incubation in groups of animals fed with 0.01% and 0.025% concentration of *S. officinalis* EO in their diet, in comparison with the control group.

Our results showed, that supplementation of the poultry diet with lower concentrations of *S. officinalis* EO had more positive effect on their antioxidant status, small intestine integrity and blood phagocytic activity than higher EO concentrations. The appropriate concentration of EO is very important for the diet composition. It is very important to find such a concentration of EO which can positively influence the physiological functions of animals and thus improve their health status.

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BA 23
CHEMICAL COMPOSITION AND ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF ESSENTIAL OILS FROM FLOWERS, HERB AND STEMS FROM LAVENDER (*LAVANDULA ANGUSTIFOLIA*)

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Keywords: Lavandula angustifolia, chemical composition, antimicrobial activity, antioxidant properties

In recent years there has been significant growth of interest in natural cosmetics. Valuable components of these products are essential oils, because they have both the aromatic and antioxidant and antimicrobial properties.

Chemical composition and antioxidant and antimicrobial properties of essential oils of lavender (*Lavandula angustifolia*) were tested. The essential oils were isolated by hydrodistillation from different parts of lavender (*Lavandula angustifolia*) - flowers, herbs and stems (KAWON-HURT, Poland).

1.55 ± 0.063 g of essential oil from fresh flowers / 100 g material, 0.575 ± 0.040 g of essential oil from fresh herb / 100 g material and 0.055 ± 0.025 g of essential oil from fresh stems / 100 g material were received. We determined 96.9%, 95.8% and 89.4% of the chemical composition of essential oils of fresh flowers, herb and stems of lavender. The main chemical compounds are linalool (11.9-45.5%), linalyl acetate (7.3 - 23.4%), borneol (1.1 - 7.2%), ocimene (1.2 - 8.9%), and terpinen-4-ol (1.8 - 4.9%). In the essential oil from fresh stems cadinol (14.1%) has been also detected in large quantities, this compound has not been detected in other essential oils. The main class of compounds are oxygenated monoterpene hydrocarbons, mainly monoterpene alcohols.

The antioxidant properties of essential oils measured by DPPH. It has been shown that the essential oil from fresh flowers and herb of lavender have similar antioxidant properties, and the essential oils from stems has much weaker antioxidant properties.

Lavender essential oils showed high activity against Gram-positive bacteria (*Bacillus subtilis, Strapchylococus aureus*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*), inhibition of growth was observed already at a concentration of 0.6 or 1.0 µL / mL (depending on the strain), and high efficacy against *Candida sp.* and *Aspergillus niger* and *Penicillium expansum*.

BA 24
CHANGES IN ESSENTIAL OIL COMPOSITION AND BIOACTIVITY OF *EREMOPHILA LONGIFOLIA* (F. MUELL) (SCROPHULARIACEAE) IN CONDITIONS SIMULATING AUSTRALIAN ABORIGINAL TRADITIONAL CEREMONIAL AND MEDICINAL SMOKING APPLICATIONS

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Eremophila longifolia is one of the most valued of the traditional medicinal plants used by Australian Aboriginal people. Though there are several reported usage modalities, the most consistently reported application involved smouldering leaves over fire embers to produce an acrid smoke, believed to have therapeutic effects broadly consistent with antimicrobial, anti-inflammatory and antifungal properties. The current study aims to examine the contribution of normal and partially pyrolysed essential oils to specific therapeutic effects in these applications.

Essential oils were produced by normal hydrodistillation and partially pyrolysed essential oils by using a part-wet, part-dry distillation leading to the production of smoke laden aerosols and some charring of the leaves. In both cases, antimicrobial activity was assessed using broth dilution, followed by a novel procedure using incremental temperature treatments simulating the effect of temperature gradients expected during exposure of body parts in the smoking procedure. GC-MS, GC-FID and HPLC-PAD were used to compare components from pyro-distilled with fresh essential oils. To compare free radical scavenging ability, 2,2'-Diphenyl-1-picrylhydrazyl in methanol was used. Results showed that pyro-distilled oils had three or more fold enhanced antimicrobial ability, in cultures warmed incrementally. Partially pyrolysed essential oils had radical scavenging ability 30-700 times greater than the corresponding non-pyrolysed oils. HPLC-PAD revealed the presence of additional constituents not present in the fresh essential oil. These results provide the first known scientific justification for the smoking ceremonies involving leaves of *E. longifolia*. During custom use, both partially pyrolysed as well as non-pyrolysed essential oils may contribute significantly to the overall desired medicinal effect.

BA 25
ESSENTIAL OILS FROM AUSTRALIAN MEDICINAL PLANTS – AN ETHNOPHARMACOLOGICAL PERSPECTIVE

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Keywords: *Aboriginal people, Rutaceae, Melaleuca, Eucalyptus, Backhousia*

In this presentation we will review research into the chemistry, bioactivity and possible pharmacological and therapeutic uses of essential oils from native Australian plants used in traditional medicinal practices of Aboriginal peoples with specific mention of recent research in our laboratory.

Australia has more endemic flora and an unusually higher proportion of total endemic species than any other region in the world. In this context the endemic genus *Eremophila* (Myoporaceae), is of particular interest to us owing to its distinctive phytochemical characteristics as well as the high degree of representation in literature detailing indigenous medical ethnobotanical practices. Members of the *Rutaceae* also yield significant levels of essential oils and are cited as sources of indigenous *materia medica*. With respect to the putative role of essential oils in achieving the intended therapeutic effects, consideration must be given to traditional useage modalities. This is of particular importance in the therapeutic application of ritualistic smoking. We have attempted to reproduce in the laboratory conditions likely to occur during the production of smoke from smouldering leaves and collected and characterised modified partially pyrolysed oils.

Comprehensive investigation into the pharmacology, clinical value and related phytochemistry of native Australian plants with supposed therapeutic activity, has largely been restricted to a small number of species of known commercial value. In particular, efforts have concentrated on steam distilled essential oils of myrtaceaeous genera with reputed antiseptic properties, the most well known and researched examples being those derived from various species of *Eucalyptus* and *Melaleuca* and more recently, citral rich oils of *Backhousia citriodora* ("lemon myrtle"). In these instances, a pre-existing market, based on popular demand, for over the counter therapeutic and cosmetic products is well established, and this has served to promote and direct research into the therapeutic value of these products.

Notwithstanding the successes which have occurred in Australian native medicinal plant research, a vast number of endemic species with rare and sometimes unique phytochemical constituents still remain to be thoroughly examined. This is particularly evident in terms of the bioactivity and pharmacological potential of essential oils and we will be presenting some of our recent research in this regard.

BA 26
INFLUENCE OF (-)-LINALOOL AND 1.8-CINEOLE ON MENTAL STRESS

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Keywords: mental stress, (-)-linalool, 1.8-cineole, R-R Interval, fragrance preference, drive behavior

It is assumed that human errors trigger traffic accidents, therefore, some researchers have focused on the effect of aroma compounds to improve drivers' conditions (1, 2).

Since this study positions as a pilot study, the measurement was performed in a test room. We focused on two aroma compounds, (-)-linalool and 1.8-cineole, which have opposite effects. The former has sedative effect and the latter has simulative one.

A result of a male participant (23 years old) is reported here. Electrocardiogram was employed in this study and RR Interval (RRI) was used as a stress index. The task of participant was to place randomized set 15 numbers into numerical order with mouse on an Excel sheet as quickly as possible. During his task an operator disturbed his operation, such as moving the cursor and clearing numbers which he had placed. There are 20 task sheets, and aroma compounds were supplied for 12th-16th sheets. These experiments were performed under 4 conditions such as high density (300µl) and low density (100µl). After 4 measurements his fragrance preference were checked.

The results of 8th-11th ,12th -16th and 17th -20th were compared. The values of RRI showed tendency to increase when high density of (-)-linalool and low density of 1.8-cienole were supplied, respectively. Therefore, it is assumed his stress were decreased by these aroma compounds, although his preference of high density of (-)-linalool was low ("-1" of 5 scales, "-2 to 2"). It seemed low density of (-)-linalool did not affect the variation of RRI and after the supply of this fragrance the RRI was decreased. The high density of 1.8-cineol tended to increase RRI.

Except low density of (-)-linalool showed tendency to decrease participant's mental stress in this study. For (-)-linalool the effect of fragrance preference was not shown, however the preference of 1.8-cineole were high, therefore, it might there is a connection between preference and the effect of decreasing the value of RRI.

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BA 27

ORGAN ACCUMULATION IN MICE AFTER INHALATION OF SINGLE OR MIXED ESSENTIAL OIL COMPOUNDS

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Keywords: organ accumulation, inhalation, volatile compound

We studied the distribution of essential oil components after inhalation of single and mixed components in mice. This research was done using four main components of *Alpinia zerumbet* (Pers.) B. L. Burtt. and R. M. Sm.: alpha-pinene, *p*-cymene, 1,8-cineole, and limonene. After inhalation of single (10 μL/L air) or mixed (40 μL/L air) components for 90 min, component levels in the brain and liver of mice were measured by GC-MS and GC-FID. The results indicated that the amount of alpha-pinene in the brain and liver was twofold greater after mixed-component inhalation than that after single-component inhalation (Figure 1). In a comparison of the components of the mixed inhalation, the ratio of alpha-pinene increased to about three times that of 1,8-cineole. It is thought that the absorption via the nasal mucus greatly influences this phenomenon. The results of this investigation of the bodily distribution of essential oil volatile components may provide clues for elucidating their action.

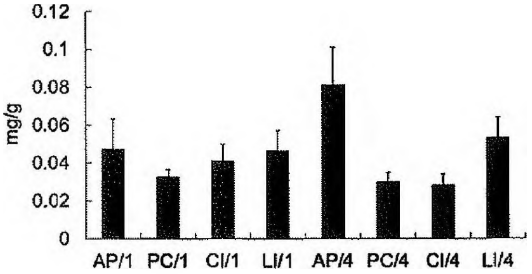

Figure 1. The distribution of alpha-pinene, *p*-cymene, 1,8-cineole, and limonene in the brain by single- (10 μL/L air) and mixed- (40 μL/L air) compound inhalation. Values are expressed as mean ± SE (mg/g organ). n=5. *p<0.05. AP/1, concentration of alpha-pinene by single inhalation; PC/1, concentration of *p*-cymene by single inhalation; CI/1, concentration of 1,8-cineole by single inhalation; LI/1, concentration of limonene by single inhalation; AP/4, concentration of alha-pinene by four-compound mixture inhalation; PC/4, concentration of *p*-cymene by four-compound mixture inhalation; CI/4, concentration of 1,8-cineole by four-compound mixture inhalation; LI/4, concentration of limonene by four-compound mixture inhalation.

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BA 28
JUNIPERUS PHOENICEA L. ESSENTIAL OILS FROM ALGERIA AS PROMISING AGENTS AGAINST NOSOCOMIAL INFECTIONS

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Keywords: *Juniperus phoenicea L., nosocomial infection, antibacterial, antifungal*

Hospital-acquired infections are responsible for nearly hundred thousand deaths a year in the US alone (1). Their continuous and incorrect therapy with antibiotics caused the development of resistant strains (2). Essential oils have shown high efficacy against such strains (3).

Juniperus phoenicea L. fresh and dry leaves as well as berries collected in the Saharan Atlas mountains were distilled using a Clevenger-type apparatus. Essential oil yields were 0.6%, 0.9% and 2.0%, respectively. Analysis done by GC-FID and GC-MS revealed the predominant components to be α-pinene (56.6%, 55.9% and 29.6%) and β-caryophyllene (1.3%, 1.6% and 2.6%) of the essential oils of juniper berries, dry and fresh leaves, respectively.

Antimicrobial activity was tested against Gram-negative bacteria *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli* and *Proteus mirabilis*, Gram-positive *Staphylococcus aureus* and the yeast *Candida albicans*. All strains were isolated from infected hospitalized patients.

Antimicrobial testing was performed once by direct contact method as well as by Vincent method. Antibiotic aromatogrammes method (4), for reference substances CA-SFM method was used. 19 standard antibiotics and antifungal agents were tested as references. All tests were performed in triplicate.

The berry essential oil was ineffective, with the exception of two strains, whereas the essential oil of dried leaves significantly inhibited all strains but *Pseudomonas aeruginosa* which turned out to be the most resistant strain overall. However, *Escherichia coli* was the most susceptible for the essential oils tested. Moreover, the essential oil of dry leaves was highly active against *Candida albicans*, outclassing even standard antifungal substances.

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BA 29
EFFECTS OF CLARY SAGE (*SALVIA SCLAREA* L.) ESSENTIAL OIL ON HUMAN SUBJECTS

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Keywords: sclareol, psychophysiology, hormones, transdermal, inhalation

The effect of essential oil of clary sage (*Salvia sclarea* L.) on psychophysiological parameters after inhalation as well as after massage was measured in three different experiments on a total of 83 human subjects (31 males). The used clary sage essential oil (Fa. KurtKitzing GmbH, Wallerstein, Germany) contained 0.28% of sclareol, the main components were linalool (22.1%) and linalyl acetate (61.3%). Due to structural similarity of sclareol with estrogen, clary sage is suggested to have hormonal functions, such as normalizing the menstrual cycle, promoting menstruation, and strengthening the uterus (1). For this reason, clary sage essential oil is commonly used in aromatherapy for treatment of dysmenorrhea (2). Sclareol is also a precious raw material for the semisynthesis of amber odorants (3) and is active against tumor growth *in vivo* against breast cancer (4).

In pilot-experiment one, 21 female subjects aged between 18 and 40 years were tested in two sessions each: in one session clary sage odor was administered (aroma lamp) for 30 minutes, the other session served as control condition with water. The order was counterbalanced. At the beginning and the end of each session blood pressure and puls rate were taken and the subjective mood state was collected by questionnaire (MDBF). Aditionally the participants had to rate the oil and water for pleasentness and familiarity. The data indicated no significant influence of clary sage on physiological parameters or blood pressure in tearms of ANOVA. Clary sage was experienced significantly more pleasant and familiar than water.

In experiment two, the influence of clary sage oil (in peanut oil) on subjective well being and blood pressure was tested in 30 participants (15 males) aged between 18 and 35 years after transdermal absorption on the right under arm. The area was then covered with plastic film. Pure peanut oil served as control condition. The other procedures were the same as in experiment one. An ANOVA revealed significant differences in heart rate between the sexes. Heart rate increased in female subjects compared to males. Clary sage oil was rated unpleasant by female and pleasant by male participants. There was also a slight trend in clary sage oil being more familiar to men.

A third experiment on 32 healty subjects (16 males) investigating the same parameters after inhalation for 40 minutes is now performed, and the results will be presented.

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BA 30

SCREENING OF THE ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS USING TLC-BIOAUTOGRAPHY

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Keywords: essential oil, antibacterial activity, TLC-bioautography, MTT, gas chromatography

Essential oils are very interesting natural products and among other qualities they possess various biological properties. The treatment of bacterial infections is presently problematic due to their antibiotic resistance. Therefore, the number of studies focusing on essential oils, as well as on their applications as new potential antimicrobial agents against human pathogenic microorganisms has recently increased (1,2,3).

The aim of the present study was the chemical characterization of some traditionally used and therapeutically relevant essential oils (sweet fennel, spearmint, rosemary and cinnamon bark) and the optimized microbiological investigation of the effect of these oils on *Staphylococcus aureus, S. epidermidis*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli, Micrococcus luteus* and *Bacillus subtilis*. Determination of the chemical composition of the oils was performed by thin layer chromatography (TLC), gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The antibacterial effect of essential oils was investigated without separation and after separation of the components using a TLC-bioautographic method. Solvents with different composition were used as mobile phases. The antibacterial activity of sweet fennel, spearmint and cinnamon bark oils, as well as their main components (*trans*-anethole, carvone and *trans*-cinnamic aldehyde) were observed against all the bacterial strains used in this study. *Escherichia coli* was the most sensitive strain. The essential oil of rosemary showed weak activity in the bioautographic system.

Undoubtingly, TLC-bioautography is a directly combined application of an analytical method with an *in situ* bioassay that facilitates a rapid identification of the active compound or compounds in a complex mixture. Further experiments focus on modelling the antibacterial effect of the essential oils involved in this study in a mouse airway inflammation model.

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BA 31
HERBICIDAL POTENTIAL OF *CUPRESSUS SEMPERVIRENS* L. ESSENTIAL OIL

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Keywords: Cupressus sempervirens, essential oil, herbicidal activity, germination, seedling length

Cupressus sempervirens L. is an ornamental tree belonging to the family Cupressaceae, native to Northern America, Africa, Southeastern Europe and Western Asia (1). It is used to make hedges. The essential oil from *C. sempervirens* has antimicrobial, virucidal (2) and repellent activities (3).

The herbicidal potential of the essential oil from a cultivated population of *C. sempervirens* growing in Valencia province (Spain) was tested against three important weeds in Mediterranean crops, *Amaranthus hybridus* L., *Portulaca oleracea* L. and *Setaria adhaerens* (Forssk.) Chiov. The essential oil was effective when applied at the two higher concentrations against *S. adhaerens* and *A. hybridus* germination, reducing them up to 83.7% and 15.6% respectively (Table 1), and it also controlled *A. hybridus* seedling growth decreasing its seedling length up to 29.4% as compared to controls (Table 2). However the essential oil was not effective towards *P. oleracea* germination and seedling growth, neither against *S. adhaerens* seedling growth (Table 1 and Table 2).

Table 1. Effect of Cupressus sempervirens essential oil against A. hybridus, P. oleracea and S. adhaerens seed germination

Concentration (µl/ml)	Germination (% ± s.e.)		
	Amaranthus hybridus	***Portulaca oleracea***	***Setaria adhaerens***
0 (control)	90.0±3.2 a	67.0±3.4 a	43.0±6.6 a
0.125	91.0±2.9 a	55.0±7.6 a	29.0±5.8 a
0.25	89.0±1.9 ab	63.8±1.3 a	30.0±5.2 a
0.5	80.0±1.6 b	73.0±8.7 a	14.0±5.1 b
1	76.0±3.3 c	72.0±4.4 a	7.0±2.5 b

Different letters in the same column indicate that the mean values are different at the 95% level of probability (P≤0.05) using Fisher's least significant difference test (LSD).

Table 2. Effect of Cupressus sempervirens essential oil against A. hybridus, P. oleracea and S. adhaerens seedling growth

Concentration (µl/ml)	Seedling length (mm ± s.e.)		
	Amaranthus hybridus	*Portulaca oleracea*	*Setaria adhaerens*
0 (control)	25.2±1.5 a	7.3±0.8 a	55.2±1.5 a
0.125	22.1±0.5 a	8.2±1.1 a	59.6±3.0 a
0.25	23.0±1.1 a	7.9±0.9 a	50.1±2.2 a
0.5	17.8±0.5 b	9.8±0.9 a	52.5±0.5 a
1	18.3±1.2 b	8.1±0.6 a	54.5 a

Different letters in the same column indicate that the mean values are different at the 95% level of probability ($P \leq 0.05$) using Fisher's least significant difference test (LSD)

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BA 32
ESSENTIAL OIL OF ALGERIAN *TETRACLINIS ARTICULATA* (VAHL) MASTERS; CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY

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Keywords: Tetraclinis articulata (Vahl) Masters, essential oil, antibacterial activity

Tetraclinis articulata or sandarac tree is an evergreen coniferous cypress endemic to the Mediterranean region. The resin (sandarac) and wood has been used for artisanal and traditional purposes since ages and other parts of the plant as remedies in folk medicine due to supposed antidiarrhoeal, antipyretic and antirheumatic properties (1). The dry or fresh leaves yield (0.1-1.0%) an essential oil (EO) that is rich in compounds such as a-pinene, camphor, borneol and bornyl acetate (2). Due to the development of multiresistant bacterial strains alternative ways of antimicrobial therapy, especially with remedies from natural, renewable sources, is crucial. Therefore the EOs were tested against selected test strains isolated from hospitalized infected patients, i.e. Gram-negative bacteria *Pseudomonas aeruginosa* (PA), *Klebsiella pneumoniae* (KP), *Escherichia coli* (EC) and *Proteus mirabilis* (PM), Gram-positive *Staphylococcus aureus* (SA), the yeast *Candida albicans* (CA) and the activity compared to 14 reference antibiotics and 5 antifungals. Inhibition zones (IZ) were determined according to the Vincent method [3] (paper discs (6 mm) impregnated with 6 µl of the EO were placed on the inoculated media surface) and minimum inhibitory concentration (MIC) by direct contact of the tested germs in test tubes filled with agar solution emulsified with different concentrations of EO (4).

All test strains were significantly inhibited by both EOs (fresh and dried leaves) with IZ ranging from 20-25 mm and MICs between 268 and 516 µg/mL and even those strains that were resistant to several antibiotics were susceptible to the EOs. It could be demonstrated that the log MIC values strongly correlate with IZDs.

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BV 33

CHEMICAL COMPOSITION AND INSECTICIDAL ACTIVITY OF OREGANO (*ORIGANUM VULGARE* SSP. *HIRTUM*) ESSENTIAL OIL AGAINST LARVAE OF *ALPHITOBIUS DIAPERINUS* PANZER (COLEOPTERA: TENEBRIONIDAE)

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Keywords: oregano oil, carvacrol, thymol, essential oils, lesser mealworm, Alphitobius diaperinus

Oregano is a herb commonly used for culinary purposes and has confirmed antimicrobial and antioxidant activity (e.g 1,2). Most of the cultivated *Origanum vulgare* ssp. *hirtum* types are rich in carvacrol which is known for insecticidal activity but there is the lack of information about insecticidal activity of pure EO with high contents of this component (e.g 2,3).

The object of this study was to estimate the chemical composition and insecticidal activity against larvae of the lesser mealworm, *Alphitobius diaperinus* Panzer of the essential oil (EO) from leaves of *O. vulgare* ssp. *hirtum* obtained by steam distillation. The EO composition was analyzed by gas chromatography (GC) coupled with mass spectrometer (MS). Twenty four compounds were identified in the volatile fraction of oregano essential oil. All identified components were identified by co-injection with authentic samples, MS libraries and their Kovats indices. In fact, the oil was characterized by high content of phenolic monoterpenoids, but finally it was classified as a carvacrol/thymol chemotype. The content of carvacrol and thymol was 84.39% and 2.39% respectively. We observed also high concentration of γ-terpinene (3.91%), *p*-cymene (3.45%) and caryophyllene (1.10%). The content of other compounds in oil was <1%.

The younger and older larval stages of the lesser mealworm were reared on diets containing 1% acetone solution of essential oil. Insecticidal activity of *Origanum* ssp. *hirtum* EO against *A. diaperinus* larvae was depended on the age of larvae. The younger larvae was much more sensitive in comparison to the older ones. The mortality of younger and older larvae was 81.25% and 46.67%, respectively. In both cases larvae were distinguished by weakly body weight gain compared with control trials.

The introduction of oregano essential oil into the diet of larvae extended their development life cycle in comparison to control and effected on the growth of cannibalism among them.

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BA 34

INTRASPECIFIC VARIABILITY OF SELF-SOWN PINE (*PINUS SILVESTRIS* L.) OCCURRING IN EASTERN AREA OF POLAND

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Key words: natural sites, populations, essentials oils, terpenes

Poland and other Baltic countries seem to be the area where Scots pine finds good environmental growth conditions and creates valuables ecotypes (1). Due to its low habitats requirements and easy adaptation to different soils, this tree occupies over 70% of Polish forests area. It results that self-sown plants of this species can be commonly found on various kinds of wastelands, often on poor and sandy sites (2). Scots pine is known as a medicinal plant providing important herbal raw materials: shoot buds and essential oil. Extracts from pine buds reveal expectorant activity and can be used in respiratory tract diseases treatment while essential oil indicates antibacterial, spasmolytic and warming properties (3,4).

The aim of the present work was to determine intraspecific variability of Scots pine occurring on natural sites in Poland, concerning the content and composition of essential oil in buds. Research covered the north-eastern region of Poland considered as comparatively ecologically clean. In 2012 pine buds were collected from self-sown plants growing wild on 27 natural sites, particularly wastelands or areas near forests, excluded from agricultural use. Geographical location of these sites was determined using GPS. The harvest took place in March when buds were still dormant and closed. Collected raw material was dried in 35°C and subjected to chemical analysis. The content of essential oil was evaluated using hydrodistillation in Deryng apparatus, according to Polish Pharmacopeia VIII. Its composition was determined by GC/MS method using Shimadzu GCMS QP-2010S gas chromatograph equipped with Phenomenex Zebron ZB-FFAP polar column.

The essential oil content in investigated buds ranged from 0.60 to 1.87%. Among 31 compounds identified in the essential oil the dominant was δ-3-carene. Other monoterpenes i.e. α-pinene, β-pinene, limonene and p-cymene-8-ol were also present in high amounts. The percentage of main compounds in essential oil ranged, as following: δ-3-carene from 29.64 to 52.86%, α-pinene from 7.19 to 16.46% and β-pinene from 3.91 to 14.0%. Obtained results show that investigated populations of Scots pine differed both in the content and chemical composition of essential oil in shoot buds.

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ANALYSIS OF THE ODOR-ACTIVE CONSTITUENTS OF ATLAS CEDARWOOD ESSENTIAL OIL

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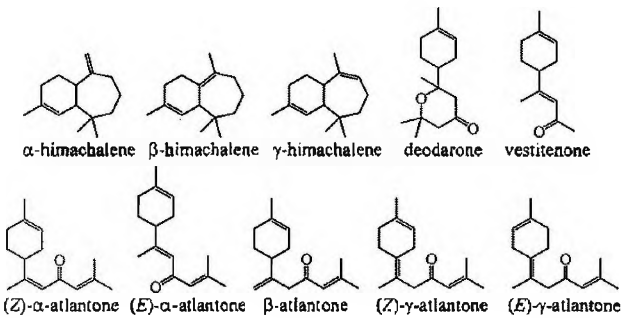
Keywords: Cedrus atlantica Manetti, Atlas Cedarwood, Gas chromatography-Olfactometry (GC-O), AEDA, odor-active constituents

Atlas cedar (*Cedrus atlantica* Manetti) is a tree native to the Atlas Mountains of Algeria and Morocco. It is used for timber production, and the sawdust produced during the wood processing is often valorised by hydrodistillation to furnish an essential oil with a typical sweet and warm odor. The composition of Atlas cedarwood essential oil is generally dominated by himachalenes and atlantones, accompanied with minor amounts of many other constituents specific of the *Cedrus* genus (see figure below).

Despite the large number of publications on this material and on the synthesis of the atlantones, the data concerning the odor-active constituents of cedarwood oil are scarce and somewhat contradictory. Pfau and Plattner (1) have reported that the mixture of α- and γ-atlantones is the aromatic principle of the oils, without mentionning the olfactory differences between each isomer and later, the odors of deodarone (2) and vestitenone (3) have been described as characteristic of the wood.

To better understand the relative olfactory contribution of the volatile constituents of Atlas cedarwood, we achieved a detailed investigation on a sample of Moroccan essential oil. We first performed a series of fractionations by means of basic liquid extraction, distillation and flash chromatography, as well as some synthetic transformations, followed by GC-MS analysis. Subsequently, GC-O analysis was performed on the raw oil and its fractions to determine the main odor active constituents. All the panelists involved in this olfactory study agreed on the fact that the contribution of the above-mentioned ketones was much less significant than that of several more volatile constituents.



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BV 36

SCENTS FROM BRAZILIAN CERRADO: CHEMICAL COMPOSITION OF THE ESSENTIAL OIL FROM THE FLOWERS AND LEAVES OF *LIPPIA STACHYOIDES* VAR. *MARTIANA* (SCHAUER) SALIMENA & MÚLGURA

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Keywords: Chemical composition, flora of the Cerrado, nerolidol, Verbenaceae

Cerrado is a savannah-like biome which occurs in Central Brazil. It is the second largest biome in Brazil, containing about 12,000 known plant species, many of which are endemic (1). It is considered one of the most important biodiversity hotspots of the world, highly threatened by anthropic action (2). A large number of these endemic species, albeit cataloged by botanists, have never been subjected to chemical investigation. Aiming to gather knowledge on the flora of the Cerrado, we have started a research project in order to investigate the chemical composition of some plants native from this biome. Herein we report the analysis of essential oils from *Lippia stachyoides* var. *martiana*, an endemic shrub, 1m tall, with white flowers growing at Cerrado field.

Flowers and leaves from five individuals of a population were collected at the ecological reserve of the Instituto Brasileiro de Geografia e Estatística (IBGE, Brasília, Brazil). A voucher specimen was deposited in the herbarium of the Genetic Resources and Biotechnology (CEN 82848). Fresh flowers (100 g) and leaves (450 g) were subjected to hydrodistillation separately in a Clevenger-type apparatus for 2 hours each. The oils were analyzed by GC/FID and GC/MS in an Agilent 7890A and an Agilent 5973N system, both fitted with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 μm). Hydrogen was used as carrier gas at a flow rate of 1.0 mL/minute for GC/FID, and helium for GC/MS. Oven temperature was programmed from 60 to 240°C at 3°C/minute. The percentage composition was obtained by normalization from FID. Essential oil constituents were identified by comparison of both mass spectra and linear retention indices with spectral library and literature (3,4).

Essential oil (EO) yields were 1.6% and 0.6% (mg EO/100g plant) for flowers and leaves, respectively. A total of 57 compounds were identified in the flowers, corresponding to 96.9% of the constituents, whereas 41 compounds were identified in the leaves (97.7% of the constituents). Both essential oils were rich in (*E*)-nerolidol (15.6, and 16.4%, respectively), delta-cadinene (15.8 and 18.5%), spathulenol (8.1 and 16.4%), caryophyllene oxide (6.6 and 7.8%) and cubebol (8.5 and 7.4%).

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BV 37
MYRTLE (*MYRTUS COMMUNIS* L.) IN THE HOUSE

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Keywords: myrtle, essential oil

It is known that growth conditions significantly affect development of plants and accumulation of various substances by them. The objective of our study was the assessment of the main components of essential oil in the plant samples of *Myrtus communis* L. received in the vegetative way of reproduction and having an identical origin. Plants were grown under indoor conditions, in a light room where illumination made near the 4000 lux, air temperature 20° C with, the photoperiod – 16 hours day and 8 hours night, and also conditions of an open ground at sufficient moistening of the soil in the conditions of the Moscow region.

The dried herb was hydrodistilled and the oil was analysed by GC-MS. The components were identified by MS libraries and based on their linear retention indices (LRIs). For registration of mass spectrograms the recommended temperature conditions were chosen (1).

We identified 17 compounds in the oil; α-pinene was the main component (31.7-50.1%). The second and third most abundant components were limonene (27.1-35.2%) and 1,8-cineol (5.2-10.6%).
All identified components were found in research of essential oil of myrtle (2, 3).

It is possible to believe that myrtle cultivation in office conditions and in the conditions of Moscow region doesn't lead to considerable changes in qualitative and quantitative composition of essential oils, therefore the plant can be used for the prevention of bacterial, viral and fungal infections.

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CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF NEW AROMATIC PLANTS OF THE GENUS ARTEMISIA FROM MONGOLIAN GOBI

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Key words: wormwoods, hydrodistillation, GC-MS

The genus Artemisia (Asteraceae) is represented in the flora of outer Mongolia by 103 species. An international literature review has revealed that the chemical composition of their essential oils have not been described in any previous study. The hydrodistilled essential oils obtained from dried plant material were analyzed by GC-MS. The results are summarized below.

Species	Collection site	Main components (% of oil)
A.xerophytica Krasch	Middle Gobi, Uush mankhan (September)	Camphor (30.7), 1.8-cineol (13.4), camphene (7.5), terpinene-4-ol (6.1), α,β-pinenes (4.2-4.9), isopinocamphene (3.4) and caryophyllene (3.0)
A.xanthochroa Krasch	Southern Gobi, mountain of Gurvan-saikhan (September)	β-pinene (11.6), 1.8-cineol (8.1), α-pinene (6.1), limonene (5.0), γ-terpinene (3.0) and sesquiterpen-hydrocarbons: δ-cadinene, β-bergamotene, caryophyllene, ylangene, humulene, bisabolene, gurjunene
A.pamirica.C. Winkl.	Southern Gobi, Gurvan-saikhan mountain (September)	Methyl chavicol (56.4), α-terpinene+limonene (15.1), sabinene+myrcene (13.4)
A.sphaerocephala Krasch	Eastern Gobi (September)	β-pinene (16.2), germacrene-D (14.5), γ-terpenene (11.6), 1.8-cineol (10.1), E,Z- β-ocimene (5.5-8.0), p-cymene (3.7) and cis-chrysanthylacetate (1.8)
A.mongolorum Krasch	Gobi-Altai mountain (September)	1.8-cineol (20.4), E-nerolidol (9.4), p-cymene (5.4), chrysanthenone (3.1)
A.subdigitata Mattf	Southern-Altai and Dzungarian Gobi (September)	Eugenol (11.2), methyl eugenol (9.4), camphor (9.0), limonene (8.1), β-pinene (6.5), 1.8-cineol (5.9), spathulenol (4.8), linalool (4.5), γ-curcumene (4.4), caryophyllene (2.8)
A.argyrophylla Ldb	Gobi-Altai mountain (September)	Lavandulol (24.3), chrysanthenol (6.9), thymol (11.9), lavandulylacetate (7.8), nerol (7.0), nerylacetate (4.7), α-terpinene (4.3), p-cymene (3.2)
A.changaica Krasch	Northern Gobi and valley of lakes (September)	E-nerolidol (65.0), sabinene (8.2), (Z)- β-ocimene (4.4), β-phellandrene (3.8), (E)- β-ocimene (2.3)
A.gracilencens Krasch	Dzungarian Gobi (September)	1.8-cineol (40.8), camphor (36.0), camphene (3.0) and terpinene-4-ol (3.7)

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ESSENTIAL OIL COMPOSITION OF SOME ANTHEMIDEAE (ASTERACEAE)

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Keywords: chamomile, Matricaria, Anthemis, oil composition, bisabolol oxide; matricaria ester, chrysanthenyl acetate

German chamomile (*Matricaria recutita*) is a widely consumed medicinal plant which is also used in various food additives and supplements. The present study compares the essential oil composition from various *Matricaria* and *Anthemis* species in order to point out characteristic volatile patterns and to assess whether they may be suitable for the authentication of the plant material.

The four German chamomile (*Matricaria recutita*) accessions analysed showed α-bisabolol oxides in varying amounts as main compounds besides en-in-dicyloethers and chamazulene. However these plants were very low in or devoid of α-bisabolol. *Trans*-β-farnesene and the en-in-dicycloethers as well as low amounts of bisabolone oxide A were also present in *Matricaria matricoides* (= *M. discoidea*). The oils from *Matricaria disciformis, Matricaria trichophylla* and *Tripleurospermum perforatum* (= *Matricaria perforata*) were characterised by polyacetylenes as matricaria acid methylesters and lachnophyllum esters. The oil of *Matricaria nigellaefolia* contained chrysanthenone, filifolone and β-pinene as major compounds.

Anthemis tinctoria had β-pinene, 1,8-cineol and various sesquiterpene hydrocarbons and oxidised sesquiterpenes in the oil. Sesquiterpene hydrocarbons as germacrene D and further sesquiterpene oxides were the characteristics of *Anthemis cotula*. In the oil of *Anthemis austriaca* cis-chrysanthenyl acetate dominated (60-70%) besides 1,8-cineol and α-pinene. *Anthemis arvensis* and *Anthemis altissima* were very low in essential oils. The former contained α-pinene, β-caryophyllene and caryophyllene oxide, the latter δ-3-carene, caryophyllene oxide and further sesquiterpenes as main compounds.

Finally the oil from *Tanacetum parthenium* was rich in camphor (48,4%), *trans*-chrysanthenyl acetate (23,1%) and camphene (13,6%).

In conclusion the oils from German chamomile appear to be well characterised by the presence of th α-bisabolol oxides sometimes accompanied by bisabolone oxide which are not found in similar looking ruderal species which might be present in food products due to unintended admixture or confusion.

The plants were raised from seeds obtained from the seed bank IPK Gaterslebern, Germany and transplanted into an experimental field. Additionally plants collected in the surroundings of Vienna, Austria were collected during flowering.

The plant material was harvested during blooming stages, dived in plant parts and dried in the ambient air. The essential oils were obtained by hydrodistillation or microdistillation and analysed by GC and GC/MS where the compounds were identified according to their mass spectra and retention indices.

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CHEMICAL COMPOSITION OF ESSENTIAL OIL *HELICHRYSUM ITALICUM* SUBSP. *ITALICUM* (ROTH) G.DON FIL CULTIVATED IN CORSICA

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Keywords: *Cultivated plant, Helichrysum italicum, essential oil, neryl acetate, β-diketones*

Helichrysum italicum (Roth) G. Don Fil., a typically Mediterranean species, is an aromatic shrub with yellow flowers (1). *H. italicum* oil is widely used in perfume industry and aromatherapy due to their flavoring properties and biological activities (anti-inflammatory, antioxidant) (2). In recent years, the culture of *Helichrysum italicum* subsp. *italicum* has been largely developed in Corsica Island in order to meet of industrial needs and to protect wild taxa. The aim of this work is to study the chemical composition of essential oil according to the age of plantation and duration of distillation process.

43 volatile components were identified in *Helichrysum italicum* subsp. *italicum* oils by GC and GC-MS analysis. The essential oils were dominated by oxygenated monoterpenes (31.7-43.6%) with neryl acetate as major compound. Other major constituents were γ-curcumene and limonene. This essential oil was also characterized by several β-diketones, unusual compounds in essential oils (3). The amount of neryl acetate has increased near to 10% during the first three years of cultivation. The content of sesquiterpene hydrocarbons has also increased during this period (closer to 5%). Conversely, the concentration of β-diketones has decreased approximately to 8% at the same time. Finally, the study showed that duration of hydrodistillation is an important parameter for the essential oil quality. For instance, the content of b-diketones increased during the first three hours of hydrodistillation.

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BV 41
DYNAMICS OF THE HEADSPACE CHEMICAL COMPONENTS OF *ROSA ALBA* L. FLOWERS

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Keywords: Rosa alba L., essential oil, headspace, dynamycs

In Bulgaria the white oil-bearing rose is the second rose species of economic importance for rose oil production after *Rosa damascena f. trigintipetala.* There is an increasing interest of perfumery and cosmetical industry towards the essential oil of *Rosa alba* L. It has raisen the necessity to evaluate the quality of different genotypes and the possibilities of the production of volatiles.

The present paper demonstrates the first results on the daily dynamics of components in the natural essential oil of a *Rosa alba* L. population.

As a whole, the population consists of four clones, which differ mainly in the number of petals and the color tint. For this monitoring we used static headspace solid microextraction technique (sHS-SPEM). 39 compounds were identified. It was established that the headspace model was a combination of the terpene alcohols: geraniol (1.57 – 41.66 %), citronelol (3.55 – 18.19 %), nerol (1.33 – 14.53 %), furthermore that of menthone (0.00 – 21.82 %) and limonene (0.93 – 13.91 %). However, the presence and quantity of compounds were highly dependent on the sampling time during the day and on the environmental factors (air temperature, relative humidity, intensity of sunlight and wind) of the relevant period.

The results show the daily variability of natural oil composition in Rosa alba L. and the significance of abiotic factors during population growth.

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MICROMORPHOLOGICAL TRAITS AND ESSENTIAL OIL CONTENTS OF *STACHYS IVA* GRISEB. AND *S. HORVATICII* MICEVSKI (LAMIACEAE)

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Keywords: non-glandular trichomes, glandular trichomes, caryophyllene oxide, hexadecanoic acid

The genus *Stachys* L. (Lamiaceae) includes 300 (–450) annual or perennial herbs and small shrubs with a nearly worldwide distribution. Fifty eight *Stachys* species have been described for Europe (1). The types and distribution of trichomes, and chemical composition of the essential oil were investigated in endemic *Stachys iva* Griseb. and *S. horvaticii* Micevski (Lamiaceae) growing in Republic of Macedonia.

Attenuate non-glandular (NG) trichomes, and two types of glandular capitate trichomes (type 1 composed of one basal epidermal cell, one stem cell and a unicellular head cell with subcuticular space; type 2 composed of one elevated basal epidermal cell, one stem cells, and a head composed of four, sometimes two small cells) were observed on leaves, calyx and the stem. NG trichomes are also present in *Stachys ehrenbergii* and *S. distans* (2). Trichomes comparable to capitate trichomes type 1 are present in *Stachys ehrenbergii* and *Micromeria fruticosa* (2,3) while type 2 are known from *Stachys cretica, S. distans, S. neurocalycina, S. nivea, S. palaestina* and *S. recta* (3,4).

Water distilled essential oils from aerial parts of investigated plants have been analysed by GC and GC/MS using VF-5ms capillary column. Total yield of all oil was 0.1% in both species, based on dry weight of samples. The essential oil of *S. iva* was characterized by a high concentration of caryophyllene oxide (24.2%), *E*-caryophyllene (9.2%) and spathulenol (8.3%). Caryophyllene oxide and *E*-caryophyllene were also identified as major compounds in the oil of *Stachys germanica, S. scardica, S. plumosa* and *S. iva* (5). The major compounds of the essential oil of *S. horvaticii* from locality Veles were hexadecanoic acid (35.7%), caryophyllene oxide (12.8%) γ-cadinene (12.2%), and δ-cadinene (9.3%). The oil of *S. horvaticii* from locality Kavadrci was characterized by a high concentration of hexadecanoic acid (48.4%). According to Conforti et al. (6), hexadecanoic acid was the major component in the oil of *Stachys cretica, S.* germanica, *S. hydrophila, S. nivea, S.palustris,* and *S. spinosa.* The present study gives additional knowledge about micromorphological traits and essential oil contents on the genus *Stachys.*

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BV 43
EFFECT OF GROWING SITE AND PLANT AGE ON THE DRUG YIELD AND QUALITY OF LOVAGE (*LEVISTICUM OFFICINALE* W.D.J.KOCH)

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***Keywords:** diversity, essential oil, α–terpinyl-acetate, β-phellandrene, (Z)-ligustilide*

1- and 2-year-old lovage populations established from the same genetic material in Soroksár were examined in 2012 in order to determine the influencing role of plant age on the leaf and root drug production and quality. Beside 1-year-old stand of Soroksár another annual population has been produced in a different growing site, in Nagydorog in order to explore the effect of growing place on the leaf and root drug yield, the essential oil content and composition of lovage.

The harvest of leaf (foliage of 5 plants/plot was cut completely) was achieved at the beginning of September, while roots were harvested at the beginning of November (5 individuals/plot). The essential oil content was determined by water distillation according to the European Pharmacopoeia (Ph.Eur.) and the component spectrum was determined by GC-MS method based on standard compounds and retention indices.

Comparing the *leaf and root drug yield* of 1- and 2-year-old populations cultivated at the same environment we found significantly higher values in the 2-year-old stand (leaf: 34.6 g/plant; root:171.8 g/plant) than in 1-year-old one (leaf:13.9 g/plant; root:45.3 g/plant). In case of annual populations grown at different places we measured greater values in the stand of Nagydorog (leaf:46.8 g/plant; root:64.8 g/plant) than in the population of Soroksár, but differences proved to be significant only in respect of leaf drug. Here the leaf yield also exceeded the 2-year-old population's values. According to this it can be ascertained that leaf yield was affected by environmental conditions (temperature, precipitation, soil properties) first of all, but root yield has increased linearly with age of the plant.

Examining the *essential oil content* of 1- and 2-year old populations of Soroksár we established that older, 2-year-old plants had higher essential oil accumulation in leaves and roots (1.83 ml/100g and 0.74 ml/100g) than 1-year-old ones (1.50 ml/100g and 0.35 ml/100g). However, significant differences were found only in case of roots' essential oil content. In point of annual populations cultivated at different growing sites we didn't experience great differences between their essential oil levels. Thus, it is presumable, that essential oil accumulation is influenced by plant age primarily mainly in case of roots and environmental conditions are only secondary in this respect.

The main *essential oil components* of leaves were α–terpinyl-acetate (31.8-36.5%), β-phellandrene (22.4-33.5%), (Z)-ligustilide (20.2-26.6%), (Z)-ocimene (8.2-9.4%) and β-myrcene (2.6-4.5%). In the essential oil of root (Z)-ligustilide was detected as main constituent (82.7-85.6%). Further minor components of roots were 1-pentyl-cyclohexa-1,3-diene (4.9-9.9%), β-phellandrene (1.5-2.8%), (E)-ligustilide (2.0-2.1%) and (Z)-3-butylidenephthalide (1.3-1.8%). There were no significant differences between populations' essential oil composition, thus, it was not influenced by either plant's age, or growing site.

BV 44
ACCUMULATION AND RELEASE OF ENDOGENOUS AROMA COMPOUNDS FROM *ROSA* 'YVES PIAGET' GROWN IN ARCHING HYDROPONIC CULTURE SYSTEM

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Keywords: rose, flower scent, emission

Rose (*Rosa hybrid* L.) is grown for use as garden shrub, cut flower, and a source of natural fragrance. Modern cut-flower rose cultivars have been selected for long vase life, flower shape, and color. Some cultivars are selected for flower fragrant, too. 'Yves Piaget' has strong floral scent and is popular fragrant variety in Japan. In rose flowers, the dominant aroma compounds are 2-phenylethanol (2PE) and 3,5-dimethoxytoluene (DMT) (1). However, there is no report about aroma compounds of 'Yves Piaget'.

When grown in an arching hydroponic culture system which is a culture procedure to use bent-down shoots on bench in an arch, the scent of 'Yves Piaget' becomes weak and the corolla is easy to be malformed shape. In this study, we aimed to elucidate the mechanisms of the flower malformation and scent weakening, which have yet to be explained. Moreover, we aimed to identify the aroma compounds present in the headspace of 'Yves Piaget' as well as their endogenous aroma compounds.

'Yves Piaget' flowers were cut when the flowers started to open, and the exogenous and endogenous aroma compounds were isolated by solid-phase micro extraction and by diethyl ether solvent extraction, respectively. The extracted compounds were quantified by gas chromatography-mass spectrometry. The components were identified by comparison against libraries of mass spectra and by their retention indices.

A total of 32 exogenous and endogenous aroma compounds were identified in quantifiable amounts from the intact and malformed cut rose flowers, and these compounds were common to both flower types. The most abundant compounds were (*E*)-geraniol, (*Z*)-geraniol, (*Z*)-3-hexenyl acetate, DMT, (R)-citronellol and 2PE. The relative concentration of exogenous compounds from intact rose flowers was 2-fold that of malformed flowers. By contrast, the relative concentration of endogenous compounds from intact rose flower was 11-fold that of malformed flowers. For all 32 endogenous compounds, the relative concentration from intact rose flowers was higher than that from malformed flowers. These results show that malformed flowers, which have a weak scent, produced and released very low concentration of aroma compounds. This suggests the possibility of a defect in the biosynthetic pathway of aroma compounds in malformed flowers.

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RT	Compounds	Exogenous compounds µg Flower /10 min ± SE		Endogenous compounds µg/g ± SE	
		the intact flower	the malformated flower	the intact flower	the malformated flower
17.0	β.-myrcene	3.22 ± 0.90	0.82 ± 0.27	10.35 ± 1.49	0.89 ± 0.26
18.2	D-limonene	0.96 ± 0.22	0.40 ± 0.15	3.59 ± 0.53	0.32 ± 0.12
19.3	(Z)-ocimene	0.63 ± 0.20	0.16 ± 0.06	3.63 ± 0.57	0.29 ± 0.09
19.9	(E)-ocimene	1.59 ± 0.39	0.61 ± 0.26	5.54 ± 0.89	0.45 ± 0.13
20.5	hexyl acetate	5.17 ± 0.84	3.48 ± 1.55	0.47 ± 0.05	0.07 ± 0.02
21.9	(z)-3-hexenyl acetate	16.33 ± 2.57	12.35 ± 5.43	1.44 ± 0.13	0.21 ± 0.07
22.4	2-hexenyl acetate	0.61 ± 0.12	0.21 ± 0.10	0.08 ± 0.01	0.04 ± 0.03
22.5	6-methyl-5-hepten-2-one	0.09 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.02 ± 0.00
22.9	1-hexanol	0.35 ± 0.07	0.33 ± 0.14	1.61 ± 0.14	0.64 ± 0.09
23.9	(Z)-3-hexene-1-ol	0.25 ± 0.04	0.33 ± 0.13	3.90 ± 0.17	1.71 ± 0.31
26.8	2-ethyl-1-hexanol	0.23 ± 0.04	0.26 ± 0.10	0.08 ± 0.03	0.01 ± 0.00
27.1	n-decanal	0.12 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.01 ± 0.00
28.2	l-linalool	0.56 ± 0.09	0.20 ± 0.08	0.59 ± 0.06	0.05 ± 0.02
29.9	caryophyllene	0.08 ± 0.01	0.10 ± 0.04	0.16 ± 0.03	0.01 ± 0.00
31.2	Citronellyl acetate	0.75 ± 0.15	0.42 ± 0.19	0.60 ± 0.06	0.07 ± 0.02
31.6	anisole	1.59 ± 0.32	0.72 ± 0.29	1.43 ± 0.16	0.15 ± 0.02
31.7	(Z)-Citral	0.75 ± 0.14	0.49 ± 0.21	1.46 ± 0.20	0.16 ± 0.08
32.0	Methyl geranate	0.31 ± 0.07	0.14 ± 0.07	0.22 ± 0.05	0.03 ± 0.01
32.6	germacrene-d	1.43 ± 0.30	0.18 ± 0.07	1.46 ± 0.18	0.21 ± 0.06
32.7	neryl acetate	1.36 ± 0.24	1.15 ± 0.47	1.01 ± 0.28	0.26 ±
32.9	(E)-Citral	1.83 ± 0.34	0.98 ± 0.44	3.72 ± 0.47	0.45 ± 0.18
33.2	.alpha.-farnesene	0.19 ± 0.04	0.20 ± 0.05	0.70 ± 0.08	0.12 ± 0.02
33.4	geranyl acetate	9.01 ± 1.59	3.31 ± 1.63	6.54 ± 0.75	0.49 ± 0.24
33.5	(R)-citronellol	6.22 ± 1.22	3.86 ± 1.99	17.27 ± 1.37	1.65 ± 0.58
34.0	γ-Isogeraniol	0.12 ± 0.03	0.14 ± 0.00	0.37 ± 0.05	0.04 ± 0.01
34.3	(Z)-geraniol	17.88 ± 3.36	8.45 ± 4.25	34.62 ± 3.81	3.24 ± 1.18
34.8	2-phenylethyl acetate	3.60 ± 0.90	1.22 ± 0.49	3.93 ± 0.29	0.30 ± 0.11
35.3	(E)-geraniol	25.84 ± 5.19	6.43 ± 3.99	114.31 ± 23.68	3.27 ± 0.15
35.4	3,5-Dimethoxytoluene	7.73 ± 1.43	4.45 ± 1.79	34.04 ± 2.69	1.86 ± 0.24
35.6	neryl acetone	0.30 ± 0.05	0.18 ± 0.04	0.26 ± 0.02	0.07 ± 0.02
36.8	phenylethyl alcohol	8.57 ± 2.16	3.86 ± 1.68	148.26 ± 18.15	16.11 ± 5.36
38.0	dihydro-.beta.-ionol	0.08 ± 0.02	0.36 ± 0.08	1.69 ± 0.18	1.46 ± 1.18
	Relative concentration	119.03 ± 21.59	56.19 ± 25.14	416.97 ± 28.05	35.12 ± 6.72

BV 45
IDENTIFICATION OF EXOGENOUS VOLATILE COMPONENTS OF HYDRANGEA QUERCIFOLIA

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Keywords: flower scent, HS-SPME, GC-MS

Many plants attract pollinators by emitting floral scent, and adaptive evolution of floral scents to attract pollinators has often been suggested. Many floral fragrance components have been analyzed and identified recently. Most of *Hydrangea* plants do not have floral fragrance; however *H. quercifolia*, which is native to North America, has floral fragrance. The corolla consists of bisexual and decorative flowers. Cultivars of *H. quercifolia* are classified by single or double petals in decorative flowers and only single-petal cultivars have floral fragrance. There is no report about identification of volatile compounds in *Hydrangea*. In this study, we analyzed the exogenous volatile components of bisexual and decorative flower from 'Back Porch' (single-petal cultivar) and 'Snow Flake' (double-petal cultivar). Exogenous volatile compounds were extracted by solid phase micro extraction method and were analyzed by GC-MS.

Totally 37 exogenous volatile components (16 terpene, 9 alcohol, 8 aldehyde, 2 acid, 2 ketone) were identified from 'Back Porch' flowers. 60% of exogenous volatile components were classified as monoterpenes, among them 3-Carene was the most abundant one (34.9%). The second and third abundant terpene components were D-Limonene (7.9%) and sabinene (6.5%), respectively. 'Snow Flake' emitted 20 exogenous volatile components (4 terpene, 9 alcohol, 7 aldehyde), but 3-Carene could not be detected. Total amount of exogenous volatile components in 'Snow Flake' was about one twentieth in 'Back Porch'.

These results indicated that, in the case of 'Snow Flake' the applied extraction method was not acceptable. We need to analyze endogenous volatiles compounds of bisexual and decorative flowers for further analysis.

BV 46
EFFECT OF ENVIRONMENTAL CONDITIONS ON MORPHOLOGY, PRODUCTION AND ESSENTIAL OIL CONTENT OF *ACHILLEA COLLINA* BECKER

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Keywords: yarrow, volatile oil, growth chamber, climatical effect

Achillea species are known as medicinal plants worldwide, some of them are cosmopolitans in the Northern hemisphere and distributed in different climatic regions. The genus shows high chemical variability, which is also influenced by different environmental conditions. The range of environmental influence was investigated in climatic chambers in a pot experiment, from February to July of 2012. For our investigations a selected strain of the azulenogenic *Achillea collina* species was chosen.

Ten, one-year old plants originating from open field collection were divided into two and these sister-individuals planted separately in pots.

„Cold" and „hot" weather conditions as treatments were installed in the growth chambers by different temperature and light settings, and the sister-plants were investigated in parallel.

The plant development and phenological features were checked in every week. Measurement of morphological characteristics and sampling the flowering shoots was carried out in full flowering. The biologically active substances of the plant material were determined from the dried herb: content of essential oil and concentration of proazulenes according to the European Pharmacopoeia (Ph. Eur.).

The blooming started after 10 weeks in hot conditions, while the cold treatment prolonged the appearance of the first inflorescences by another 9 weeks. The number of inflorescences per plant proved to be similar in both treatments (12-13 flower heads), while the number of the branches on the floral stems was higher under hot conditions (10.3/plant). The cold treatment stimulated plant growth: the plant height was significantly bigger, approximately by 50 % (0.82 m as a mean) compared to the ones in the hot treatment (0.54 m). The leaf-size was also bigger and the stems were covered with leaves more densely.

In connection with the above mentioned characteristics, yarrow produced significantly higher amounts of fresh flowering stem yield (0.117 kg/plant) in cold conditions, in contrast to the hot treatment (0.81 kg/plant). This difference manifested itself in the drug production, too. Weather conditions effected also the ratio of the plant organs, hot conditions resulted in higher flower proportion (20.4 %) in the herbal drug.

The essential oil content was higher under hot conditions (0.160 % v/w versus 0.159 % v/w), while the proazulene content was elevated by the cold treatment (0.106 % versus 0.071 %).

BV 47
INTRASPECIFIC VARIABILITY OF LITTLE-LEAVED LINDEN (*TILIA CORDATA MILL.*) OCCURRING IN EASTERN POLAND

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Keywords: natural sites, populations, essentials oils, nonanal

Little-leaved linden is considered as a valuable medicinal plant. In Poland, the species grows wild on natural sites in hail forests (*Tilio-Carpinetum*) but it is also often planted along the streets, in parks and gardens (1). Raw materials obtained from this plant are flowers rich in flavonoids and mucus (2), containing also a small amount of volatile compounds responsible for its specific flavor (3,4). Flowers reveal diaphoretic, anti-inflammatory and sedative activities and could be used in cold and respiratory diseases treatment (5). Due to increasing demand on this herbal raw material, the evaluation of its quality including chemical variability of wild growing populations seem to be important.

The aim of investigation was to determine intraspecific variability of little-leaved linden occurring on natural sites in Poland. The research was focused on the composition of essential oil in flowers. In 2012, the raw material was collected from trees growing wild on 21 natural sites located in eastern area of Poland. Geographical location of these sites was determined using GPS apparatus. The harvest of flowers took place in July, during its full blooming time. Collected raw material was dried in 35°C and subjected to chemical analysis. The essential oil was obtained using hydrodistillation method (with xylene addition), according to Polish Pharmacopeia VIII. The composition of essential oil was determined by GC/MS method using Shimadzu GCMS QP-2010S gas chromatograph equipped with Phenomenex Zebron ZB-FFAP polar column.

Among 24 compounds identified in the essentials oil, the dominants ones were: nonanoic acid, nonanal and acetophenone. Other major compounds were higher alkanes i.e. heneicosane, tricosane and pentacosane. Terpenes were represented mainly by monoterpenes: linalool, geraniol, alpha-terpineol and sesquiterpenes: nerolidol, farnesyl acetate and tetrahydrofarnesyl acetate. The percentage of main essential oil compounds ranged, as follows: nonanoic acid from 2.57 to 18.24%, nonanal from 1.30 to 7.76% and acetophenone from 3.05 to 7.42%.

Obtained results indicate that little-leaved linden flowers from investigated populations differed significantly in respect of essential oil composition and consequently may present different sensory profile.

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BV 48
INFLUENCE OF GROWING SUBSTRATES AND FERTILIZATION IN BIOMASS AMOUNT AND ESSENTIAL OIL YIELD AND COMPOSITION OF *MENTHA PIPERITA* L

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Keywords: Mentha piperita, essential oil, substrates, fertilization, yield, linalool, linalyl acetate

Peppermint (*Mentha piperita* L.) is one of the most economically valuable *Mentha* species both because it is widely used for medicinal and food purposes, and easily grown in many different environmental conditions (1). Although its most typical essential oil (EO) compounds are menthol and derivatives, some varieties are characterized by the predominance of linalool metabolic pathway. Such is the case of *Mentha x piperita "lavanduliodora"* (2), which contain valuable compounds as linalool and linalyl acetate, as has been found in this study.

Many research have been devoted to study of agronomic factors affecting peppermint crops: fertilization, harvest time, propagation way (1,3,4,5), biosolids use (6), etc. The aim of this contribution is to study the influence of fertilization and use of some typical substrates in biomass amount and essential oil yield and composition in *Mentha x piperita "lavanduliodora"* growing. For this purpose, a total amount of 36 plants were bred from stolons in pots. The following treatments, with and without fertilization, were applied in triplicate: (1) Natural sandy soil (NS), (2) universal substrate (US), (3) peat (P), (4) Peat + perlite (P-P) 1:1, (5) P-P 2:1 and (6) Peat + perlite + natural soil (P-P-NS) (1:1:1).

After 10 weeks, the plants were raised and their biomass weighed. Likewise, the essential oil from fresh leaves was extracted by hydrodistillation with a Clevenger apparatus and the yield % (V/w) measured. The EO composition was determined by GC/MS and GC/FID analysis. The identification was performed from Kovats retention index calculation and by computer matching mass spectral data with those from NIST 2.0 library. The quantification was carried out by *peak area normalization* method (GC/FID). The major compounds were: linalool: 51,4 ± 10,4 %, linalyl acetate: 26,7 ± 8,4 %, 1,8-cineol: 5,6 ± 2,1 % and α-terpineol acetate: 4,5 ± 1,0 %

Regarding the fertilization and substrate treatments effect on biomass and EO yield and composition, a two-way analysis of variance (*ANOVA*) test was performed by means of Statgraphics 5.1 software. With regard to EO composition, no significant differences ($P<0.05$) were found except for sesquiterpenoid fraction whose % was higher for no fertilized plants (2,9-3,7 %). The biomass yield was significantly higher for US and NS treatments. The EO yield referred to fresh leaves showed a range of 0,33-0,80 mL/100 g. Significantly higher EO yield values were found for peat and peat-perlite mixtures with regard to universal substrate and natural soil. No significant differences were found when the EO yield was referred to total plant biomass.

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BV 49
SEASONAL VARIATION OF ESSENTIAL OIL COMPOSITION IN A POPULATION OF *ARTEMISIA ABSINTHIUM* L. FROM TERUEL (SPAIN): INDIVIDUAL SAMPLING VS. INDIVIDUAL MONITORING

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Keywords: seasonal variation, sampling, Artemisia absinthium, chemotype,

The high intrapopulational variability in essential oil (EO) composition is an important obstacle in order to obtain reliable data both for chemotaxonomy purposes and to select and propagate individuals with a specific composition. This way, sampling of individuals is considered as a more suitable procedure for taking into account this variability (1, 2). One of the most important factors affecting EO chemical composition is its seasonal variation. To study it, different sampling methods have been applied in some *Artemisia* species: to gather together material from randomly selected plants on a population (3,4), to collect a number of individual plants which are analyzed separately (individual sampling) (5,1) and the individual monitoring by means of taking material from the same individuals over a period of time (2). The aim of this contribution is to compare these last two methods in *Artemisia absinthium* EO composition. For this purpose, five wild plants from a population in Teruel (Spain) were monitored taking 10 g samples of aerial parts over their vegetative period until after flowering (individual monitoring). The samples were extracted by means of a SDE apparatus. In the same sampling dates, five individuals from the same population were randomly selected and their whole aerial parts submitted to hydrodistillation with a Clevenger apparatus. In both cases, the EO analysis was performed by GC/MS (identification) and GC/FID to quantify it by peak normalization areas.

The most wide range for the major compounds was observed with individual randomly sampling: cis-epoxyocimene (39.4-80.8 %), chrysanthemyl acetate (0.0-33.1 %), camphor (0.0-13.8 %) and linalool (0.0-12.8 %). Regarding seasonal variations, both ways of sampling showed similar profiles, but narrower LSD intervals were appreciated with the individual monitoring procedure, giving therefore more significant differences. The highest level of epoxyocimene (cis + trans) takes place when the full vegetative development is achieved, before flowering, and decreases from that time on. The opposite evolution is shown by the other major compound: cis-chrysanthemyl acetate, in accordance with the previous research (2). Linalool increases in a regular way over the vegetative cycle. When the compounds are gathered according their molecular skeleton or chemical nature, the differences are determined by the major compounds occurrence.

In summary, some individual results show how odd samplings (not taking into account the vegetative cycle) may lead to erroneous chemotypes definition. Indeed, the results lead us to consider the co-occurrence of two chemotypes in the studied population: cis-epoxyocimene and cis-epoxyocimene + chrysanthemyl acetate.

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BV 50
SEASONAL STUDY OF *LIPPIA ORIGANOIDES* FROM CARAJÁS NATIONAL FOREST, PA, BRAZIL, BASED ON THE ANALYSIS OF ITS ESSENTIAL OIL

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Keywords: Lippia origanoides, essential oil variation, seasonal study, 1,8-cineole, (E)-methyl cinnamate, (E)-nerolidol

The interaction in the environmental conditions that occur throughout the day or month, can influence directly or indirectly the process of secondary metabolism, resulting in quantitative and qualitative variations in the essential oils. In ISEO 2012 were showed some data from the circadian study of *Lippia origanoides* Kunth, based on the analysis of its essential oil. Now, the data of seasonal study of the plant was completed. *Lippia origanoides* Kunth is a small shrub up to 3 m, showing variable aromatic characteristics and some known chemotypes. This species occurs in countries of Central and South America, especially in the Brazilian Amazon, and it is used for culinary and medicinal purposes. The oils of the present study were analyzed by GC and GC-MS. The leaf oil yields in the rainy season (from December to May) were 1.7%, 2.2%, 2.5%, 2.6%, 2.7% and 2.8%, respectively. In the dry season (from June to November) were 4.6%, 4.2%, 3.8%, 2.8%, 2.1% and 2.1%, respectively. In the rainy season, the main constituents of the oils (over 10%) (from December to May) were (*E*)-nerolidol (29.2%) and (*E*)-methyl cinnamate (16.7%); (*E*)-methyl cinnamate (16.5%) and (*E*)-nerolidol (16.3%); (*E*)-methyl cinnamate (24.7%) and (*E*)-nerolidol (15.9%); 1,8-cineole (19.2%), carvacrol (15.0%) and (*E*)-caryophyllene (11.8%); (*E*)-nerolidol (25.1%) and 1,8-cineole (11.1%); and 1,8-cineole (31.9%) and (*E*)-nerolidol (16.2%), respectively. In the dry season (from June to November), the major components were carvacrol (22.6%), 1,8-cineole (18.8%) and γ-terpinene (10.4%); (E)-methyl cinnamate (5.9%), (E)-nerolidol (12.5%), carvacrol (11.4%) and 1,8-cineole (10.1%); (*E*)-methyl cinnamate (52.4%) and (*E*)-nerolidol (23.2%); (*E*)-methyl cinnamate (26.6%), (*E*)-nerolidol (16.9%) and 1,8-cineole (9.9%); 1,8-cineole (18.5%), (*E*)-methyl cinnamate (13.8%) and (*E*)-nerolidol (16.9%); and (*E*)-methyl cinnamate (18.7%), α-pinene (16.8%), (*E*)-nerolidol (14.0%) and 1,8-cineole (12.2%), respectively. These data will contribute to a better commercial exploitation of the plant.

Acknowledgments: this work was supported by BIONORTE/CNPq and FAPESPA/PA.

BV 51

EVALUATION OF CHEMOTYPIC DIFFERENTIATION AND VOLATILE OIL CONTENT IN INDIGENOUS POPULATIONS OF *FERULA GUMMOSA* BOISS. GROWN IN IRAN

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Keywords: Ferula gumosa, essential oils, gas chromatography, chemotypes

Essential oil components and yield of areal and underground parts of an endemic wild growing *Ferula gummosa* Boiss, in different bioclimates of Iran, has been investigated using GC-MS analysis. The highest essential oil versus fruit dry weight was recorded in the samples from Sorkh Abad (2.64%) and the lowest amount (1.45%) was measured in the samples from Angouran. According to GC-MS analysis, monoterpene hydrocabons were the predominant components in the oils of *F. gummosa* among which the major monoterpenes were α-pinene (17-41.29%), β-pinene (10.44-37.04%), δ-3-carene (9.16-10.75%) and limonene (0-13.23 %). The high variability in the oil content and composition in different populations showed a remarkable difference such as in monoterpene content variation from 74.96 to 86.48% of the oil composition. It is interesting to note that the highest concentration of α-pinene (41.29%) was in the roots sampled from Ebrahim Abad followed by β-pinene having the highest concentration (37.04%) from the roots sampled in Angouran. The sesquiterpene fraction was composed mainly of α-cadinol (0.76–3.09%), guaiol (0.49–2.50%), germacrene D (1.13-2.05) and δ-cadinene (0.67–1.84%) in all samples. The main finding of the study was the highest percentage of oxygenated sesquiterpenes in oils of fruit samples in comparison with root samples of *F. gummosa* in all locations. There was a considerable chemo-variation in the oils observed appears to be environmentally determined, the study is useful as a base for selection of this medicinal plants in breeding programs to obtain a proper line with advantageous compound as natural source for pharmaceutical, cosmetic, agricultural and food industries.

BV 52

INTER POPULATION CHEMICAL COMPOSITION VARIABILTY IN ESSENTIAL OILS OF SAINT JOHN'S WORT (*HYPERICUM PERFORATUM L.*) FROM NORTHWESTERN OF IRAN

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Keywords: Hypericum perforatum L., essential oil, chemotypes

The composition of the volatile oils from the aerial parts of *Hypericum perforatum* L., a wild growing species of the Iranian flora collected from the different populations in three localities from northwestern Iran. The essential oils were obtained by hydrodistillation and were analyzed by GC–MS. A comparison essential oils composition of *H. perforatum* revealed minor variations among the three populations. There were significant differences in content and composition of *Hypericum perforatum* L. essential oil and the highest concentration of monoterpene hydrocarbon was found in the Ebrahim Abad and Sorkh Abad locations; 87.92, 87.36 respectively. The most abundant constituents were monoterpene hydrocarbons including α–pinene (54.29-69.5%), β-pinene (4.36-7.97%), β-myrcene (2.42-3.43%) and n-nonane (1.23-3.90%). Moreover, a high concentration of sesquiterpenes was characteristic for Sorkh Abad area (5.52%) in which differences were attributed to the main components of sesquiterpene hydrocarbons in essential oil of all accessions by the following: the highest germacrene D (0.8-2.11%) and δ-cadinene (0.48-0.90%). Wherase, moreover α-cadinol (0.41%) were present only in the oxygenated sesquiterpenes of essential oils of *H. perforatum* L. in Angouran site. The composition of essential oils obtained from areal parts in 3 accessions of *Hypericum perforatum* L. revealed that that the environmental factors play a role in composition pattern in essential oils of studied *Hypericum* plants.

BV 53

VARIATION OF SCENT EMISSION RATES IN TWO SPECIES OF *ADANSONIA*: IMPACT IN A PLANT-POLLINATOR SYSTEM

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Keywords: Baobab, Adansonia, floral scent, pollination, signal evolution, Madagascar

Floral odor is an important trait used by many plants to attract pollinators and it plays a major role in the specificity of the relation plant-pollinator (1). The baobab (*Adansonia*) is mainly pollinated by hawkmoths, bats, lemurs and bees (2, 3). Little information is known about floral scents emitted by baobabs. The aim of this study is to determine the influence of the biological functions of the floral scent components to attract pollinators especially in *A. rubrostipa* (Longitubae) and *A. grandidieri* (Brevitubae). The scent emitted by the flowers was sampled every three hour after the flower opening. For each sample, both variables were recorded to the visitor behavior. High relative amounts of aromatic compounds (80-88%) were found simultaneously during the flower opening in *A. rubrostipa*: 2-phenyl acetonitrile, (Z)-hex-3-enyl tiglate and hexyl tiglate (4) were the most characteristic aromatic molecules identified. Their amount decreased (30-35%) along the time whereas the aliphatic compounds ratio increased. On the other hand, nonan-2-one and heptadec-8-ene were prominent compounds in the scent emission of *A. grandidieri* (Brevitubae) during the first six hours after the flowers opening. During our observations, the hawkmoths activity was positively correlated with the peak emission of aromatic molecules (19H-21H) while bees' behavior seemed to be rather influenced by the pollens availability. The visit of other pollinators such as bats and lemurs seemed to be less related to the flowers emission.

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BV 54

STUDY OF THE CHEMICAL VARIABILITY OF LEAF ESSENTIAL OILS OF *LIPPIA MULTIFLORA MOLD.* GROWN IN SAVANNA AND FOREST AREAS IN IVORY COAST

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Keywords: *Lippia multiflora, essential oils, chemical variability*

Lippia multiflora Moldenke (Verbenaceae), an aromatic plant called "Gambia tea", is naturally widespread in subtropical Africa. It has a double interest because of its food and therapeutic uses. A study of the variability of the chemical composition of the essential oils obtained from leaves of cultivated *Lippia multiflora* was carried out in different ecological areas of Ivory Coast. The oil samples were obtained by steam distillation according to the methodology recommended by the European Pharmacopoeia and they were analyzed by GC / FID and GC / MS.

Two chemotypes were identified: the essential oils samples collected in the south (Abidjan) and central regions (Toumodi) of Ivory Coast were characterized by similar chemical compositions, dominated by geranial (29.1 – 29.0%) and neral (21.9- 21.0%), along with thymol (6.5 - 9.1%) and minor content carvacrol. On the other hand, the essential oils from plants harvested in the north-easterm Ivory Coast (Bondoukou) contained high amounts of aromatic terpene constituents such as thymol (56.6%), thymyl acetate (10.7%) and carvacrol (6.9%); citral could not be detected in this chemotype. The other constituents, in particular sesquiterpenes, were similar in all samples. This change in the chemical composition of the plant essential oil will affect its pharmacological activity and a chemical control will be necessary before using for therapeutic purpose.

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BV 55
ESSENTIAL OILS FROM NIGERIAN MEDICINAL PLANTS

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Keywords: Phyllantus reticulatus, Hibiscus surattensis, Croton zambesicus, sesquiterpenes, monoterpenes

The chemistry of essential oils obtained by hydrodistillation of the leaves of *Phyllantus reticulatus* Poiret (Euphorbiaceae), *Hibiscus surattensis* L. (Malvaceae) and *Croton zambesicus* Muell. Arg. (Euphorbiaceae) growing in Nigeria has been studied. The air-dried plants were hydrodistilled (PhEur) and the oils were analyzed by GC and GC-MS. The components were identified by MS libraries and their estimated linear retention indices (LRI). The essential oil contents varied between 0.10% and 0.15% (v/w) on a dry weight basis. Monoterpenes and sesquiterpenes were the dominant classes of compounds present in the sample. The major constituents identified in the oil of *P. reticulatus* were β-pinene (18.1%), β-caryophyllene (11.9%), germacrene D (8.6%), camphor (7.7%), sabinene (7.6%), linalool (6.9%) and α-pinene (6.0%). The major oil constituents of *H. surattensis* were β-caryophyllene (12.9%), menthol (10.6%), methyl salicylate (9.7%) and camphor (9.2%). There were significant amounts of germacrene D (5.5%), hexadecanoic acid (4.3%), α-humulene (4.0%), 1, 8-cineole (3.0%) and menthone (3.0%). On the other hand, β-pinene (15.1%), β-caryophyllene (12.6%), germacrene D (10.9%), camphor (7.3%), linalool (7.0%), sabinene (6.4%) and α-pinene (5.2%) were identified in higher amounts from *C. zambesicus*. These results are in continuation of our research on the chemical composition and biological activities of poorly studied Nigerian flora (1-4).

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BV 56
CHEMICAL COMPOSITIONS OF ESSENTIAL OILS FROM CORSICAN PROPOLIS AND *POPULUS NIGRA* VAR. *ITALICA* BUDS

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Keywords: essential oil, propolis, Populus nigra, Guaiol, Bulnesol

Propolis is a resinous hive product collected by honeybees from buds and/or barks of some plants like *Populus* sp., *Betula* sp., *Alnus* sp. It is used by honeybees to protect the colony from diseases and parasites and more particularly to inhibit bacterial growth (1). In addition, propolis is considered as a natural product which has antibacterial, antioxidative and antifungal activities (1, 2). *Populus nigra* var. *italica* (family: Salicaceae) was known as an important raw resource of propolis. The previous studies on solvent extracts of the genus *Populus* reported the presence of terpenoids and phenolic compounds (3). However, only few studies focused on the volatile compounds of *Populus nigra* buds (4, 5). Otherwise, the essential oil of Corsican propolis was never been studied. The objective of this study was to characterize the essential oil compositions of Corsican propolis and its possible raw material; the resineous secretion of buds from *Populus nigra* var. *italica*. The chemical relationships between the hive propolis and plant material were also established on the basis of volatile composition.

In this work, the analysis of essential oil composition of five samples of propolis and five samples *of P. nigra* var. *italica* buds was carried out using GC-FID and GC-MS. The propolis essential oil was characterized by 107 volatile compounds. This oil was dominated by oxygenated sesquiterpenic compounds (27.9 – 36.0 %) with guaiol (5.8 %), β-eudesmol (3.6 %) and bulnesol (3.5 %) as main components. The three same main constituents were reported in essential oil of *P. nigra* var. *italica* buds amounting to 19.4 %, 3.8 % and 20.9 % of the total oil, respectively. 43 other common volatile compounds such as α-bisabolol, hinesol, γ-eudesmol were also reported on propolis and *P. nigra* var. *italica* oils. These results suggested that the buds of *P. nigra* var. *italica* are one of the principal raw material for the production of Corsican propolis by honeybee. The study of chemical variability of propolis and corresponding plant material could be used to establish the complex botanical origins of propolis.

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BV 57
CHEMOTAXONOMIC INVESTIGATIONS IN *CITRUS* GENUS BASED ON FLOWER ESSENTIAL OIL COMPOSITIONS

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Keywords: flower essential oils, Citrus genus, Chemotaxonomy, GC-MS, volatile compounds

The taxonomic classification of *Citrus* species has been extensively discussed in scientific literature (1-3) and some chemotaxonomic investigations of *Citrus* genus have been reported (4). Several studies have been performed on the chemical composition of the peel and leaf oil of *Citrus* species (5), but their reported data are not easily comparable because the extraction conditions were quite different (cold-pressure, solvent extraction, steam distillation). The fragrance of *Citrus* flowers due to the volatile components is probably the main characteristic that has contributed to the uses of orange flower essential oil (*Citrus sinensis* L.) in perfumery and cosmetic industry. However, to our knowledge, little attention has been paid to assay the chemical variability of *Citrus* flowers and no data are available on the flower essential oil from various *Citrus* species. The aim of the present work was to characterize for the first time the chemical compositions of flowers oils of 40 varieties from eight *Citrus* species (*C. medica*, *C. aurantifolia*, *C. maxima*, *C. limon*, *C. sinensis*, *C. reticulata*, *C. paradisi*, *C. aurantium*) in order to consider a potential utilization of these oils, and to establish a chemotaxonomic relationship between *Citrus* species.

The chemical compositions of flowers essential oils, obtained from 40 *Citrus* cultivars were established by GC and GC/MS. Combined analysis of essential oils led to the identification of 92 components amounting to 84.5-98.0% of the total oil. The flowers oils of the eight studied species showed important qualitative and quantitative differences. To synthesize the chemical composition data, statistical analysis was applied to examine the relative distribution of species/varieties according to their production of volatile compounds. The data analysis suggested the existence of three chemotypes: - the first was characterized by limonene (*C. aurantifolia*) followed by γ-terpinene (*C. medica*) or β-pinene (*C. limon and C. maxima*); - the second characterized by sabinene associated with linalool (*C. sinensis* and *C. reticulata*) or limonene (*C. paradisi*); - the third (*C. aurantifolia*) characterized by oxygenated monoterpenes: linalool, linalyl acetate and citronellal. Such result is important for the development of conservation programs (characterisation of the core collection) of *Citrus* diversity and for the selection of the most appropriate chemical traits to the possible valorisation of *Citrus* flowers.

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BV 58

EFFECT OF TWO IRRIGATION REGIMES ON THE PRODUCTION AND SECONDARY METABOLITES OF SUMMER SAVORY (*SATUREJA HORTENSIS* L. 'BUDAKALÁSZI')

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Keywords: savory, stress, drought, water supply, SPAD

As consequence of the documented climatic changes analysis of the effects of high temperature and drought stress on different plant species seems to be essential. In our present study summer savory (*Satureja hortensis* L. 'Budakalászi') was investigated in pot experiment. Each pot contained 10 L soil. 3 plants per pots were planted. Two irrigation regimes were set while natural precipitation was locked out. In the first treatment (T1) plants were irrigated with 1 L water when the soil volumetric water content (SVWC) decreased below 20% while in the second treatment (T2) the plants were irrigated with 0,5 L water if the SVWC decreased below 10%. To measure the SVWC HH2 moisture meter and ML2x theta probe were used. During the vegetation period the chlorophyll content (SPAD-502) was measured. The plants were harvested once in full flowering phenophase. Fresh and dry mass was determined. The essential oil content was measured by hydro distillation from dry leaves. The essential oil composition was identified by GC-MS.

The effect of the different irrigation regimes was obvious to the majority of the examined traits. The chlorophyll content increased (T1:44,78±2,06 SPAD unit; T2: 55,74±3,02 SPAD) significantly with decreasing water supply. The fresh weight of the plants decreased (T1: 81,60±2,70/pot; T2: 41,20±4,15 g/pot) and the dry weight as well (T1: 24,78 ± 0,92 g/pot; T2: 11,26±1,49 g/pot). As a result of the low water supply higher essential oil content was measured (T1:3,63±0,10 ml/100g dw; T2: 4,20±0,08 ml/100g dw).

The main components of the essential oil were *carvacrol* and *γ-terpinene*. Their concentration did not show significant changes.

The results show that the water supply of medicinal plants may significantly influence the efficacy of the production and quality of drugs as well.

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BV 59
SCENTS FROM BRAZILIAN CERRADO: CHEMICAL COMPOSITION OF THE ESSENTIAL OIL FROM THE LEAVES OF *HYPTIS LYTHROIDES* POHL *EX* BENTH. (LAMIACEAE)

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Keywords: Hyptis lythroides, spathulenol, Brazilian Cerrado, Lamiaceae

Brazil is known to harbour the richest flora of the planet, about one-sixth of the total of number of plant species. Its flora is divided in several biomes, such as the savannah area, called Brazilian Cerrado. It is the second larger Brazilian biome and contains *circa* 12 000 plant species, most of them never submitted to phytochemical studies (1,2). This work is part of a research project aimed to investigate the chemical composition of aromatic plants native from Brazilian Cerrado. Herein we report the analysis of the essential oil from *Hyptis lythroides* (=*Oocephalus lythroides* (Pohl ex Benth.) Harley & J.F.B.Pastore - Lamiaceae), an endemic Brazilian herb, with purple flowers, growing at forest borders.

Leaves from several individuals of a population were collected at ecological Park Dom Bosco, Brasília, Brazil. A voucher specimen was deposited in the herbarium of Embrapa Genetic Resources and Biotechnology (CEN 82851). Dried leaves (129.3 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 2 hours. The oil was analyzed by GC/FID and GC/MS using an Agilent 7890A GC and an Agilent 5973N MSD system, both fitted with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 μm). Carrier gas was kept at a flow of 1.0 mL/minute (hydrogen for GC/FID and helium for GC/MS). Oven temperature was programmed from 60 to 240°C at 3°C/minute. The percentage composition was obtained by normalization from FID. Essential oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature (3, 4).

The essential oil yield was 0.84%, which can be interesting considering a wild species. Seventy-three compounds were identified, corresponding to 95.6% of the total essential oil. The major compounds found were spathulenol (14.5%), β-pinene (12.1%), bicyclogermacrene (11.9%), (*E*)-sesquisabinene hydrate (8.6%) and α-thujene (5.8%).

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BV 60

SCENTS FROM BRAZILIAN CERRADO: ANALYSIS OF THE CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM LEAVES OF *MYRCIA LINEARIFOLIA* CAMBESS. (MYRTACEAE)

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Keywords: Myrcia linearifolia, β-pinene, Myrtaceae, Brazilian Cerrado

Brazil is known to harbour the richest flora of the planet, about one-sixth of the total of species. Its flora is divided in several biomes, such as the savannah area, called Brazilian Cerrado. It is the second larger Brazilian biome and contains *circa* 12 000 plant species, most of them never submitted to phytochemical studies (1,2). This work is part of a research project aimed to investigate the chemical composition of aromatic plants native from Brazilian Cerrado. Herein we report the analysis of essential oil from *Myrcia linearifolia* Cambess. (Myrtaceae), an endemic herb, 50-80 cm tall, with white to pink flowers, occurring frequently in the Cerrado of Tocantins and Goias States of Brazil (3).

Leaves from several individuals of a population were collected at the ecological reserve of the Instituto Brasileiro de Geografia e Estatística (IBGE), Brasilia, Brazil. A voucher specimen was deposited in the herbarium of the Genetic Resources and Biotechnology (CEN 82846). Dried leaves (628.7 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 2 hours. The oil was analyzed by GC/FID and GC/MS using an Agilent 7890A GC and an Agilent 5973N MSD system, both fitted with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 μm). Carrier gas was kept at a flow of 1.0 mL/minute (hydrogen for GC/FID and helium for GC/MS). Oven temperature was programmed from 60 to 240°C at 3°C/minute. The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature (4,5).

Oil yield was 0.27%. Fifty-eight compounds were identified, corresponding to 93.8% of the oil. The major components were β-pinene (24.2%), α-pinene (14.0%), *para*-menta-2,4(8)-diene (7.1%) and spathulenol (5.6%).

Acknowledgments: *CNPq, CAPES, Embrapa.*

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BV 61

CYTOGEOGRAPHY OF *EREMOPHILA LONGIFOLIA* (F. MUELL) (SCROPHULARIACEAE) ESSENTIAL OIL CHEMOTYPES IN AUSTRALIA

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Keywords: Aboriginal medicinal plant, chemotypes, cytometric analysis

Previous studies have demonstrated that the Australian Aboriginal medicinal plant *Eremophila longifolia* displays an extensive variety of essential oil types. These range from those exhibiting exceptionally high oil yields to those yielding no oil at all. In the 1960's the reputation of *E. longifolia* as a medicinal plant, was sullied somewhat when essential oil analysis of a population in remote Western Australia revealed a high yielding phenylpropanoid rich oil (5.5% w/w of wet leaves), made up predominantly of safrole and methyl eugenol ether, both of which are suspected hepatotoxic carcinogens. Subsequent analysis since that time has revealed at least 4 other chemotypes in New South Wales: type A, made up of isomenthone/menthone with a yield of 4-7% of wet leaves ; type B, made up of karahanaenone with a yield of 0.5-5%; type C, made up of various monoterpenes and monoterpenols including borneol and fenchol, with a yield of 0.2-0.7%; and type D, made up of various monoterpenes such as α-pinene, sabinene and limonene, with a yield of 0.0-0.2%. Recently relative flow cytometric analysis, coupled with chromosome counts conducted earlier, has demonstrated that chemotypes with exceptionally high essential oil yields, such as the type A and B chemotypes in New South Wales, and the phenylpropanoid chemotype in Western Australia, are diploid whereas all others are tetraploid. Of particular interest, the higher yielding type B specimens, found in a significant geographically confined area, are diploid and the lower yielding specimens found elsewhere are tetraploid. We hypothesise that ploidy analysis is a significant area for further research in essential oil biosynthesis.

BV 62
BIOLOGICAL VARIABILITY OF HUNGARIAN WILD MARJORAM (*ORIGANUM VULGARE* L.) POPULATIONS

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Keywords: *diversity, genotype, environment, sesquiterpenes*

Chemical diversity of wild marjoram populations in Hungary was analysed for three years in natural habitats and in plantations. We aimed to evaluate the determining factors (genotype, environment) of the essential oil composition by comparing the samples of the same accessions collected in the wild and that of their established progeny populations.

We surveyed 9 wild populations in 5 counties of the northern part of Hungary, at the surroundings of 7 townships during the summer of 2010 and 2011. We established plantations from the propagation materials of these wild populations at the research farm of our Department in 2011 and sampled them as well in 2011 and 2012. All samples were collected in full flowering phenophase. The essential oil content was determined according to the Hungarian Pharmacopoeia (Ph.Hg.VII.) using a Clevenger type apparatus. GC-MS 6890 N gas-chromatograph was used to evaluate the essential oil composition.

The studied populations can be regarded as poor in essential oil content based on the results of all samplings. The composition of essential oil was similar in both years in the case of the wild populations and that of the plantations in 2011. Presence of acyclic compounds was variable and between 1.85 and 21.31 %. The three main constituents were sesquiterpenes: β-caryophyllene (9.96-28.58%), germacrene D (11.04-35.3%) and β-caryophyllene oxide (4.99-16.61%) accompanied in some cases (populations Fertőrákos and Felsőtárkány – Nagy-Közép-bérc) with higher ratio of sabinene and/or linalool.

In 2012 a clear shift towards the extreme high ratio of β-caryophyllene oxide (45.85-80.45%) were proved in all samples collected at the research field. This change was more specific in the case of population Felsőtárkány – Nagy-Közép-bérc, where the typically present (14.6-21.25%) acyclic monoterpenes disappeared. The difference experienced in the essential oil compositions of the plantation in 2012 proves the high influencing effect of the environmental factors. Although the genetically determined characteristic essential oil profile (e.g.: sesquiterpenes more than 30% (1)) remained in all samplings and for all accessions.

Data on essential oil content and composition confirm the infraspecific classification of Hungarian populations under the category of *Origanum vulgare* subsp. *vulgare*. Within the characteristic sesquiterpene-type of oil the ratio of β-caryophyllene oxide increased in all accessions due to the extreme dry summer of 2012.

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BV 63

TWO CHEMOTYPES OF MARJORAM (*ORIGANUM MAJORANA* L.) IN THE FLORA OF ANTALYA

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Keywords: marjoram, carvacrol, chemotype, diversity

Marjoram (*Origanum majorana* L.) is one of the important wild oregano species in Turkey. The most important characteristic of the species is high essential oil content and high carvacrol rate in the essential oil. It is intensely collected from the natural flora of Antalya province in Turkey and used for essential oil production. The aim of the study was to characterize *O. majorana* populations in terms of essential oil contents. For this aim, plant samples were collected from three different locations (Akseki, Gazipaşa and Alanya) of Antalya. Altitudes of the plant collection sites were 582 m, 1372 m and 1041 m, respectively. Then, three individual plants from each location were sampled for essential oil analyses. Essential oils were distilled from aerial parts of the plants using an micro distiller. Then, EOs were analysed by capillary GC and GC/MS using a Agilent GC-MSD system. According to the results, three plant samples from Akseki location gave 94.0%, 87.4%, 81.3 linalool and 1.8%, 1.1%, 5.1% carvacrol; Gazipaşa location gave 77.8%, 81.6%, 2.8% carvacrol and 0%, 0%, 93.1% linalool; Alanya location gave 97.6%, 97%, 96.2% linalool and 0.8%, 0.7%, 0.1% carvacrol. As it is seen, there are two distinct chemotypes called high carvacrol and high linalool types within the wild marjoram populations grown in the flora of Antalya.

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EFFECT OF DIFFERENT HABITAT AND HARVEST METHOD ON THE ESSENTIAL OIL CONTENT AND COMPOSITION IN THE COMMON JUNIPER (*JUNIPERUS COMMUIS* L.) CONE BERRIES

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Keywords: α-pinene, Cupressaceae, Sabinene

Common juniper is a coniferous evergreen shrub that belongs to the *Cupressaceae* family. It is native to Europe and occurs all over the Carpathian basin. The pharmacological effect of the juniper cone berries is well studied; however, the optimal and sustainable harvesting method is less evaluated. This brought us - within the frame of the Traditional and wild project – to observe the effect of different harvesting techniques on the quality parameters of juniper cone berries collected in two Hungarian populations. During our study the main goal was to analyse the effect of the different harvest techniques on the quantitative and qualitative parameters of the drug (yield, essential oil amount and composition). Samples were taken in November, 2012, in the Botanical Garden of Soroksár, and near to the village of Kunadacs by using three different harvesting techniques in three replications. The applied methods were the following: comb harvesting (1), branch hitting (2), berry crumbling (3). In each case the collection of the berries was done in 5 minutes. From the collected material alien plant parts were removed, then the berries were dried in room temperature. The dried plant material was hydrodistilled according to the description of the European Pharmacopoeia (Ph. Eur., VII.) in three replications. The oil composition was analysed by GC-MS. For the statistical evaluation Microsoft Office Excel 2007 was used.

Comb harvesting resulted in 53.4-139.4 g/5min, the hitting gave 138.4-571.1 g/5min and the crumbling produced 72.9-153.7 g/5min cone berries per tree. The second technology was characterised by significantly the highest results (p=0.006; SD 5%= 128.4 g/tree). Crumbling gave the lowest relative variance (26.4%), while the hitting produced to highest differences in the drug yield (CV%=56.4). According to our results hitting of the branches can give higher yields, however, because of the technique, the variance is relatively high. On the other hand crumbling is less productive, but at the same time the collected drug yields are more homogeneous.

The average essential oil content was 1.71±0.4 ml/100 g in the samples collected in Soroksár and 1.61±0.4 ml/100 g in those ones originated from Kunadacs. The maximum oil content was 2.28 ml/100g, the minimum 1.02 ml/100g, therefore each sample exceeded the minimum requirement of the 7th European Pharmacopoeia (1.00 ml/100g). Neither the different harvesting methods (p=0,561), nor the different natural habitats (p=0,612) influenced significantly the berries essential oil content. Therefore, this parameter should be neglected to choose the optimal harvesting technique, none of the applied collection techniques caused significant damage to the berries.

The essential oil composition was very variable. In the Kunadacs samples α-pinene was the main component (37.8-57.7%), significant difference were not between the trees (p=0.16). Notable minor component were the Sabinene (2.9-22.2%). In the Soroksár population from the three samples only in one was the α-pinene content higher as Sabinen. The heterogeneity was very dominant in this population, every sample was significantly different (p=0.0001). Therefore quality reason this parameter should be consider before collecting the raw materials.

Since further studies should be done in the future to give proper advice to the collectors our study will be continued also in 2013.

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THE AROMA CHARACTERISTIC OF DRIED PLANT EXTRACTS

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Keywords: volatiles, SPME/GC/MS, profile sensory analysis

Many crops are rich in active ingredients. These properties can be used to develop a technology and market of a completely new food, which would be designed for a specific healthy activities. Dried plant extracts obtained by aqueous extraction (from white mulberry, buckwheat hulls, yellow tea, artichoke herb, fenugreek seeds and nettle) rich in pure bioactive components are designed to enrich foods.

The aim of this study was aroma characteristic of prepared dried extracts.

The volatiles isolation was performed by SPME/DVB/CAR/PDMS, the identification of volatiles was done on GC/MS apparatus 5975C VL MSD equipped with a HP-5MS capillary column (Agilent Technologies) and NIST05 library.

In order to highlight the advantages and disadvantages of sensory attributes of the extracts the profile sensory analysis was conducted. The flavor descriptors were selected in several previous experiments according to the sensory international dictionary of terms (1). Results of the profile sensory analysis have been useful for selecting the groups of food products in which bioactive dried extracts may be used. The profiles of yellow tea extract described as tart, artichoke extract described as bitter, herbal and fenugreek extract as spicy, broth-like, had imposed some restrictions on the use of these materials.

Descriptors in sensory profile corresponded with some volatile compounds identified in plant extracts e.g. herbal attribute with ß-myrcene and ß-caryophyllene in artichoke extract.

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BV 66
GC/MS AND GC/O AROMA ANALYSIS OF CHICORY COFFEE COMPONENTS

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Keywords: chicory coffee, aroma, SPME/GC/MS, GC/O

Aroma attributes of chicory coffee is coming not only from the raw material, but most of all, from roasting process which leads to considerable changes in chemical composition of the product.

The aim of the study was to investigate the aroma characteristic of traditional raw materials (roasted chicory, barley, sugar beets roots) of chicory coffee.

The identification of volatile aroma compounds of roasted raw materials were performed by using solid phase microextraction/gas chromatography/mass spectrometry (SPME/GC/MS) by using DVB/CAR/PDMS fibre. GC-MS equipment was: GC 5975C VL MSD equipped with a HP-5MS capillary column (Agilent Technologies), the identification of the compounds was done by using NIST05 mass spectra library. Isolation of volatiles for GC/O analysis simultaneous distillation extraction method (SDE) in Likens-Nickerson apparatus was used. The determination of key aroma compounds was performed by olfactometry (GC/O) which was conducted on HP 5890 gas chromatograph with an inlet splitter and a smelling port with DB-5 column (Supelco). Separated fractions were smelled in successive double dilutions of analyzed distillates, until the last detectable aroma disappeared. In this way aroma was referred to respective retention indices and dilution factors (FD) for individual fractions.

In the aroma of roasted chicory, barley, ray and sugar beet 36, 30, 25, 15 compounds were identified, respectively. Therefore, roasted chicory has the most complex aroma, while the weakest one was detected in the case of sugar beet.

The GC/O analysis showed that the primary compound, responsible for the aroma in roasted chicory, was 2,6-dihydroxyacetophenone with a sweet, spicy fragrance, for which FD value was 512, followed by 2-ethyl-3-methylpyrazine and 2-ethyl-3,5-dimethylpyrazine. The roasted barley key compounds were 2-ethyl-3,5-dimethylpyrazine and maltol which had the highest FD value (256). The analysis of roasted rye distillate showed the most decisive compound with peanut-like aroma and FD 256 which was identified on the basis of retention time as 2-methyl-3,5-dimethylpyrazine.

The most intensive aroma component of roasted sugar beets was 5-methylfurfural; however, all components of roasted beet distillate showed very low FD, so it can be determined that roasted sugar beet root doesn't have a significant influence on the aroma of chicory coffee.

The knowledge about key volatiles of particular raw materials of chicory coffee may be useful in recipe preparation (composition of roasted ingredients).

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BV 67
ANALYSIS OF ESSENTIAL OILS IN HERBS GROWN UNDER THE EXPERIMENTAL CONDITIONS

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Keywords: experimental growing, fluorescence light, essential oils volatiles, enantiomers

The aroma of selected herbs (basil, melissa and dill) grown under experimental conditions were analyzed. All herbs were exposured to fluorescent light with different ratio of red (640nm) and blue (455nm) light. The growth took about 4 weeks.

The aim of the work was the estimation of essential oils components in relation to various irradiation conditions.

The essential oil content as well as enantiomeric ratio of some chiral compounds was determined. The volatile compounds of essential oils obtained by distillation in Deryng apparatus were identified using HP 6890 chromatograph with a FID detector, Rt-βDEXsm column (30 m x 0.32 mm x 0.25 μm). Separation conditions: carrier gas helium, flow rate 1 ml/min, programmed oven temperature: 40°C-200°C (2°C/min). Enantiomeric ratios for individual compounds were determined in percent on the basis of the area of their peaks, assuming the sum of the areas for both enantiomers as 100%.

It was observed that irradiation conditions to some extent influenced the plants growth (highest, leaf surface). The lowest growth intensity occurred at 40 and 50% blue light. Quality composition of essential oils was very similar in all experimental conditions and significant differences between samples with different exposure to light were not observed. However, in some cases e.g. in melissa, the highest concentration of volatile was noticed at 30% of blue light.

In melissa there were identified 17 compounds (the main was geranial), in basil there were 24 compounds with terpineol as dominated one and in dill only 13 compounds, mostly dill ether, benzealdehyde and carvone. These compounds are known from literature concerning essential oils of the same spices naturally growing. Enantiomeric ratio of chiral compounds did not depend on conditions of experiment.

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BV 68
CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *MICROLICIA CRENULATA*

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Keywords: Microlicia crenulata, antimicrobial activity, essential oil

The chemical compositions of essential oils extracted by hydrodistillation and of volatile substances extracted by HS-SPME from aerial parts of the Microlicia crenulata plant were determined by GC-MS and GC-FID. The antimicrobial activity of the essential oil was investigated. The essential oil of M. crenulata consisted of a complex mixture, consisting mainly of terpenoid compounds. The principal class of compounds was that of the oxygen-containing monoterpenes, especially trans-pinocarveol, ⊛ż-terpineol e myrtenal. The volatile compounds extracted from the plant by HS-SPME consisted principally of monoterpene hydrocarbons, the principal component being ⊛R-Pinene (61.65%). There was a slight variation in the yield and chemical composition of the essential oil according to the phenological stages of the year. The antibacterial activity was determined by the disk diffusion test against the Gram-positive strains (Bacillus cereus var. mycoides ATCC 11778 and Staphylococcus aureus ATCC 25923) and the Gram-negative bacteria (Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922). The antifungal susceptibility against Sacharomices cerevisiae, Aspergillus flavus and Aspergillus niger was evaluated by the broth microdilution assay according to the international standards established by the CLSI. ATCC microorganisms and clinically isolated organisms were used in the tests for determination of the MICs. The oil presented moderate inhibitory activity against P. aeruginosa (128 ⊛Eg/mL), but did not inhibit the growth of B. cereus, E, coli and S. aureus. The oil showed moderate antimicrobial activity against S. cerevisiae and A. flavus (250 to 500 ⊛Eg/mL), weak activity against A. niger (500 to 1000 ⊛Eg/mL) and was not active against species of Candida and Cryptococcus at the highest concentration tested (256 ⊛Eg mL-1). This study is the first to report the composition and biological properties of the essential oil from M. crenulata.

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VARIATION IN COMPOSITION OF THE ESSENTIAL OILS FROM *EREMANTHUS ERYTHROPAPPUS* (DC) MACLEISH WITH PLANT AGE

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Keywords: *Eremanthus erythropappus (DC) MacLeish, candeia, essential oil, biosynthesis*

The chemical composition of the essential oils from the stem, branch and flowers of the candeia plant [*Eremanthus erythropappus* (DC) MacLeish] were determined at different stages of their development. The samples of *E. erythropappus* were collected in the Rola Moça State Park near the city of Belo Horizonte, Minas Gerais, Brazil, at coordinates 20º 01' 39.74" S, 43º 50' 53.75" W, at an elevation of 1200 m above sea level. Plant materials were collected from individuals with trunk diameters of 7.5, 12.5 17.5 and 22.5 cm. This measurement was made at 1.5 m above the surface of the ground; each sample consisted of plant material from 10 individuals. The plant material – leaves, branches and trunk – were collected between July 27 and 31, 2009, between 7:00 and 10:30 a.m. The essential oils were extracted using a Clevenger apparatus. They were analyzed using a gas chromatograph coupled to a mass detector and by GC-FID. The identification of the constituents of the oil was performed using the NIST library and the Kovats index. The main biosynthetic route of the essential oil extracted from the trunk and branch was determined. We also found a significant level of α-bisabolol in the leaves of the candeia plant. The principal compounds in the trunk and branches were α-bisabolol and α-bisabolol oxido B. Those in the leaves were α-copaene, β-caryophyllene, germacreno D, δ-cadinene and elixene The general compositions of the essential oils extracted from the candeia branches, leaves and trunks did not change significantly throughout the stages of its development.

Acknowledgements: CNPq; CAPES, FAPEMIG

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VARIATION IN THE CHEMICAL COMPOSITION OF THE OIL FROM CULTIVATED *EREMANTHUS ERYTHROPAPPUS* ACCORDING TO THE EXTRACTION TIME

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Keywords: Eremanthus erythropappus, Asteraceace, Compositae, candeia, essential oil

The species *Eremanthus erythropappus*, Asteraceae, popularly known as "candeia" is native to Southeastern Brazil. It has great economic significance historically, being a major source of α-bisabolol, a component widely used in pharmaceuticals and cosmetic industries. It has been obtained from the native species through forest management. Currently, we seek to develop and evaluate studies that allow the planting of the candeia, both to meet the needs of the market, reducing the pressure on the native forests, and as an alternative for generating more income for small farmers.

The present study sought to determine the variation in the chemical composition according to the time of extraction of the essential oil from planted *E. erythropappus*. Samples were collected from the trunk of 64 nine-year-old trees from a plantation in the town of Carrancas, Minas Gerais, Brazil. The material was ground and the essential oil was extracted by hydrodistillation; the distillate was continuously collected at intervals of two hours, up to a total of eight hours of extraction. The chemical composition of the oil was determined by GC-MS. The compounds identified in all the samples at all the extraction times and in the highest concentrations were α-bisabolol oxide, α-bisabolol, eremanthin and spathulenol. The yields of α-bisabolol and spathulenol decreased with the time of extraction, but the yield of α-bisabolol oxide was lower in the first two hours than at the four-hour time interval. Its yield decreased in the intervals of four to six hours and six to eight hours. The yield of eremanthin (vanilosmim) was directly proportional to the time of extraction. The yield of the essential oil was 1.05%, based on the dry weight. It was also noted that the α-bisabolol was the major component (89.02% in 8 hours of extraction), and that, even in the range of six to eight hours of extraction, the yield (82.42%) was still considerable, although smaller than that obtained at the beginning of the distillation (93.83%, with two hours of extraction). This fact confirms the strong potential of the cultivated candeia.

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GC-AROMALYSER: A USEFUL TOOL FOR THE ROUTINE ANALYSIS OF ESSENTIAL OILS

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Keywords: gas chromatography, database, essential oils, retention index, LRI

GC-Aromalyser is a data collecting software with automated retention index calculation function, specially designed for the routine analysis of complex matrices such as essential oils, flavours and fragrances by dual channel capillary gas chromatography. The software is compatible with all the operating Windows systems (XP, Vista, 7) and collects the chromatographic data of the common manufacturers of chromatographs (Agilent, Perkin-Elmer, Shimadzu etc.).

Although the identification of essential oil constituents in gas chromatography is usually performed by mass spectrometry (1), many laboratories still use the retention time based identification of compounds, especially for standard applications where a certain chromatographic pattern is expected. However, the absolute retention time is not useful for peak identification, since it is influenced by several operational parameters (2). In order to overcome this limit, the retention index (RI) system has been introduced by measuring retention of compounds relative to a homologous series, usually of n-alkanes. The method is based on the formulas developed for isothermal conditions (Kováts Index, KI) as well as for column temperature programmes (Linear Retention Index, LRI)(3,4).

For the creation of a retention index database, pure chemicals, essential oils and fragrances of known composition have to be used. *GC-Aromalyser* automatically calculates the RI for every compound of a matrix of volatiles on polar and non-polar columns, and performs an automated library search by using an adjustable RI range (by default ± 5 units) and RI libraries linked to the characteristics of the used columns. Moreover, for standard applications, method-specific libraries with a predefined set of compounds can be established. An enhanced search algorithm that incorporates the RI filter in combination with an area range filter for each compound significantly increases the accuracy of peak identification.

GC-Aromalyser has been tested on a vast range of essential oils such as lavender, thyme, sage, coriander, fir and pine needle oil. Based on the LRI approach, the software permits a fully automated, fast and reliable identification of the main compounds of an essential oil of known composition, using only gas chromatographic data. In addition, the analytical chemist is provided with a set of useful tools for the quality control of essential oils, including automatic matching and comparison of chromatograms (sample against reference), conformity check of selected compounds against a given range of concentrations, automatic generation of reports. Furthermore, when run under similar analytical conditions, chromatograms recorded on different instruments and at different times can be easily compared.

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AN 72
CHEMICAL COMPOSITION AND VARIABLITY OF THE *SENECIO ANGULATUS* ESSENTIAL OILS

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Keywords: Senecio angulatus, essential oil, chemical variability, GC, GC/MS

The genus *Senecio* is one of the largest of the Asteraceae family that includes more than 1500 species all around the world (1). In Corsica Island, 10 species including 3 endemics are reported (2). Among them *S. angulatus* (creeping groundsel) are a perennial climbing plant 1-5 m high that bloom from October to March, native of South Africa and considerate as an invasive species. Only one study reports the composition of the plant solvent extract (3) in which angularine, a pyrolizidic alkaloid has been identified. However to our knowledge, no report deals with the essential oil chemical composition. The aim of the work was to investigate the chemical composition of *S. angulatus* essential oil using Gas Chromatography (GC-FID and GC-MS) and to characterize the intraspecies variations of essential oils from 25 Corsican sample locations. The study of chemical variability was performed using Principal Component Analysis (PCA) and Cluster Analysis (CA). Analysis of *S. angulatus* essential oils allowed the identification of 65 components accounting to 90.2-97.1 % of the total oils. Among them, the main components were α-pinene (6.1-17.6%), limonene (9.0-13.6%), viridiflorol (6.3-12.1%), germacrene-D (3.0-11.0%) and camphene (1.2-8.6%). Statistical analyses allowed distinguishing two main groups GI and GII according to the chemical composition of the essential oil samples and the locations of harvest. GI includes all the oil samples from the East Corsica in which viridiflorol was higher (10.9%-12.1%) and GII includes all the oil samples from the West Corsica in which viridiflorol was lower (6.3%-9.9%).This geographical distribution of *S. angulatus* specimens could be linked to the both spots of introduction on Corsica Island, the one from the Tyrrhenian Sea (East Coast) and the other from the Mediterranean Sea (West Coast).

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AN 73
MICROWAVE ASSISTED EXTRACTION OF ESSENTIAL OIL FROM STAR ANISE FRUITS (*ILLICIUM VERUM HOOK. F.*)

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Keywords: microwave assisted Soxhlet extraction (MASC), extraction, essential oil, Illicium verum Hook. f., star anise fruits, Doehlert matrices

The objective of this study is to determine and optimize microwave extraction parameters to obtain the best yield of essential oil. The influence of microwave power (W), irradiation time (h) and particle size (P) on the yield and composition of essential oil from *Illicium verum* Hook.f from Vietnam has been assessed by means of a new experimental design. The combined effects of these three factors on the yield of essential oil Y% (r.m) have been determined using Doehlert matrices in the range of 100-400 W for microwave power, 10-40 min for irradiation time and 0.4-2.0 mm for particle size. In these conditions, a second order polynomial relationship between Y% (r.m) and these factors has been established with regression coefficients close to 0.93.

The major effect of microwave power and particle size on yield has been highlighted. In the extraction conditions as follows - 325 W microwave power, 20 min irradiation time and 0.4 mm particle sizes - the highest essential oil yield by microwave assisted soxhlet extraction was 8.3%.

The chemical components α-pinene, β-mycrene and α-phellandrene were absent from the essential oil extracted from *Illicium verum* Hook. f. by use of MASE. (E)-anethole was the most important compound.

AN 74

ANALYSIS OF TURKISH ORANGE (*CITRUS SINENSIS* (L.) OSBECK) PEEL OILS OBTAINED BY CUPERCRITICAL CO_2 EXTRACTION, COLD PRESSING AND HYDRODESTILLATION METHOD

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Keywords: *Citrus sinensis (L.) Osbeck, orange peel oil, cold press, hydrodistillation, supercritical CO2 extraction, GC/MS*

Turkey is representing the 2.5% of total world citrus production and 48% of this production is orange. Orange essential oils used to give the aroma and flavor of orange to different kind of products like carbonated drinks, perfumes, cakes, ice-creams (1-2). Recently, Kirbaslar et al. reviewed cold-pressed orange peel oil studies in the literature (3-4).

The way of producing essential oils is important because it effects the composition of oil. There are some conventional ways to produce essential oils like cold pres and hydro distillation. However researchers still working on new technologies for producing more valuable essential oils (5). In our study Turkish sweet orange peel essential oil produced by hydrodistillation, cold-pressing and supercritical CO_2 extraction techniques in purpose of to see the difference between production methods of essential oils.

The volatile constituents of essential oil was determined by GC/MS analysis and 48 compounds identified. Identification of individual constituents was based on comparison of their GC Retention indices (RI) on the apolar column determined relative to the retention time of a homolog series of n-alkanes (C_8-C_{18}) with linear interpolation, with those of authentic compounds and computer matching with the MS database [National Institute of Standards and Technology (NIST) and Wiley libraries] commercial spectral libraries of the GC/MS, as well as by the comparison of the fragmentation pattern in the mass spectra with those reported by Adams (6). Main component groups which are monoterpene hydrocarbon, sesquiterpene hydrocarbon and oxygenated compounds were found in CP products as 97. 77%, 0.33% and 1.80%, whereas they are in SFE products 97.32 %, 0.50% and 2.12% and for HD products 98.21%, 0.05%, 1.44% respectively. The major monoterpenes of the orange oils were found limonene (93.99, 94.16, 95.26 %), myrcene (1.57- 1.48 , 1.71%) and sabinene (0.85 - 0.73, 0.41%). The major sesquiterpene component was Z-β-farnecene (0.12, 0.12, 0.05%). The major oxygenated components of the oils were found; aldehyde components; decanal(0.35,0.40, 0.31 %), geranial (0.12, 0.13, 0.11%), alcohol component; linalool (0.25, 0.50, 0.37%), and esther components; geranyl acetate (0.09, 0.09, 0.09%) and neryl acetate (0.06, 0.06, 0.06%).

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AN 75
THERMAL VARIATION OF THE AROMA PROFILE OF MYRRH

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Keywords: thermal variation, thermal instability, myrrh, myrrh-like odor, (E) beta-ocimene

We examined the thermal variation of the characteristic odor of myrrh. NMR analysis showed that the constituents of the natural material and its hexane extract were thermally unstable; this thermal instability caused the constituents and odor to change over time. Myrrh was extracted with hexane, leaving the constituents of the extract unaltered. The hexane extract was used to determine the initial odor characteristics of myrrh. The main constituent—(*E*)-beta-ocimene (group A)—and the other constituents (group B) were separated from this oil by bulb-to-bulb distillation. The constituents and odors in both groups were analyzed over time. The characteristic odor of the oil depended on the (*E*)-beta-ocimene content which contributed to the fresh odor of myrrh. The fresh odor of the oil decreased gradually. The group B constituents had a myrrh-like odor similar to the odor of original material. Six main components were isolated from group B, all of which were terpenes with similar molecular structures: alpha-santalene, alpha-bergamotene, (*Z*)-alpha-bisabolene, (*E*)-alpha-santalal, alpha-photosantalol, and camphernol.

Gas chromatography-olfactometry analysis showed that the odors of these compounds were not myrrh-like individually, indicating that all the group B compounds were essential for producing the myrrh-like odor. We demonstrated that the aroma profile of myrrh depended on the thermal instability of (*E*)-beta-ocimene and the combination of the six thermally stable terpenes.

AN 76
INVESTIGATION OF COMPOSITION OF GRAPEFRUIT PEEL OIL OBTAINED BY SUPERCRITICAL CO_2 EXTRACTION, COLD PRESSING AND HYDRODISTILLATION METHODS

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Keywords: grapefruit oil, chemical composition, limonene, neryl acetate, geranyl acetate

Grapefruit oil is a valuable ingredient in foods and beverages, as well as perfumery and cosmetic product. Several reviews comparing the volatile chemical composition of grape fruit and other citrus oils can be found in literature (1-3).

The Star Ruby grapefruit variety (*Citrus paradisi* Macfayden) peel oil was obtained supercritical CO_2 extraction (SFE), cold-pressing (CP) and hydrodistillation (HD) methods. The grapefruit samples used in the present study were planted at Batı Akdeniz Tarımsal Araştırma Enstitüsü "BATEM" located at Antalya, South Turkey, in december 2010.

The chemical compositions of the three different peel oil samples were analyzed by gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS). 35 components were identified by mass spectra (4), linear retention indices . Grapefruit oil has high content of monoterpene hydrocarbons (95.55, 95.95 and 96.17 %) with limonene (91.12, 91.75 and 91.79 %) myrcene (2.44, 2.56 and 2.72 %), α-pinene (1.08, 0.77 and 0.77 %) and β-pinene (0.66, 0.64 and 0.65 %) being the major monoterpene hydrocarbons in the SFE, CP and HD methods, respectively. The major sesquiterpene hydrocarbons (1.20, 1.24 and 0.74 %) were (E)-caryophyllene (0.38, 0.36 and 0.24 %) and δ-cadinene (0.24, 0.26 and 0.24 %) in the SFE, CP and HD oils, respectively. The major oxygenated components (3.04, 2.77 and 2.91 %) of the oils were found as the following carbonyl components (1.76, 1.38 and 1.40 %): decanal (0.59, 0.63 and 0.75 %) and octanal (0.31, 0.32 and 0.23 %); alcohol components (0.61, 0.60 and 0.88 %): linalool (0.36, 0.35 and 0.47 %) and α-terpineol (0.19, 0.19 and 0.25 %); ester components (0.46, 0.58 and 0.52 %): neryl acetate (0.33, 0.35 and 0.35 %) and geranyl acetate (0.13, 0.23 and 0.17 %) in the SFE, CP and HD oils, respectively.

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AN 77
FAST GAS CHROMATOGRAPHY COMBINED WITH A HIGH-SPEED TRIPLE QUADRUPOLE MASS SPECTROMETER FOR THE ANALYSIS OF UNKNOWN AND TARGET CITRUS ESSENTIAL OIL VOLATILES

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Keywords: fast gas chromatography, triple quadrupole mass spectrometry, food analysis, Citrus essential oil

The present work is focused on the off-line combination of high performance liquid chromatography (HPLC) and comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry (GC×GC-quadMS), and its application to the detailed qualitative analysis of of two genuine *Citrus* essential oils, bergamot and sweet orange. Specifically, a silica column was exploited for the separation of the essential oil constituents in two groups, namely hydrocarbon and oxygenated compounds. After, each HPLC-fraction was reduced in volume, and then subjected to cryogenically-modulated GC×GC-quadMS analysis. The volatiles were separated on a normal-phase GC×GC column train set, and identified through database matching and linear retention index information. Two analyses were performed for monoterpenes and sesquiterpenes, higher injection volume for the second family allowed the detection of trace sesquiterpenes, without the monoterpene interferences. The concentrated HPLC fractions gave origin to unexpectably-crowded chromatograms, due to two fundamental GC×GC characteristics, namely the enhanced separation power and sensitivity. The results attained were particularly stimulating with regards to the oxygenated compounds, namely those constituents which contribute most to the essential oil aroma, and are of more use for the evaluation of quality and genuineness. The present research description is focused on the evaluation of a high-speed triple quadrupole mass spectrometer, carried out under moderately-fast gas chromatography conditions (analysis time: 17 min). The mass spectrometric system is capable of operation under high speed GC conditions, in both full-scan (maximum scan speed: 20,000 amu/sec) and multiple reaction monitoring modalities. Furthermore, the triple quadrupole system can generate full scan and multiple reaction monitoring data simultaneously, also in a very rapid manner.

A fast method was developed for the: I) qualitative analysis of untargeted *Citrus* essential oil compounds, and II) the quali/quantitative analysis of targeted ones, namely three preservatives (*o*-phenyl phenol, butylated hydroxytoluene, butylated hydroxyanisole). The mass spectrometric system generated a more-than-sufficient number of data points/peak for both identification and quantification purposes. The level of sensitivity, reached through the multiple-reaction-monitoring side of the analysis, widely exceeded the requirements of current legislation. Method validation, related to the targeted analysis, was performed considering precision of retention times, peak areas and ion ratios; limits of detection/quantification, and accuracy were also measured.

AN 78

A NEW POWERFUL APPROACH FOR HIGHLY-DETAILED ESSENTIAL OIL ANALYSIS: THE OFF-LINE COMBINATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Keywords: comprehensive two-dimensional gas chromatography, quadrupole mass spectrometry, Citrus essential oil

The present work is focused on the off-line combination of high performance liquid chromatography (HPLC) and comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry (GC×GC-quadMS), and its application to the detailed qualitative analysis of of two genuine *Citrus* essential oils, bergamot and sweet orange. Specifically, a silica column was exploited for the separation of the essential oil constituents in two groups, namely hydrocarbon and oxygenated compounds. After, each HPLC-fraction was reduced in volume, and then subjected to cryogenically-modulated GC×GC-quadMS analysis. The volatiles were separated on a normal-phase GC×GC column train set, and identified through database matching and linear retention index information. Two analyses were performed for monoterpenes and sesquiterpenes, higher injection volume for the second family allowed the detection of trace sesquiterpenes, without the monoterpene interferences. The concentrated HPLC fractions gave origin to unexpectably-crowded chromatograms, due to two fundamental GC×GC characteristics, namely the enhanced separation power and sensitivity. The results attained were particularly stimulating with regards to the oxygenated compounds, namely those constituents which contribute most to the essential oil aroma, and are of more use for the evaluation of quality and genuineness.

AN 79
QUALITATIVE AND QUANTITATIVE ANALYSIS OF TUNISIAN *ROSA* SPP. ESSENTIAL OILS BY MEANS OF GAS CHROMATOGRAPHY (GC-FID) AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

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Keywords: Rosa canina L., Rosa sempervirens L., Tunisia, GC-MS, retention index, chemical variability

Rosa canina L. is a spiny shrub which grows in the wild and produces fleshy red fruits. Dried fruits, but also roots and leaves, are used in therapy as diuretic, antinflammatory, antiallergic and antioxidant (for the high content of vitamin C). Very few reports are present in literature about *R. sempervirens* L., which is a shrub with white flowers and fleshy fruits [1].

Objectives of the present work were to determine the chemical variability: i) interspecific level; ii) intraspecific level, among different parts of the plants; iii) among different geographic origins. The volatile extracts were isolated from leaves and stems by either hydrodistillation or liquid-liquid extraction. GC-MS analyses were performed with a high sensitive quadrupole-mass spectrometer, which allows for high-speed scanning (up to 20,000 *u/s*). The identification process was based on: i) mass spectral matching; ii) retention index matching. In particular, the two steps were merged in only one by using a dedicated GC-MS library, along with a very versatile and interactive software. The library lists reference spectra from the flavor and fragrance field, each provided with retention index, that is used interactively by the software in order to filter the list of candidates produced by the searching process [2].

Major compounds were of *R. sempervirens* were caryophyllene oxide (10.4%), terpinen-4-ol (8.0%) and germacrene D (7.08%). In *R. canina* we found: linalool (7.34%), caryophyllene oxide (20.9%) and α-terpineol (1.9%).

Considerations were made about the compositional differences caused by the variables investigated.

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BV 80

TRADITIONAL AND WILD: FAIRWILD STANDARD FOR THE SUSTAINABLE WILD COLLECTION AND TRADE OF PLANTS IN CENTRAL EUROPE

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Keywords: wild plants, traditional knowledge, sustainable harvesting, FairWild Standard

Wild medicinal and aromatic plants (MAPs) are important for their intrinsic biodiversity value, their contribution to rural incomes, and use in local and global healthcare and industries. Unfortunately, many of the estimated 60,000 MAP species used globally are threatened by overharvesting and trade. In Central Europe, traditional knowledge relating to collection of wild plants passed down from generation to generation, started being lost in the 20th century, owing to urbanization and changes in land ownership and lifestyle. A project *Traditional and Wild*, which aims to prevent the disappearance of this historical wild collection knowledge and help improve the livelihoods of vulnerable wild-collectors groups in rural parts of Central Europe runs between 2011-2014. The project includes academic and research institutions, local authorities, chamber of commerce, and NGOs from Hungary, Czech Republic, Slovenia and Poland.

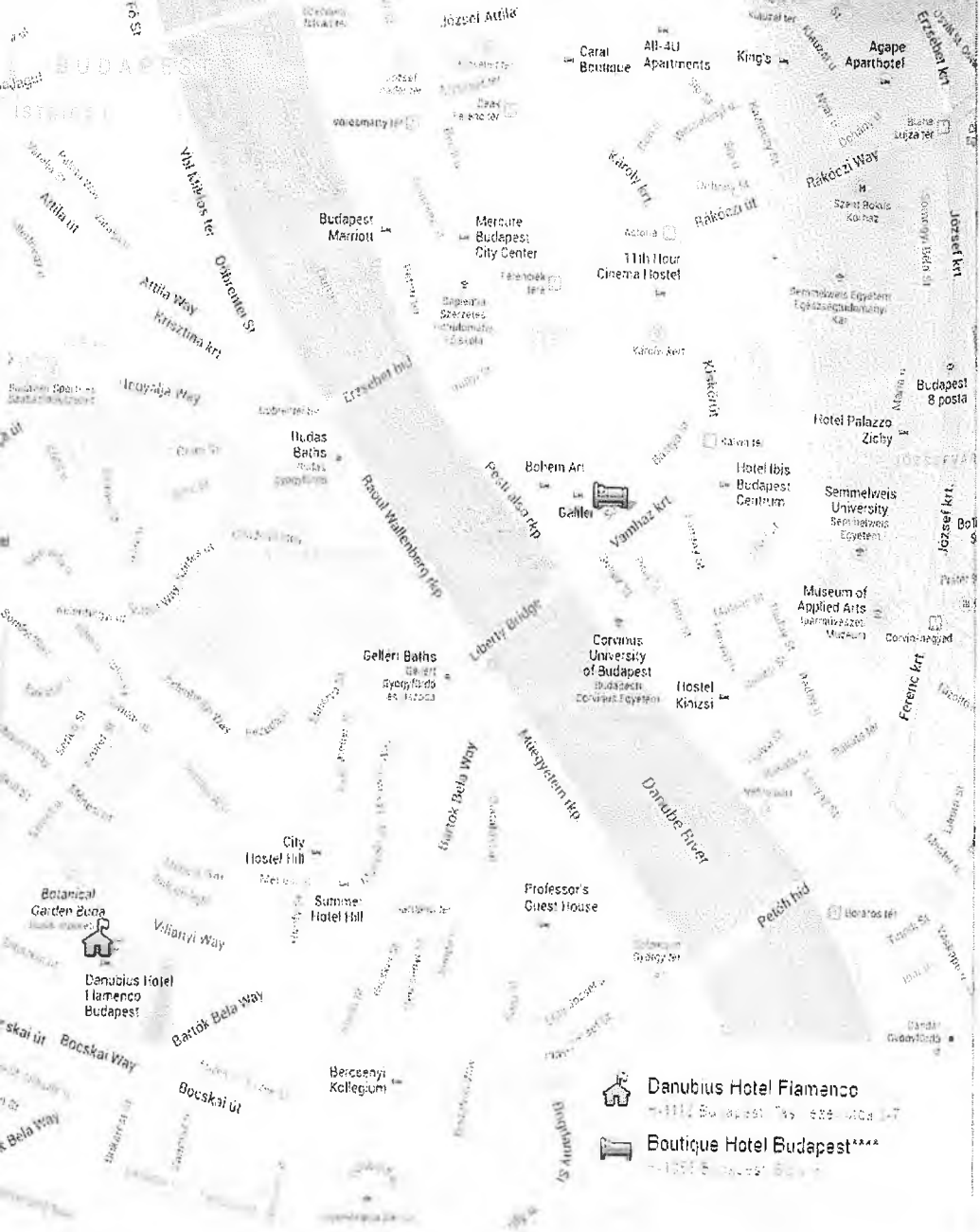
Traditional and wild focuses on the sustainable harvest of and trade in wild plants in four aforementioned countries through the implementation of the FairWild Standard, the best practice standard for the sustainable harvesting and fair trade in wild collected plants. The purpose of the FairWild Standard is to ensure the continued use and long-term survival of wild species and populations in their habitats, while respecting the traditions and cultures, and supporting the livelihoods of all stakeholders, in particular collectors and workers, ensuring fair conditions of labour. While the main purpose of the FairWild Standard is to provide verifiable set of principles to companies involved in harvesting and trade of wild MAPs, it can also support the development of government policies, and community resource management strategies. Applicable to industry, the FairWild Standard applies to wild plant collection companies wishing to demonstrate their commitment to sustainable collection, social responsibility and fair trade principles.

Collection and processing of *Traditional and wild* target plants, that include species producing essential oils (e.g. Juniper *Juniperus communis*) growing in the project implementation areas in Hungary (Felső-Kiskunság and and Ormánság regions), Poland (Podkarpackie province), Czech Republic (South Moravia) and Slovenia (Kozjansko and Dravinjsko area and Northern Primorska area) is developed following the sustainability principles of the FairWild Standard.

The application of the FairWild Standard is relevant to essential oils supply chains and production sector, as proportion of raw materials are sourced from the wild. The application of the Standard through the supply chain allows ensuring quality and traceability of the sourcing in addition to the sustainability of supply, and social sustainability of the company. A number of essential oil products are FairWild certified, including the Frankincense essential oils produced from harvested resin of *Commiphora confusa* and *Boswellia neglecta* in Kenya, resulting in the product by a UK cosmetics company.

Ecological and social sustainability of wild plants harvesting and trade supports the development of sustainable productive sector. FairWild Standard is the best practice tool applicable to the wild-harvesting companies, including the essential oils sector, openning opportunities for sustainable operations.

AUTHORS' INDEX



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