43rd ISEO

International Symposium on Essential Oils

5 to 8 September 2012
Lisbon – Portugal

CBV | Centro de Biotecnologia Vegetal
PBRG | Plant Biotechnology Research Group
INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING

iseo2012.fc.ul.pt

5 to 8 September 2012
Lisboa • Portugal

Program, Book of Abstracts and Participants List

Organized by

Prof. Dr Ana Cristina Figueiredo
Prof. Dr José Gonçalves Barroso
Prof. Dr Luis Gaspar Pedro

43rd International Symposium on Essential Oils (ISEO2012)

Faculty of Sciences of Lisbon, 5 to 8 September 2012, Lisbon, Portugal

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and Participants List

Editors
Ana Cristina Figueiredo
José Gonçalves Barroso
Luis Gaspar Pedro

Publisher
Centro de Biotecnologia Vegetal - Fundação da Faculdade de Ciências de Lisboa

Printer
Europress

Cover
In9

Legal deposit: 347181/12

ISBN: 978-989-20-3188-0

Additional copies can be obtained from the Editors at Faculdade de Ciências de Lisboa, C2, Piso 1, Campo Grande, 1749-016 Lisboa, Portugal

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Welcome Addresses

Welcome

On behalf of the *Faculdade de Ciências da Universidade de Lisboa* I am quite pleased to welcome you to our University and to the *43rd International Symposium on Essential Oils*.

This is the second time that such an important meeting takes place in Portugal at our facilities. I believe that is a clear indication that happy memories remain from the first occasion and we are very honoured for hosting you again. Also, I want to congratulate the organizers of the Symposium, specially my colleagues Ana Cristina Figueiredo, José Gonçalves Barroso and Luis Gaspar Pedro who made a great effort for putting things together, timely and efficiently.

Looking at your scientific programme, I realize that you planned an intense activity with several plenary lectures and working sessions for oral communications and posters presentations. Hence, I anticipate four days of fruitful scientific exchange but plenty of social contact among all the participants. In particular, I hope that you still have some spare time to visit our beautiful city and to enjoy the almost mythical light of Lisbon.

I wish you all a very pleasant stay in Portugal.

Prof. Dr. José Manuel Pinto Paixão
Dean of the *Faculdade de Ciências da Universidade de Lisboa*

Welcome

As Director of the *Laboratório Associado Institute for Biotechnology and Bioengineering (IBB)*, a network of research units in Portugal, in which the Plant Biotechnology Research Group (Centro de Biotecnologia Vegetal, CBV), that organizes this event, is integrated, I am delighted to welcome you all to Lisbon to contribute and attend the *43rd International Symposium on Essential Oils* (ISEO2012).

The organization of the ISEO2012, is not only timely and relevant, but also of utmost importance for both the CBV and to IBB in view of the increasing importance of this research field in Portugal and abroad. The scientific program of this meeting promises compelling science in the field of essential oils as well as in related fields, namely ecology, odour perception, biological activities, among others. The outstanding level of the invited speakers as well as of many oral and poster contributions warrants the success of the Symposium.

I would like to express my hearty greetings and thanks to scientists and researchers, coming from all around the world, who brought to this venue their know-how and the result of years of studies, to share with the scientific community, the general public, and the media.

I am certain that the ISEO2012 is the right forum for further consolidation and development of the medicinal and aromatic plants field, in general, and essential oils and volatile compounds in particular.

Despite the tight schedule of the scientific program, I hope you can find out some time to enjoy the social and cultural wealth and allurements of our beautiful city of Lisbon.

I therefore renew my welcome, and wish you all a pleasant and fruitful stay.

Prof. Dr Joaquim M. Sampaio Cabral
The Director of the *Laboratório Associado Institute for Biotechnology and Bioengineering (IBB)*

Acknowledgements

The *43rd International Symposium on Essential Oils* was generously sponsored by:

- Faculdade de Ciências da Universidade de Lisboa
- Centro de Biotecnologia Vegetal (CBV), Institute for Biotechnology and Bioengineering (IBB)
- International Federation of Essential Oils and Aroma Trades (IFEAT)
- Fundação Calouste Gulbenkian
- Estética Viva
- John Wiley & Sons Limited
- Milestone
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- ILC-Instrumentos de Laboratório e Científicos, Lda
- Fundação para a Ciência e a Tecnologia (FCT) under CBV / IBB plurianual funding and research contract PTDC/AGR-CFL/117026/2010

The generous obligingness of the following entities is gratefully acknowledged:

- Bacardi Martini Portugal, Lda
- Ach. Brito & C.ª, S.A.
- Quinta Picos do Couto Lda
- Gorreana Chás
- Universidade de Lisboa
- Câmara Municipal de Lisboa
- Planalto Dourado
- Associação Portuguesa de Horticultura

Gratitude is given to all those that contributed to make this event public:

- Centro de Informática da Faculdade de Ciências da Universidade de Lisboa
- Gabinete de Comunicação, Imagem e Cultura da Faculdade de Ciências da Universidade de Lisboa
- Câmara Municipal de Lisboa
- Estética Viva
- Associação Portuguesa de Horticultura
- John Wiley & Sons Limited

ISEO2012 Organizing Committee deeply acknowledges the Young Scientists Fellowships Selection Committee for the excellent and invaluable involvement in the evaluation of Young Scientists CVs and abstracts contributions. Gratitude is also given to the ISEO permanent Scientific Committee and ISEO2012 Scientific Committee for their commitment.

Young Students Fellowships

Thanks to the support of the *International Federation of Essential Oils and Aroma Trades* (IFEAT) (14) together with the ISEO2012 Organizing Committee (6), a total of twenty *Registration Fellowships* to assist young researchers to attend ISEO2012, could be available.

The Young Scientists ISEO2012 *Registration Fellowship* Selection Committee has accepted 37 out of the 40 application proposals. Candidates from seventeen countries (Algeria, Brazil, China, Czech Republic, Germany, Iran, Italy, Japan, Poland, Portugal, Romania, Serbia, South Africa, Spain, The Netherlands, USA and Yemen) submitted an appropriate application.

In total 20 high quality abstracts and applicants CVs were recomended by the Selection Committee as *Registration Fellowship* award winners. Due to the tight symposium schedule not all works could be presented as oral communications. Thus, of the selected 20 contributions, 14 were set as poster presentations. The remaining 6 were chosen for oral presentations, in a special session devoted to Young Scientists ISEO2012.

The *Registration Fellowship* award consists of the student registration fee reimbursement (€265). The awardees will also be entitled to a certificate stating their prize and the awardees names will be listed in the ISEO2012 book of abstracts.

During the conference, the Fellowships Selection Committee will announce and reimburse the awardees with the pecuniary prize (€265) and prize certificate. The first author of the selected 20 contributions will receive the award during the special session devoted to Young Scientists ISEO2012. Six awardees will additionally be offered the Symposium dinner, supported by *John Wiley & Sons*, *Milestone*, *Taylor & Francis* and *Estética Viva*.

- The International Federation of Essential Oils and Aroma Trades (IFEAT) and the Organizing Committee supported the Registration fees of the following students:

Andrea Occhipinti - University of Turin, Italy.
Filomena Silva - Universidade da Beira Interior, Portugal.
Maxleene Sandasi - Tshwane University of Technology, South Africa.
Patrícia Costa - Universidade do Algarve, Portugal.
Makiko Kitao - Kinki University, Japan.
Rachel Thompson - University of Alabama in Huntsville, USA.
Jakub Smid - Czech University of Life Sciences, Czech Republic.
Jorge Faria - Faculdade de Ciências da Universidade de Lisboa, Portugal.
Nikola Stojanovic - University of Niš, Serbia.
Adela Frankova - Czech University of Life Sciences, Czech Republic.
Amel Bouzabata - University of Corsica, France.
Satoshi Asada - Meijo University, Japan.
Inés Méndez Tovar - Agricultural Technical Intitute of Castilla y León, Spain.
Jennifer Bufalo - Universidade Estadual Paulista, Brazil.
Michelle Cristiane Bufalo - Universidade Estadual Paulista, Brazil.
Beata Zawitowska - Nicolaus Copernicus University, Poland.
Agustina Nurcahyanti - Heidelberg University, Germany.
Miljana Đorđević - University of Niš, Serbia.
Prabodh Satyal - University of Alabama in Huntsville, USA.
Ana Miltojevic - University of Niš, Serbia.

Organization

ISEO2012 Organizing Committee

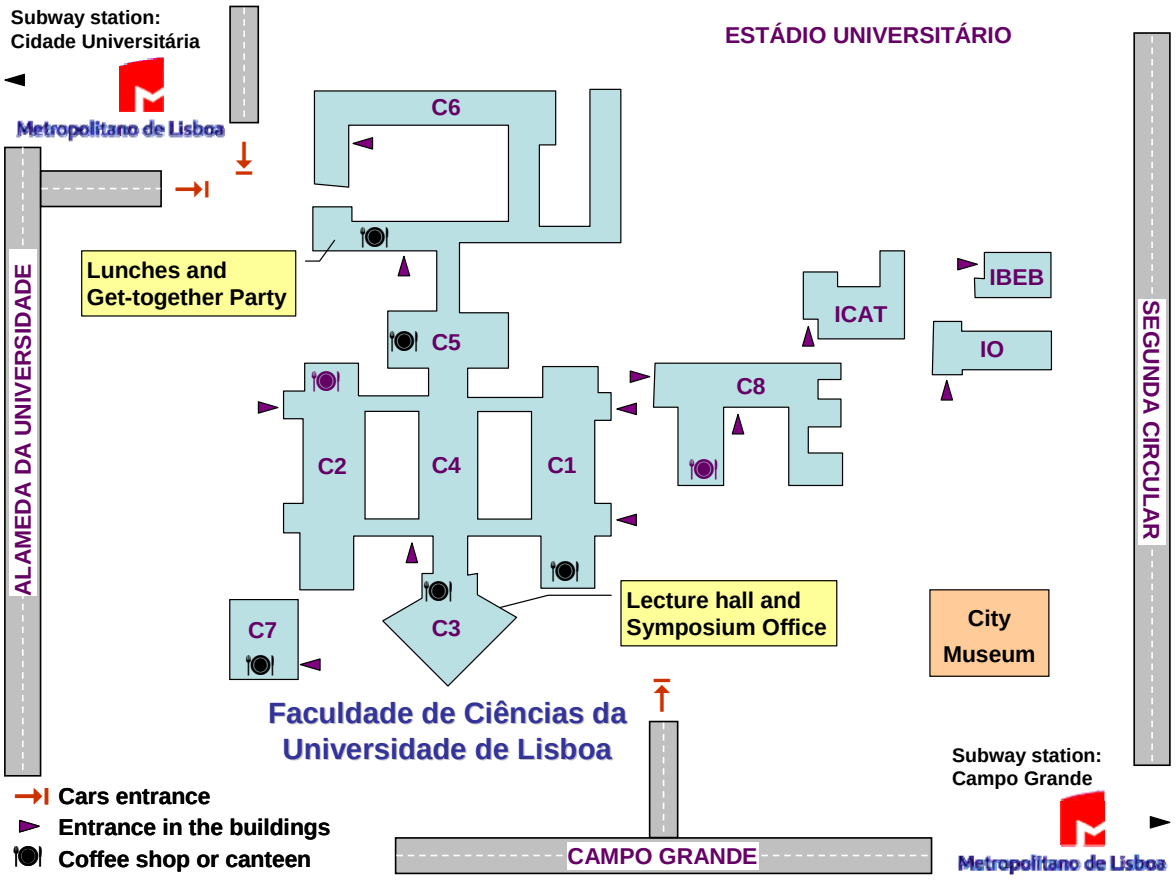
Prof. Dr Ana Cristina Figueiredo
Prof. Dr José Gonçalves Barroso
Prof. Dr Luis Gaspar Pedro

ISEO2012 Scientific Committee

Prof. Dr Ana Cristina Figueiredo
Prof. Dr Carlos Cavaleiro
Prof. Dr Hans Scheffer
Prof. Dr Helena Trindade
Prof. Dr José Gonçalves Barroso
Prof. Dr Lígia Salgueiro
Prof. Dr Luis Gaspar Pedro

Symposium Venue

The Faculty of Sciences of Lisbon hosts the *43rd International Symposium on Essential Oils*.



WWW Information

http://iseo2012.fc.ul.pt/

International Symposium on Essential Oils (ISEO) Permanent Scientific Committee

Dr Agnieszka Ludwiczuk
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Prof. Dr Alvaro Viljoen
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General Information

Symposium Office

The Symposium Office will be located at the Foyer of the Lecture Halls in the building C3 of the Faculty of Sciences of Lisbon.

Registration and Opening Hours:

Wednesday, September 5:	14:00 – 19:00
Thursday, September 6 and Friday, September 7:	09:00 – 18:00
Saturday, September 8:	09:00 – 13:00

Symposium Language

English will be the official language of the ISEO2012. There will be no simultaneous translation.

Badges

Participants are requested to wear their Symposium badges for identification and admittance to Scientific and Social venues. Symposium badges are required at the lunch's canteen.

Plenary Lectures and Oral Communications

The Plenary Lectures are limited to 50 min, Oral Communications to 10 min, all with plus 5 min for discussion. A room for presentation preview will be available. MS-Power Point presentation facilities are available. Presentation files should be handed at the Symposium Office, in the Foyer of the Lecture Halls, with the name of the presenting author at least 1h prior to the beginning of the respective session.

Posters

All posters will remain exhibited throughout the symposium and the authors are recommended to be present at their posters during posters sessions.

Two poster sessions will be scheduled, odd numbers on Thursday and even numbers on Friday.

Poster boards measuring 90 cm wide and 120 cm height will be available for poster presentation with portrait orientation and A0 size (84.1 cm X 118.9 cm). The Registration Office provides fixation facilities.

Meals

Two lunches, of Thursday, September 6, and Friday, September 7, 2012 are included in the registration fee of regular and student participants.

The lunches will be served at the C6 building Canteen (information on location provided at *Symposium Venue* section).

The Canteen serves meals from 12:00 to 14:00. Personal badges are required at the Canteen. Accompanying persons lunch vouchers can be purchased at the Symposium Office. Neither the Canteen nor the Campus coffee shops accept foreign currency.

There are also several restaurants, snack bars and coffee shops in the area around the University Campus.

Currency

All payments should be done in Euro.

Means of conveyance to the Symposium venue

796 - 778 - 777 - 768 - 767 - 764 - 755 - 747 - 738 - 735 - 701 - 207 - 108 - 36

Cidade Universitária - Campo Grande

Insurance / Liability

The Organizers are in no respect responsible for any accommodation problem, nor for any accident, injury, theft, loss and property damage to any person during the Symposium.

Scientific Program

Wednesday, September 5, 2012

All lectures, the poster presentations and the symposium office will be located at the building C3 of the Faculty of Sciences of Lisbon (information on location provided at *Symposium Venue* section).

The Get-together party will be held at the building C6 of the Faculty of Sciences of Lisbon (information on location provided at *Symposium Venue* section).

14.00 - 19.00	**Registration at the Symposium Office**	Foyer of the Lecture Halls, C3
17.00 - 17.45	**Opening Ceremony** ***Welcome Addresses*** Performance of the *Vicentuna*	Main Lecture Hall
18.00 - 19.00	**Plenary Lecture** Chair: Prof. Dr Karl-Heinz Kubeczka ***Umami and Kokumi: latest developments in taste perception*** *Dr Chris Winkel*, Givaudan, Flavour Ingredients Research, the Netherlands	Main Lecture Hall
19.00 - 21.00	**Get-together Party**	C6 building canteen

Thursday, September 6, 2012

09.00 – 10.00	**Plenary Lecture** Chairs: Prof. Dr Johannes Scheffer & Prof. Dr Lígia Salgueiro ***The "invisible hand" of floral volatiles in pollination ecology: dosage, context and signal evolution*** *Prof. Dr Robert Raguso*, Cornell University, USA	Main Lecture Hall
10.00 – 10.15	**Oral Communications** ***Essential oils as allelochemicals – the mode of action and their potential use as bio-herbicides*** *Dr Nativ Dudai*, Division of Aromatic and Medicinal Plants, Israel	Main Lecture Hall
10.15 – 11.00	**Coffee Break**	Foyer of the Lecture Halls
11.00 – 12.00	**Plenary Lecture** Chair: Prof. Dr Agnieszka Ludwiczuk ***Ecological role of essential oils and volatiles in plants*** *Prof. Dr Anna-Karin Borg-Karlson*, KTH, Stockholm	Main Lecture Hall
12.00 – 14.00	**Lunch**	C6 Canteen/Local Restaurants
14.00 – 15.00	**Plenary Lecture** Chair: Prof. Dr Chlodwig Franz ***Aroma compounds in tomato flavour*** *Prof. Dr Denise Teman*, University of Florida, USA	Main Lecture Hall
15.00 – 16.00	**Oral Communications** Chairs: Prof. Dr Patrizia Rubiolo & Prof. Dr Stanislaw Lochynski	Main Lecture Hall
15.00 – 15.15	***Qualitative and quantitative analysis of Vetiver oils by comprehensive two-dimensional Gas-Chromatography*** *Dr Jean-Jacques Filippi*, Université de Nice-Sophia Antipolis, France	
15.15 – 15.30	***Dealing with phthalic-acid ester (PAE) residues in raw materials and compounded flavourings*** *Dr Thierry Cachet*, International Organization of the Flavor Industry (IOFI), Belgium	
15.30 – 15.45	***Kinetics of isolation of essential oils from plants*** *Dr Helena Sovova*, ASCR, Czech Republic	
16.00 – 17.00	**Coffee Break**	Foyer of the Lecture Halls
16.00 – 19.00	**Poster Session (Odd Numbers)**	Foyers of the Lecture Halls

Friday, September 7, 2012

09.00 – 10.00	**Plenary Lecture**	Main Lecture Hall
	Chairs: Prof. Dr Helena Trindade & Prof. Dr Eva Nemeth	
	The molecular mechanisms of essential oil composition *Prof. Dr Jörg Degenhardt*, Martin Luther University Halle-Wittenberg, Germany	
10.00 – 10.15	**Oral Communications**	Main Lecture Hall
	Structural factors in the odor of α-santalol derivatives *Dr Toshio Hasegawa*, Saitama University, Japan	
10.15 – 11.00	**Coffee Break**	Foyer of the Lecture Halls
11.00 – 12.00	**Plenary Lecture**	Main Lecture Hall
	Chair: Prof. Dr Carlo Bicchi	
	Chemical ecology of essential oils: analytical methods and biological activity *Prof. Dr Alěs Svatǒs*, Max Planck Institute, Germany	
12.00 – 14.00	**Lunch**	C6 Canteen/Local Restaurants
14.00 – 15.00	**Plenary Lecture**	Main Lecture Hall
	Chair: Prof. Dr Jan Karlsen	
	Are essential oils natural products? *Dr Daniel Joulain*, SCBZ, France	
15.00 – 16.30	**Young Scietistis Symposium**	Main Lecture Hall
	Chairs: Prof. Dr Alvaro Viljoen and Dr Jan Demyttenaere	
15.00 – 15.10	***Effects of PSBS gene silencing by RNAi on volatile compound biosynthesis and accumulation in tomato (Solanum lycopersicum)*** *Dr Andrea Occhipinti*, University of Turin, Italy	
15.15 – 15.25	***Coriander oil antimicrobial activity: A flow cytometric study*** *Dr Filomena Silva*, CICS-UBI, Portugal	
15.30 – 15.40	***Application of vibrational spectroscopy and chemometrics as a rapid and low cost alternative in the quality assessment of indigenous South African essential oils*** *Dr Maxleene Sandasi*, Tshwane University of Technology, South Africa	
15.45 – 15.55	***Effect of hydroxypropyl-β-cyclodextrins on the antioxidant activity of Lavandula essential oils*** *Dr Patrícia Costa*, University of Algarve, Portugal	
16.00 – 16.10	***Classification of Lavender essential oils for the evaluation of sedative effects on Human*** *Dr Makiko Kitao*, Kinki University, Japan	
16.15 – 16.25	***Essential oils do not inhibit Escherichia coli peptidyl-tRNA hydrolase*** *Dr Rachel Thompson*, University of Alabama in Huntsville, USA	
16.30 – 17.30	**Coffee Break**	Foyer of the Lecture Halls
17.00 - 19.00	**Poster Session (Even Numbers)**	Foyers of the Lecture Halls
19.30 – 23.00	**Symposium Dinner**	*Casa do Leão*, at *Castelo de S. Jorge*

Saturday, September 8, 2012

09.00 – 10.00	**Plenary Lecture**	Main Lecture Hall
	Chairs: Prof. Dr Gerhard Buchbauer & Prof. Dr Paola Dugo	
	Propolis volatiles: chemical diversity and biological activity *Prof. Dr Vassya Bankova*, Institute of Organic Chemistry, Bulgaria	
10.00 – 10.15	**Oral Communications**	Main Lecture Hall
	Cryptic speciation in liverworts: Conocephalum conicum, Reboulia hemisphaerica and Marchantia polymorpha *Prof. Dr Agnieszka Ludwiczuk*, Medical University of Lublin, Poland	
10.15 – 11.00	**Coffee Break**	Foyer of the Lecture Halls

11.00 – 12.00	**Oral Communications**	Main Lecture Hall
	Chairs: Prof. Dr Yoshinori Asakawa & Prof. Dr Carlos Cavaleiro	
11.00 – 11.15	***Synergistic biological activity and chemodiversity of Himalayan medicinal plants from Nepal*** *Dr Prabodh Satyal*, University of Alabama in Huntsville, USA	
10.15 – 11.30	***Screening of essential oils for inhibitory effects on the activity of BACE-1, the β-secretase involved in Alzheimer's disease*** *Dr Rita Videira*, University of Coimbra, Portugal	
10.30 – 11.45	***Portuguese Lavenders as a source of anti-inflammatory drugs*** *Dr Mónica Zuzarte*, University of Coimbra, Portugal	
12.00 – 13.00	**Closing Ceremony**	Main Lecture Hall
	Performance of the *Vicentuna*	

Social Program

Get-together Party

Wednesday – 5 September

The Get-together Party is included in the registration fee. The Get-together party will be held at the building C6 of the Faculty of Sciences of Lisbon (information on location provided at *Symposium Venue* section).

Symposium Dinner

Friday – 7 September

The Symposium dinner will take place at *Casa do Leão*, located at *Castelo de S. Jorge*, downtown Lisbon.

The Symposium dinner is not included in the registration fee, and participants wishing to join it may register to take part in it at the Symposium Office.

Tours

The Secretariat makes available different tours programs. For Tours booking, contact the ISEO2012 Secretariat.

Plenary Lectures Abstracts

PL 1. Umami and Kokumi: latest developments in taste perception

Winkel C

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Chris.winkel@givaudan.com

Keywords: Umami, Kokumi, Taste

Since health has become one of the main drivers in nutrition, the scientific interest in taste perception is high. Academia as well as food and flavour companies aim to make food more healthy but not less appetizing. A good example is salt (NaCl) reduction. Salt has a pleasant taste but too much sodium is considered unhealthy. Salt reduction per se will therefore lead to less attractive food. Currently there is no good replacer for sodium chloride. To compensate for the loss in salty taste, umami taste and kokumi taste can help.

This review will give a general overview on savoury taste with a focus on umami taste, kokumi taste and syneristic effects of odour and taste molecules.

Umami is now generally accepted to be a primary taste and the umami receptor is known. Many new molecules that have umami taste or umami enhancing properties have been discovered.

Kokumi is a much less defined taste property. Kokumi has been linked to mouthfeelness, complexity and authenticity. Only recently the first papers dealing with this effect have been published

Synergy of odour and taste is known for some time for sweet tastants. Recently this effect has been studied for savoury tastants as well.

PL 2. The "invisible hand" of floral volatiles in pollination ecology: dosage, context and signal evolution

Raguso RA[1], Moré M[2]; Svensson G[3]

[1]*Cornell University, USA;*
[2]*Universidad Nacional de Córdoba, Argentina;*
[3]*Lund University, Sweden*

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Keywords: Bioassays, Deception, Floral scent, Foraging, Mutualism

Floral volatiles have long been appreciated as pollinator attractants in specialized tropical plants or those engaged in obligate mutualisms (e.g. figs with fig wasps). In contrast, generalized plant-pollinator interactions more commonly studied in ecological or agricultural contexts typically are considered visually-mediated phenomena, with scant reference to floral chemistry. Here I discuss the evidence challenging this perception, focusing on how context determines the ecological functions of plant volatiles. I will use common floral volatiles – 2-phenylethanol (2PE), dimethyl disulfide (DMDS) and (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) – to illustrate my thesis.

Volatiles were analyzed using static (SPME) or dynamic headspace collection coupled with GC-MS, and behavioural bioassays monitoring pollinator responses to flowers whose odours were manipulated using extracts, emittors or traps.

2PE is common in diurnal wildflowers pollinated by bees. As a low dosage pollen odour, 2PE attracts andrenid bees to *Fragaria* flowers (1), but at higher dosage in floral nectar, it repels ants and limits bumblebee visits to *Polemonium* flowers, reducing nectar thievery and optimizing selection on floral shape (2). DMDS is a universal component of deceptive flowers that mimic carrion. When present in diurnal, temperate flowers or boreal mosses, it attracts flies that disperse the plants' pollen or spores (3, 4). However, when DMDS is emitted by nocturnal, nectar rich flowers of Neotropical vines, it attracts bats as pollinators (5). Finally, DMNT is a common herbivore-induced vegetative volatile in maize, cotton and other crop plants, attracting parasitic wasps as an indirect defense. However, DMNT is the primary floral attractant in two specialized systems, a South American bee-pollinated orchid (6) and an obligate North American yucca-yucca moth interaction (7). Concentration, timing and community context determine the functional roles of these three common floral volatiles.

1. TL Ashman et al. (2005) Ecology 86: 2099-2105.
2. C Galen et al. (2011) Am. Nat. 177: 258-272.
3. M Moré et al. (2012) Ann. Bot. (in review).
4. P Marino et al. (2009) Symbiosis 47: 61-76.
5. von Helversen et al. (2000) J. Comp. Physiol. (A) 186: 143-153.
6. A Wiemer et al. (2009) Plant Biology 11: 506-514.
7. Svensson et al. (2011) Oikos 120: 1577–1583.

PL 3. Ecological role of essential oils and volatiles in plants

Borg-Karlson A-K

KTH, School of Chemical Science and Engineering, Department of Chemistry, Ecological Chemistry group, 100 44 Stockholm

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Keywords: Volatiles, Terpenoids, Chirality, Behavior

Essential oils are important and sustainable sources for bioactive compounds, especially for those with complex molecular structures that are difficult or expensive to synthesize. Plants compounds are often used for control of insects and microorganisms as they function as repellents, antifeedants, inhibitors, bactericides, and fungicides (1). Some of these compounds have more than one biological function and can in specific cases attract and/ or repel various insects. Attacking insects or microorganisms induce the biosynthesis of the plant which then starts to produce more of specific compounds or synthesize new which function as physical and biological barriers towards the infestations (2). A large number of the plant compounds are chiral and the importance of knowing the different function of the enantiomers will be exemplified (4,5,6). Recent findings and ideas of how to utilize essential oils for controlling insects and other organisms will be discussed.

1. A. Sofrata, et al. (2011) Plos One 11
2. T. Zhao, et al. (2011) Oecologia: Volume 167, Issue 3, Page 691-699
3. K. Pålsson. Et al. (2008) J Med Entom. 45,1,88-93(6)
4. A.-K.-Borg-Karlson, et al. (2003) J.Chem. Ecol. 29,1,1-14.
5. M. Stranden, et al. (2002) Chemical Senses. 27:2,142-152
6. A. Wibe, et al. (1998) J. Chem Ecol. 24, 2

PL 4. Aroma compounds in tomato flavour

Tieman DM, Klee HJ

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Keywords: Tomato, *Solanum lycopersicum*, Solanaceae, Aroma, Volatiles

Flavour of modern tomato varieties is generally considered to need improvement. Older heirloom varieties are considered to have better flavour, but do not have the agronomic properties for commercial production. Tomato flavour is a result of interactions between sugars, acids and aroma compounds. Analysis of heirloom tomato aroma volatiles indicated that these varieties have surprising diversity in aroma compound levels. This diversity allows us to determine the aroma volatiles important for good tasting tomatoes. Traditional methods have focused on aroma compounds that are above the threshold of detection by the human olfactory system; however, this method does not account for interactions between compounds in the mouth and nose. We have analyzed the biochemical components of flavour in over 60 heirloom varieties. Correlations between aroma compound levels and ratings from consumer taste panels have allowed us to define the components of a good tasting tomato. Some aroma volatiles that were identified as important for tomato flavour using the odour threshold method are shown to not contribute to tomato flavour using these methods. In addition, we have identified volatiles that add to the perceived sweetness of tomato fruit. Using this approach we can identify targets for breeding better tasting tomatoes.

PL 5. The molecular mechanisms of essential oil composition

Degenhardt J, Schimmel J, Krause S

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Keywords: Thyme, Chemotype regulation, Terpene biosynthesis

Essential oils are usually complex mixtures of monoterpenes, sesquiterpenes and simple phenylpropanoids. The economic value of an essential oil depends on its composition, especially the presence of compounds that are pharmaceutically active, antimicrobial, or impart a desired scent or flavour. On the other hand, compounds with toxic and irritant properties or undesired flavours need to be absent from the oil. For many centuries, selection and classical breeding of essential oil plants have been used to optimize oil production. However, some components of the oils are difficult to separate from each other and the essential oil yield of a plant is hard to raise. In recent years, the biosynthesis of essential oils has been studied with molecular and biochemical methods. These efforts provide a better understanding of the biosynthesis of the essential oils and can reveal new strategies for the breeding of high-value plants.

Common thyme (*Thymus vulgaris*) is an excellent system to study the biosynthesis of essential oils and its regulation. Thyme forms several chemotypes which are morphologically identical but differ in the terpene content of their essential oils. Each chemotype is characterized by its dominant monoterpene alcohol, which can be geraniol, alpha-terpineol, sabinene hydrate, linalool, carvacrol, or thymol. Two of these monoterpenes, the phenolic monoterpene alcohols thymol and carvacrol, are especially valuable due to their antibacterial, antiseptic and spasmolytical effects. In all chemotypes, we characterized the key enzymes of terpene biosynthesis, the class of terpene synthases. Since most of these terpene synthases form multiple products, relatively few enzymes are sufficient to generate the complex terpene blends of each of the chemotypes. While each chemotype appears to have a complete set of terpene synthase genes coded in its genome, only the genes necessary for the chemotype-specific terpenes are activated. The terpene synthase TPS1 is expressed in the carvacrol and thymol types. The major product of TPS1 is γ-terpinene, which can be converted to carvacrol and thymol. We identified a family of cytochrome P450 monooxygenases that might catalyse this conversion *in planta*. Elucidation of the biosynthetic pathways and the enzymes involved in the biosynthesis of essential oil components allows developing molecular markers for directed breeding efforts to optimize oil production.

PL 6. Chemical ecology of essential oils: analytical methods and biological activity

Svatoš A

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Keywords: Structure elucidation, Mass spectrometry, Volatiles, Imaging

Essential oils (EO) are mixtures of individual compounds which are typically found in terpenoid skeletons; EOs have widespread industrial application. However, in nature, plants produce terpenoids for their own protection, survival, and reproduction [1]. To understand the biochemical processes behind EO production, first the chemical structures of these mixtures need to be identified. Due to its high sensitivity, modern mass spectrometry (MS) is an important tool for the structural elucidation of EOs. Additionally, nuclear magnetic resonance (NMR) is used to refine stereochemistry of the compounds.

In this lecture I will describe theoretical and practical aspects of the MS of terpenoids, placing specific emphasis on their biosynthesis and biological activity. Typically, EO mixtures are separated by gas-phase liquid chromatography, and the eluted peaks are analyzed by electron-ionization mass spectrometry (EI-MS) using databases of MS spectra [2]. However, the mass spectra of EO components are highly similar and more detailed analysis is typically needed to identify the compound. Additional chemical reactions (hydrogenation, ozonization, methylthiolation) should be performed to obtain additional structural information [3,4]. Very recently, a new computer algorithm ("fragmentation trees" [5]) was developed to assist researchers in high-throughput structure elucidation.

Dramatic developments in ambient MS technology now allow for the rapid sampling of compounds emitted from plant surfaces. Our current interest is to follow emission of compounds from individual cells in plants' organs. This method, called MS imaging, has already increased our understanding of the spatial distribution of non-volatile compounds [6,7] and of the function of these molecules in the chemical ecology of plants.

Acknowledgments: Financial and technical support from Max Planck Society is acknowledged with pleasure. Work of current and previous members of our group and of co-operating partners' is highly recognized and appreciated.

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PL 7. Are essential oils natural products?

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Keywords: Essential oils, Naturalness

During the last two decades, mainly by the vector of the communication on the Internet, we observed a considerable tendency to promote essential oils as beneficial natural products in many aspects of well-being, and in various domains such as cosmetic ingredients, aromatherapy, “aromachology”, various more or less defined therapeutic applications, and foods, to cite only a few.

In considering on the one hand the strict definition of essential oils according to the ISO standard, and on the other hand the accepted and/or enforced definitions of natural products in various countries, one can observe a disparity of judgments, and often obvious inaccuracies, which account for public ignorance, and even of some professional or official organizations.

Regardless, the relevance of these more or less supported claims in relation with their alleged benefits, one can wonder whether essential oils as complex chemicals, are entirely constituted of the same substances which exist naturally in the plant raw material from which they are derived. As a result, one may question the relevance of their labelling as “natural” or “100% natural” or “100% pure and natural” and so on. In contrast, products obtained by other techniques often recover more faithfully the bulk of constituents originally present in the plant material, but are often deprived of the “natural´´ label by certain governmental or private organizations.

Examples will be presented to illustrate these considerations, with the aim of correcting inaccurate declarations or exaggerated claims.

PL 8. Propolis volatiles: chemical diversity and biological activity

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Keywords: Propolis, Essential oils, Biological activity, Plant origin

Propolis (bee glue) is a sticky material collected by bees from plants, and serves dual purposes in the hive: as building material and as defensive substance. It has been used as a remedy in Europe since ancient times for treatment of wounds and burns, sore throat, etc. Nowadays, propolis use in over-the-counter preparations, “bio”-cosmetics and functional foods and for other purposes continues to increase.

Modern science has confirmed the antimicrobial action of bee glue and has discovered many other beneficial pharmacological properties: immunostimulating, antitumor, anti-inflammatory, antiulcer, local anaesthetic, hepatoprotective, antioxidant. Volatile compounds are found in low concentrations in propolis, but their aroma and significant biological activity make them of importance for the characterisation of propolis. As bee glue is a plant derived product, its chemical composition is highly variable and depends on the local flora at the side of collection and on the choice of propolis plant sources made by bees. For this reason it offers a significant chemical diversity and the study of propolis from different locations often reveals new constituents and new bioactive compounds. The available data about chemical composition of propolis essential oils from different geographic regions is reviewed. The role of propolis volatiles in identification of its plant origin is also discussed. The contribution of essential oils and their constituents to the diverse biological activities of propolis is considered. Propolis essential oils have demonstrated antibacterial, antifungal, antiprotozoan activity, they were found to inhibit the proliferation of cancer cells by inducing apoptosis and to have immunostimulating effect in humans, etc. Future perspectives in research on propolis essential oils are outlined.

Oral Communications Abstracts

OC 1. Essential oils as allelochemicals – the mode of action and their potential use as bio-herbicides

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Keywords: Essential oils, Monoterpenes, Allelopathy, Allelochemicals

Allelopathic activity of aromatic plants has been described frequently. Essential oils inhibit germination and growth of various plants. Residues of leaves of aromatic plants in soil inhibit the growth and the emergence of seedlings. We have developed a good quantitative assay to test for the inhibitory activity of volatiles on seed germination and have screened dozens of essential oils and their components. The major active allelochemicals were found to be aldehydic and ketonic monoterpenes, having a α-β unsaturated double bond, such as citral, (geranial and neral) and pulegone. Exposure of seeds for 4h to the compounds was enough to cause inhibition of the rate of germination and of seedling development in wheat, *Amaranthus palmeri* and *Brassica nigra*. Using histochemistry, we observed that citral is absorbed by wheat seed through the abscission layer, and that it reaches highest concentration in the embryo, where it accumulated in the aleurone, scutellum and parts of the endosperm. After exposure of wheat seeds to pure components of essential oils, GC-MS analysis of extracts of the seeds showed the presence of new products in the endosperm and embryo. These were probably formed by the metabolic detoxification of the inhibitors by the seed. Detoxification occurs in both embryo and endosperm during the first day of imbibition. In further work it was found that microtubules of Arabidopsis or wheat seedlings were disrupted within minutes after exposure to very small concentrations of some monoterpenes in the gaseous phase, whereas actin filaments remained intact. In field studies, in which known amounts of residues of aromatic plants were mixed with soil in which wheat seeds sown, some components of the essential oil and their derivatives were found in the seed embryo and endosperm. The agro-technology aspects will be discussed and further work of formulation and application of essential oils as bio-herbicides will be present.

OC 2. Qualitative and quantitative analysis of *Vetiver* oils by comprehensive two-dimensional Gas-Chromatography

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Keywords: Vetiver; GCxGC; quantitative analysis

Vetiver oil is among the main basic ingredients of every modern perfumer [1]. However, vetiver oil has been lately incorporated in western fragrances, although being known for centuries in India. In the 1960's, many fragrance manufacturers started to include vetiver in perfume compositions and from that time, the worldwide production has increased so as to reach ~300-400t/year nowadays [2]. Since more than 200 constituents have already been described in the literature, mostly consisting of sesquiterpenoid derivatives, vetiver oil is actually considered as one of the most complex essential oils [3]. Indeed, such a complexity has always been a barrier to the fast and direct quantitative analysis. We thus aimed at using GC×GC (with both FID and MS detection) as the main tool for the identification and quantitation of vetiver oils constituents.

The correct understanding of 1D- and 2D-chromatographic data is the first step of an analytical process leading to the identification of odour impact compounds in vetiver extracts.

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OC 3. Dealing with phthalic-acid ester (PAE) residues in raw materials and compounded flavourings

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Keywords: Phthalic-acid esters, Phthalates, DEHP, Flavourings, Contaminants

Phthalic-acid esters (PAE, often referred to simply as “phthalates”) are a group of diesters of ortho-phthalic acid.

PAE have become ubiquitous environmental contaminants due to leaching from their widespread applications as plasticisers to soften polyvinyl chloride (PVC) and other widely-used polymeric materials. However, except for the use of diethyl phthalate which is accepted as a carrier for fragrances for use in cosmetics and personal care products, there is NO authorized use of PAE in flavour and fragrance raw materials and compounded flavourings. The illegal use of di-(2-ethylhexyl)phthalate (DEHP) in specific food flavourings in Taiwan uncovered early 2011 has triggered a major food supply chain issue in Asia with massive food recalls, particularly in the P.R. China.

As a consequence of the adulteration issue the China Health Ministry set limits on the presence of DEHP, di-isononyl phthalate (DINP) and di-n-butyl phthalate (DBP) of 1.5 mg/kg (DEHP), 9.0 mg/kg (DINP) and 0.3 mg/kg (DBP) in food and food additives, with no distinction between final food and food ingredients, including flavourings. These limits have subsequently been qualified by another rule specifying that food flavourings, in which the total sum of phthalates does not exceed 60 mg/kg, are permitted for use in production, for sale and applications.

IOFI reacted to this issue by initiating several projects. One project focused on reviewing the presence of PAE, and in particular of DEHP, in food from the use of flavourings. The objective of this effort was to be able to present evidence that the contribution of DEHP to the final food that may be contributed by the flavourings is negligible compared to overall human exposure. The results of this review will be presented.

In another project the IOFI Working Group on Methods of Analysis (WGMA) reviewed the extensive literature on the analysis of PAE in food and food components. From this review the WGMA has developed a set of recommendations for the analysis of PAE in flavourings. These recommendations were used for initiating additional method evaluation work to detect PAE in spiked and unspiked essential oils and extracts with the support of a large number of flavour industry analytical laboratories. Initial results of the method evaluation will be provided and discussed along with recommendations for the reduction of PAE residues in raw materials from natural and synthetic sources and in compounded flavourings.

OC 4. Kinetics of isolation of essential oils from plants

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Keywords: Essential oils, Distillation, Extraction, Kinetics, Plant microstructure

The rate of isolation of essential oils from plants depends on the method used, on operation conditions and on the plant pre-treatment. From chemical engineering point of view, the rate of the process is determined by phase equilibrium, mass transfer resistances, and flow pattern, whatever method is used – steam distillation, hydro-distillation [1], solvent extraction, supercritical fluid extraction [2] or the extraction with subcritical water.

The aim of this contribution was to compare these processes on the basis of their kinetic parameters and with respect to microstructure of plant materials, as significant progress was made recently in modelling the extraction of essential oils from different types of glands visualised by scanning electron microscopy [3–6].

Kinetic data and mathematical models are taken from the literature on different types of distillation and extraction techniques. It is often necessary to distinguish between the easily accessible oil and the oil captured in a low-permeable tissue or to take into account loss of volatile substances during the plant pre-treatment in order to explain different yields of essential oil obtained using different techniques.

The results show how the extraction or distillation rate depends on equilibrium and mass transfer parameters, being controlled first by equilibrium and then by mass transfer resistance. It is also shown that the parameters of geometry and microstructure of a given plant material determined for one of these separation techniques are applicable in the models for other separation techniques so that a prediction of extraction or distillation rate is possible.

Acknowledgements This work was supported by Technology Agency of the Czech Republic (project TA01010578).

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OC 5. Structural factors in the odor of α-santalol derivatives

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Keywords: α-Santalol, Structure–odour relation, *E/Z*-isomers, Derivatives

α-Santalol and β-santalol are the main constituents that give sandalwood (*Santalum album* L.) its distinctive woody odour. Other santalol derivatives with a formyl group have been identified as minor components that notably contribute to the sandalwood fragrance [1][2]. Here, we examine how structural factors affect the odour of α-santalol derivatives by comparing the odour of their *E/Z*-isomers and saturated analogues. α-Santalol is a sesquiterpene with a polycyclic moiety and a hydroxyl group. These parts were replaced with other structures, and we compared the odours of the *E/Z*-isomers and their saturated analogue for the prepared derivatives. We have previously studied the structure–odour relations of α-santalols bearing various functional groups (hydroxyl, formyl, formyloxy, and acetoxy), and found a similarity in odour between the *Z*-isomer and its saturated analogue [3]. Next we selected α-santalols with a benzyl group in place of a hydroxyl group. Many benzyl compounds have strong characteristic odours, suggesting that a benzyl group should strongly affect the odor of these compounds. We found similar odours between the *E*-isomer and its saturated analogue, in contrast to the case of an α-santalol derivative with a hydroxyl, formyl, formyloxy, or acetoxy group. In addition, we investigated the influence of the bulky polycyclic moiety on the odor of the derivatives. The odour was evaluated when the polycyclic moiety was replaced with a linear alkyl chain. The polycyclic moiety was found to be the most important structural factor in the characteristic sandalwood odour. Through the synthesis of derivatives and the evaluation of their odour, we could identify key structural factors in α-santalol odour.

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OC 6. Cryptic speciation in liverworts: *Conocephalum conicum*, *Reboulia hemisphaerica* and *Marchantia polymorpha*

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Keywords: Volatile components, Liverworts, Cryptic speciation, Chemotypes, GC/MS technique

Cryptic speciation is a concept that has been adopted along with the use of molecular methods in studies of intraspecific phylogenies. It refers to cases where two species or populations are clearly genetically differentiated, but show little or no morphological differences. Cryptic species have been also identified in several liverworts, and these may be either allopatric or sympatric [1,2].

The aim of this work was to apply GC/MS technique as a simple method in identification of cryptic species in liverworts. The volatile components of different collections of three liverwort species; *Conocephalum conicum*, *Reboulia hemisphaerica*, and *Marchantia polymorpha* were analyzed. On the basis of GC/MS data, all analyzed *C. conicum* samples can be divided into 5 chemical groups, which correspond to 5 cryptic species found with use of isozymes and psbA sequencing [3]. The formerly single species, *Reboulia hemisphaerica*, recently was subdivided into several subspecies [4]. The analysis of the volatiles of the Japanese collections of this liverwort shows the occurrence of two very distinct chemotypes. One chemotype is characterized by the presence of cyclomyltaylane and chamigrane sesquiterpenoids, and the second one produces mainly gymnomitranes. Studies of enzyme polymorphism of the European colonies of *Marchantia polymorpha* indicated the presence of three subspecies, which can be distinguished mainly on the basis of isozyme and ecological preferences [5]. On the basis of the chemical composition of Tahitian *M. polymorpha*, this liverwort can be divided into 4 chemotypes. Each of the chemotype is characterized by the presence of their own peculiar components, but all of them are identical from morphological point of view.

In conclusion, the analyzed liverwort species are characterized by high diversity of lipophilic terpenoids, especially sesquiterpenoids, and these secondary metabolites can assist in taxonomic differentiation and identification of cryptic species within one liverwort species.

Acknowledgments: The authors gratefully acknowledge Prof. I.J. Odrzykoski (Poland) for C. conicum samples and Prof. P. Raharivelomanana and Prof. J.-P. Bianchini (French Polynesia) for M. polymorpha collections.

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OC 7. Synergistic biological activity and chemodiversity of Himalayan medicinal plants from Nepal

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Keywords: Essential oil, Himalayan range, Biological activity, Chemotypes, natural medicines, Volatile chemicals

Nepal is a small country, located between two emerging giants of the Asian continent: India and China. However, like the Himalayan range, Nepal´s biodiversity, a mosaic of species, is an unmatched giant in its own right. In coordination with a botanist and a chemist from Tribhuvan University in Nepal, and the Natural Products Group at University of Alabama in Huntsville, around 100 essential oil samples were collected and further analyzed using GC-MS for chemical composition as well as tested for biological activity including cytotoxicity, antimicrobial, brine shrimp lethality, allelopathy, larvicidal, and insecticidal activity. In addition, separate testing of essential oil components was conducted to determine if any single component was responsible for the biological activity recorded in our samples. The results could not attribute any one compound responsible for the observed activities in the samples and we therefore conclude the activities to be a result of synergistic effect amongst the components in the samples.

Aside from biological testing, the result of our systematic and detailed analyses about plant chemical composition has revealed new chemotaxonomic divisions for some of the different species. Plant chemotypes are not only dependent on genetic difference between species but vary among geographical and climatic conditions. This was studied in some cases through comparison of oil compositions of the same species which were collected from different locations.

In conclusion, through international collaboration this project was undertaken with the sole goal of identifying potential biologically active essential oils in Nepalese plants. However our efforts have also translated into a wider understanding of chemodiversity and species differentiation. Through our research we believe that the Himalayan range has the potential to be one of the most important sources for alternate natural medicine.

Acknowledgments: We would also like to express our gratitude to Tilak Gautam for identification of plants and to Dr. Bernhard Vogler for technical assistance.

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OC 8. Screening of essential oils for inhibitory effects on the activity of BACE-1, the β-secretase involved in Alzheimer's disease

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Keywords: BACE-1; cell-permeable inhibitors; *Lavandula luisieri*; essential oil; Alzheimer's disease

One of the most important features in Alzheimer's disease is the generation and deposition of neurotoxic β-amyloid peptide (Aβ) outside and around brain neurons. The inhibition of BACE-1, a key enzyme in Aβ formation, is pointed as a promising therapeutic alternative for this disease. A crucial attribute for the *in vivo* effectiveness of BACE-1 inhibitors is the ability to crossover blood-brain barrier to reach brain targets. Pharmacokinetic theory points hydrophobicity and low molecular weight as advantageous attributes of drug candidates considering cellular membranes and barriers crossover. Recognizing such physicochemical feature in the ordinary components of essential oils we undertook a screening of essential oils for their ability to inhibit recombinant proBACE-1.

Among dozens of essential oils tested, the oil from *Lavandula luisieri* was signalized as inhibiting the enzyme (IC50 ≈120μg.ml^{-1}), showing an effect comparable to those of standard inhibitors used for research purposes. Moreover, the oil of *L. luisieri* was tested on the endogenous BACE-1 in cultured CHO cells, being responsible for a reduction in Aβ production (EC50 ≈ 86μg.ml^{-1}) with no significant toxicity. The composition of the oil was established by combination of GC and GC-MS data, with the identification of approximately 100 constituents, representing 88.4% of the composition. It is mainly composed of oxygen-containing monoterpenes (62.3%), including 33.0% of necrodane skeleton compounds.

Results prove the effectiveness of the essential oil of *L. luisieri* as inhibitor of BACE-1 activity, both in enzymatic and cellular assays, and supported the bioguided fractioning of the oil towards the isolation of a new BACE-1 inhibitor.

Acknowledgements: Authors are grateful to Fundação para a Ciência e Tecnologia (FCT), POCI 2010/FEDER for financial support. R. Videira was founded through the PhD grant SFRH / BD / 19199 / 2004.

OC 9. Portuguese Lavenders as a source of anti-inflammatory drugs

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Keywords: *Lavandula* spp., Essential oils, Anti-inflammatory, Cytotoxicity, iNOS, COX-2

Inflammatory diseases are a major burden on humanity, despite recent successes with biopharmaceuticals. The lack of responsiveness and drug resistance, delivery problems and the cost of biopharmaceuticals justify the search for effective and less toxic anti-inflammatory agents. As part of our ongoing work on the valorization of Portuguese lavenders [1, 2, 3, 4] we now report their anti-inflammatory potential.

The essential oils were isolated by hydrodistillation and analysed by GC and GC/MS using fused silica capillary columns with two different stationary phases. The main constituents of the oils were carvacrol (42.8%) and *cis-β*-ocimene (27.4%) for *L. multifida*; 1,8-cineole (33.9%), fenchone (18.2%), camphor (2.2%) and rare necrodane derivates for *L. luisieri*; fenchone (42.1%), camphor (17.9%), and 1,8-cineol (10.4%) for *Lavandula pedunculata;* and 1,8-cineol (34.5%), camphor (13.4%), *α*-pinene (9.0%) and linalool (7.9%) for *Lavandula viridis*.

To evaluate the anti-inflammatory potential of the oils, the *in vitro* model of lipopolysaccharide (LPS)-stimulated macrophages was used. The following inflammatory parameters were evaluated: nitrite oxide (NO) production by Griess reaction and inducible NO synthase (iNOS) and cycloxygenase-2 (COX-2) expression by western blot analysis using specific antibodies.

All the oils (0.64µL/mL), excluding *L. multifida* were able to significantly ($p<0.001$) inhibit NO production without affecting cell viability. *L. luisieri* and *L. viridis* were the most effective, showing an inhibitory activity around 100%. Regarding the inhibition of iNOS and COX-2 expression, *L. viridis* was the most effective, inhibiting completely iNOS expression and COX-2 expression around 50%. *L. luisieri* and *L. pedunculata* were also effective on the inhibition of iNOS expression.

These promising results highlight the potential of Portuguese lavenders as anti-inflammatory drugs, thus justifying further *in vivo* assays to confirm the effectiveness of the *in vitro* results.

Acknowledgments: This work was funded through national funds from FCT – Fundação para a Ciência e a Tecnologia under the project CEF/POCI2010/FEDER and a PhD fellowship to Mónica R. Zuzarte (SFRH/BD/40218/2007).

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Young Scientists (YS) Oral Communications Abstracts

YSOC 1. Effects of PSBS gene silencing by RNAi on volatile compound biosynthesis and accumulation in tomato (*Solanum lycopersicum*)

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Keywords: Gene silencing, Solanaceae, Nonphotochemical quenching, GC-MS, qPCR, Terpene synthases

Photosynthetic organisms convert light energy into chemical energy, however, high light irradiation can lead to the production of Reactive Oxygen Species (ROS) if the light energy is not dissipated safely. ROS burst can damage cell membranes (photoinhibition). In order to reduce photoinhibition, nonphotochemical quenching (NPQ) is the main regulated mechanism used by plants to dissipate the excess of light energy. NPQ in higher plants is strictly dependent on the presence of a Lhc (Light Harvesting Complex)-like protein, PsbS which does not bind pigments, unlike the other Lhc proteins, and stimulates NPQ interacting with Photosystem II antenna proteins. This mechanism is very efficient, inducing thermal dissipation up to 80% of absorbed light even at moderate irradiance, suggesting that evolution lead higher plants to fully protect themselves at the expense of partial a loss of light energy conversion efficiency. In this work, we produced different transformed lines of *Solanum lycopersicum* with RNA interference (RNAi) construct in order to silence *PsbS* gene. Here we report the effects on biosynthesis and accumulation of volatile compounds (VOCs) in mutant plants. Leaf and fruit tissue extracts were analyzed by GC-MS, whereas quantitative gene expression analysis was carried out through qPCR for genes involved in terpene biosynthesis.

The metabolic and biochemical analyses revealed an increased accumulation in *psbs* mutants of some monoterpenes. The results suggest that deletion of this photoprotective mechanism might have an industrial application for plants cultivated in control conditions as in greenhouses, where the light can be artificially modulated without impairing the growth and fruit production, but increased accumulation of volatiles compounds that should influence fruit flavour and increase the resistance to herbivores.

YSOC 2. Coriander oil antimicrobial activity: A flow cytometric study

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Keywords: Coriander oil, Antibacterial activity, Antifungal activity, Flow cytometry, Germ tube, Synergism

The aim of this work was to study the antimicrobial effect and mechanism of action of coriander essential oil against Gram-positive, Gram-negative bacteria and *Candida* yeast cells.

Antibacterial and antifungal susceptibilities were evaluated using classical microbiological techniques with MIC and MLC determination. The effect or coriander oil on respiratory activity, membrane polarization, efflux activity, cell cycle progression and membrane permeability was evaluated by flow cytometry. Furthermore, in Candida cells, the coriander oil effect on germ tube formation and the potential synergism of coriander oil with amphotericin B were also studied.

Our results showed that coriander oil has an effective antibacterial activity against all bacteria tested with a potent bactericidal activity against almost all bacteria tested, with the exception of *B. cereus* and *E. faecalis* [1]. For fungal cells, coriander oil also revealed a fungicidal activity against the Candida strains tested with MLC values equal to the MIC value [2]. Propidium iodide (PI) incorporation and concomitant loss of all other cellular functions such as efflux activity, respiratory activity and membrane potential seem to suggest that the primary mechanism of action of coriander oil on both Gram-positive and Gram-negative bacteria as well as in *Candida* cells is membrane damage, which leads to cell death [1,2]. Also, concentrations bellow the MIC value caused a marked reduction in the percentage of germ tube formation for *C. albicans* strains with a synergetic effect between coriander oil and amphotericin B also observed for *C. albicans* strains [2].

These results, showing a potent antibacterial and antifungal activity due to membrane permeability, are noteworthy and justify the use of this plant not only as a food flavouring agent, but also as a food preservative and even in medical devices in order to prevent fungal infections.

Acknowledgments: This study was supported by grants SFRH/BD/41521/2007 and SFRH/BD/66857/2009 from the Portuguese Foundation of Science and Technology.

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YSOC 3. Application of vibrational spectroscopy and chemometrics as a rapid and low cost alternative in the quality assessment of indigenous South African essential oils

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Keywords: Vibrational spectroscopy, Chemometrics, Buchu, Sage, Lippia, Cape chamomile

As the market for herbal products continues to grow, concerns over the safety of some products has been raised. Adulteration especially in essential oils has become a point of great concern which compromises the quality of the product and ultimately the safety of the consumer. Current analytical methods are based on the identification and quantification of marker constituents which in some cases may not be biologically active. Due to the complexity and variation in chemical composition of these mixtures, it is important to profile the whole metabolome. Spectroscopic techniques that include mid- and near- infrared spectroscopy (MIR and NIR) have been identified as potential quality control alternatives.

Four commercially important essential oils from South Africa (Buchu, Cape chamomile, Sage and *Lippia* oils) were used to demonstrate the application of vibrational spectroscopy and chemometrics in multivariate classification and class predictions. Additionally calibration models were developed for marker constituents that would be used to predict these constituents in future samples.

Plant material was obtained from cultivation sites and the wild. The essential oils were isolated by hydrodistillation and reference analysis performed using GC-MS. The oils were also scanned on MIR and NIR spectrometers for spectral acquisition. For identification and classification, orthogonal projections to latent structures (OPLS-DA) were employed and the models built were used to further predict the botanical origin of commercial oils. Partial least squares regression (PLSR) analysis was used to develop multivariate calibration models that were also used to predict the quantities of marker constituents in future samples.

The results showed that OPLS-DA is a useful tool in the differentiation of essential oils from different species. Class prediction of unknown oil samples was possible with high predictive power. The PLS calibration models developed showed the best model performance with very high correlation coefficients of > 90% and low errors of prediction for marker constituents. The feasibility of using vibrational spectroscopy as an alternative quality assessment technique has been demonstrated.

Acknowledgement: The authors would like to thank the National research Foundation (NRF) of South Africa and Tshwane University of Technology for financial support.

YSOC 4. Effect of hydroxypropyl-β-cyclodextrins on the antioxidant activity of *Lavandula* essential oils

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Keywords: Antioxidant activity, Cyclodextrins, Essential oils, ORAC assay

Excess free radicals are produced under stress and in response to pathogens, often resulting in oxidative damage to lipids, proteins and nucleic acids. In humans, this damage is implicated in ageing and in numerous diseases [1]. The ability of natural antioxidants to protect cells from oxidative stress has been previously demonstrated [2] and several methods have been proposed to evaluate the antioxidant activity of natural products [3]. However, many assays are conducted in an aqueous system and, therefore, must be adapted to measure lipophilic natural products, such as essential oils. Cyclodextrins are a group of naturally occurring cyclic oligosaccharides with a lipophilic central cavity and a hydrophilic outer surface [4], which have been used as solubilizing agents for hydrophobic volatile oils [5]. In this work, the antioxidant activities of the essential oils from *Lavandula viridis* L'Hér and *Lavandula pedunculata* subsp. *lusitanica* (Chaytor) Franco, endemic species from Portugal (Algarve region), were assessed in the absence and presence of hydroxypropyl-*β*-cyclodextrins (HP-*β*-CD) by means of the oxygen radical absorbance capacity (ORAC) assay [6]. The results showed that the peroxyl radical scavenging activity increased in the presence of HP-*β*-CD in both species, however the *L. viridis* essential oil exhibited the highest ORAC values. The results obtained demonstrated that the addition of HP-*β*-CD improves the solubility of *Lavandula* essential oils and, consequently, their chain-breaking antioxidant activities.

Acknowledgements: C. Patrícia, S. Gonçalves acknowledge a grant from Portuguese Science and Technology Foundation (FCT, SFRH/BD/63505/2009 and SFRH/BPD/31534/2006).

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YSOC 5. Classification of Lavender essential oils for the evaluation of sedative effects on Human

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Keywords: Lavender, Principal Component Analysis, Aroma component composition, Enantioselective GC-MS, Optical isomer

Lavender essential oils are used for aromatherapy. Linalool is the main aroma component and thought to have a sedative effect on human [1]. The other components must affect the autonomic nervous system and then the whole effect of an essential oil may be determined by the component composition. Two steps are important for aromatherapy: the categorization of essential oils and the proper use based on scientific evaluation. In this study, lavender essential oils were categorized by principal component analysis of aroma component compositions.

Twenty-seven types of lavender essential oils, which include several *Lavandula* species, were purchased in markets in Japan. These essential oils were analyzed by GC-MS. Furthermore, optical isomer compositions of some aroma components were also determined using enantioselective GC-MS. Principal component analysis was conducted based on the data obtained by GC-MS.

High amount of linalool was commonly found in the lavender essential oils (20-50%) except *L. stoechas*. Linalyl acetate was also predominantly contained but not in *L. stoechas* and *L. spica*. *L. angustifolia* oils showed lower camphor content. Optical resolution analysis revealed the (R) form optical isomers are predominant in most of the essential oils, although (S) formula of borneol was abundant in some essential oils. The essential oils tested in this experiment could be divided into several groups by principal component analysis according to the PC1 and PC2. Loading factors indicated that the components which mainly contributed to the PC1 were camphor, alpha-pinene and eucalyptol.

From the chemical profile analysis of lavender essential oils, some oils including *L. stoechas* were selected according to their unique compositions. Principal component analysis results might make it possible to reveal the relationships between the compositions and sedative effects on human.

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YSOC 6. Essential oils do not inhibit *Escherichia coli* peptidyl-tRNA hydrolase

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Keywords: Peptidyl-tRNA hydrolase, Essential oil inhibition, *Escherichia coli* Pth

The purpose of the study was to purify peptidyl-tRNA hydrolase (Pth) and peptidyl-tRNA from bacterial cells in order to determine the inhibitory activity of essential oils from aromatic medicinal plants located in Nigeria, South Africa, and Tajikistan. *E. coli* Pth was expressed and purified using metal chelation chromatography and gel shift assays were used to determine the inhibitory factor of the essential oils against Pth. Throughout all assays, the peptide was cleaved off from peptidyl-tRNA. Thus, enzymatic activity was observed from Pth when incubated with each essential oil and peptidyl-tRNAs in solution. As a result, essential oils show no sign of inhibitory activity against Pth. These findings are beneficial for future antibacterial research because they prevent further time and money exhausted and allows for the focus on other plant extracts that display inhibitory activity against Pth. The plant extracts that show inhibitory activity were not essential oils. Future studies include analysis by gas chromatography and mass spectrophotometry in order to determine what compound is responsible for inhibition. Plant extracts show great promise for the future development of antibacterials.

Acknowledgments: I greatly appreciate the McFeeters lab group in the Biochemistry department at The University of Alabama in Huntsville. Also, thanks to the Research Experience for Undergraduates Program at the University of Alabama Huntsville as well as Alabama Space Grant Consortium, President´s office of the University of Alabama in Huntsville, and Funds from the Department of Chemistry Patent account.

Posters Abstracts

P 1. Chemical composition, antifungal activity and citotoxicity of *Otanthus maritimus* (L.) Hoffmanns. & link from Portugal

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Keywords: *Otanthus maritimus*; essential oil; GC-MS; antifungal activity; cytotoxicity

Otanthus maritimus (L.) Hoffmanns. & Link belongs to the Asteraceae family that includes well-known aromatic and medicinal species such as chamomile, yarrow, wormwood and feverfew [1]. The use of *O. maritimus* has been reported in traditional medicine for various purposes including the treatment of toothache, asthmatic bronchitis, dysentery and inflammation of the urinary bladder [2-4]. The present work aimed to characterize the chemical composition of the essential oils obtained from the aerial parts of *O. maritimus* collected in Portugal, as well as to evaluate the antifungal activity of the oil at concentrations without skin cytotoxicity.

The oils were isolated by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Broth macrodilution methods based on the Clinical and Laboratoty Standards Institute (CLSI) reference protocols were used to determine minimum inhibitory concentrations (MICs) and minimum lethal concentrations (MLCs) of the essential oils. Cytotoxicity was tested in HaCaT keratinocytes through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

The main compounds of the oils are chrysanthenone (40.4-57.2%), filifolone (12.2-15.5%), *cis*-chrysantenyl acetate (10.1-12.2%) and *α*-pinene (6.7-7.2%). Results concerning the antifungal activity demonstrated the potential of *Otanthus* oil against dermatophytes. Furthermore, evaluation of cell viability showed no significant cytotoxicity in HaCaT keratinocytes at concentrations between 0.16 and 0.32 µL/mL.

These findings add significant information to the knowledge on the pharmacological activity of *O. maritimus* essential oils, specifically due to its antifungal properties. This evidence of the therapeutic effects of *O. maritimus* essential oil, suggest that doses with antifungal activity with very low detrimental effect on keratinocytes combined with its pleasant odor could be of great value to cosmetic and pharmaceutical industries.

Acknowledgements. Our thanks to CEF/POCI2010/FEDER, for financial support.

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P 2. Physical and chemical characterization of inclusion complexes: essential oil from *Ocimum basilicum* and β-cyclodextrin

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Keywords: inclusion complexes, β-cyclodextrin, *Ocimum basilicum*

Several *Ocimum* species (Lamiaceae) are used to treat central nervous system (CNS) disorders in various parts of the world and its anticonvulsant activity is frequently reported [1,2]. *Ocimum basilicum* L. essential oil (OBEO) is rich in monoterpenes, such as δ-cadinol (10.2%), estragole (22.6%), and linalool (47.3%) [3]. Thus, essential oils have shown potential biology activity, but its insolubility makes it difficult to use in therapeutic systems. Accordingly, this study aimed to characterize the physical-chemical properties and assess the thermal properties of inclusion complexes of *Ocimum basilicum* with β-cyclodextrin (β-CD). Inclusion complexes were prepared by three different procedures. A physical mixture (PM) was prepared by the addition of OBEO to a powdered β-CD under manual agitation. Paste complexation (PC) was carried out by homogenization of β-CD with water (1:1 molar, 10 mL) under constant manual agitation. Then, the material was dried at room temperature (in a desiccator) till the formation of a glass film, which was removed by manual trituration and stored in airtight glass containers. Co-evaporated (CE) was carried out by the addition of water to a beaker containing β-CD (1:1 molar, 20 mL) and stirred for 36 h by a magnetic stirring device operating at 400 rpm. DSC curves can be observed that the preparations PM, PC and CE showed three events endothermic (25-400ºC), followed by elimination of the carbonaceous material. The thermoanalytical curve of PM was a superposition of the guest and host curves, which indicates a lower evidence of inclusion and significant interaction between the host and guest molecules. On the other hand, the thermal curves of PC and CE showed in the interval from 130 to 275°C a gradual mass loss attributed to oil release. The FTIR spectra also suggest the complexation, since the bands for the oil were present also in three different preparations. Further studies should be performed to confirm these results.

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P 3. Antimicrobial and antioxidant activities of volatile oils of three species of Piperaceae grown in Cuba

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Keywords: *Piper aduncum, Piper auritum, Piper angustifolium;* essential oil; antioxidant activity; antimicrobial activity.

The Piperaceae family is recognized in the traditional medicine in all America for the use of its oils as astringent, digestive, diuretic and antimicrobial [1-4]. The present study has as purpose evaluate the antimicrobial and antioxidant activities by different *in vitro* techniques of volatile oils obtained by hydrodistillation of dried leaves of three species of Piperaceas grown in Cuba.

The antioxidant activity was evaluated by several assays such as free radical DPPH activity, reducing rower and complementary antioxidant activity in linoleic acid system (FTC method).The antioxidant activity of these essential oils was also evaluated against unrefined crude *Cucurbita* seed oil by peroxide, thiobarbituric acid and *p*-anisidine methods.

The essential oil *Piper auritum* showed antioxidant activity similar to natural antioxidants in some cases and better results than *Piper angustifolium* and *Piper aduncum oils*. The *Piper auritium* showed a stronger antioxidant activity than BHA and BHT but lower than that of propyl gallate. Moreover, this antioxidant activity was supported by the complementary antioxidant assay in linoleic acid system and 2, 2′-diphenyl-1-picrylhydrazyl. In other hand the essential oils showed good to moderate inhibitory effects against three microorganisms evaluated, *Klebsiella pneumoniae* ATCC 13833*, Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 18231 but these didn't had activity with *Staphylococus aureus* ATCC 25932 and *Pseudomona aeruginosa* ATCC 27853.

This study gives a wide view on the composition of Piperaceas essential oils produced during the entire productive season, useful to identify quality parameters for the analytical characterization of this product.

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P 4. Antimicrobial activity of essential oils from *Lavandula luisieri* and cineol against *Botrytis cinerea*

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Keywords: Essential oils; *Botrytis cinerea;* food spoilage; *Lavandula luisieri;* antimicrobial activity;

The microbiological contamination of strawberry, caused by filamentous fungi, leads to high fruit stock losses during his transport and storage period. The use of edible films with essential oils from *Lavandula luisieri* may be a powerful solution. So, the main goal of this work was to test the antifungal activity of essential oils (EO) of *L. luisieri* and cineol against *Botrytis cinerea*, a common spoilage mold of strawberry.

In order to test the antifungal activity of essential oils against *Botrytis cinerea* the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) were determined, respectively, by the broth microdilution method [1] and by the drop plate method onto solid medium [2]. The suspensions, $0.4x10^4$-$5.0x10^4$ spores/ml, were performed according NCCLS [3] and Alizadeh-Salteh [4].

The MIC values of essential oils against *Botrytis cinerea* were between 0,38% (v/v) and 1,5% (v/v) and the MFC values were between 1,25% (v/v) and 3% (v/v). Cineol was less effective in mold inhibition than the essential oils of *L. luisieri.*

The results showed an effective antifungal activity of essential oils of *L. luisieri.*

Acknowledgements: CERNAS/IPC is supported by FCT, *Fundação para a Ciência e a Tecnologia* (Science and Technology National Foundation)

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P 5. Morphological and essential oil variability of Portuguese fennel (*Foeniculum vulgare* Mill.)

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Keywords: *Foeniculum vulgare*; Apiaceae; Portugal; descriptors; essential oils; morphological characterization

Previous studies on Portuguese fennels (*Foeniculum vulgare* Mill.) essential oils [1-3], showed the existence of chemotypes (anethole, anethole / fenchone, anethole / methyl chavicol) that diverge from international standards [4,5]. In the present work, twenty accessions of *F. vulgare* consisting of wild material and fruits' progeny were assessed for diversity through a combined morphochemical evaluation.

The accessions, collected in 2005 and 2006, were morphologically characterized using 34 qualitative and quantitative variables scored on 20 randomly selected plants per population [3]. The essential oils were isolated by hydrodistillation from the fruits (seeds) progeny and analysed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). The percentage composition of the essential oils was used to determine the relationship between the different oil samples by cluster analysis as in [3].

Essential oils analysis defined two main clusters: cluster I, composed of 9 samples, included *trans*-anethole (43-70%) and fenchone (18-33%) rich oils, and cluster II, with 11 samples, included methyl chavicol (= estragole) (15-44%), *trans*-anethole (14-37%) and fenchone (19-39%) rich oils.

Combined morphological and essential oil assessment, showed that the *trans*-anethole dominant cluster I included plants with larger umbels, larger leaves and higher number of umbels per plant. Accessions whose essential oils gathered in cluster II were morphologically characterized by being taller plants, with higher stems number, larger petiole length and distance between 1st and 2nd leaf segment pairs, small umbels with lower number of rays/umbel and short leaflets.

Two subspecies of *F. vulgare* Mill. (= *F. officinale* All.) occur in Portugal, *F. vulgare* Mill. subsp. *capillaceum* (= *F. vulgare* Mill. subsp. *vulgare*) and *F. vulgare* Mill. subsp. *piperitum* [references in 3]. The latter subspecies is differentiated from subsp. *capillaceum* by smaller umbels with lower number of rays/umbel. The chemical analysis allowed a taxonomical clarification between the two subspecies, despite their morphological similarity.

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P 6. Anti-hypernociceptive activity of citronellol, a monoterpene presents in lemongrass essential oil, involves periaqueductal gray and piriform cortex

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Keywords: Monoterpenes, citronellol, hypernociception, pain

Citronellol (CT) is a monoterpene present in lemongrass essential oil. We investigated the anti-hypernociceptive and central nervous system (CNS) effects of CT on rodents.

Male Swiss mice (n=6/group) were treated with CT (25, 50 or 100 mg/kg; i.p.) or saline, 0.5h after the plantar injection of carrageenan (CG; 300 µg/paw), TNF-α (100 pg/paw), prostaglandin (PGE_2; 100 ng/paw) or dopamine (DA; 30 µg/paw). The hypernociception was evaluated at times of 0.5, 1, 2, and 3h after the injection of agents, using the digital analgesymeter (von Frey). To evaluate the central effects, the animals were perfused 90 min after the treatment, the brains were removed, frozen, cut (20 um) and subjected to immunofluorescence for Fos. The protocols were approved by Animal Ethics Committee at the UFS (CEPA/UFS: 72/11).The data were analyzed by ANOVA (one-way) followed by Tukey's test ($p<0.05$).

CT significantly ($p<0.001$) reversed the hypernociception induced by CG at 100 mg/kg (0.5h: 5.9±0.6, 1h: 6.3±0.6, 2h: 6.4±0.6, 3h: 6.2±0.4) comparing with vehicle group (0.5h: 1.5±0.1, 1h: 2.1±0.2, 2h: 1.9±0.2, 3h: 1.9±0.2). The hypernociception induced by TNF-α was significantly ($p<0.001$) reversed at 25 (0.5h: 5.0±0.2, 1h: 5.0±0.3, 2h: 4.8±0.4, 3h: 5.9±0.2), 50 (0.5h: 4.6±0.5, 1h: 5.0±0.4, 2h: 5.1±0.5, 3h: 5.6±0.5) and 100 (0.5h: 4.7±0.2, 1h: 4.9±0.3, 2h: 4.9±0.2, 3h: 4.7±0.3) comparing with vehicle (0.5h: 1.9±0.4, 1h: 2.0±0.4, 2h: 1.8±0.3, 3h: 2.1±0.3). The hypernociception induced by PGE_2 was also significantly ($p<0.001$) reversed at 25 (0.5h: 3.7±0.3, 1h: 3.8±0.3, 2h: 4.6±0.4, 3h: 4.1±0.2), 50 (0.5h: 4.2±0.3, 1h: 4.8±0.1, 2h: 4.7±0.3, 3h: 4.6±0.4) and 100 (0.5h: 4.4±0.3, 1h: 4.8±0.2, 2h: 5.1±0.1, 3h: 5.2±0.3) when compared with vehicle (0.5h: 1.9±0.2, 1h: 1.8±0.05, 2h: 1.9±0.2, 3h: 1.7±0.2). The hypernociception induced by DA was significantly ($p<0.001$) reversed at 25 (2h: 5.4±0.4, 3h: 5.0±0.2) and 100 (3h: 5.4±0.1) comparing with vehicle (0.5h: 3.4±0.2, 1h: 3.4±0.2, 2h: 2.6±0.3, 3h: 3.2±0.1). The immunofluorescence assay demonstrated a significant increase in the number of Fos positive cells in the piriform cortex (25: 8±1.4, 50: 23.7±4.3, 100: 24±4.8, vehicle: 1.2±0.2) and in the periaqueductal gray (25: 12.2±1.2, 50: 25.7±2.8, 100: 26±3.4, vehicle: 1.5±0.8).

Our results suggest an anti-hypernociceptive effect of CT, probably by the activation of the piriform cortex and periaqueductal gray CNS regions.

Acknowledgments: CAPES, CNPq, FAPITEC-SE.

P 7. Citronellol, a monoterpene alcohol, reduces the orofacial nociceptive behavior in mice

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Keywords: Monoterpenes, citronellol, orofacial pain, analgesic

Citronellol (CT) is a monoterpenoid alcohol present in the essential oil of many plants, as the *Cymbopogom citratus*. We evaluated the effects of CT on orofacial nociceptive behavior in mice.

Male Swiss mice (n=6/group) were treated intraperitoneally with CT (25, 50 or 100 mg/kg) or saline (control). Orofacial nociception was induced by injection (s.c.) of 20 ul of 2% formalin, 20 ul (2.5 ug) of capsaicin or 40 ul of glutamate (25 uM), 0.5 h before the treatment. The quantification of nociception was performed by measuring the time (seconds) that the animal spent face-rubbing in the injected area with its fore or hindpaws. In the formalin test, the animals were observed at periods of 0 to 5 minutes (first phase) and 15 to 40 minutes (second phase) after formalin injection, in the capsaicin test, at periods of 10 to 20 minutes after capsaicin injection, and in the glutamate test, at for 15 minutes following the glutamate injection. The protocols were approved by Animal Ethics Committee at the UFS (CEPA/UFS: 72/11).The data were analyzed by ANOVA (one-way) followed by Tukey's test ($p<0.05$).

CT significantly ($p < 0.001$) reduced, in both phases, the orofacial pain in the formalin test. In the first phase at doses of 25 (42.8 ± 6.7), 50 (42.5 ± 7.2) and 100 mg/kg (26.3 ± 2.0) when compared with control (93.5 ± 6.3), and in the second phase at 25 (47.3 ± 18.8), 50 (21.6 ± 10.2) and 100 (2.0 ± 1.3) when compared with control (168.7 ± 33.0). The orofacial pain induced by capsaicin was, also, significantly ($p < 0.001$) reversed at 25 (67.1 ± 14.4), 50 (64.3 ± 15.2) and 100 mg/kg (42.3 ± 17.4) comparing with control (193.7 ± 16.2). In the glutamate-induced nociception, the CT significantly ($p < 0.01$ or $p < 0.001$) reduced the orofacial pain at doses of 25 (48.3 ± 8.1), 50 (37.3 ± 7.4) and 100 mg/kg (24.1 ± 7.0) comparing with control (86.1 ± 8.7).

Our results suggest that CT reduces the orofacial nociceptive behavior in mice.

Acknowledgments: CAPES, CNPq, FAPITEC-SE.

P 8. Chemical composition of the leaf essential oils from lavandin transformed with limonene synthase gene

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Keywords: lavandin, limonene synthase, transgenic plant, cyclic monoterpenes

Lavender is a well known aromatic plant, whose essential oil from florets comprises several monoterpenes, including linalool, linalyl acetate, limonene, and lavandulol [1]. The leaves of lavender also produce monoterpenes such as 1,8-cineol and camphor, whose fragrance differs from that of florets. These compounds are produced via an enzymatic reaction from the common precursor geranyl diphosphate [2]. We hypothesized that it should be possible to produce floret-specific monoterpenes in the leaves using gene transfer. In this study, we transformed the limonene synthase gene driven by a constitutive promoter into lavender plants and analyzed essential oil components in the leaves.

Lavandin, the interspecific hybrid between true lavender and spike lavender, was used as the plant material. Leaf-derived callus was inoculated with *Agrobacterium tumefaciens* harboring the binary plasmid, pRI101AN/LIMS, into which the limonene synthase gene of true lavender (*LIMS*) was driven by the 35S promoter within the T-DNA region and then regenerated using the method of Tsuro et al [3]. The essential oils of the leaves from regenerates were extracted with *n*-hexane and analyzed by GC and GC-MS.

Three regenerated transformants, expressing 2.4- to 8.8-times higher levels of LIMS mRNA than non-transgenic control, were obtained. Comparative analysis of the essential oil components identified ten compounds: eight terpenoids and two aromatics. All the transformants produced significantly more borneol than did the non-transgenic control. Furthermore, significantly higher levels of the terpenoids of 1,8-cineol and limonene, α-pinene and camphor, were detected in the two transformants than the non-transgenic control. From these results, limonene synthase was suggested to act as both a limonene synthase and a cyclic monoterpene synthase. We are now evaluating their growth habit and essential oil components of florets.

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P 9. Antinociceptive effect of *Xylopia laevigata* leaf essential oil in mice – evidence of involvement of periaqueductal gray area

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Keywords: *Xylopia laevigata*, pain, periaqueductal gray

Xylopia laevigata (Annonaceae) is commonly known as 'meiú' or 'pindaíba', and it is a plant used in folk medicine of Brazilian Northeast to treat painful and inflammatory disorders. Thus, we investigated the possible antinociceptive effect of *Xylopia laevigata* leaf essential oil (EOX) and trying to describe the possible involvement of central nervous system (CNS) areas.

EOX was extracted by hydrodistillation of fresh leaves of the *X. laevigata*. The identification of the components was made through comparison of substance mass spectrum with the database of the GC-MS, literature and retention index. The antinociceptive effect of EOX was examined using the acetic acid (0.85%) writhing reflex and formalin (20 µl of 1%)-induced nociception tests. The motor coordination was also evaluated using Rota rod (7 rpm, 180 s). Animals were pretreated with EOX at the doses of 12.5, 25 or 50 mg/kg (i.p.) 1 h before injection or vehicle (saline + 2 drops of Tween 80 0.2%). Experimental protocols were approved by the animal care and use Committee (CEPA/UFS: 16/11) at the Federal University of Sergipe. The obtained data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test.

The phytochemical analysis of EOX showed the presence of g-muurolene (17.78%), δ-cadinene (12.23%), bicyclogermacrene (7.77%), α-copaene (7.17%), germacrene D (6.54%) and (*E*)-caryophyllene (5.87%) as the main compounds. In mice, the EOX also significantly produced ($p < 0.05$ or $p < 0.001$) an antinociceptive effect by reduction in the early and late phases of paw licking and by a reduced writhing reflex (formalin and writhing tests, respectively) (Table 1). Such results were unlikely to be provoked by motor abnormality (Data no shown). The immunofluorescence essay demonstrated that acute treatment with EOX produces a significant ($p<0.01$ or $p<0.001$) increase in the number of Fos positive cells in the piriform cortex and periaqueductal gray CNS areas (Figure 1).

Altogether, the results provide evidence for the use of *X. laevigata* by traditional medicine practitioners in the management of pain and inflammatory disorders.

Acknowledgments: FAPITEC-SE, CNPq and CAPES, Brazil.

P 10. Composition of the essential oil from the flowers of *Solandra maxima* (Sessé & Moc.) P.S. green

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Keywords: *Solandra maxima*; Solanaceae; essential oil composition

Solandra is a genus of flowering plants in the nightshade family (Solanaceae) having phytochemical, ethno botanical and ornamental importance. They have very large, attractively scented flowers and are native to the Caribbean, Mexico and South America. To the best of our knowledge, no data dealing with its volatile compounds have been yet reported. In this study, the dried flowers of *S. maxima* cultivated in Austria under greenhouse conditions were hydrodistilled yielding 0,28% (w/w) of essential oil; alternatively, the flowers were subjected to enzymatic hydrolysis using beta-glucosidase prior to hydrodistillation, yielding 0,34% (w/w) of essential oil. The two samples were subjected to a comparative investigation by GC-FID and GC/MS. More than 70 compounds were identified in both samples, representing more than 90% of the total. The oil is dominated by oxygenated compounds: alcohols, phenols and esters were the major classes, the concentration of terpene alcohols being generally higher in the sample obtained after enzymatic hydrolysis. The main constituents in the two samples were linalool (12,4% and 17,9%), methyl salicylate (9,1% and 11,4%), safranal (3,8% and 5,7%), alpha-terpineol (2,0% and 4,5%), geraniol (2,6% and 3,6%), carvacrol (17,8% and 11,5%), (E)-nerolidol (3,4% and 4,3%) and benzyl salicylate (11,1% and 7,1%). This for a tropical flower rather unusual composition corresponds to a complex odour profile that can be described as heavy-floral, spicy-phenolic and rounded up by tobacco notes.

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P 11. Antimicrobial activity of essential oil of *Satureja montana* spp. *pisidica* (Lamiaceae)

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Keywords: *Satureja montana* spp. *pisidica*; essential oil; antimicrobial activity

Satureja montana spp. *pisidica* (Wettst.) Šilic (Lamiaceae) is an aromatic semi-shrub that grows only in FYR of Macedonia as an endemic species. All species of the genus *Satureja* contain more than 0.5% of essential oil with strong antimicrobial activity [1,2]. The aim of this work was to study the composition and antimicrobial activity of essential oils of *S. montana* spp. *pisidica* from two different locations.

Plant material was collected on the mountains Galičica and Korab in July 2011 and subjected to hydrodistillation on Clevenger apparatus. The composition of essential oils was determined using GC/MS and GC/FID, while antimicrobial activity was evaluated using broth microdillution method and expressed as minimal inhibitory concentration (MICs, µg/ml).

Thirty six components were identified in the essential oil of *S. montana* from Galičica, which represents about 95% of the oil. Forthy two components were identified in the samle from Korab or about 94% of the oil. In both oils, carvacrol was the most abundant component (Galičica 37.6%; Korab 20.9%). Antimicrobial activity was tested against selected bacteria and fungi: *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans*. Tested essential oils exhibited strong antibacterial activity, especially against G(+) bacteria, and lower antifungal activity. MICs values ranged from 12.5-50.0 µg/ml (bacteria), and 25.0-50.0 µg/ml (fungi). The essential oil of the sample from Galičica was demonstrated the best activity against *S. epidermidis* (12.5 µg/ml). The most resistant was *P. aeruginosa* (50.0 µg/ml). This effect can be attributed to the monoterpene components thymol, carvacrol and p-cymene.

Acknowledgments: Ministry of Education and Science of Republic of Serbia (P173021).

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P 12. The volatile oil profile of *Salvia officinalis* cultivated in Arad county, Romania

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Keywords: *Salvia officinalis*; GC-MS; cineole; bornyl acetate

The volatile oil is the main compound of the species of *Salvia officinalis* L. (Lamiaceae). This study presents the evaluation of chemical composition of the Salvia essential oil obtained from plants cultivated and harvested in Arad county, Romania.

The volatile oil of *Salvia officinalis* was obtained by hydrodistillation (57 ml/kg) by means of the special device described in the European Pharmacopoeia. The distillation took place in 4 hours at a speed of 2-3 mL/minute. 0.6 mL volatile oil was brought to 10 mL with hexane (Merck, Germany). A TLC and GC-MS determination followed on the thus obtained volatile oil. The qualitative analysis by thin layer chromatography was performed using the chromatographic conditions mentioned in the monographs for *Salvia officinalis* from the German Homeopathic Pharmacopoeia respectively the European Pharmacopoeia. Based on the Rf values and the aspect of the bands, compared to those of the standards applied on the plate, we could identify in the volatile oil of common sage: thujon, cineole and bornyl acetate. In the chromatogram there are still other bands in different shades of violet and of different intensities which may correspond to some terpenic compounds.

We can notice that the volatile oil contains small quantities of thujon, a neurotoxic compound. For a more precise determination of the terpenic compounds, the GC-MS analysis was performed. The obtained results confirm the data of the TLC analysis and show the possibility of identifying 16 terpenic compounds having different concentrations from 0,09 to 13,40 %. In the volatile oil of common sage we could identify as main compounds: 1,8-cineole (13.40 %), camphor (8.08 %); alpha-pinene (5.81 %); camphene (3.70 %) and thujon (2.76 %).

The results shown that the studied common sage contains less thujon (2.76 %) and camphor (8.08%), than is found in reference data (20-60 % for thujon, 14-37% for camphor) respectively a higher concentration of 1,8-cineole (13.40 %), that is almost at the maximum found in reference data (6-16%) [1].

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P 13. Morphoanatomical and chemical composition of leaf essential oil of two *Lippia alba* morphotypes

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Keywords: morphology; leaf anatomy; Verbenaceae

Lippia alba is commonly known in south of Bahia, Brazil, as “cidreira” or “erva-cidreira” and used as a tea in the treatment of respiratory infections and stomach problems. In this same region is possible to distinguish two morphotypes of *L. alba* with a degree of polymorphism, used irrespective by the population for the same indications, although only one is chemically known. The objective of this work was to characterize and compare the morphology of these plants, the leaf secretory structures, and the essential oil produced. Studies on medicinal and aromatic plants indicate that it is common in some species the occurrence of accesses with morphological and phytochemicals variations (1).

The morphotypes were called "cidreira" and "melissa" due to their popular name originally associated. "Cidreira" is more high, erect and has larger leaves than "melissa". Although some authors (2) reported there are not important anatomic variations between chemotypes of this specie, we found differences in the number of layers of palisade parenchyma and the types of trichomes. They present identical anatomical pattern except for the number of layers of palisade parenchyma, which is biserial in "cidreira" and uniseriate on "melissa". The two morphotypes have glandular and no glandular trichomes on both leaf surfaces, and the last confirmed the accumulation of essential oils by histochemical test using Nadi reagent (3). The glandular trichomes were described and classified into six types distributed differently in the two morphotypes. There were differences in the content and chemical composition of essential oil, with citral as the major component of "cidreira" and citral, limonene and carvone in "melissa". This comparative study allowed us to distinguish two morphotypes of *Lippia alba* through morphological differences as plant size, leaf area, number of layers of palisade parenchyma and types of trichomes. Differences were also found in the content and chemical composition of essential oil.

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P 14. Allelopathic effect of *Cymbopogon winterianus* Jowit essential oil on respiratory metabolism of *Euphorbia heterophylla*

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Keywords: Geraniol; citronellal; citronellol; bioherbicide

Allelopathic effect of *Cymbopogon winterianus* Jowitt essential oil on respiration of *Euphorbia heterophylla* (dairy) was evaluated. Seeds were treated with essential oil of C. *winterianus* (0 to 0.25% with 2% DMSO) and placed randomly on germination chamber (Type BOD) with 12h photoperiod at 25°C for 7 days. Effects of essential oil on respiration of embryos and mitochondria isolated pretreated with essential oil were measured. Direct effect of essential oil on mitochondrial respiration also was determined. The data were analyzed by ANOVA and the variables analyzed by regression at 5% probability. Total respiration and cyanide-sensitive respiration (SHAM) were affected by pretreatment of embryos with the essential oil of all species. Essential oil of *C. winterianus* significantly reduced mitochondrial oxidative phosphorylation measured by O2 consumption during ADP phosphorylation (state III) and for ADP/O when using NADH or malate as respiratory substrate. Basal, state IV and respiratory control (RC) did not differ significantly from control. The results demonstrate that the essential oil dramatically affects both the total respiration and the sensitive to KCN one. It´s probably that essential oil interferes on ATP-synthase complex activity and/or adenylate (e.g ADP) supply. Therefore less adenylates may reach mitochondrial matrix to be phosphorylated. Thus, essential oil of citronella has high potential to be used as bioherbicide.

Acknowledgments: The authors thank to Fundação Araucária, CAPES and Fundação para a Ciência e Tecnologia (FCT), POCI 2010/FEDER for financial support to this work.

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P 15. Effect of *Pinus elliottii* essential oil on initial growth of *Ipomoea grandifolia* L.

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Keywords: α-pinene, β-pinene; geraniol; citronellol; allelopathy

The weed *Ipomoea grandifolia* cause large reductions in productivity in some crops such as soybeans. The control of this weed with herbicide may cause several problems to the environment. Thus, aim of this study was to evaluate the effect of essential oil of *Pinus elliottii* (bioherbicida) on germination and growth of weed *Ipomoea grandifolia* L. In petri dish containing 15 seeds of sorghum in a circular distributed were added different petri dishes were placed in a growth chamber (type BOD), temperature of 25^0C (2 ±). The concentrations of pine essential oil of each petri dish varied from 0 to 1% (v/v). Control was consisted of DMSO (dimethyl sulfoxide – 2%). The germination variables were analyzed during period of 72h. Germination percentage (%G=∑ni.Ni).100), germination average time (GAT=∑ni.ti/∑ni), germination average speed (GAS) and germination speed index (GSI) were evaluated. In addition, biometric variables such as root system and shoot growth, fresh and dry weight of seedlings were evaluated.

The essential oil of pine affected the kinetic variables and the percentage of germination. Concentrations of 0.6 and 1% dramatically inhibited the germination percentage (%G). One hundred percent germination was inhibited in a concentration of 1%. GAT, GAS and GSI variables were highly affected by the increase in the concentration of essential oil. The effect on these variables was proportional to the concentration of essential oil tested. All biometric variables were also affected by the essential oil in a dose-dependent mode. Major compounds in pine essential oil are α-pinene (14,7%) and β-pinene (8,4%), geraniol (8,3%) and citronellol (7,9%). These compounds seem to act on the plant energy metabolism so that could explain the results presented here[1,2]. Thus, it appears that this essential oil has great potential as bioherbicide. Our results suggest that the pine essential oil demonstrates high potential as bioherbicide to control the weed *Ipomoea grandifolia* L.

Acknowledgments: The authors thank to Fundação Araucária, CAPES and Fundação para a Ciência e Tecnologia (FCT), POCI 2010/FEDER for financial support to this work.

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P 16. Essential oil of *Baccharis dracunculifolia*: antitumoral and antiviral activities

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Keywords: essential oil; *Baccharis dracunculifolia*; HEp-2 cells; poliovirus

Baccharis dracunculifolia D. C., a native plant from Brazil commonly known as "alecrim-do-campo", has been indicated as the most important botanical source of a Brazilian propolis [1]. Since propolis is known for its antiviral and antitumoral activities [2], the goal of this work was to evaluate the effect of essential oil of *B. dracunculifolia* against poliovirus and human laryngeal epidermoid carcinoma (HEp-2) cells in vitro.

The essential oil of the dried leaves (500 g) was extracted by hydro-distillation using a Clevenger type apparatus. HEp-2 cells were incubated with different concentrations (5, 10, 25, 50 and 100 µg/well) of essential oil for different time periods (24, 48 and 72h), and the percentage of viable cells were analysed by crystal violet test. The effects of 0.2% DMSO were also analysed (essential oil solvent). For the antiviral assay, HEp-2 cells were incubated with 100DICT50 of virus suspension and stimulated with non-cytotoxic concentrations of essential oil for 48h. The viral quantification of poliovirus was evaluated using real-time PCR.

Data showed that 5, 10 and 25 µg/well of essential oil of *Baccharis* extracts were not cytotoxic to HEp-2 cells ($p>0.05$). However, higher concentrations of *Baccharis* extracts (50 and 100 µg/well) had an efficient cytotoxic effect against these cells ($p<0.05$). 0.2% DMSO showed no effects in the number of viable cells, evidencing that the cytotoxic effects were exclusively due to essential oil components. For the antiviral activities, 25 µg/100 µl the essential oil showed the lower relative viral quantification in real-time PCR.

Essential oil of *B. dracunculifolia* showed an antitumoral activity in a dose- and time-dependent manner, and important antiviral action. Further assays in vivo are still needed in order to explore a better comprehension of antiviral and antitumoral properties of *Baccharis dracunculifolia.*

Acknowledgments: Financial support: CNPq

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P 17. Essential oil content and composition of some oregano species cultured in Denizli district, oregano production center of Turkey

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Keywords: Turkey, Denizli district, Oregano, cultivation, essential oil

Turkey supplies more than 50% of the world's exported oregano (*Origanum* types). Denizli province is the primary Turkish oregano (*Origanum onites* L.) cultivation center in Turkey with a ratio of over 80% in both production and exporting. According to the registrations of Denizli Provincial Directorate of Agriculture, oregano cultivation area and the amount of production in 2009 were 7839 ha and 10660 tones, resectively. Recently *Origanum vulgare* subsp. *hirtum* (Link) Iestwaart (Greek or Istanbul oregano) has also started to cultivate in limited field areas. *O. sipyleum* L. (Anatolian oregano) is an endemic species of the Western Anatolia, Turkey. In this research, essential oil content and composition of these species grown in Denizli district at about 1200 m altitude during flowering period on July, 2011 were determined by Clevenger type hydro-distillation apparatus and by GC/MS system, respectively. Plant samples from the locations were analyzed in terms of leaf, flower and stem ratios in the dried herb. The soil samples were analyzed in terms of their physical and chemical properties. According to the results of chemical soil analysis, oregano soils in the region were generally medium-textured and non-salinity, but high alkaline and excessively calcareous, and with low levels of organic matter. The main compound in the essential oil of the all oregano species examined in this study was carvacrol. Carvacrol percentages in the leaf and flower essential oils were 84.03 and 87.83% in *O. onites*, 78.23 and 81.96% in *O. vulgare* subsp. *hirtum*, 60.74 and 85.71% in *O. sipyleum*, respectively. Essential oils distilled from the dried flowering tops contained more carvacrol than that of the essential oils distilled from the dried leaves. Essential oil ratio of oregano should be at least 2% according to ASTA standards. When these standards are taken into consideration, Turkish and Greek oreganos grown in non-irrigated, loamy and alkali soils of Denizli district were found to be higher than the standards.

P 18. Yield and chemical composition of the essential oil of Moroccan chamomile [C*ladanthus mixtus* (L.) Chevall.] growing wild in different areas of Morocco

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Keywords: *Cladanthus mixtus*; *Ormenis mixta*; *Chamaemelum mixtum*; *Anthemis mixta*; Asteraceae; Compositae; Moroccan chamomile

Recent floristic literature adopted new names for genera of Anthemideae (Compositae, Asteraceae). Based on phylogenetic analysis of *Chamaemelum* Mill. [1,2], the genus *Ormenis* is now named *Cladanthus* and therefore *Cladanthus mixtus* (L.) Chevall. is the newly adopted scientific name for the Moroccan chamomile [2].

Moroccan chamomile essential oil production ranks 9th in the Top 20 of Moroccan produced essential oils [3]. Given its commercial worth, the chemical variability of the essential oils isolated from Moroccan chamomile was evaluated.

Flowering aerial parts from *C. mixtus* populations were collected at nine Moroccan regions, Benguerir (Be), Bouznika (Bo), Chefchaouane (C), Kenitra (K), Meknes (M), Oujda (O), Settat (S), Sidi Alal lbahraoui (SAI) and Tamesna (T). The essential oils were isolated by hydrodistillation and analyzed by GC and GC-MS as in [4]. The essential oils yields ranged between 0.1-0.8% (v/d.w.). Only five out of the nine essential oil samples analyzed showed a good correlation after agglomerative cluster analysis based on the essential oils chemical composition. These samples (Be, K, S, M and T) were characterized by the dominance of camphor (14-27%), β-myrcene (3-17%) and santolina triene (3-15%). All these, and C essential oils showed blue colour, due to the presence of chamazulene (<1%), whereas O, Bo and SAI essential oils were yellow. β-Myrcene (3-17%), *trans*-β-farnesene (18%) and 2-tridecanone (16%) dominated C essential oil, whereas *trans*-β-farnesene (43%) was the main component in O essential oil. 2-Methyl-2,*trans*-butenyl methacrylate (34%) dominated Bo sample, and santolina alcohol and 1,8-cineole (17% and 12%, respectively) were the main components in SAI essential oil.

The presence of some characteristic volatile components suggests that Moroccan chamomile essential oils variability may result from the fact that under the same common chamomile name different species may be collected, and reinforces the importance of avoiding the wild population's harvest. Being the chemical composition so diverse, this will reflect negatively in the essential oil quality and biological activity.

Acknowledgments: Partially funded by the Transnational Cooperation research contract FCT/CNRST - 6818.

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P 19. New esters of long chain alcohols and isobutanoic and isovaleric acids from the essential oil of *Scandix pecten-veneris* L. (Apiaceae)

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Keywords: *Scandix pecten-veneris* ssp. *brachycarpa*; volatiles; synthesis; C13-C18, C21 and C23 alcohols; isobutanoate; isovalerate

Scandix pecten-veneris L. (Apiaceae) is a wild-growing plant species native to Eurasia. This species is commonly divided into three different subspecies - *brachycarpa* (Guss.) Thell, *pecten-veneris* L. and *macrorhyncha* (C. A. Meyer) Rouy & Camus.[1]. The young stem tops of the plant have been used to stop bleeding, amenorrhea and for bladder affections [2]. Since the volatiles of this plant species have been the subject of only two previous studies, that identified 12 and 10 constituents in total [2,3], we decided to perform a more detailed analysis of the aerial parts and roots essential oil of *S. pecten-veneris* ssp. *brachycarpa* from Southeastern Serbia (in total 7 samples). Among one hundred-nine constituents identified by GC (FID) and GC/MS, tridecane (8.5-52.8%), pentadecane (1.3-23.5%) and b-caryophyllene (0.1-18.5%) were found to be the most abundant ones. The analysis also pointed to the presence of 14 additional minor compounds (up to 0.9%), that were, according to their mass spectral fragmentation, tentatively identified as the isomeric tricosanyl-, heneicosanyl-, tri-, tetra-, penta-, hexa-, hepta- and octadecyl pentanoates and butanoates. In order to corroborate the tentative identification of these esters, and obtain sufficient quantities for biological activity assays, a synthesis was undertaken of 17 esters of isobutanoic, isovaleric and 2-methylbutanoic acids with appropriate alcohols. All synthesized esters (of which 9 entirely new and 13 found for the first time as plant secondary metabolites) were spectrally characterized (IR, MS, NMR) and separately GC co-injected with the essential oils. Except for tri-, penta- and heptadecyl 2-methylbutanoates, all other synthesized compounds were detected in the analyzed oils of *S. pecten-veneris*. The content of the new esters was determined from the appropriate GC-FID calibration curves of excellent linearity (R^2=0.9999) and varied within the range 0.01-9.33 mg / 100 g of dry plant material. The testing of the oils and of the herein synthesized compounds for antimicrobial activity is under way.

Acknowledgments: This work was funded by the Ministry of Education and Science of Serbia (Project 172061).

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P 20. Volatile secondary metabolites of *Senecio vernalis* Waldst. & Kit.

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Keywords: *Senecio vernalis* Waldst. & Kit.; Asteraceae; essential oil; roots; above-ground parts; chemotypification

Senecio vernalis Waldst. & Kit., Asteraceae, (local Serbian name: žutenika) is a plant species widely distributed in Asia and SE Europe [1]. However, limited data on the chemical composition of the corresponding essential oil exist [1,2]. For this reason, detailed chemical analyses of the volatile secondary metabolites of several populations of *S. vernalis* were conducted in order to provide an insight into the chemotypification of this species in Serbia. Analyses (by GC and GC-MS) of 8 essential oils hydrodistilled from dry above-ground parts and rhizomes of *S. vernalis* (4 different populations) enabled the identification of 60 different constituents, representing 95.5-99.4% of the total oils. The most abundant constituents of the oils obtained from the above-ground parts (all populations) were α-pinene (26.7-38.5%) and β-pinene (23.0-24.8%), whereas the most dominant constituents of the rhizome oils were either α-humulene (40.9-57.0%, 3 samples) or nonacosane (33.3%, 1 sample). Hence, the biosynthesis and/or accumulation of volatile metabolites in *S. vernalis* is strongly plant organ dependent. This was additionally corroborated by agglomerative hierarchical cluster analysis (AHC) and principal component analysis (PCA). Both AHC and PCA analyses of 30 different essential oils (18 different *Senecio* taxa; data from the literature and the present study) were performed using four different types of variables.

Acknowledgments. This work was funded by the Ministry of Education and Science of Serbia (Project 172061). PB was supported by UNESCO-L´OREAL National Fellowships Programme for Young Women in Life Sciences.

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P 21. Long chain 3-methyl-2-alkanones from the essential oil of *Inula helenium* L. (Asteraceae)

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Keywords: *Inula helenium* L.; Asteraceae; root essential oil; 3-methyl-2-alkanones; antistaphylococcal activity; 3-methyl-2-tetradecanone

Inula helenium L. (Compositae) is a perennial herb originally native to Southeastern Europe. In Serbia, it is a widespread plant, used in folk medicine mostly for the treatment of respiratory conditions, disorders of digestion, urinary infections, and for skin disorders. Very recently, we reported that *I. helenium* root essential oil possesses a very potent antistaphylococcal activity, with obvious membrane-damaging effects. Bioassay guided fractionation of the oil pointed to alantolactone, isoalantolactone and diplophyllin as the main carriers of the observed activity [1].

Herein, we report the identification of additional nine constituents of the oil from a fraction with a low MIC value (0.8 mg/ml). Mass spectral fragmentation pattern (*e.g*. base peak at *m/z* 72) of these compounds suggested their possible identity - long chain 3-methyl-2-alkanones. The difference of 14 amu (one CH_2 group) between the molecular ions of two consecutive compounds in the gas chromatogram, separated by *ca*. 100 RI units, pointed to the existence of a C_{10}-C_{18} homologous series. These 3-methyl-2-alkanones were synthesized in three reaction steps with the overall yields of 45-50% starting from an appropriate halogen alkane. Gas co-chromatography of the obtained compounds with the fraction of *I. helenium* root oil unequivocally corroborated the original assumption. The construction of GC-FID calibration curves (R^2=0.9997-0.9999) enabled us to determine the content of the ketones in the plant material: 0.05-25.1 mg/100 g of dry roots. The synthetic 3-methyl-2-alkanones were also screened for in vitro antimicrobial activity. These long chain 3-methyl-2-alkanones have a rather restricted occurrence in samples of natural origin: 3-methyl-2-decanone was found in the territorial marking fluid of the male Bengal tiger, *Panthera tigris* [2], while 3-methyl-2-tridecanone, 3-methyl-2-tetradecanone and 3-methyl-2-pentadecanone were detected in Dufour glands secretions of the species of desert-dwelling ants of the *Cataglyphis bicolor* group [3]. Hence, this is the very first report of long chain 3-methyl-2-alkanones as the plant secondary metabolites.

Acknowledgements: The authors acknowledge the Ministry of Education and Science of Serbia for the financial support (Project No 172061).

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P 22. Chemical composition of tuber essential oil from *Helianthus tuberosus* L. (Asteraceae)

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Keywords: *Helianthus tuberosus*; Jerusalem artichoke; tubers; essential oil; helianthol A; dihydroeuparin

The genus *Helianthus* L. (Asteraceae) comprises about 50 annual and perennial sunflower species. *Helianthus tuberosus* L. (Jerusalem artichoke) is cultivated in Europe and other parts of the world as a crop and ornamental plant. The volatile oils of the aerial parts of *H. tuberosus* were analyzed in 1982, but no study has been carried out to date to determine the constituents of the tuber essential oil [1]. Herein, we describe the first analysis by GC and GC-MS of the hydrodistilled essential oil of the Jerusalem artichoke tubers. The fresh plant material, collected in Serbia, yielded only a small amount of the oil (0.0014%, w/w). One hundred-one constituents were identified in total, representing 89% of the oil. The main constituents were β-bisabolene (18.8%), undecanal (10.4%), α-pinene (6.2%), kauran-16-ol (5.7%), 2-pentylfuran (4.7%) and (2*E*)-tetradecenal (4.0%). Several rare compounds characteristic for *Helianthus* sp. were also detected: helianthol A (0.9%), desmethoxy encecalin (0.2%), desmethyl isoencecalin (trace amount), encecalin (trace amount), dihydroeuparin (1.9%), euparin (0.2%) and eupatoriochromene (0.3%). The tuber essential oil and the aerial parts one share a common major component - β-bisabolene.

Acknowledgments: This work was funded by the Ministry of Education and Science of Serbia (Project 172061).

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P 23. Antimicrobial volatile oil of *Lycopus europaeus* L. (Lamiaceae)

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Keywords: *Lycopus europaeus* L.; gypsywort; Lamiaceae; essential oil; antimicrobial activity; hotrienol

Lycopus europaeus L. (Lamiaceae), commonly known as gypsywort, is a perennial plant native to Eurasia. Gypsywort´s most important medicinal properties come from the stem and leaves and these were traditionally used as astringents, sedatives, tonics and narcotics, as well as in the treatment of hemorrhages, nervous heart palpitations, goiter and Graves´ disease [1, 2]. On only one previous occasion the essential oil of this taxon was investigated [3]. However, this work was undertaken some 40 years ago and is lacking the cutting edge of modern analytical techniques. This provoked us to carry out a detailed study of *L. europaeus* essential oils from the blooming (sample A) and fruit forming stages (sample B), and to screen these for *in vitro* antimicrobial activity in order to determine if some of these constituents may contribute to the plant's renowned ethnomedicinal uses.

GC and GC/MS analysis of the oils allowed the identification of 199 constituents. The oils consisted mainly of sesquiterpenoids, the main one being b-caryophyllene (13.9% and 25.7%) in both cases. Other important contributors were shyobunol (9.7% of sample A) and caryophyllene oxide (2.4% and 12.5%, in samples A and B, respectively). In addition to this rather unequal distribution of the main sesquiterpenoids, another significant feature that distinguished the two samples was the inverted ratio of the mono- (15.6% against 1.6%) and diterpenoids (24.6% against 8.8%). The monoterpenoid fraction was characterized by the high content of oxygenated acyclic derivatives among which the most predominant one was the rare monoterpenol (*E*)-hotrienol. Screening of *L*. *europaeus* essential oil for its *in vitro* antimicrobial activity showed that this oil possesses a selective action against two Gram negative bacterial strains, *Escherichia coli* and *Klebsiella pneumoniae*.

Acknowledgements: The authors acknowledge the Ministry of Education and Science of Serbia for the financial support (Project No 172061).

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P 24. Determination of the absolute stereochemistry of limonene and α-santalol by both vibrational circular dichroism and raman optical activity spectroscopies

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Keywords: absolute configuration; Vibrational circular dichroism (VCD); Raman optical activity (ROA); limonene; α-santalol

Chirality is an important factor affecting the odor characteristics of fragrance compounds. Thus, it is fundamentally important to determine the absolute configuration of chiral odor compounds. Although X-ray crystallographic analysis is a useful and powerful method for determining the stereochemistry of crystalline organic compounds, it is necessary to obtain the good-quality crystals of the target molecule. Many types of terpenoids, especially monoterpenes and sesquiterpenes, with chiral centers are known. These compounds are important in fragrance, medicinal, and food chemistry. However, most of these compounds are liquids, and so evaluating their absolute configuration is difficult. Vibrational circular dichroism (VCD) and Raman optical activity (ROA) spectroscopies should be useful in solving this problem. Recently, we reported the preliminary results by using these two methods [1]. We measured infrared, Raman, VCD, and ROA spectra of (*R)*-(+)-limonene, (*S*)-(-)-limonene, and (*E*)-(+)-α-santalol. The optimized structures and infrared, Raman, VCD, and ROA spectra were calculated for these compounds by DFT method at B3LYP/6-31G* and B3LYP/6-311+G** levels. We compared the observed infrared, Raman, VCD, and ROA spectra of the compounds with those calculated by DFT method. We succeded in the determination of the absolute configurations of (*R)*-(+)-limonene, (*S*)-(-)-limonene, and (*E*)-(+)-α-santalol.

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P 25. Antimicrobial activity of essential oil isolated from young twigs of Macedonian pine (*Pinus peuce* Griseb., Pinaceae)

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Keywords: *Pinus peuce*; essential oil; antimicrobial activity.

Essential oils isolated from pine species have shown variable antimicrobial activity against different microorganisms [1, 2, 3]. Up to present, there is a lack of data that are related to the antimicrobial activity of *Pinus peuce*, thus the aim of this study was to assess antimicrobial activity of the essential oil isolated from young twigs of *Pinus peuce* from Macedonian flora. Essential oil was isolated by hydrodistilation in Clevenger type apparatus. Analysis of the essential oil composition by GC/FID/MS method reviled α- and β-pinene as dominant components, beside limonene+β-phellandrene, bornyl acetate, *trans*-caryophyllene and germacrene D. Antimicrobial screening of the essential oil was made by hole-plate diffusion and broth dilution method against 13 bacterial isolates representing both Gram positive and Gram negative bacteria and one strain of *Candida albicans*. The most sensitive bacteria against tested *Pinus peuce* essential oil were *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Enterococcus*, *Candida albicans*, encompassing *Staphylococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter* spp*., Escherichia coli* and *Salmonella enteritidis.*Minimal inhibitory concentrations (MICs) of the oil ranged from 7.5 - 125 µl/ml. As this essential oil has shown promising activity towards the above mentioned microorganisms, it could be considered as antimicrobial agent for inhalation for the infections of upper respiratory tract, for treatment of urinary and gastrointestinal infection as well as different skin conditions.

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P 26. Essential oils composition of *Hyptis* species with occurrence in the Brazilian Amazon

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Keywords: *Hyptis crenata*; *H. recurvata*; *H. parkeri*; *H. dilatata*; *H. suaveolens*; *H. alutacea*; essential oil composition

The genus *Hyptis* (Lamiaceae) is composed of 400 species occurring in tropical America. The species *H. suaveolens*, *H. mutabilis*, *H. goyazensis* and *H. crenata* were previously studied by us [1-4]. The essential oils of leaves and fine stems of *Hyptis crenata* Pohl ex Benth. (1), *H. recurvata* Poit. (2), *H. parkeri* Benth. (3), *H. dilatata* Benth. (4), *H. suaveolens* (L.) Poit (5). and *H. alutacea* Pohl ex Benth. (6) were analyzed by GC and GC-MS. The plants were collected in Eastern Amazon, in the municipality of São Geraldo do Araguaia (1,2), Pará state, and in the municipalities of São Felix de Balsas (3) and Mirador (4-6), Maranhão state. The main constituents identified in the oil of *H. crenata* were 1,8-cineole (43.8%), α-pinene (20.7%) and ß-pinene (8.0%); in the oil of *H. recurvata* were ß-elemeno (15.5%), (E)-caryophyllene (14.0%), α-selinene (10.2%), ß-selinene (5.6%), γ-elemene (4.9%), germacrene D (4.8%) and caryophyllene oxide (4.6%); in the oil of *H. parkeri* were spathulenol (27.9%), δ-elemene (7.5%), bicyclogermacrene (7.2%) and caryophyllene oxide (6.1%); in the oil of *H. dilatata* were camphor (32.6%), 1,8-cineole (19.9%), p-cymene (5.2%), γ-terpinene (5.4%) and α-pinene (4.3%); in the oil of *H. suaveolens* were (*E*)-caryophyllene (18.1%), bicyclogermacrene (11.2%), δ-elemene (12.3%), 1,8-cineole (9.1%) and spathulenol (8.1%); and in the oil of *H. alutacea* were γ-cadinene (19.1%), δ-cadinene (10.5%), α-muurolene (8.0%) and (*E*)-caryophyllene (7.2%).

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

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P 27. Essential oils composition of some Myrtaceae species from Eastern Amazon, Brazil

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Keywords: Myrtaceae species; essential oil variation; Eastern Amazon

The Myrtaceae is comprised of about 80 genera and 3,000 species distributed in warm climates, with centers of occurrence in Australia and America. As part of the survey of Amazon aromatic flora, this work presents the chemical composition of essential oils of the following species of Myrtaceae: *Psidium myrsinites* Mart. ex DC. (1), *Myrcia fallax* (Rich.) DC. (2), *M. atramentifera* Barb. Rodr. (3), *M. bracteata* (Rich.) DC. (4), *Calycolpus goetheanus* (DC.) O. Berg (5), *Eugenia punicifolia* (Kunth) DC. (6), *E. patrisii* Vahl (7) and *E. piauhiensis* O. Berg (8). These species were collected in eastern Amazon, comprising the municipality of São Geraldo do Araguaia (1-4), Pará state, and the municipalities of São Domingos do Azeitão (5) and Mirador (6-8), Maranhão state. The essential oils of these species were hydrodistilled and analyzed by GC and GC-MS. The main constituents (over 4%) of *P. myrsinites* oil were (*E*)-nerolidol (18.6%), 1,8-cineole (14.6%), α-pinene (11.0%), (*E*)-caryophyllene (10.4%), *p*-cymene (4.5%) and carvacrol (4.0%); in the *M. fallax* oil were α-cadinol (11.1%), 1-*epi*-cubenol (7.7%), *epi*-α-muurolol (6.9%), guaiol (6.5%), viridiflorol (5.9%), δ-elemene (4.9%) and selin-11-en-4α-ol (4.8%); in the *M. atramentifera* oil were α-cadinol (12.9%), δ-cadinene (10.6%), *epi*-α-muurolol (6.5%), α-copaene (6.3%) and *epi*-α-cadinol (4.3%); in the *M. bracteata* oil were caryophyllene oxide (10.1%), α-cadinol (10.0%), δ-elemene (6.8%), (*E*)-caryophyllene (6.6%), *epi*-α-muurolol (5.5%), α-calacorene (5.0%) and germacrene D (4.5%); in the *C. goetheanus* oil were (*E*)-caryophyllene (31.0%), caryophyllene oxide (14.7%) and α-humulene (9.7%); in the *E. punicifolia* oil were (*E*)-caryophyllene (18.8%), caryophyllene oxide (12.3%), 1-*epi*-cubenol (8.3%), α-humulene (5.3%), *trans*-calamenene (4.7%), α-cadinol (4.4%) and α-copaene (4.2%); in the *E. patrisii* oil were germacrene D (23.5%). (*E*)-caryophyllene (10.9%), δ-elemene (7.9%), β-copaene (5.7%), bicyclogermacrene (5.7%) and δ-cadinene (5.6%); in the *E. piauhiensis* oil were (*E*)-caryophyllene (19.7%), γ-elemene (14.7%) and δ-elemene (4.5%).

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

P 28. Nematoxic effect of essential oils and their fractions against the pinewood nematode, *Bursaphelenchus xylophilus*

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Keywords: *Bursaphelenchus xylophilus*; *Pinus pinaster*; essential oils; monoterpenes; nematicidals; 2-undecanone

The pinewood nematode (PWN) *Bursaphelenchus xylophilus* is a highly pathogenic plant parasite that greatly affects pine forests. In Portugal, the most affected species is *Pinus pinaster* Aiton. Despite great efforts, since its fist detection in 1999, the PWN has spread through the country, including Madeira Island, having been recently detected in Spain [1,2]. Containing this pest is of the utmost importance for European pine forest safeguard.

Since most synthetic chemicals used to control phytoparasites are toxic to humans and animals, and can accumulate in the soil and in food plants [3], in the present work, the nematoxic potential of over 80 essential oils (EOs), isolated from the Portuguese flora, were assessed against the PWN. EOs were isolated by hydrodistillation and analysed by GC and GC-MS [3]. EOs hydrocarbon and oxygen-containing fractions were obtained as in [4]. Direct-contact assays, adapted from [3], were performed by adding EOs/methanol stock-solutions to 50-100 mixed-stage PWN suspensions. After 24h in darkness, dead and live nematodes were counted under an inverted microscope. Assays were repeated at least 10 times in two series.

Mortalities ≥96% were obtained with 2µL/mL of the EOs isolated from *Cymbopogon citratus*, *Eucalyptus citriodora*, *Mentha arvensis*, *Origanum virens*, *Origanum vulgare*, *Ruta graveolens*, *Satureja montana*, *Syzygium aromaticum*, *Thymbra capitata*, *Thymus caespititius* (carvacrol and/or thymol-rich), *Thymus vulgaris* and *Thymus zygis*. These EOs were further tested at 1, 0.5 and 0.25µL/mL. Minimum lethal concentrations (LC100) <0.4µL/mL, were obtained for the 2-undecanone-rich *R. graveolens* EO and the carvacrol and γ-terpinene-rich *S. montana* and *T. capitata* EOs. Assays with EO fractions revealed that the monoterpene-rich nematoxic EOs control PWN through their combined hydrocarbon and oxygen-containing fractions through additive and/or synergic relations.

As complex mixtures of active components, EOs may prove to be effective nematoxic agents.

Acknowledgments: Jorge Faria is grateful to the Fundação para a Ciência e a Tecnologia (FCT) for the PhD grant SFRH/BD/43738/2008. This study was partially funded by FCT, under research contract PTDC/AGR-CFL/117026/2010.

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P 29. Chemotypification of *Astrantia major* L. (Apiaceae) inferred from its fruit essential oil profile

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Keywords: Essential oils; *Astrantia major*; Apiaceae; Saniculoideae; Multivariate statistical analyses; Chemotaxonomy

Astrantia major L. (family Apiaceae, subfamily Saniculoideae) is a perennial plant species found in the Pyrenees, Carpathian Mountains, Balkan Peninsula and Western Asia [1]. The only previous study [2] dealing with the chemical composition of the essential oil of this plant species reported the isolation and structural elucidation of three oxygenated sesquiterpenes (β-sinensal, β-sinensol and β-sinensyl acetate). We decided to reanalyze, in much more detail, the essential oil of the up to now uninvestigated populations of *A. major*. In total, seventy-six constituents identified by GC and GC/MS accounted for 92.7-94.0% of the oils from two populations (Serbia and Poland). The oils differed significantly - the wild-growing population from Serbia contained zingiberene (47.9%), β-bisabolene (9.7%) and β-sesquiphellandrene (7.9%), whereas the one from Poland (botanical gardens) was sesquiterpene-poor with oleic acid (38.6%), nonacosane (15.4%) and linoleic acid (5.1%) as the major contributors. Motivated by the unresolved taxonomical relations between the Saniculoideae and Apioideae subfamilies, we performed multivariate statistical analyses (agglomerative hierarchical cluster analysis (AHC) and principal component analysis (PCA)) on the compositional data of these A. major samples and additional 15 Saniculoideae and 31 Apioideae taxa. This allowed us to assess the chemotaxonomical usefulness of such chemical data in differentiating taxa from these two Apiaceae subfamilies and to corroborate the existence of at least two *A. major* chemotypes.

Acknowledgement: This work was funded by the *Ministry of Education and Science of Serbia* (Project 172061).

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P 30. Phenological variability of the alkaloids content in the essential oil of *Conium maculatum* L.

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Keywords: *Conium maculatum*, essential oil, conmaculatin, 2-pentyl-3,4,5,6-tetrahydropyridine, coniceine, coniine.

Conium maculatum L. (Apiaceae), hemlock, is a worldwide distributed, highly poisonous weed. Up to now ten volatile alkaloids have been identified from this plant species [1,2]. Coniine and γ-coniceine are generally the most abundant ones and they account for the most of the plant's acute and chronic toxicity [2]. Recently, we reported the existence of a dihomologue of coniine (2-pentylpiperidine, conmaculatin) in the fruit essential oil of hemlock [3]. In this work, the content of coniine, γ-coniceine and their dihomologues was determined by detailed GC-MS analyses of twelve samples of hemlock and tracked during different phenological phases. The highest levels of γ-coniceine and 2-pentyl-3,4,5,6-tetrahydropyridine were detected in the first stages of phenological development (11.0-47.8% and 0.5-3.7%, respectively), and at the same time coniine (7.0-15.8%) and conmaculatin (0-0.7%) were present in significantly lower amounts. During fruit ripening, coniine (15.4-35.6%) and conmaculatin (0.7-1.3%) content increased substantially, whereas that of γ-coniceine (0.4-7.1%) and its dihomologue (0.1-0.4%) dropped accordingly. These results indicate that an analogous biosynthetical relationship between conmaculatin and 2-pentyl-3,4,5,6-tetrahydropyridine exist as for the coniine/γ-coniceine pair.

Acknowledgement: This work was funded by the *Ministry of Education and Science of Serbia* (Project 172061).

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P 31. *Myrtus communis* from Algeria: chemical composition and variability

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Keywords: *Myrtus communis*; Algeria; chemical composition; variability.

The aim of the present study was to evidence either an homogeneity or a chemical variability within oil samples isolated from aerial parts of *Myrtus communis* L. growing wild in Algeria.

The chemical composition of 55 oil samples of *Myrtus communis* isolated from aerial parts harvested, at the flowering stage, in 16 locations from East to West Algeria was investigated by GC (FID), in combination with retention indices and by ^{13}C NMR spectroscopy. The results were submitted to statistical analysis.

The major components were α-pinene (27.4-59.2%) and 1, 8-cineole (6.1-34.3%), and were characterized by the lack of myrtenyl acetate. Statistical analysis allowed the distinction of two groups including each one two sub-groups. Groups I (78% of the samples) and II may be distinguished on the basis of the content of α-pinene, linalool and linalyl acetate. Subgroups IA and IB could be distinguished by their contents of α-pinene and 1,8-cineole. Subgroups IIA and IIB differed substantially by their contents in cineole and limonene.

According to the present and previous results, there is no correlation between pedo-climatic factors and the chemical composition of Algerian myrtle leaf oil. Taking into account these results as well as literature data on the composition of myrtle oils from North Africa, we remark that all the reported Moroccan oil samples contained myrtenyl acetate, and in contrast, oils samples from Algeria and Tunisia were characterized by the lack of myrtenyl acetate. As far the information was provided, it appeared that all the Moroccan myrtle oils were isolated from plants collected in the west part of Morocco and therefore, it could be hypothesized that Atlas Mountains constitute the natural limit between myrtle characterized by the presence or by the lack of myrtenyl acetate.

Acknowledgments: A. Bouzabata wishes to thank Dr G. De Belaire (Badji-Mokhtar University, Annaba, Algeria) for identification of the plant.

P 32. Determination of minimal inhibitory and microbicidal concentrations of essential oils produced by Mediterranean plants

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Keywords: essential oils; antimicrobial effect; pathogenic microorganisms

The aim of this work was to evaluate the antimicrobial effect of essential oils, for its possible application on salads to control the contaminant microbial flora. Methods: Essential oils of oregano (*Origanum vulgare*), three different sages (*Salvia officinalis, Salvia lavandulaefolia* and *Salvia sclarea*) and rosemary (*Rosmarinus officinalis*) were analyzed to evaluate its antimicrobial action. These oils were tested by two methods against *Yarrowia lipolytica*, *Pseudomonas aeruginosa* and *Listeria monocytogenes.* First it was determined the minimum inhibitory concentration (MIC) of -all the essential oils, by the micro dilution method on 96 well plates, at 25 or 37ºC respectivelly. The determination of MIC was done by measuring the growth absorbance at 540 nm in all micro plates. To evaluate the most suitable oils for future work, statistical analyses were performed using ANOVA and Tukey tests with the R software. After MIC determination, for results scaling up purposes, it was necessary to study the minimum microbicidal concentration (MMC) by the dilution method in liquid medium. The MMC were tested using three of the five initial essential oils that presented the best performance: *Origanum vulgare, Rosmarinus officinalis* and *Salvia lavandulaefolia*. Spreading inoculations were done on agar plates for surviving cells count and the variability of the obtained calculations was evaluated. The inhibitory concentrations of the essential oils tested are discussed.

All the microbial strains demonstrated the most sensitivity to the *Origanum vulgare* essential oil and the least sensitivity to the *Salvia* spp. essential oils. The antimicrobial activity of *Origanum vulgare* oil is effective against *Yarrowia lipolytica*, its MMC correspond to 0.235% (v/v). However, it was found that *Pseudomonas aeruginosa* has a low sensitivity to the antimicrobial effect of these essential oils. The results obtained with *Listeria monocytogenes* for *Rosmarinus officinalis* showed a MMC of 3.75% (v/v).

The results obtained are promising for the intended application but further studies should be done.

P 33. Chemical composition and antinociceptive properties of the essential oil of *Ferula ovina* (Umbelliferae)

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Keywords: *Ferula ovina*; essential oil; vanillates; Umbelliferae

Ferula L. is a genus of about 170 species of Umbelliferous flowering plants native to the Mediterranean region and east to central Asia. The Iranian flora comprises 30 species of this genus, one of them being *Ferula ovina* (Boiss.) Boiss. The aqueous extracts of *F. ovina* was found to possess antispasmodic, anticholinergic and smooth muscle relaxant activities [1]. Although the essential oil composition of *F. ovina* was previously reported [2,3], the potential pharmacological activity of this oil remains unknown up to date. Having this and the ethnopharmacological use of this plant species in mind, we decided to assess the antinociceptive activity of the essential oil of the aerial parts of *F. ovina* (collected in Iran) using the hot plate and tail immersion methods for central and the abdominal writhing test for peripheral analgesia in BALB/c mice. In the highest applied dose, the oil showed a strong peripheral analgesic activity by causing a 92.4% decrease in the number of writhes induced by acetic acid. On the other hand, the oil exerted only moderate central analgesic activity, reaching 55.8% of inhibition in the tail immersion test. These interesting results provoked us to perform detailed compositional analyses of the oil. GC and GC-MS analyses allowed the identification of 120 constituents. The oil consisted mainly of monoterpene hydrocarbons. The major constituents of this oil were camphene (18.0%), limonene (14.3%) and myrcene (13.5%). The oil also contained a number of rare esters of borneol. The structures of these esters were corroborated by synthesis. Bornyl *p*-methoxybenzoate turned out to be a new natural compound. Currently, additional work is underway to determine the active principles of this essential oil.

Acknowledgments: This work was funded by the Ministry of Education and Science of Serbia (Project 172061).

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P 34. Effect of *Origanum vulgare* essential oil on *Penicillium digitatum* using warm air flow

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Keywords: oregano; essential oil; antimicrobial; postharvest diseases; lemon; shelf-life

Antimicrobial activity of essential oils (EOs) is well known, but still is not widespread in crop products treatment. Because of high costs for treatment with EOs, new and cheaper methods are recommended. *Penicillium digitatum* is one of the main fungus causing storage loses on citruses and *Origanum vulgare* EO is known for its antimicrobial properties [1]. Method for treatment with essential oils vapours was greatly improved. Gas chromatograph (5890A GAS Chromatograph, Hewlett Packard) was modified as a warm air flow treating box (oven temperature: 40°C). Glass cube was inserted into the oven and the detector heater was ejected into the oven and used for vaporization of EO (detector temperature: 150°C). Antimicrobial activity of *O. vulgare* EO was tested in vitro and in model system on *Citrus limon* fruits. Inoculum was made from fourteen days old cultures of *P. digitatum* dissolving in Tris buffered saline with tween 80.

Six inoculated Petri dishes (55 mm diameter) or six *C. limon* fruits inoculated with *P. digitatum* in three artificial wounds (5 mm diameter) were placed on the bottom of glass cube. Both were kept in warm air flow for five minutes. EO from *O. vulgare* in concentrations 0.5; 2; 4 and 8 $\mu l.l^{-1}$ of air was used. Carvacrol (64,6%), para – cymene (5,2%) and thymol (2,9%) are the main components identified by GC-MS. The radial growth of *P. digitatum* on *C. limon* fruits were measured and Petri dishes were checked for *P. digitatum* growth after 4 days of incubation in 25°C. All tests were conducted in triplicate. Growth of *P. digitatum* on Petri dishes was totally inhibited by EO concentration 4 $\mu l.l^{-1}$. EO in 8, 4, 2 and 0.5 $\mu l.l^{-1}$ concentrations reduced *P.digitatum* radial growth on *C. limon* fruits to 39, 46, 70 and 79 % respectively.

Previous in vitro experiments show inhibition in concentrations about 400 $\mu l.l^{-1}$[2]. The method using activation of EO with temperature and air flow rapidly reduces effective concentration (100x). Moreover this method could be improved for mass production and is suitable for any fruits and vegetables.

Acknowledgement: This presentation is supported by University-wide internal grant agency - CIGA 20122008 – Plant products treatment with essential oil vapours under different physical conditions.

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P 35. Essential oil composition of juniper berry (*Juniperus communis* L., Cupressaceae) from Macedonian flora

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Keywords: *Juniperus communis*; essential oil composition; GC/FID/MS.

The berries of common juniper, *Juniperus communis* L. (Cupressaceae), is used for isolation of juniper oil, which has multi-purpose medicinal use as diuretic, antiseptic, stomachic, antireumathic, *etc*. [1]. Among medicinal, there is also a large commercial use in food industry and cosmetic and perfume production. The aim of the present study was determination of the chemical composition of Macedonian juniper berry oil in correlation to the areas of collection. The samples of berries were collected on twelve different locations in Republic of Macedonia. The essential oils were isolated by steam-distillation in the Clevenger-type apparatus using official method from European pharmacopoeia [2]. The chemical composition of essential oil samples was analyzed by gas chromatography system equipped with flame ionization detector and mass spectrometer as well as capillary flow technology. For that purpose, HP-5ms capillary column was used. Seventy-four components were identified representing 91.08 to 99.83% of the entire oils. The predominant fractions of the oils were monoterpene hydrocarbons presented from 39.11 to 73.38% of total oil composition. Great variability in the chemical composition and the content of some components was registered, depending of the area of collection. The most variable were α-pinene (15.59-43.19%), β-pinene (1.65%-5.35%), β-myrcene (2.89%-26.50%), sabinene (2.80-11.77%) and limonene (2.90-4.46%). In the fraction of oxygen-containing monoterpenes the most abundant was terpene-4-ol (0.02-6.32%), while in the fraction of sesquiterpenes germacrene D (2.03-10.22%), β-elemene (1.43-6.40%) and *trans*-(E)-caryophyllene (1.8%-4.05%). The differences in the environmental conditions of plant growth and the characteristics of the locations where the collections were made, were considered as important factors for the variability in the essential oil composition of Macedonian juniper.

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P 36. Glandular trichomes, histochemical localization of secretion and essential oil composition in *Plectranthus grandidentatus*

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Keywords: *Plectranthus grandidentatus*; Lamiaceae; glandular trichomes; histochemistry; essential oils

A large number of *Plectranthus* species are well known for their medicinal uses, while others are very popular as ornamentals [1]. *Plectranthus grandidentatus* Gürke is an herbaceous perennial herb commonly used as groundcover due to its procumbent growth habit and pleasant scented leaves. Although this species was not reported as medicinal, their abietane diterpenoids showed activity against methicillin- and vancomycin-resistent bacteria [2].

As part of a ongoing study on *Plectranthus* species [3,4,5], we report herewith the morphology and histochemistry of the glandular trichomes, and the essential oils composition from *P. grandidentatus* growing in Portugal.

The types of glandular trichomes and their pattern of distribution on the leaves and flowers were studied by LM and SEM as in [3]. The stems and leaves of *P. grandidentatus* are covered by a tomentose indumentum composed of long, uniseriate and multicellular nonglandular trichomes. Five distinct types of glandular trichomes were identified. Peltate trichomes showed an orange characteristic colour and never developed a large subcuticular space. Capitate trichomes were divided in three subtypes according to the dimension of the glandular head, length of stalk and secretion process. The flower exhibited also uncommon conoidal trichomes. The histochemical characterization of the secretion revealed the presence of terpenoids and flavonoids as the main constituents.

The essential oils, isolated by hydrodistillation of dried leaves and stems, were analyzed by GC and GC-MS as in [6]. The oils yields ranged from 0.02 to 0.05% (v/w). A total of 70 components were identified, representing more than 84% of the oils. Sesquiterpenes constituted the dominant fraction (58-68%), being β-caryophyllene, α-santalene and α-humulene the most abundant hydrocarbons and β-caryophyllene oxide and ledol the major oxygen-containing compounds. Monoterpene fraction did not exceed 10% and only α-pinene and *trans*-β-ocimene reached percentages above 1%.

The data obtained showed the high diversity of *P. grandidentatus* glandular trichomes and, as far as we know, this is the first report on their secretory structures and essential oils.

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P 37. Chemical and statistical analysis of *Achillea clypeolata* Sibth. & Sm. essential oils: plant organ-dependent biosynthesis/accumulation of the volatiles

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Keywords: *Achillea clypeolata* Sibth. & Sm.; Asteraceae; essential oil; above-ground parts; multivariate statistical analysis

Achillea L. (Asteraceae) is a genus of about 85 flowering plants, commonly referred to as *yarrows* that occur in Europe and temperate areas of Asia. A number of *Achillea* species were the subject of extensive phytochemical studies, mainly due to their (ethno)pharmacological importance [1-4]. *Achillea clypeolata* Sibth. & Sm., an endemic species to the Balkan Peninsula and Asia Minor, is among those members of this genus that have received little attention and only several papers can be found in the literature dealing with *A. clypeolata* volatile secondary metabolites [1-3]. It has been previously reported that collections of *A. clypeolata* growing in different regions of Bulgaria differ in their chemical constituents to such an extent that the existence of chemotypes might be suggested [4]. Bearing this in mind, and in continuation of our chemical investigations of Asteraceae species used in folk medicine, we have now studied five Serbian populations of *A. clypeolata*. Analyses (GC and GC-MS) of 10 essential oil samples hydrodistilled from dry flowers and vegetative above-ground parts of *A. clypeolata* enabled the identification of more than 100 different constituents, representing 90-95% of the total oils. The most abundant constituents of the obtained oils were 1,8-cineole (0.1-34.8%), *trans*-verbenol (0-38.2%), camphor (0-17.9%), borneol (0-6.9%), *p*-mentha-1,5-dien-8-ol (0-9.5%), 4-terpineol (1.2-8.1%), germacrene D (1.3-9.8%), elemol (0.4-14.85%), g-eudesmol (trace-8.8%) and b-eudesmol (1.4-13.3%). As revealed by chemical analyses and a multivariate statistical treatment of the oils (agglomerative hierarchical cluster analysis and principal component analysis), the biosynthesis and/or accumulation of volatile metabolites in *A. clypeolata* is strongly plant organ–dependent. In addition, the obtained results showed that the studied populations do not belong to a single *A. clypeolata* chemotype. Moreover, they suggest that the volatile profile of the studied species is susceptible to environmental and climatic factors.

Acknowledgments: This work was funded by the Ministry of Education and Science of Serbia (Project 172061). PB was supported by UNESCO-L´OREAL National Fellowships Programme in Serbia.

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P 38. Effects of methyl and isopropyl *n*-methylanthranilates from the essential oil of *Choisya ternata* Kunth (Rutaceae) on experimental anxiety and depression in mice

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Keywords: methyl N-methylanthranilates; isopropyl N-methylanthranilates; *Choisya ternata* Kunth; essential oil; light/dark test; tail suspension test

Choisya ternata Kunth (Rutaceae) is a plant species whose leaves are used in Mexican folk medicine for its antispasmodic and stimulative properties. Up to now, the essential oil of this plant species (Mexican orange) has been investigated on one previous occasion with only eighteen reported components [1]. This motivated us to perform detailed GC and GC-MS analyses of the hydrodistillate from *C. ternata* leaves. The essential oil turned out to be a complex mixture of compounds of which 157 were identified (the extent of identification of components being more than 98% of the total detected GC peak areas). A characteristic of the oil was the presence of two alkaloids - isopropyl and methyl *N*-methylanthranilates. Their structures were confirmed by GC co-injection of the synthesized standards with the essential oil of Mexican orange. Motivated by the ethnopharmacological use and availability through synthesis, we decided to evaluate the anxiolytic and antidepressant effects of the two volatile anthranilates in open field, horizontal wire, light/dark, forced swimming and tail suspension tests, as well as their effect on the onset and duration of diazepam-induced sleep in BALB/c mice. The volatile alkaloids (50-200 mg/kg, *i.p.*), without having a muscle relaxant effect, caused a significant decrease in the time the animals spent in an unsecured and putatively dangerous area when compared with the control group, but had no effect on the number of crossings between the light/dark compartments. In addition to this anxiolytic activity, a significant antidepressant-like effect was apparent at all tested doses, which was not due to an increase in locomotive activity. The anthranilates administered on their own did not induce sleep in mice, but significantly prolonged the diazepam-induced sleep, in a dose dependent way, suggesting an interaction with the GABA receptor complex. The obtained data at least partially provide a justification for the ethnomedicinal usage of Mexican orange and point to the two volatile alkaloids as the active principles from the essential oil.

Acknowledgments - This work was funded by the Ministry of Education and Science of Serbia (Project 172061).

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P 39. Chemotaxonomy of Balkan *Geranium* and *Erodium* species inferred from the essential oil chemical composition

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Keywords: Geraniaceae; *Geranium*; *Erodium*; Essential oil; Chemotaxonomy; Multivariate statistical analysis

The genus *Geranium* L. (Geraniaceae) comprises *ca.* 250 species, distributed mainly in the temperate region of the northern hemisphere, but no comprehensive taxonomic treatment is nowadays available [1]. A closely related genus of this plant family, *Erodium* L' Hérit. includes more than 50 species, of which, about 40 are widespread across the Mediterranean region. According to Janković [1], nineteen *Geranium* and two *Erodium* species can be found in the Serbian flora.

Very little attention was paid to the species of these genera with respect to their volatile constituents. In general, reports regarding the essential oils of only few species exist in the literature: *G. macrorrhizum* [2-3], *G. robertianum* [4], *G. phaeum* [2] and *E. cicutarium* (leaf hexane extract [5]).

Prompted by the lack of such compositional data, we decided to perform detailed GC-MS analyses of the essential oils isolated from seven *Geranium* (*G. macrorrhizum*, *G. robertianum*, *G. sanguineum, G. columbinum, G. lucidum, G. purpureum* and *G. phaeum*) and three *Erodium* (*E. cicutarium*, *E. ciconium* and *E. absinthoides*) species from Serbia and Macedonia. This resulted in the identification of 551 and 233 constituents in total, respectively. The significance of the variations in essential oil composition/production was determined using multivariate statistical analyses (agglomerative hierarchical clustering analysis and principal component analysis). The main conclusion, based on the dendograms and biplots obtained as the results of the performed analyses, using relative abundances of the identified oil constituents, consists in that there is no great intergeneric variability in the oil compositions, *i.e.* the chemical composition of the investigated *Geranium* and *Erodium* taxa was generally very similar. These results confirm the close phylogenetic relationship between the two genera.

Acknowledgment: This work was supported by the Ministry of Education and Science of Serbia [Project No. 172061].

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P 40. Antimicrobial volatile glucosinolate autolysis products from *Hornungia petraea* (L.) Rchb. (Brassicaceae)

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Keywords: Brassicaceae; *Hornungia petraea*; Glucosinolate; Isothiocyanate; Antimicrobial activity

The genus *Hornungia* comprises approx. 10 species, distributed in Europe, North Africa and west Asia. In Serbia, the genus is represented by only one species – *Hornungia petraea* (L.) Rchb. (syn. *Hutchinsia petraea* (L.) R. Br.) [1].

In general, only two previous references on the phytochemistry of *Hornungia* and the closely related *Hutchinsia* taxa can be found regarding the analysis of glucosinolates (GLS) and their volatile breakdown products in *Hutchinsia alpina* [2,3].

Recently we reported the results of a study of volatile GLS autolysis metabolites in plant samples of *H. petraea* [4]. GC–MS analysis of the autolysate and the synthesis of a series (12 compounds) of possible GLS breakdown products revealed/corroborated the presence of glucoaubrietin, glucolimnanthin, glucolepigramin and glucotropaeolin in this species as the most likely ‘‘mustard oil’’ precursors.

According to the classification of GLS-containing species based on GLS profiles (subdivision of the crucifers based on their GLS content into four groups) [3], this taxon and *Hutchinsia alpina*, a species closely related to *H. petraea*, the only studied from this genus, could be recognized as crucifers with the predominance of simple aromatic GLSs. The similarity of the GLS profile of the two phylogenetically close taxa provides support for the suggestion of Appel and Al-Shehbaz [5] to reduce the genus *Hutchinsia* (=*Pritzelago*) to synonymy of *Hornungia*, i.e. to treat *H. alpina* as a species of the genus *Hornungia*.

GLS degradation products identified in the autolysate of *H. petraea*, benzyl, 3- and 4-methoxybenzyl isothiocyanate, along with several other structurally related compounds were evaluated for antimicrobial activity in order to possibly pinpoint the role of the latter secondary metabolites in the plant tissues. The assays showed a very high antibacterial activity of the tested isothiocyanates against *Sarcina lutea* and an antifungal effect against *Aspergillus fumigatus* and *Candida albicans* with MIC values in the order of 1 mg/ml value.

Acknowledgment: This work was supported by the Ministry of Education and Science of Serbia [Project No. 172061].

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P 41. Essential oils composition of some Asteraceae species from Eastern Amazon, Brazil

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Keywords: Asteraceae species, essential oil variation, Eastern Amazon

In Brazil, mainly in the Amazon, the Asteraceae are represented by ca. 180 genera, most of which consists of herbaceous plants. The family is considered one of the most important sources of species with therapeutic value, many of which have been studied chemically and pharmacologically. Specimens of *Tilesia baccata* (L.) Pruski (1), *Vernonia scabra* Pers (2), *Ichthyothere cunabi* Mart. (3), *Blainvillea dichotoma* (Murray) A. stewart. (4) *Ageratum conyzoides* L. (5), *Unxia camphorata* L. (6), *Riencourtia angustifolia* Gardner (7), *Eupatorium squalidum* DC. (8) and *Ichthyothere rufa* Gardner (9) were collected in the municipality of São Geraldo do Araguaia (1-5) and Carajás National Forest (6,7), Pará state, and in the municipalities of Taquaruçu (8) and Palmas (9), Tocantins state. The essential oils of these species were hydrodistilled and analyzed by GC and GC-MS. The main constituents (over 4%) of *T. baccata* oil were β-bisabolene (16.0%), β-caryophyllene (7.9%), γ-elemene (7.6%) and spathulenol (6.8%); in the *V. scabra* oil were caryophlyllene oxide (17.1%), (*E*)-caryophyllene (16.9%), germacrene D (8.7%), α-humulene and (6.0%); in the *I. cunabi* oil were dehydrosesquicineole (17.9%), δ-elemene (11.0%), bicyclogermacrene (10.0%), spathulenol (5.7%), germacrene D (5.5%) and β-elemene (4,4%); in the *B. dichotoma* oil were (*E*)-caryophyllene (17.9%), spathulenol (5.9%), caryophlyllene oxide (7.3%), (*E*)-nerolidol (5.5%), δ-cadinene (5.1%), γ-elemene (4.5%) and bicyclogermacrene (4.1%); in the *A. conyzoides* oil were *epi*-α-cadinol (31.3%), *trans*-β-guaiene (11.7%), α-cadinol (10.7%) and α-santalene (7,0%); in the *U. camphorata* oil were α-phellandrene (27.6%), camphor (15.0%), (*E*)-caryophyllene (11.6%), thymol methyl ether (7.0%) and germacrene D (4.4%); in the *R. angustifolia* oil were δ-elemene (15.4%), bicyclogermacrene (12.9%), geranyl acetate (10.0%), geraniol (9.5%), (*E*)-caryophyllene (7.3%), terpinolene (5.2%), germacrene D (5.2%) and *epi*-α-cadinol (4.9%); in the *E. squalidum* oil were β-elemene (21.6%), caryophlyllene oxide (5.8%), α-humulene (5.3%), humulene epoxide II (4.9%); and in the *I. rufa* oil were sabinene (33.1%), terpinen-4-ol (28.1%), limonene (6.7%) and γ-terpinene (6.4%).

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

P 42. The minor constituents of modern roses

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Keywords: Ambridge rose, Cymbeline, Lady Hillingdon, Alkylpyridines, 6-hydroxydihydrothespirane

The volatile constituents of English roses (Ambridge rose & Cymbeline) were analyzed using Aromascope® technology followed by GC-MS analysis.

As the major components of both of the flowers, 4-methoxystyrene, 1,3-dimethoxy-5-methylbenzene(DMMB), geraniol, nerol, citronellol, cis-3-hexenyl acetate, anisaldehyde, and citral (neral & geranial) were detected and from Ambridge rose total 118 components then from Cymbeline total 154 components were identified.

Among them, three kinds of alkylpyridines, such as 3-ethylpyridine(1), 5-ethyl-2-methylpyridine (2) and 2,6-dimethylpyridine (3) the nitrogen containing components were identified from both of English roses for the first time. These alkylpyridines have already reported as the one of the key components from the basic fraction in peppermint oil[1)] and jasmine absolute[2)].

Then, the small addition of these alkylpyridine derivatives 1-3 to the rosy fragrance made to enhance the natural flowerscent and a good effect for the rosy fragrance.

In addition, from Lady Hillingdon (the most popular representative tea-scented modern rose), 6-hydroxydihydrothespiranes (2,6,6,10-tetramethyl-1-oxaspiro[4,5]decan-10-ols) (4), considering as the precursor of the theaspiranes or their intermediate from β-ionol, β-ionone and dihydro-β-ionol , were identified for the first time from this rose flower. The compounds 4 have already found in the *Osmanthus* absolute[3)], black tea[4)] and green tea[5)] as racemate .

These compounds 4 showed the woody camphorous and very strong earthy note. In this study, only two enantiomers were detected on GC and chiral GC analysis.

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P 43. Influence of leaf drying and storage of the essential oil of *Ocimum basilicum* cultivar Maria Bonita on its chemical constituents

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Keywords: basil; essential oil; post-harvest; linalool

The objective of this study was to evaluate the influence of leaf drying and oil storage on the content and chemical composition of the essential oil of the Maria Bonita cultivar of basil. In the first trial, the effect of the drying time of leaves in an oven with forced-air circulation was evaluated at a temperature of 40°C. In the second trial, the effect of storage time was evaluated (0, 15, 30, 60, 90, 120, 150, 210, 240 and 270 days) at two temperatures [room (±27°C) and freezer (-20°C) temperature]. The essential oil was obtained from dry leaves by hydrodistillation using a Clevenger apparatus. Qualitative analysis of the chemical composition of the essential oils was performed using a gas chromatograph coupled to a mass spectrometer (GC-MS). A quantitative analysis of the essential oil components was conducted in a gas chromatograph equipped with a flame ionization detector (FID). The drying process at 40°C was efficient, reducing the moisture content of basil from 84.5% to 1.3% over a period of eight days. There was a linear reduction in the essential oil content from 6.0% to 3.9% during the process of leaf drying at 40°C. We also observed that the drying time proportioned decrease of linalool content from 76.38% to 74.09%, and increase of the content of á–*trans*-bergamotene (from 1.1% to 1.8%) and epi-á-cadinol (from 1.57% to 1.77%). In the second trial, we noted increase of the linalool content from 76.99% to 79.40% after 210 days of storage at room temperature and to 79.82% after 240 days of storage in freezer. We can conclude that basil essential oil can be stored for up to seven months at room temperature and eight months in freezer.

Acknowledgments: The authors thank FAPITEC/SE, CNPq and CAPES for their financial support of this work.

P 44. Influence of time and temperature of storage of the essential oil of *Pogostemon cablin* on its chemical constituents

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Keywords: patchouli; aromatic plant; volatile oils; postharvest; patchoulol

The aim of this work was to investigate the effect of the time (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 months) and temperature (room (± 27°C) and freezer (-20°C) temperature) of storage on the chemical composition of patchouli (*Pogostemon cablin* (Blanco) Benth.) essential oil. For this purpose, the essential oil of patchouli accession POG-002 was extracted from dry leaves at room temperature by steam distillation. The chemical composition was determined by gas chromatography-mass spectrometry (GC-MS), and the essential oil was quantified by gas chromatography with flame ionization detection (GC-FID). Fifteen of the components present in the essential oil were identified. Patchoulol was the main component of the oil; its concentration ranged from 36.36% to 42.12% at room temperature and from 35.19% to 42.93% at -20°C. These results indicate that the essential oil of patchouli may be stored at room temperature and at -20°C for 11 months without affecting its quality.

Acknowledgments: The authors thank FAPITEC/SE, CNPq and CAPES for their financial support of this work.

P 45. Antifungal activity and analysis of volatile components of *Origanum virens* Hoffmans & Link

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Keywords: *Origanum virens*; antifungal activity; essential oils

Origanum virens Hoffmans & Link is an aromatic plant which grows wild in some regions from Portugal. In the present paper, we analysed the chemical composition of essential oils from fresh and dried leaves of *O. virens* and their antifungal activity against strains of *Aspergilus niger*, *Penicilium* sp and *Fusarium oxysporum* isolated from soils.

O. virens was colected in Central Eastern of Portugal during the flowering period. Plant material was identified by a plant taxonomist and voucher specimens wew deposited in the Herbarium of ESACB (Escola Suerior Agrária de Castelo Branco). Aerial parts of the plant were separated into two lots, one of them was imediately submited to hydrodestilation and other one was dried at room temperature during a month.

Yellowish essential oils were obtained from O. virens in a yiels of 1.8% and 2.4% (v/W) to fresh and dry plant, respectivly. The essential oil presented as major components linalool and tymol.

Our results showed that although the essemtial oil yield of dry plant is greather than those of fresh plant.

In order to the antifungal activity, both essential oils of *O. virens* studied by us, on doses of 10 µL, inhibited totaly the growth of the tested fungi. Doses of 5 µL of each essential oil also showed activity against the fungi stains used in this work, in particular against *Penicilium* sp and *Aspergilus niger*.

P 46. Identification of methyl 2-hydroxy-3-methylhexanoate - a new headspace constituent of *Galanthus nivalis* L. (Amaryllidaceae)

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Keywords: *Galanthus nivalis* L.; Amaryllidaceae; snowdrop; headspace; synthesis; methyl 2-hydroxy-3-methylhexanoate

Galanthus nivalis L. (Amaryllidaceae), snowdrop, is an early spring flower native to a large area of Europe, highly admired for its delicate beauty. It has attracted considerable attention due to its capability to biosynthesize pharmaceutically important compounds, especially alkaloids such as galanthamine [1]. Despite its popularity, the volatiles of this species have never been previously investigated.

Detailed GC and GC/MS analyses of the headspace constituents of intact *G. nivalis* flowers revealed, among others, the presence of a number of methyl esters of 2-hydroxycarboxylic acids known to be olfactively important trace components of some flowers absolutes, highly valued in perfumery [2]. In order to corroborate the tentative mass spectral identification of these headspace constituents, a synthesis of several methyl esters of different α-hydroxycarboxylic acids was performed, utilising two different synthetic approaches. The stereospecific one involved the diazotisation and hydrolysis of L-amino acids to the corresponding L-α-hydroxycarboxylic acids, whereas the other approach included the addition of HCN to an aldehyde and the hydrolysis of the obtained cyanohydrin. In both cases the obtained α-hydroxycarboxylic acids were esterified by ethereal diazomethane. One of the headspace constituents was shown to be the diastereisomerically pure methyl 2-hydroxy-3-methylhexanoate (1). Compound 1, which can be regarded as a new plant secondary metabolite, was obtained in gram quantities starting from 2-methylvaleraldehyde (total yield 55%), in order to perform its full spectral characterisation (MS, IR, 1D and 2D – NMR, ^{1}H and ^{13}C) and for further testing of its biological activity and olfactory properties.

Acknowledgements: Authors are grateful to the Ministry of Education and Science of the Republic of Serbia (Project No. 172061) for financial support of this work.

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P 47. Chemical composition and *in vitro* biological activity of the volatile and fixed oils of *Nigella sativa*

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Keywords: *Nigella sativa*; essential and fixed oil; antibacterial; antifungal; antituberculosis; supercritical extraction

Nigella sativa L. (Ranunculaceae) grows in Mediterranean and western Asian countries. *N*. *sativa* seeds have traditionally been used in folk medicine as a natural remedy for various diseases as well as a spice. The seeds of *N*. *sativa* have been subjected to a range of pharmacological, phytochemical and nutritional investigations. The seeds contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activity of the seeds has been shown to be due to thymoquinone, the major component of the essential oil. The fixed oil is composed mainly of unsaturated fatty acids, including the unusual C20:2 eicosadienoic acid. Isolation of volatile and fixed oils from *N. sativa* seed of Turkey and Egypt have been obtained by supercritical fractioned extraction with carbon dioxide. Extraction experiments were carried out at pressures of 90 and 250 bar and temperature of 40 °C. The extraction step performed at 90 bar produced a volatile fraction mainly formed by tymoquinone (79-86%) and o-cymene (5-11%). The oil yield relative to this step of the process was 0.1-0.3% by weight of the charge. The last extraction step at 250 bar produced a fixed oil. The yield of this step was 21-26% by weight. The most represented fatty acids of fixed oil from *N. sativa* were 18:2 n-6 (54-55%), 18:1 n-9 (22-23%), 16:0 (12-13%), 18:0 (3%), and 20:2 (2-3%). The fixed oils obtained from *N. sativa* were evaluated for the antibacterial activity by employing standard strains of *E*. *coli*, *P. aeroginosa*, *A. baumannii*, *S. aureus*, *E. faecalis* via broth microdilution method. In vitro antifungal activity of the derivatives against *C. albicans, C. tropicalis,* and *C. krusei* were screened by using ketoconazole, and fluconazole as control agents. Anti-mycobacterium activity the breakpoint concentration (µg mL-1) was determined against standard strains of *M. tuberculosis H37Rv* and *M. avium (ATCC 15769)* by using the colorimetric resazurin microtiter assay (REMA). The compounds displayed antimicrobial activity towards all of the standard (ATCC, RSKK) strains of the tested bacteria at MIC values of 8-64µg ml-1 and were revealed to be ineffective against isolated strains (MIC;>256µg ml-1). The fixed oils emerged as the most effective against the bacteria of *M avium* with MIC values between 2 and 8 µg ml−1. On the other hand, the compounds exhibited better antifungal effect against *C. krusei* at 64 µg ml−1, which is close to the effect of the control fluconazole.

P 48. Carvacrol attenuates cancer pain in mice

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Keywords: Monoterpene, carvacrol, cancer, pain

The purpose of the present study was to evaluate the activity of carvacrol (CARV), a phenolic monoterpene, on hypernociception induced by Sarcoma 180 (S180) in mice.

Swiss male mice (20-30 g) were divided into groups (n = 12/grupo) which were treated (s.c.) after tumor induction with vehicle (saline + cremophor) CARV (12.5, 25 or 50 mg/kg) or morphine (15 mg/kg) daily for 15 days. For tumor induction a suspension of 10^6 cells (25 mL) was injected into the right hindpaw of the mice. Mechanical hypernociception was evaluated using digital Von Frey apparatus (Insight®, Brazil), and the paw withdrawal threshold was determined in grams and expressed as delta (Δ = force (g) after cells implantation - the force (g) before cells implantation). The spontaneous nociception and limb use was assessed guarding and flinching behaviors were measured during a 10 min observation period. Tumor growth assessed by paw volume was measured using a plethysmometer. All of these parameters were evaluated at days 1, 3, 5, 7, 9, 13 and 15 after tumor induction. The data were statistically analyzed by ANOVA followed by Tukey test. Protocols were approved for the UFS Ethic Committee (CEPA: 71/10).

In mechanical hypernociception induced by S180, CARV (50 mg/kg) was able to increase significantly ($p < 0.05$) the paw withdrawal threshold in 9 (0.37 ± 0.50 g), 11 (0.85 ± 0.65 g), 13 (1.48 ± 0.65 g) and 15 (1.63 ± 0.62 g) days compared to vehicle (2.50 ± 0.34 g, 3.87 ± 0.46 g, 3.82 ± 0.69 g, 4.24 ± 0.60 g, respectively). This same dose was able to reduce ($p < 0.05$) the spontaneous nociception on days 11 (0.17 ± 0.11 s), 13 (0.17 ± 0.17 s) and 15 (0.25 ± 0.18 s) days, different from vehicle (1.08 ± 0.43 s, 1.17 s ± 0.37, 1.08 ± 0.29 s, respectively). Besides, all doses improved the limb use in 9 (12.5 mg/kg: 3.91 ± 0.08, 25 mg/kg: 4.00 ± 0.00; 50 mg/kg: 3.92 ± 0.08) and 11 (12.5 mg/kg: 3.75 ± 0.13, 25 mg/kg: 3.75 ± 0.13, 50 mg/kg: 3.83 ± 0.11) days, when compared to vehicle (9 days: 3.58 ± 0.15, day 11: 3.17 ± 0.24). In 13 (3.58 ± 0.19) and 15 days (3.50 ± 0.19) only the highest dose promoted significant changes ($p < 0.05$) compared it with the control (day 13: 2.91 ± 0.23, day 15: 2.83 ± 0.24). However, no significant change of paw volume was observed.

The results show that CARV have antinociceptive activity on nociception induced sarcoma 180, suggesting that this compound might be a promising candidate for the treatment of cancer pain.

Acknowledgements: CNPq and FAPITEC-SE

P 49. Comparison of essential oil composition from *Artemisia* species: *A. dracunculus*, *A. absinthium*, *A. abrotanum*, *A. vulgaris*

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Keywords: *Artemisia dracunculus*; *Artemisia absinthium*; *Artemisia abrotanum*; *Artemisia vulgaris*; essential oil compounds; GC

Artemisia is a large and diverse genus of plants belonging to the family *Asteraceae* [1]. *Artemisia* species are used as spices in the culinary tradition as well as herbal medicine, in particular its essential oil, in folk medicine [2, 3].

The objective of the study is to compare the essential oil composition of four *Artemisia* species wild grown in the Botanical Garden of the Institute for Applied Botany and Pharmacognosy (Vienna, Austria).

A representative sample of leaves of *A. dracunculus, A. absinthium, A. abrotanum, and A. vulgaris* were collected, air dried and distilled in the Eppendorf MicroDistiller. The analyses of the essential oils were performed by GC-MS and GC-FID. The compounds were identified according to their mass spectra and retention indices [4].

A. dracunculus and *A. absinthium* present a simple essential oil composition where the main compound is estragole with 79,5% and *β*-thujone with 78,1%, respectively. The main compounds of the essential oil of *A. abrotanum* are davone derivates, while the *A. vulgaris* essential oil composition shows cineol (36,0%), sabinene (17,4%) and terpinene-4-ol (11,0%) as main compounds.

The study highlights that each four *Artemisia* species investigated present different main compounds enabling their differentiation.

Acknowledgments: This project is carried out within the PlantLIBRA project (www.plantlibra.eu) that has received funding from the European Community´s FP7/2007-2013 under grant agreement n°245199.

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P 50. Comparative evaluation of fruit volatiles from two pear (*Pyrus communis* L.) Portuguese varieties

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Keywords: *Pyrus communis*, Rosaceae, fruit, pear, Rocha pear, volatiles

With about 10969 ha of pears and an average annual production of about 176870 t, the pear (*Pyrus communis* L.) is the second most cultivated fruit crop in Portugal [1]. FAO statistics reveal that Portugal ranked 11 among the Top 20 pear-fruit exporters in 2009 [2]. Many local pear varieties are produced throughout Portugal, but one variety, Rocha pear (*pêra Rocha*), makes up about 95% of total production and is distinguished with a Protected Designation of Origin, or PDO [3]. As part of a wider research aiming to characterize a pear cultivar resulting from a chance seedling originated on a farm located at Tábua, Portugal, this work compares these pear fruit volatiles with those from Rocha pear.

The fruits from the pear under study (Tp) were collected and kept at 6ºC in normal atmosphere for 4 months. Rocha pear fruits (Rp) were obtained commercially. The volatiles were isolated by hydrodistillation and analysed by Gas Chromatography and Gas Chromatography-Mass Spectrometry [4].

The same yield, <0.05% (v/f.w.) was obtained for both fruit volatiles, hexyl acetate (51% in Tp, 37% in Rp), butyl acetate (12% in Tp, 18% in Rp) and *trans*,*trans*-α-farnesene (4% in Tp, 15% in Rp) being the dominant components. Although Tp and Rp fruit volatiles shared similar main compounds, some major differences were detected in the minor compounds (<5%). The presence of hexyl acetate in both Portuguese fruits varieties, as well as in other pears varieties volatiles, such as William´s [5], does not allow using this compound as a diagnostic character to differentiate among varieties. Further studies are being carried out to evaluate the differences in volatile profile at harvest date and after storage, as volatiles, in addition to appearance, flesh texture and grit stone content, are determinant in pear quality.

Acknowledgments: Eng. Justina Franco (ESAC), Eng. Rui Sousa (INRB), Eng. Arminda Lopes (ESAV), Sr Fernando Tavares e Dr Mário Vicente (Picos de Couto). Partially supported by Picos de Couto, Portugal.

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P 51. Composition of volatile compounds of horseradish roots (*Armoracia rusticana* L.) depending on genotype

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Keywords: SPME; horseradish; allyl isothiocyanate; genotype

Horseradish (*Armoracia rusticana L.*) is a member of the family *Brassicaceae*. It is a perennial plant, with a particularly pungent flavour and significant antioxidant properties. Horseradish has about 0.2% to 1.0% essential oil, mainly sinigrin, sinigrin-derived allyl isothiocyanate, diallyl sulfide. The present study was carried out to determine composition of volatile compounds of *A. rusticana* depending on genotype. Nine genotypes of horseradish were collected at the end of August 2011 at Pure Horticultural Research Centre (Latvia) collection field. Volatiles from fresh horseradish roots were extracted using solid phase microextraction (SPME) with DVB/Car/PDMS fibre and analysed by gas chromatography-mass spectrometry. Compounds were identified by comparison of their mass spectra with mass spectral libraries (Nist98), and by calculation of linear retention indexes. The studied horseradish genotypes differed both in quantitative and qualitative content of aroma compounds and the highest content of horseradish volatile compounds in genotype 'Turku' was detected. A total of 15 volatile compounds were identified. The main aroma compound of all horseradish samples was allyl isothiocyanate forming 61-82% of total identified volatile compounds and these results are comparable with those found in literature. Isothiocyanates are formed during breakdown of sulfur-containing compounds – glucosinolates that are in cruciferous vegetables. Also all horseradish samples contained significant amounts of phenylethyl isothiocyanate (4-18%) that is formed from glucosinolate - gluconasturtiin. This study revealed the great influence of genotype on the content of volatiles in horseradish roots.

Acknowledgement This research has been performed within the framework of the "Osmose" between Latvia University of Agriculture and Institut Polytechnique de Toulouse. Authors acknowledge Pure Horticultural Research Centre for supply of horseradish roots.

P 52. Alkamides from *Achillea serbica* Nym. (Asteraceae) root essential oil

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Keywords: *Achillea serbica* Nym; Asteraceae; yarrow; root; essential oil; alkamide

An extensive amount of work has been done on the aerial parts volatiles of many yarrow species (*Achillea* L., Asteraceae) [1]. Contrary to that, and despite the fact that root oil ducts exist in a number of *Achillea* taxa, the underground parts were generally neglected as a potential source of essential oils [1]. The volatile oils of the above-ground parts of *Achillea serbica* Nym. (syn. *A. ageratifolia* (Sibth. & Sm.) Boiss. subsp. *serbica* (Nyman) Heimerl and *A. ageratifolia* (Sibth. & Sm.) Boiss. subsp. *ageratifolia* var. *serbica* (Nyman) Hayek) were analyzed on two previous occasions [2,3], but no data on the corresponding root oil exist. For that reason and due to the fact that the roots of this yarrow are a valued folk herbal remedy used as a general stimulant, we have performed detailed chemical analyzes (gas chromatography and gas chromatography/mass spectrometry) of the hydrodistilled *A. serbica* root oil. Interestingly, the oil was characterized by the presence of five different (akl)amides (pyrrolides) containing the C_{12} and olefinic C_{16} acid moieties: 3,6-epoxydodecanoic (1, 3.4%), (*E*,*Z*)-hexadeca-2,7-dien-10-ynoic (2, 21.8%), (*Z*)-hexadeca-7-en-10-ynoic (3, 8.6%), (*E*,*E*,*E*)-hexadeca-2,6,8-trien-10-ynoic (4, 3.6%), and (*E*,*Z*)-hexadeca-2,7-dienoic (5, 3.0%), which made 40.4% of the total oil. These were detected for the first time in an essential oil sample. The different patterns of unsaturation of the acid residues suggest close biogenetic connections with oleic and crepenynic acids [4]. Compounds 1-5 were previously reported as constituents of *A. serbica* root extract. Worth mentioning is the fact that alkamides are generally considered as unstable and that there are only two previous studies reporting this type of compounds as essential oil constituents, and both in *Achillea* species (*A. ptarmica* and *A. distans*) [5].

Acknowledgments: This work was funded by the Ministry of Education and Science of Serbia (Project 172061). PB was supported by UNESCO-L´OREAL National Fellowships Programme for Young Women in Life Sciences.

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P 53. Genetic and chemical variation in Mexican oregano essential oil

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Keywords: AFLP; chemotype; genetic diversity; genetic differentiation; *Lippia graveolens*.

Mexican oregano is an economically important aromatic plant. In order to understand the importance of environmental and genetic factors in essential oil production, in this study we estimated the genetic diversity and structure for 14 wild populations of *Lippia graveolens* located in four different bioclimatic regions in southeastern Mexico using AFLP (amplified fragment length polymorphism) markers. Essential oil composition from each individual (total n = 95) was characterized using GC and GC-MS [1]. Based on the definition of chemotype by previous authors [2] we identified three different chemotypes: (a) thymol and (b) carvacrol, characterized by high concentration of these monoterpenes and (c) sesquiterpene with high concentrations of a and b-caryophyllene. Two primer combinations produced a total of 87 polymorphic bands. The overall genetic diversity of *L. graveolens* described as the percentage of polymorphic loci (PPL= 60.9%) and Nei's gene diversity (H_j =0.17) was moderate but was not associated to bioclimatic conditions. Genetic variation was analyzed at different levels. Regarding chemotypes, thymol had the highest genetic diversity (PPL = 82.8% and H_j = 0.22). PCoA revealed that chemotypes exhibit certain level of genetic differentiation. Maximum parsimony dendogram showed that some individuals from the same chemotype clustered together. Bayesian analyses revealed a low but significant differentiation among chemotypes (q_{II} =0.008). Regarding populations, gene diversity showed significant differences ($F_{13,1204}$ = 22.8, P < 0.001), the populations 4, 5 and 8 (predominantly thymol individuals) showed the highest gene diversity (H_j = 0.31-0.25), while population 3 (exclusively sesquiterpene chemotype) showed the lowest value (H_j =0.058). Cluster (PCoA and maximum parsimony) and Bayesian analyses (q_{II} =0.027) revealed a low level of genetic differentiation among populations.

Acknowledgments: This study was financially supported by CONACyT, doctoral scholarship to DMN and grant given to LCI (CB-2007, 82873). We thank Sara V. Good, University of Winnipeg, for laboratory facilities and Dulce Linares and Felix Martínez-Nuñez for technical assistance in laboratory work.

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P 54. A survey of the essential oil composition of rose-scented geranium grown in South Africa

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Keywords: *Pelargonium* sp; chemical composition; variability; climatic diversity

Rose-scented geranium (*Pelargonium* sp) essential oil was received from different provinces of South Africa from 2003 to 2011 and its chemical composition determined by gas chromatography (GC) and GC/MS. The variability of the major constituents of the essential oil between provinces and within regions of the KwaZulu-Natal province was determined. South African climate presents great diversity between and within provinces [1]. The seasonal effect on the oil composition from the KwaZulu-Natal regions was investigated. With the exception of 6,9-guaiadiene and citronellol which varied significantly at $P \leq 0.05$ between some of the provinces, the major constituents of the oil analysed were not significantly different. Within the KwaZulu-Natal province, linalool and citronellyl formate were found to differ significantly. Interactions between season and KuaZulu-Natal regions were not significant. The mean values of the major constituents of South African oil, expressed as percentages of the total constituents were: isomenthone 4.78, linalool 3.03, 6,9-guaiadiene 7.17, citronellyl formate 15.26, geranyl formate 8.25, citronellol 24.08 and geraniol 14.81. The rose-sented geranium essential oil received from different provinces of South Africa was found to have a lower linalool and a higher citronellyl formate than oils from other parts of the world [2]. These characteristics can be used to identify rose-scented geranium oil of South African origin.

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P 55. Essential oils composition of *Croton* species with occurrence in the Brazilian Amazon

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Keywords: *Croton*; essential oil composition; cluster analysis

Croton is a genus of Euphorbiaceae comprising about 1300 species widespread in Africa, Asia and South America. Many species are used in the traditional medicine of these continents, especially to treat cancer, diabetes, malaria, ulcers, among other diseases [1]. The essential oils of leaves and fine stems of *Croton campestris* A. St,-Hil. (0.5%), *C. chaetocalyx* Mull. Arg. (1.1%), *C. draconoides* Mull. Arg. (0.8%), *C. eriocladus* Mull. Arg. (0.6%) and *C. glandulosus* Mull. Arg. (0.7%) were analyzed by GC and GC-MS. The plants were collected in Eastern Amazon and their compositions are rich in terpene compounds. The main constituents identified in the oil of *C. campestris* were (*E*)-caryophyllene (23.0%), γ-elemene (13.9%), germacrene D (13.7%), β-elemene (7.1%) and δ-elemene (6.0%); in the oil of *C. chaetocalyx* were bicyclogermacrene (13.9%), δ-elemene (13.5%), germacrene D (9.3%), spathulenol (9.0%), δ-cadinene (8.0%) and (E)-caryophyllene (7.1%); in the oil of *C. draconoides* were β-pinene (16.9%), α-pinene (16.5%), curzerene (12.8%) and germacrene D (9.0%); in the oil of *C. eriocladus* were (*E*)-caryophyllene (24.1%), germacrene D (17.9%), α-humulene (6.2%), bicyclogermacrene (5.2%) and δ-elemene (5.0%); and in the oil of *C. glandulosus* were spathulenol (19.7%), bicyclogermacrene (9.6%), (*E*)-caryophyllene (8.9%) and δ-elemene (8.8%). A hierarchical cluster analysis using the compositional profile of the major constituents (below 5%) has been carried out. Complete linkage and absolute correlation coefficient distance was selected as measure of similarity. The oils were divided into three groups: *C. campestris* and *C. eriocladus* (92% similarity), *C. chaetocalyx* and *C. glandulosus* (88% similarity), and *C. draconoides* maintained separately.

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

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P 56. Antifungal activity of *Piper* essential oils from the Amazon against the *Fusarium* disease in pepper

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Keywords: *Piper nigrum*, *P. callosum*, *P. divaricatum*; *P. krukoffii*; *Fusarium solani* f. sp. *piperis*; antifungal activity

The pepper (*Piper nigrum* L.), originary from southeastern Asia, is a famous spice used in the entire world to aromatize many traditional and regional dishes. This species is very susceptible to the disease known as fusariosis, with restricted occurrence to Brazil. The attack of phytopathogen *Fusarium solani* f. sp. piperis Alb. causes root rot and large losses for the plant culture. The essential oils obtained from *Piper callosum* Ruiz & Pav, *P. divaricatum* G. Mey. and *P. krukoffii* Yunck. Were analyzed by GC and GC-MS and submitted to antifungal activity [2] against *Fusarium solani* f. sp. *piperis*. The oil compositions of *P. callosum*, *P. divaricatum* and *P. krukoffii* were rich in phenylpropanoid compounds. The main constituent identified in the oil of *P. callosum* were safrol (78.0%), methyeugenol (8.7%) and 1,8-cineole (2.6%); in the oil of *P. divaricatum* were methyleugenol (84.0%) and eugenol (8.7%); and in the oil of *P. krukoffii* were myristicin (40.3%), apiole (25.4%) and â-elemene (8.2%). The inhibition percent of growth of *Fusarium solani* f. sp. *Piperis* in the oil of *P. callosum* was 53.0%. The inhibition percent varied from 78.9 to 100% in the oil of *P. divaricatum*. The oil of *P. krukoffii* was only able to inhibit the fungus growth in 24.5%. The inhibition percent data were submitted to analysis of variance (ANOVA, $p < 0.05$). The differences were found to be extremely significant to *P. divaricatum* ($P = 0.000$), moderate to *P. callosum* ($P = 0.0023$) and not significant to *P. krukoffii* ($P = 0.733$).

Acknowledgments: This work was funded by BIONORTE/CNPq and FAPESPA/PA and supported by UFPA/New Doctors Program.

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P 57. Essential oils composition of Annonaceae species with occurrence in the Brazilian Amazon

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Keywords: essential oil composition; Annonaceae

The Annonaceae comprises tropical and sub-tropical species that are widespread in South and Central America, Africa, Asia and Australia. Early phytochemical review has estimated *ca* 120 genera and more than 2000 species in this family [1]. Economically, it is of appreciable importance as a source of edible fruits, raw material for cosmetics and perfumery and medicinal plants. As part an ongoing survey of the Amazon aromatic plants we are reporting the volatile composition of some Annonaceae species collected in Eastern Amazon, municipality of São Geraldo do Araguaia, Pará state, Brazil. The oils were analyzed by GC and GC-MS. The main constituents identified in the oil of *Xylopia aromatica* (Mart.) Lam. were spathulenol (21.7%), limonene (17.8%), δ-elemene (10.2%) and bicyclogermacrene (9.4%); in the oil of *Annona paludosa* Aubl. were spathulenol (18.0%), caryophyllene oxide (14.7%), (*E*)-caryophyllene (7.6%) and germacrene D (5.8%); in the oil of *Annona crassiflora* Mart. were γ-elemene (21.0%), δ-elemene (14.3%), (*E*)-caryophyllene (14.0%), germacrene D (13.4%) and bicyclogermacrene (9.6%); in the oil of *Guattteria schomburgkiana* Mart. were δ-cadinene (14.7%), spathulenol (11.2%), α-cadinol (10.9%), caryophyllene oxide (4.9%) and zonarene (4.0%); in the oil of *Duguetia echinophora* R.E.Fr were spathulenol (37.4%), caryophyllene oxide (7.1%), (*E*)-caryophyllene (5.8%) and germacrene D (5.7%). Eearly papers indicated that spathulenol and caryophyllene oxide could be considered as chemotaxonomic markers for Annonaceae species occurring in the Amazon [2,3]. Excepting the oil of *A. crassiflora*, the other oils showed significant amounts of spathulenol and caryophyllene oxide, which confirm this hypothesis.

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

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P 58. Antioxidant and antifungal activity and composition of the oils from *Lippia gracilis* and *L. origanoides*

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Keywords: *Lippia gracilis*; *L. origanoides*; antioxidant and antifungal activity; oil composition

The present study was conducted to evaluate the antioxidant and antifungal properties of the essential oil of *Lippia gracilis* Schauer and *L. origanoides* Kunth, collected in the locality of São Felix das Balsas (February 2011), Maranhão state, and in the National Forest of Carajás (May 2011), Pará state, respectively. The chemical composition of hydrodistilled oils was analyzed by GC and GC-MS. The main constituents in the oil of *L. gracilis* were thymol (72.5%), *p*-cymene (9.3%) and thymol methyl ether (5.4%) and in the oil of *L. origanoides* were 1,8-cineol (31.9%), nerolidol (16.2%) and α-terpineol (7.5%). The antioxidant potential of the samples was evaluated using the method of inhibition of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The essential oils were able to reduce the stable radical DPPH showing a significant antioxidant activity to *L. gracilis* (IC_{50} = 35.7 ± 3.3 µg.mL^{-1}), while for *L. origanoides* it was very low (IC_{50} > 500 µg.mL^{-1}). The antifungal assay showed that both essentials oils inhibited the growth of the fungus *Cladosporium sphaerospermum*, with detection limit of 25 µg to *L. gracilis* and 50 µg to *L. origanoides*. The results showed that, at least, the essential oil of *L. gracilis* could be used as a natural preservative ingredient in food and pharmaceutical industries.

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

P 59. Composition and antifungal activity of *Sphagneticola trilobata* Pruski (Asteraceae) volatile oils collected in different biomes

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Keywords: Wedelia; essential oil; Atlantic Rain Forest; Brazilian Cerrado; *Cladosporium sphaerospermum*

Sphagneticola trilobata (Asteraceae) is a native Brazilian herbaceous plant, commonly known as vedélia, used in folk medicine to treat a variety of illnesses, such as headaches, fevers, infections and respiratory pathologies. The geographical origin, soil characteristics and climate differences may influence in quantitatively and/or qualitatively the essential oils chemical composition. The present work analysed the composition and antifungal activity of the volatile oils from *S. trilobata* collected at two different biomes from São Paulo state: two populations from Atlantic Rain Forest (São Paulo [SP] and Paranapiacaba [PARN]) and one from the Brazilian Cerrado (Mogi-Guaçu [MGÇ]). Fresh aerial parts were hydrodistilled in a Clevenger apparatus during four hours and analysed by GC and GCMS. The antifungal activity was determined by the bioautography method as described by Homans & Fuchs [1] with the fungus *Cladosporium sphaerospermum* (Penzig). The yields (w/w) were 0.21, 0.11 and 0.09% for SP, PARN and MGÇ respectively. The chemical composition of the volatile oils obtained from these three locations was quantitatively distinct. The main components of the oils were: SP - β-Pinene 45.2%, α-Phellandrene 16.0%, α-Pinene 15.7% and Limonene 8.8%; PARN - α-Pinene 29.8%, β-Pinene 22.0% and α-Phellandrene 14.3% and MGÇ - α-Pinene 28.7%, α-Phellandrene 18.4% and Limonene 15.5%. The oil from the population of the Brazilian Cerrado showed strong inhibitory activity against the fungus tested. The oil from the Atlantic Rain Forest at PARN showed lower activity and SP did not show growth inhibition. The highest percentage of limonene in the composition of the volatile oil from MGÇ plants might have had influence in the activity found, either for intrinsic activity or synergism with other compounds in the oil.

Acknowledgments: Pos-graduate program in Biodiversidade Vegetal e Meio Ambiente and CNPq

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P 60. Antioxidant activity of essential oils from *Satureja* growing in Spain

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Keywords: *Satureja*, essential oil, antioxidant activity, total phenolic compounds

The phenolic content and the antioxidant activity of five essential oils obtained from *S. montana*, *S. innota*, *S. cuneifolia* and *S. intricate* growing in Spain were measured in order to find new potential sources of natural antioxidant.

Satureja is a genus of the perennial or annual semi-bushy species native to warm temperate regions. The aerial parts of five wild perennial populations of *Satureja* species were collected from the phytogeographic area of this genus in the Comunidad Valenciana (Spain) in September 2010. One population of each species was studied, except in the case of *S. innota*, for which two populations were analysed due to their different ecological conditions.

Essential oils were obtained by hydro-distillation using a Clevenger-type apparatus. Total phenolic compounds and antioxidant activity of the essential oils were determined according to Folin-Ciocalteu [2] and to FRAP assays [3, 4], respectively.

The total phenolic content of the essential oils varied between *Satureja* species. Significant differences were found between *S. montana* and others *Satureja* species, being its content in total phenolic compounds much higher. Data also provided no significant differences between both populations of *S. innota.*

The results showed a relationship between total phenolic compounds and antioxidant activity.

S. montana had the highest antioxidant activity. The GC/MS analysis [5] indicates that this essential oil was characterized by a higher carvacrol content compared to the others *Satureja* essential oils.

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P 61. Individual variability of wormwood (*Artemisia absynthium* L.) esssential oil composition

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Keywords: chemotype, diversity, thujone, myrcene, sabinene

Wormwood has gained a contradictious reputation by its thujone content and potential toxicity which is heavily disputed until today (1). In the last decades, composition of the essential oil of several populations of different origin has been studied and numerous chemotypes identified (e.g. 2,3,4). However, these results always represented the quality of bulk samples which is not able to reveal the natural variability of the species. In the recent investigations we studied the essential oil content and composition of 30 individual plants in a common cultivated genotype in Hungary. Shoot samples were taken at the beginning of flowering (July). The dried herb was hydrodistilled (PhEur) and the oil analysed by GC-MS. The components were identified by MS libraries and their LRIs.

The essential oil content varied between 0.110 and 0.962% d.w. We identified 49 compounds in the oil. β-Thujone was the main component (in 55-71% of the oil) in 53% of the plants. However, in 9 plants it has been found only in traces. The linear correlation coefficient between essential oil content and its β-thujone proportion was r= 0.5764.

The second and third most abundant components were β-myrcene (4-34% in oil) and sabinene (2-32% in oil) both being main compounds in 13% of the samples. Besides, in 20% of the oils, they were found in appr. equal proportions. A single sample contained trans-chrysanthenol and another one an unidentified sesquiterpene (RT: 44.63; LRI 1985) as main component. Sabinene and trans-chrysanthenol chemotypes of wormwood have not been reported before. On the other side, compounds described as main components in the populations of other regions like p-cymene, bornyl-acetate, cis-epoxyocimene, cis-chrysanthenyl-acetate (2,3,4) have not been found in our samples. Minor compounds present in each sample were p-cymol, β-caryophyllene, linalyl-butanoate, α-curcumene, neryl-isobutanoate, lavandulyl-isovalerate, neryl-isovalerate, caryophyllene-oxide, geranyl-isovalerate. Each oil consisted higher number of sesquiterpene compounds than monoterpenes.

The results demonstrate the significance of individual sampling for chemotaxonomical surveys. Selection of unique chemotypes may contribute to development of new products.

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P 62. Chemical composition, antioxidant and antibacterial activity of the essential oils of *Pimpinella pruatjan*, an endemic species from Java Island, Indonesia

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Keywords: antioxidant activity, antibacterial activity, *Pimpinella pruatjan*, essential oil, chemical composition

The essential oil from root and aerial parts of native *Pimpinella* species growing in a restricted mountain area in Java was studied concerning chemical composition, antioxidant and antibacterial activities. Using high resolution GLC-MS, the essential oil isolated by hydrodistillation was separated and components were identified [1]. The antioxidant activity was determined by conjugated diene assay and analysis of scavenging free radicals activity [2,3]. The antibacterial activity of the essential oil was assessed against some pathogenic bacteria [4]. The essential oil contains monoterpenes, sesquiterpenes, and phenylpropanoids. Antioxidant and antibacterial properties (MIC and MBC) against Gram negative and Gram positive bacteria will be presented. The composition of the essential oils from *Pimpinella* species growing in tropical climate region will be compared with known *Pimpinella* species which are widely distributed in the temperate zone of Europe and Iran.

Acknowledgments: We are grateful to Dr. Ireng Darwati Kadarso (Indonesian Medicinal and Aromatic Crops Research Institute, Indonesia) for providing *Pimpinella pruatjan*.

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P 63. The composition of the essential oil from "candeia da serra" (*Eremanthus erythropapus*) determined by injection of the oil and by SPME of the entrapped vapor, as well as its antibacterial activity

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Keywords: candeia; composition; essential oil; antibacterial activity

The essential oil from candeia da serra (*Eremanthus erythropapus*) was isolated by hydrodistillation. The composition of the oil was determined by injection of the oil onto a 30 m X 0.25 mm DB-5 column in a Shimadzu model GC-17 gas chromatograph coupled to Shimadzu QP5050 mass selective detector. The composition of the headspace above the essential oil was also determined by adsorption onto a DVB/PDMS-coated fiber and desorption in the injection chamber of the chromatograph. The potential of the essential oil from the leaves of candeia to inhibit the growth of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) in foods was also assessed. The composition of the essential oil was confirmed by the Kovats index and the NIST library. A total of 43 compounds were isolated, mostly terpenes. The study of the antimicrobial activity was performed employing the disk diffusion method standardized by the Subcommittee for Antimicrobial Susceptibility Tests (NCCLS) [1]. Isolated colonies of bacteria were standardized in sterile saline solution according to the 0.5 McFarland scale. After immersing the swab in this solution, the microorganism was inoculated on Mueller-Hinton agar. Filter paper discs (6 mm), previously coated with 10 uL of sample at 50 mg/mL were placed on the surface of the agar. A disk impregnated with ethanol was used as a negative control, and a disk impregnated with 10 mg of Ampicillin was used as a positive control. Tests were performed in six replicates. After incubation of the plate in an oven at 35 °C for 18 hours, the formation of inhibition zones around the disks was assessed. An inhibition zone ≥7 mm was considered to be positive for herbal extracts [2]. The *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) showed resistance in the in vitro test, indicating that the essential oil from candeia leaves does not inhibit the growth of these microorganisms.

Acknowledgements: The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and scholarships.

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P 64. Seasonal variations in essential oil composition of roots and plants of *Artemisia absinthium* L in a population from Teruel (Spain)

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Keywords: *Artemisia absinthium*; essential oil; seasonal variations; root; chemotype

The essential oil of *Artemisia absinthium* L has been extensively studied because of its many applications as to prepare absinth beverages as for the etnopharmacological point of view [1, 2]. Seven chemotypes have been characterized in the Iberian Peninsula, three of them lacking in the neurotoxic compound beta-thujone [1]. Nevertheless, less attention has been paid to root essential oil composition [3, 4, 5]. The aim of this research is to study the plant and root essential oil composition of a (*Z*)-epoxyocimene-chrysantenyl acetate chemotype of an *Artemisia absinthium* L population from Teruel (Spain) over its vegetative period (may-august). For this purpose, three sampling were performed. In each one of them, five plants were collected and the essential oils from the fresh aerial parts and roots were obtained by means of a Clevenger type and a Likens-Nickerson apparatus, respectively. They were analyzed by GC and GC-MS with the same column (DB-5) and operating conditions.

Concerning the aerial parts, the results confirm their chemotype and show a predominance of individual variations with regard to seasonal ones. The sesquiterpenoid fraction is noticeable (4-10 %) and a significant occurrence of C12 compounds, such as (*8E,10E*)-dodeca-8,10-dien-1-ol, was found at the beginning of vegetative period. Regarding the root extracts composition, their main components are monoterpenes (35-77 %), mainly, alpha-fenchene and beta-myrcene, and monoterpenoids esters (15-55 %), mainly bornyl acetate, while the sesquiterpenoid fraction and other compounds amount is almost negligible. These results are similar to the ones obtained in a previous research [5] whose aerial part chemotype was different (with a high amount of beta-thujone). The conclusions of these results lead to define two trends to pursue this research: firstly, to evaluate separately individual and seasonal variability, monitoring the aerial parts composition in individual plants over the vegetative cycle. Secondly, to go deeply into the secondary metabolites role of plants and roots to explain their qualitative differences.

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P 65. Diurnal and environmental variations in essential oil composition of *Mentha suaveolens* L and *Mentha longifolia* L populations from the Eastern Iberian Peninsula

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Keywords: *Mentha longifolia*, *Mentha suaveolens*; essential oil; chemotypes; diurnal variations; environmental factors

Many researches dealing with the geographical chemical variability [1, 2] and biological applications [3, 4] of *Mentha longifolia* L and *Mentha suaveolens* L have been developed. Nevertheless, a limited attention has been paid to environmental factors affecting their essential oil composition [5]. The goal of this research is to contribute to knowledge of diurnal and organ variations and soil characteristics in essential oil of *Mentha suaveolens* L and *Mentha longifolia* L from six populations in the Eastern Iberian Peninsula.

For this purpose, a total amount of 79 samples were processed and the following variables were studied: location and climate (Coastal or Continental Mediterranean), morning or evening harvest. organ (leaves or flowers) and soil characteristics. The plant material was frozen and kept at -40ºC until its extraction by means of a Likens-Nickerson apparatus. The extracts were analyzed by GC and GC-MS with the same column (DB-5) and operating conditions. In addition, 24 soil samples were obtained and pH, conductivity, organic matter, carbonates, etc. were determined.

Piperitenone oxide was the main compound (around 80 %) in two populations of *Mentha suaveolens* L, although the occurrence of pulegone instead of piperitenone oxide was found in some samples. In the other location, its composition is closer to the reported for *Mentha suaveolens* ssp. *Insularis* (Req.) [3], with piperitone oxide as a main compound. Some diurnal significant differences ($P<0.05$) have been found regarding the total oxygenated monoterpenes, both for leafs and flowers. With regard to *Mentha longifolia* L, the main compounds were alpha –terpineol acetate (30-45 %) and carvone acetate (30-40 %). This composition has not been found from literature [6]. This fact should be object of further investigations.

Despite of these significant differences, these results show a great variability among samples which can be interpreted as a greater predominance of genetic factors over environmental ones. Consequently, this study will be extended to other populations by means of individual analysis.

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P 66. Chemical characterization of the essential oil from the leaves and blossoms of "escova de garrafa" (*Callistemon viminalis*) from Minas Gerais, Brazil

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Keywords: escova de garrafa; *Callistemon viminalis*; essential oil

Callistemom viminalis (Myrtaceae; Callistemon) is an ornamental plant from Australia and is commonly known as "escova de garrafa" (bottle brush). It is a shrub that reaches 3-7 feet in height. The hydroalcoholic extract has antimicrobial and insecticidal activity [1,2]. The aim of the present work was to identify and quantify the chemical constituents present in the essential oils from the leaves and flowers of *C. viminalis* by GC. The collection of plant material was performed in the Garden of Medicinal Plants of the Federal University of Lavras (UFLA/MG).The essential oil was isolated by hydrodistillation from the wood and the aerial parts of *C. viminalis*. The characterization of the essential oil was performed at the Department of Plant Biology, Faculty of Science, University of Lisbon, Portugal, by gas chromatography coupled with mass spectrometry and gas chromatography with a flame ionization detector as in [3]. In the analysis of the essential oil from the leaves (fo) and flowers (fl) of *C. viminalis*, 99.82 and 99.46%, respectively, of the constituents were identified. The same major constituents were found in both leaves and flowers in different proportions (%):1,8-cineole (fo - 69.14, fl - 66.93), α-pinene (fo - 18.91, fl - 16.00),limonene (fo - 5.89, fl - 10.04) and α-terpineol (fo - 1.70; fl - 2.19). The components or combination of components responsible for the biológical activities are yet to be determined.

Acknowledgments: Partially supported by CNPq, CAPES, Fapemig.

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P 67. Composition of volatile oil of *Mosiera bullata* species grown in Cuba.

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Keywords: *Mosiera bullata*; Myrtaceae; essential oil composition; cineol

Myrtaceae family (121 genera, 3800–5800 spp.) is one of the most important families in tropical forests. Several members of this family are used in folk medicine as antidiarrheal, antimicrobial, antioxidant, cleanser, antirheumatic, and anti-inflammatory agent and to decrease the blood cholesterol. [1-5] Objective: The aim of the study was to determine the composition of essential oil of Mosiera bullata leaves collected in botanical garden, Cuba, being examined by GC/MS and to evaluate the antioxidant activity of essential oil by in vitro techniques. Methods: The volatile oil was obtained from dried Mosiera bullata obtained by hydrodistillation and was carried out the identification of its main component by GC–MS analyses. Kováts indices, mass spectra and standard compounds were used to identify a total of 14 individual compounds. The major component found was 1.8 cineol (ca. 62%). The potential antioxidant activity was also investigated and the sample tested by 2, 2′-diphenyl-1-picrylhydrazyl (DPPH), reducing power and linoleic acid assay. Results: The sample tested was found to interact slightly with the stable free radical DPPH in a time dependent manner and this antioxidant activity was supported by the complementary antioxidant assay in linoleic acid system and TBA method. The total amount of phenolic compounds in the essential oil was determined as gallic acid equivalents. Conclusions: This research is essential to identify quality parameters of this oil and is useful for quality control in the future industrial production and for the use of essential oil as antioxidant in the pharmaceutical industry.

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P 68. Essential oil and glandular trichomes of *Ocimum campechianum* in South Eastern Mexico

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Keywords: aridity index; *Ocimum campechianum*; sesquiterpenes; Yucatan

Ocimum campechianum Mill. is an important aromatic herb traditionally used for different medicinal purposes in Mayan communities [1], particularly gastrointestinal disorders [2]. Despite the economic and therapeutic importance of *Ocimum campechianum* there is no information regarding secretory structures and the effect of environmental factors on the essential oil characteristics. The main goal of this study was to describe the morphology of glandular trichomes in *Ocimum campechianum*, and assess the variation in glandular trichome and essential oil characteristics across six populations of this species, found at sites with varying levels of aridity at southeastern Mexico. The morphology and density of glandular trichomes were investigated using a combination of stereo microscopy and SEM. Essential oil was extracted using steam distillation and was characterized with GC, GC-MS and commercial standards. Correlation analysis between essential oil yield and aridity was performed. *Ocimum campechianum* presented one type of glandular trichome, peltate, on both sides of the leaf: Peltate trichomes were clearly distinct in size and morphology. Higher size trichomes (*ca* 50 µm diameter) with 8 cell head, were more abundant than smaller peltate trichomes (*ca* 10 µm de diámeter) with a two cell head. Trichomes were uniformly distributed on both leaf surfaces. Average essential oil yield was considerable, 2.2% (w/w) and showed important variation among populations. Spearman rank correlation showed no clear effect of aridity on essential oil yield. *Ocimum campechianum* essential oil was dominated by sesquiterpenes, the main components were isoeugenol, caryophyllene (E-), camphor, germacrene D, bicyclogermacrene, a-humulene, spathulenol and β-elemene.

Acknowledgments: This study was financially supported by CONACyT grant given to LCI (YUC-2008 C06-108231). We thank Fabiola Escalante Erosa, Lilia Can and Cindy Cachón, for technical assistance in laboratory work.

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P 69. Combined treatment of mung sprouts contaminated with salmonella with vapors of oregano essential oil and low pressure

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Keywords: mung sprouts; *Salmonella enteritidis*; oregano essential oil; low pressure treatment

Seed sprouts are in general recognised as a good source of vitamins and minerals in human diet. On the other hand, due to the way they are processed, they present potentially high health risk for the consumers as also identified in 2073/2005 EC [1]. High temperature, humidity, and lack of special treatment during the seed sprouting makes ideal conditions for the growth of bacterial flora including the pathogenic one (e.g. *Escherichia coli, Salmonella enteritidis, Listeria monocytogenes*). Moreover, recent outbreak (*E. coli* in sprouts) has proved the need for the introduction of the new steps in the production chain, which will minimize the risk of pathogenic bacteria presence in final products. Attempts to treat the seeds before sprouting with different types of chemicals as chlorine or essential oils (EOs), high temperature and pressure sometimes brought promising results, however, so far there is no reliable method which would lead to the reduction of all pathogenic bacteria in the sprouts[2].

Thus the aim of this study was to evaluate if treatment of mung sprouts with vapor phase of oregano EO in combination with low pressure can significantly reduce the presence of *Salmonella* in the sprouted seeds.

Seeds of mung bean (*Vigna radiata*) were inoculated with 7 log $CFUg^{-1}$ of *Salmonella enteritidis* ATCC 13076 and sprouted for 4 days. After that they were treated with oregano EO (1 and 0.1 µl/ml) in combination with low pressure and fast evaporation of the EO supported by heating. Time of the treatment was 5, 15 and 60 min. The same tests series was also done under atmospheric pressure. The numbers of *S. enteritidis* was investigated immediately and fourth day after the treatment. Reduction of the *S. enteritidis* ranged from 0.3 to 3 log $CFUg^{-1}$. The most significant reduction of the bacteria was observed when the sprouts were treated for 5 and 15 min under the low pressure with heated EO.

The results have shown that combination of the EO, low pressure and heat can lead to significant and in compare to other studies quick reduction of *S. enteritidis* in final product. Thus it has a potential to be used in the real sprout production.

Acknowledgements: This study was supported by CIGA 21160/1313/3103 and S grant of MSMT CR

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P 70. Effect of *Cymbopogon winterianus* essential oil on germination and growth of *Euphorbia heterophylla*

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Keywords: Geraniol; citronellal; citronellol; germination inhibition; bioherbicide

Euphorbia heterophylla L. weed is an annual cycle plant, belonging to the Euphorbiaceae family widely distributed in tropical and subtropical America. This weed has a center of origin in South America (Brazil-Paraguay), which confers great genetic variability. It is currently one of the plants that cause most losses in soybeans, and show resistance to some herbicides as inhibitors of acetolactase synthase enzyme currently employed in agriculture [1]. The objective of this study was to evaluate the potential bioherbicide the essential oil of *Cymbopogon winterianus.* Fifty seeds of *E. heterophylla* L. were placed in Petri dishes containing two germitest sheets previously autoclaved and moistened with solutions (10 mL) of essential oil of *C. winterianus* ((0.03, 0.06, 0.12 and 0.25% [v / v]) diluted in Dimethyl Sulfoxide (DMSO [2% v/ v]) [2]. Four repetitions were placed randomly on germination chamber (Type BOD) with 12h photoperiod at 25°C for 7 days. Essential oil analysis was carried out by gas chromatography (GC) and by gas chromatography-mass spectroscopy (GC/MS). Major compounds were geraniol (23,9%), citronellal (32,4%), citronellol (14,6%), geranial (3,3%) and limonene (3,3%). Germination percentage (%G=∑ni.Ni).100), germination average time (GAT=∑ni.ti/∑ni), germination average speed (GAS) and germination speed index (GSI), root system and shoot growth, fresh and dry weight of seedlings were all affected by essntial oil of *Cymbopogon winterianus*. The results indicate that one or more monoterpenes present in essential oil directly inhibits the germination of *E. heterophylla* and has potential bioherbicide effect.

Acknowledgments: The authors thank to Fundação Araucária, CAPES and Fundação para a Ciência e Tecnologia (FCT), POCI 2010/FEDER for financial support to this work.

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P 71. Biological properties and essential oil composition of leaves of *Syzygium cumini* (L.) skeels from São Luís-Maranhão, Brazil

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Keywords: larvicidal; molluscicidal; leishmanicidal; *Syzygium cumini*

Syzygium cumini (Myrtaceae) is usually known as black plum or jambolan. In this work the essential oil of leaves of *S. cumini* was tested against *Biomphalaria glabrata* snails, *Aedes aegypti* larvae, that are schistosomiasis and dengue vectors, respectively and an aquatic non-target organism. In addition, potential anti-*Leishmania* was evaluated as well as the hemolytic activity and the chemical composition of this essential oil. The essential oil was obtained by hydrodistillation and then analyzed by GC/MS. For the larvicidal activity, solutions of 25 to 200mg/L from the oil were tested against 4th instar larvae of *A. aegypti* [1]. For the molluscicidal activity, the snails were exposed to solutions of 25 to 100 mg/L [2]. For toxicity evaluation on a non-target organism, 2nd instar larvae of *Artemia salina* were used [3]. For leishmanicidal activity, the oil was tested against *Leishmania amazonensis* at concentrations of 3.1 to 400 mg/L [4]. The hemolytic activity was evaluated using human erythrocytes [5]. All the tests were carried out in triplicate and the results are shown as LCs and percent of hemolysis. The leaves of *S. cumini* provided oil, with a yield of 0.5%. The analysis of this oil showed the identification of 11 compounds, representing 99.98% of the total oil fraction, with monoterpenes comprising 92.46% and sesquiterpenes 7.52%. The major compounds were the monoterpene hydrocarbons α-Pinene (31.85%), *cis*-β-Ocimene (28.98%) and *trans*-β-Ocimene (11.71%). In the larvicidal test, the oil was not lethal even at the highest concentration. In the molluscicidal test, toxicity tests to a non-target organism and leishmanicidal tests, the LC_{50} were 90.4mg/l, 175.4mg/l and 60.34mg/l, respectively. In the hemolytic test, 7.5, 4.7 and 3.7% of hemolysis were observed at the concentrations of 400, 200 and 100 mg/l, respectively. The essential oil of the leaves of *S. cumini* showed promising activity as a molluscicide and leishmanicide.

Acknowledgments: CAPES, FAPEMA

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P 72. Chemical composition and larvicidal activity of leaf essential oils from *Mentha pulegium* and *Foeniculum vulgare*

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Keywords: *Aedes aegypti*; dengue: vector control; aromatic plants

Dengue is a potentially fatal mosquito-borne infection with 50 million cases annually, and 2.5 billion of people vulnerable to the disease, transmitted mainly by *Aedes aegypti* (Linnaeus, 1762). This major public health problem has epidemics in Brazil and recently in Cape Verde [1]. The lack of anti-viral treatment or vaccine against dengue makes mosquito vector control the only option to prevent or reduce virus transmission. The constituents of plant essential oils can affect insect's behavior being potentially effective in pest control [2,3,4]. In our studies we intended to contribute to control the development of earlier *Ae. Aegypti* instars. Essential oils (EOs) obtained by hydrodistillation of the aerial parts of *M. pulegium* and *F. vulgare*, were assayed on third instar larvae.

For EOs larvicidal activity four concentrations were assayed. Tween 20 was used to obtain emulsions of the EOs in water. The lethal concentrations LC_{50} and LC_{90} were determined by probit regression (SPSS for Windows®). The ^{13}C-NMR technique provides reliable qualitative information of the main chemical constituents of the total oil content. The ^{13}C-NMR analysis of *M. pulegium* oil showed that pulegone was the major monoterpene ketone identified with traces of menthone, whereas trans-anethole and limonene were the main constituents of *F. vulgare*. The EOs of these plants showed strong larvicidal activity 24h after exposure. 100 % mortality of 3rd instar larvae of *Ae. aegypti* was achieved with 0.060 ml/L of the wild fennel (*F. vulgare*) essential oil. Preliminary bioassays showed that 3rd instar larvae of *A. aegypti* are more susceptible to *F. vulgare* EO than to that of *M. pulegium*.

Acknowledgments: Authors are grateful to Fundação Calouste Gulbenkian for the PhD grant and to FCT for financial support of UPMM and project PEst-OE/QUI/UI0612/2011.

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P 73. Thyme and juniper essential oils screening for light dependent antifungal properties

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Keywords: Essential oils; photoactivity; phytopathogenic fungi, antifungal activity

Plant screening for light activated bioactivity is an issue increasing importance. Essential oils of *Juniperus communis spp. alpina* and *Thymus zygis spp. zygis* were analyzed for photoactive antifungal properties. Phytopatogenic fungi *Botrytis cinerea, Cladosporium cucumerinum* and *Fusarium culmorum,* reported to be harmful toxin-producers, were used as biological targets.

For both plant oils 1:10, 1:50 and 1:100 v/v solutions were prepared in n-hexane. Fungi were grown and maintained in culture on PDA (Difco Lab) at 25ºC. For the antifungal tests fungi suspensions were prepared by disintegrating the mycelium in a specific liquid medium. Each fungus suspension was filtered under vacuum through a nylon net (100 µm pores) on a Buckner funnel, adding liquid media until a spore count of about 5-7,5 x 10^4 per ml was achieved for each fungus. Stock solutions (10-4 M) of 8-methoxypsoralen (8-MOP) and 8-methoxytionepsoralen (8-MOPT) were used as standards respectively for the irradiation with the UVA and VIS spectrum of light.For the antifungal tests performed on TLC plates 25 and 50 µl of each concentration of the plant oil, and 25 ml of the solution of the standards were applied. Control spots only with the solvent were also assayed. Fungi were inoculated by spraying the TLC plates with the fungal suspension. Incubation was performed in humid chambers at 23±2ºC for 4-5 days. Inhibition areas were revealed as spots without fungus growth. The quantitative evaluation was based on the area of the spots free of fungus growth. Photoactivity was determined by comparing the results achieved in the dark to those of the correspondent sample irradiated. A set of five fluorescent lamps (Osram L18w/10 Daylight, emission range ~350-730 nm,λ_{max}= 485nm with 0,62W/m^2 fluence) or a set of five UVA lamps (Phylips TLK 40W/09N; emission range ~310-440nm; λ_{max}=354nm, 0,44W/m^2 fluence) were used as VIS and UVA light sources, respectively.

Relevant antifungal effects were obtained with both plant oils on the different fungi, however EO were slightly less efficient then the standards 8-MOP and 8-TMOP in relation to UVA or VIS light irradiation, respectively. As a whole EO photoactivity was higher after irradiation with UVA light then with VIS light. Best photoactivity was observed with Juniper oil (~72.3% UVA and VIS) on *C.cucumerinum* and *B.cinerea*, and Thyme oil (80.3% (UVA) and 73.8 (VIS) on *C.cucumerinum.*

Acknowledgements: Authors thank Prof. António Maçanita, from IST-UTL, for supplying the standards.

P 74. Citral, a natural acyclic monoterpene, reduces orofacial nociception in mice

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Keywords: Monoterpenes, citral, orofacial pain, analgesic

The purpose of the present study was to evaluate the antinociceptive effect of citral (CIT) using orofacial nociception induced by -formalin, -capsaicin and –glutamate on mice.

Swiss male mice (26-32g) were pretreated with CIT (50, 100 or 200 mg/kg, i.p.), morphine (5 mg/kg, i.p.) or vehicle (distilled water + one drop of Tween 80 0.2%) 1 h before formalin (20 µl, 2%), capsaicin (20 µl, 2.5 µg) or glutamate (40 ìl, 25 ìM) injection into the right upper lip of animals. The motor coordination was also evaluated using Rota rod apparatus (8 rpm, 180 s). Experimental protocols were approved for the UFS Ethic Committee (CEPA # 26/09). The data were statistically analyzed by ANOVA followed by Dunnett test ($p<0.05$).

Our results revealed that i.p. pretreatment with CIT was effective in reducing nociceptive face-rubbing behaviour in both phases on formalin test ($p<0.01$ or $P<0.001$) and also produced significant antinociceptive effect at all doses in the capsaicin- and glutamate- induced orofacial nociception tests ($p<0.001$). Such results were unlikely to be provoked by motor abnormality.

Our results suggest that citral, a natural acyclic monoterpenes, might represent important tool for management and/or treatment of orofacial pain.

Acknowledgements: FAPITEC/SE/Brazil; CNPQ/Brazil.

P 75. Chemical characterization of the essential oil of *Hyptis pectinata* germplasm

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Keywords: Lamiaceae; medicinal and aromatic plant; accessions; chemical constituents; volatile oil

The aim of this study was to characterize chemically the essential oil of 14 accessions of *Hyptis pectinata* L. Poit from the Active Germplasm Bank of the Federal University of Sergipe, Brazil. The field experiment was conducted at the "Campus Rural da UFS" Research Farm of the Federal University of Sergipe, located in São Cristóvão, Sergipe, Brazil. The essential oil was obtained from dry leaves by hydrodistillation using a Clevenger apparatus. Qualitative analysis of the chemical composition of the essential oils was performed using a gas chromatograph coupled to a mass spectrometer. A quantitative analysis of the essential oils was conducted in a gas chromatograph equipped with a flame ionization detector. We do not observed significative differences between accessions for essential yield. Variations for chemical constituents of the essential oil from *H. pectinata* accessions were observed. Compounds encountered in major quantity in the essential oils of the accessions were ß-elemene, (E)-caryophyllene, biciclogermacrene, trans cadina-1,4-diene, espatulenol, caryophyllene oxide and calamusenone. Major percentages of (E)-caryophyllene were observed in the accessions SAM-014 (43.38%) and SAM-015 (31.70%). Highest percentages of caryophyllene oxide were observed in the accessions SAM-010 (44.89%) and SAM-008 (44.69%) and major percentages of calamusenone were observed in the accessions SAM-017 (37.00%) and SAM-018 (29.04%). The multivariate analysis showed the formation of four groups according to the chemical composition of the essential oil of *H. pectinata* accessions.

Acknowledgments: The authors thank FAPITEC/SE, CNPq and CAPES for their financial support.

P 76. Phytochemical analyses and antinociceptive effect of *Aristolochia trilobata* L. stem essential oil and 6-methyl-5-hepten-2yl acetate in rodents

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Keywords: *Aristolochia trilobata*, essential oil, 6-methyl-5-hepten-2yl acetate (sulcatyl acetate), pain

A. trilobata L. (Lamiaceae), popularly known as 'mil-homens', produces an essential oil that is generally sold at popular Brazilian markets to treatment of colic, diarrhea, and dysentery. Present study evaluated phytochemical composition of *A. trilobata* stem essential oil (EO) and antinociceptive property of EO and 6-methyl-5-hepten-2yl acetate (sulcatyl acetate), mainly compound, in two models of pain.

EO was extracted by hydrodistillation of dried stem of the *A. trilobata* (UFS Herbarium voucher specimens, ASE#23.161). The identification of the components was made through comparison of substance mass spectrum with the database of the GC-MS, literature and retention index. Sulcatyl acetate (SA) was isolated and indentified from OE. The antinociceptive effect of EO and SA were examined using the acetic acid (0.85%) writhing reflex and formalin (1%)-induced nociceptive behavior in mice. Male mice (34-38g) (n=8, per group) were pretreated with vehicle (saline + tween 80 0.2%) or EO (25, 50 or 100 mg/kg, i.p.) or SA (25 or 50 mg/kg, i.p.) 1 h before experiments. Protocols were approved by the animal care and use Committee (CEPA/UFS # 16/12) at the UFS. Data were evaluated by one-way ANOVA followed by Tukey's test ($p < 0.05$).

The phytochemical analysis of EO showed the presence of 6-methyl-5-hepten-2yl acetate (sulcatyl acetate) (25.45%), limonene (12.07%), linalool (7.34%) and p-cymene (7.14%) as the main compounds. The presence of sulcatyl acetate in EO was confirmed by IR, MS and 1D (1H and 13C) and 2D HSQC and HMBC NMR experiments. Our results revealed that i.p. pretreatment with EO and SA were effective in reducing nociceptive behavior in writhing reflex test ($p<0.001$) and both phases on formalin test ($p<0.01$ or $p<0.001$). Such results were unlikely to be provoked by motor abnormality.

Our results suggest that EO and SA might represent important tool for management and/or treatment of pain.

Acknowledgments: FAPITEC/SE/Brazil, CAPES/Brazil, CNPq/Brazil.

P 77. Impact of hybridization in *Ocimum basilicum* on the volatile and sensory profile.

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Keywords: basil; essential oil; quantitative descriptive analysis; principle component analysis

The aim of this study was to develop the volatile and sensory profile by Quantitative Descriptive Analysis (QDA) using essential oil of three basil (*Ocimum basilicum* L.) hybrids ('Cinnamom' x 'Maria Bonita'; 'Sweet Dani' x 'Cinnamom'; 'Sweet Dani' x 'Maria Bonita'). The plants were cultivated at the "Campus Rural da UFS" Research Farm of the Federal University of Sergipe, located in São Cristóvão, Sergipe, Brazil. The essential oils were obtained from dry leaves by hydrodistillation using a Clevenger apparatus. Qualitative analysis of the chemical composition of the essential oils was performed using a gas chromatograph coupled to a mass spectrometer (GC-MS). A quantitative analysis of the essential oil components was conducted in a gas chromatograph equipped with a flame ionization detector (FID). Twelve descriptive terms were developed by a team of selected judges, which also led to the definition of each term and the reference samples. Data were analyzed by ANOVA, Tukey test and principle component analysis. The sample ´Cinnamom´ x ´Maria Bonita´ had stronger overall aroma intensity than the others and less citrus aroma. Hybridization favored the appearance of new compounds in the essential oil of hybrid ´Cinnamom´ x ´Maria Bonita´, such as trans-linalool oxide, (E)-methyl cinnamate, (Z)-methyl cinnamate, which does not exists in the parent's essential oil. For the hybrids ´Sweet Dani´ x ´Cinnamom´ and ´Sweet Dani´ x ´Maria Bonita´ the new compound was trans-linalool oxide.

Acknowledgments: The authors thank FAPITEC/SE, CNPq and CAPES for their financial support.

P 78. Comparative study of volatile oil profile of different *Rosmarinus officinalis* L. extracts

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Keywords: *Rosmarinus officinalis*; volatile oil profile; GC-MS; fresh plant; dry plant

The Rosemary (*Rosmarinus officinalis*) is a well known medicinal and culinary herb [1]. The purpose of this study was to compare the volatile oil profile of different Rosemary extracts obtained from dry and fresh herb, highlighting the differences between the extracts used in aroma-, phyto- and gemmotherapy.The main topic of present study is to highlight the differences between the dry and fresh Rosemary´s volatile oil profile and the specific volatile oil profile of the special gemmotherapic Rosemary extract.

There were studied the volatile oil separated by hydrodistillation from dry plant, the hydroalcoholic extracts (1:5 – dry plant:solvent) obtained from fresh respectively dry plant and the gemmotherapic extract obtained from fresh plant (1:20 – dry plant:solvent) [2]. The volatile oil profile was evaluated by GC-MS using headspace injection at 85^0C, for 15 min, ZB-5MS 50 m x 0,32 mm x 0,25 microm capillary column respectively helium as carrier gas. The separated compounds were identified using an MS spectra library. The quantitative determination was performed by normalization.

The results shown the separation of 38 compounds from volatile oil, 33 from fresh and 13 from dry plant hydroalcoholic extracts respectively 28 from the gemmotherapic extract. The main separated compounds were alpha-pinene, 1,8-cineol, camphene, d-limonene and cymene. It could be observed a significant difference between the 4 volatile oil profile. The gemmotherapic extract has higher content in 1,8-cineol than in alpha-pinene. Generally, the fresh plant extracts contain more 1,8-cineole, cymene, d-limonene and less alpha-pinene or camphore than the dry plant extract.

This study showed a significant difference between the extracts from dry, respectively fresh Rosemary. The fresh plant extracts are more rich in volatile oil components than the dry plant extracts. The gemmotherapic Rosemary extract has a high content of volatile oil in comparison with its extraction ratio. These differences in the volatile oil profiles of the studied extracts are due by the degradation processes that occur during the drying of plants or the extraction. This shows the importance of using fresh plants in the extraction, having undestroyed active compounds.

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P 79. Chemical composition of *Xylopia rubescens* leaf oil: structure elucidation of new compounds

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Keywords: furanoguai-1,4-diene; (8Z,11Z,14Z)-8,11,14-heptadecatrien-2-ol; structure elucidation; *Xylopia rubescens*; leaf oil

The aim of this work was to investigate the chemical composition of *Xylopia rubescens* leaf oil. The structure of two new compounds has been elucidated.

Analysis of the essential oil and structural elucidation were carried out using a combination of chromatographic (CC, GC with retention indices) and spectroscopic techniques (MS, ^{13}C NMR, 2D NMR).

Fifty components, accounting for 91.9% of the whole composition, were identified including various compounds whose spectroscopic data were absent on commercial computerized MS libraries. The structure of two new compounds, furanoguai-1,4-diene A and (8Z,11Z,14Z)-8,11,14-heptadecatrien-2-ol B, has been elucidated using two-dimensional NMR spectroscopy (Figure 1). Among the minor compounds, 1βH,7αH,10βH-guaia-4,11-diene is reported for the first time as natural compound.

The composition of *Xylopia rubescens* oil, dominated by furanoguai-1,4-diene (36.4%) A, differs drastically from the composition of leaf oils of other *Xylopia* species.

Acknowledgements: Thanks to Pr Aké Assi (identification of the plant), Dr Casabianca (MS-TOF spectra) and the Ministère de l'Enseignement Supérieur de Côte d'Ivoire (Research grant to T.A.Yapi).

P 80. Identification and quantification of germacrenes A, B and C in *Cleistopholis patens* (Benth.) Engler & Diels essential oils

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Keywords: *Cleistopholis patens*; essential oil; heat-sensitive compounds; quantification; ^{13}C NMR

Identification of individual components of essential oils is generally achieved by GC-MS, in combination with retention indices. Some difficulties may occur when heat-sensitive compounds are present in the oil. For instance, it has been reported that 1,5-germacradiene derivatives isomerise thermally to the corresponding elemene through a [3.3] sigmatropic reaction. During GC(FID) and GC-MS analysis, isomerisation of germacradiene may occur or not, depending of temperature of injector, the length of the column (25-60m) and the temperature program (2-5°C/min). The aim of this study was to quantify three heat-sensitive germacrenes A, B and C using ^{13}C NMR in combination with GC(FID).

The chemical composition of one leaf oil and two trunk bark oil samples from *Cleistopholis patens* was investigated by GC (RI), by GC-MS and by ^{13}C NMR.

In the leaf oil sample, 35 compounds accounting for 90.9% of the composition have been identified. β-Elemene was identified by GC-MS and by ^{13}C NMR and quantified by GC(FID) (6.4%). According to CG(FID), germacrene A accounted for 0.1%. With respect to NMR analysis (mean intensity of signals), the ratio germacrene A/β-elemene was evaluated as 35/65 and the compounds accounted for 2.2/4.2% in the essential oil. Obviously, in our hands (GC columns, BP-1 and BP-20, 50m, 2°C/min), germacrene A rearranged to β-elemene and therefore, the content of β-elemene was overestimated.

Concerning trunk bark oils, 45 compounds accounting for 94.9% (sample I) and 98.7% (sample II) have been identified. In sample I, germacrene B and γ-elemene (13.5% and 6.1%, GC(FID), respectively) were identified by GC-MS, but only the signals of germacrene B were observed on the ^{13}C NMR spectrum. The thermal rearrangement of germacrene B to γ-elemene during GC analysis is evidenced.

In the sample II, δ-elemene (8.2%, GC(FID) was identified by GC-MS and by ^{13}C NMR, while germacrene C was identified only by ^{13}C NMR. One again, germacrene C is not detected by GC due to its thermal rearrangement to δ-elemene who is overestimated (δ-élémène: 5.6%, germacrène C: 2.6%).

From these results, it may be concluded that germacrenes A, B and C may undergo a thermal rearrangement to β-, γ- and δ-elemene, respectively, during GC(FID) or GC-MS analyses. Accurate quantification of the three heat-sensitive compounds was achieved by the combination of GC(FID) and ^{13}C NMR spectroscopy.

P 81. Application of dosy for the structural characterization of bergamot essential oil

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Keywords: NMR; DOSY; bergamot; essential oil

High resolution diffusion-ordered NMR spectroscopy allows the separation of signals from different compounds based on their diffusion coefficients. The diffusion coefficients of the components should be significantly different and without overlapping signals in ^{1}H NMR spectrum. Modification of the solvent matrix in which a sample is dissolved can change the diffusion coefficients and ^{1}H NMR chemical shifts of the components. It has been demonstrated that diffusion behavior in DOSY experiments can be manipulated by adding micellar surfactant in the solution, allowing better resolution ("matrix-assisted DOSY") [1,2].

In this study, a possible application of DOSY for the structural characterization of commercial available bergamot essential oil (Frey + Lau, Germany) has been considered. The main components of the oil were limonene, linalool acetate, and linalool, as determined previously by GC-MS. In order to obtain better resolution in DOSY spectrum, various NMR solvents were used. Additionally, normal and reverse micelles were created by adding surfactants such as Triton X-100 or cetyltrimethylammonium bromide (CTAB) in order to further vary diffusion behavior of the essential oil components. The matrices used were dimethyl sulfoxide-d_6/Triton X-100, dimethyl sulfoxide-d_6/D_2O, $CDCl_3$, $CDCl_3$/D_2O/CTAB, D_2O/Triton X-100, acetone-d_6, hexane-d_{14}, cyclohexane-d_{12}, cyclohexane-d_{12}/Triton X-100, toluene-d_8, and toluene-d_8/Triton X-100.

The DOSY spectra recorded in this study revealed that the best results can be achieved if $CDCl_3$ or toluene-d_8 are used as solvents. Addition of the surfactants had an impact on diffusion coefficients; however, the resolution in DOSY axis has not been improved.

Acknowledgments: The authors acknowledge their gratitude to the Ministry of Education and Science of Serbia for the financial support (Project No. 46013).

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P 82. Chemical variability of the leaf essential oil of *Xylopia aethiopica* (Dunal) A. Rich. from Côte d'Ivoire

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Keywords: *Xylopia aethiopica*; Côte d'Ivoire; chemical composition; variability

The aim of the present study was to evidence either homogeneity or a chemical variability within 48 oil samples of *Xylopia aethiopica* isolated from leaves harvested in six Ivoirian forests.

The chemical composition of 48 leaves oil samples of *Xylopia aethiopica* was investigated by GC(FID), in combination with retention indices and by ^{13}C-NMR. Statistical analysis was obtained by combination of hierarchical clustering dendrogram and principal components analysis.

Twenty-three components accounting for 82.5-96.1% of the oil composition were identified. The composition was dominated by monoterpene hydrocarbons,β-pinene (up to 61.1%), α-pinene, (up to 18.6%), and sesquiterpene hydrocarbons (germacrene D, up to 28.7%). Statistical analysis allowed the distinction of two groups on the basis of β-pinene and germacrene D contents. The chemical composition of Group I (38 oil samples) was dominated by β-pinene, while the Group II (10 samples) was characterized by the association β-pinene and germacrene D. The monoterpenes, 4,4-dimethyl-2-vinylcyclohexene B (up to 5.1%) and 3,3-dimethyl-1-vinylcyclohexene A (up to 3.5%), previously found for the first time as natural components from *X. aethiopica* root oil [1] were identified in 28 out of 48 samples.

It could be pointed out that the four forests that produce β-pinene-rich *X. aethiopica* leaf oil are inland forests while oil samples containing appreciable amounts of β-pinene and germacrene D are located near the littoral.

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P 83. Comparison of different extraction methods for volatile compounds of *Thymus mastichina*

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Keywords: *Thymus mastichina*; volatile compounds; extraction methods; GC-MS; GC-FID

Thyme is a genus conformed by 220 species distributed throughout most of Eurasia. *Thymus mastichina* is an endemic plant of the Iberia peninsula and is traditionally used in that region [1]. Nowadays the essential oil of *Thymus mastichina* is an important product for the perfumery industry [2].

The objective of the study is to compare three different methods for extracting volatile compounds: microdistillation, hydrodistillation and solvent extraction.

A representative sample of flowers and leaves of *Thymus mastichina*, collected in the region of Castilla and Leon (Spain), was extracted with dichloromethane, distilled in a standard Clevenger type apparatus or in the Eppendorf MicroDistiller. All extraction methods were performed in four replications. The quantitative analysis of the volatile compounds was carried out by GC-MS and GC-FID with biphenyl as internal standard.

The statistical evaluation by multivariate analysis of variance shows that the profile of essential oil compounds is significantly influenced by the extraction method with most of the compounds contributing to this effect but not reacting in the same way. Not only the solvent extract was different compared to the two distillation methods, but also the results between standard Clevenger type distillation and microdistillation differed in most of the compounds. The volatile fraction of *Thymus mastichina* presents a complex volatile composition, where the main compounds are 1,8-cineol and linalool. The average amounts of 1,8-cineol were 13.6, 22.3 and 14.1 mg/g plant material for hydrodistillation, microdistillation and solvent extraction respectively, the amounts of linalool 1.3, 1.5 and 0.9 mg/g, respectively.

The study shows that the extraction method influences the extracted amounts of the single volatile compounds. Although the microdistillation presents the best results, the hydrodistillation that is commonly used in practice shows satisfactory results.

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P 84. Possibilities of use rugosa hybrid roses grown in Latvia as a source of volatile oil

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Keywords: *Rosa rugosa*; volatiles; DPPH

The diploid species *Rosa rugosa* Thunb. and its hybrids have a wide range of uses. When *R. rugosa* species became known in Europe in the middle of 19th century, it was heralded as the forbear of a new race of garden roses [1]. In China the petals of full flowering *Rosa rugosa* and its hybrids are used for a wide range of medicinal products and as a source of rose oil [2]. In contrast, on coastlines of Europe and North America *Rosa rugosa* is considered as invasive species, which rapidly spreads and destroys native plant communities [3]. The aim of this work is to understand possibilities of using Latvia grown Rugosa hybrids for production of volatile oil. The composition of volatile compounds and free radical scavenging activity (1,1-diphenyl-2-picryl-hydrazil (DPPH) % inhibition) was measured in the petals of the varieties ´Ritausma´, ´Sniedze´, ´Zaiga´, ´Liga´, ´Frau Dagmar Hastrup´ [4], *R. rugosa* and *R. rugosa* ´Plena´. Volatiles from roses were extracted using solid phase microextraction with subsequent separation by gas chromatography and identification by comparison of their mass spectra with mass spectral libraries. The hydrogen atom or electron donation abilities of the corresponding extracts were measured from the bleaching of the purple-colored methanol solution of DPPH [5]. The absorption was read against a blank at 517 nm.More than forty volatile aroma compounds were identified in petals. Among those, phenylethylalcohol, betacitronelol and nerol were predominant, but the composition of compounds varied among varieties. The variety ´Sniedze´ scavenged ~ 90% of free radicals, which was the best result for Rugosa hybrids. *R. rugosa* ´Plena´ had the highest content of volatiles, but the pedigree of all varieties, *R. rugosa*, exhibited the highest free radical scavenging activity (91.26 % of total amount). Thus, in addition to its ornamental value in Latvia *Rosa rugosa* appeared to be preferable for use as a source of volatile oil.

Acknowledgements: European Social Fund within the project «Support for Doctoral Studies at University of Latvia», National Botanic garden, PhD Dzidra Rieksta.

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P 85. Volatiles stability of field propagated *Thymus caespititius* chemotypes

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Keywords: *Thymus caespititius*; Chemotypes

Endemic of the NW Iberian Peninsula, including Azores and Madeira archipelagos, *Thymus caespititius* Brot. (Lamiaceae) is an aromatic plant with well characterized essential oils and possessing several chemotypes, namely thymol, carvacrol, sabinene and α-terpineol [1-3]. Although genetically controlled, the biosynthesis of essential oils is strongly affected by environmental and agronomic conditions of the growing place and by the developmental stage of the plant material, among other physiological factors [4]. The present work reports a two-year study on volatiles stability of field propagated *T. caespititius* chemotypes.

A total of ten chemically different individual plant samples, originally collected at the Azores archipelago (Terceira, S. Jorge and Flores islands) and mainland north of Portugal, were grown at Escola Superior Agrária de Coimbra since 2008. Throughout 2010 and 2011, aerial part samples were collected during the vegetative and the flowering periods. Volatiles were isolated by distillation-extraction and chemical analysis performed by GC and GC-MS, as previously reported [2].

Although fluctuations were recorded in the relative amount of some oil components over the two-year study, eight of the ten individuals maintained their original chemotype, while two changed the volatile profile. Plant D1, characterized by having high amounts of sabinene (85%) in the original collection site (S. Jorge, Azores) revealed a chemical profile with sabinene (6-40%) and high amounts of carvacrol (24-47%) following field establishment at Coimbra. Chemotype F in its original collection site (S. Jorge, Azores) showed no carvacrol in the essential oil, while the field plant established at Coimbra was rich in carvacrol (43-57%) and thymol (21-26 %).

Both biotic and abiotic factors can contribute to these chemical modifications. To understand these changes, the isolation of genes involved in scent production on different chemotypes is in progress.

Acknowledgments: Partially supported by FCT - PTDC/AGR-GPL/101334/2008.

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P 86. Chemical composition and insecticidal activity of *Thuja occidentalis* and *Tanacetum vulgare* essential oils against *Alphitobius diaperinus* Panzer.

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Keywords: *Alphitobius diaperinus*; essential oils

Plant extracts, including essential oils have a great potential for pest management, but their insecticidal activity varies[1]. The essential oils (EOs) from the leaves of *Thuja occidentalis* and flowers of *Tanacetum vulgare* were obtained by steam distillation. The isolation, identification and quantification of the volatile compounds was performed using a gas chromatograph (GC) coupled to a mass spectrometer (MS), a Saturn 2000 MS Varian Chrompack [2] The major components of *T. occidentalis* EO were *α*-thujone (69,8%) followed by *β*-thujone (9.47 %) and 1-terpinen-4-ol (2.67%); the main components of *T. vulgare* were *β*-thujone (61%) and camphor (12.99%).

The insecticidal activity of the EOs and their major components, *α*- and *β*-thujone against different larval stages of the lesser mealworm, *Alphitobius diaperinus* Panzer, a cosmopolitan pest inhabiting chicken and broiler houses in vast numbers, was evaluated under laboratory conditions. The earlier (10 days old) and later (30 days old) larval stages were reared on diets containing 1 % acetone solutions of tested compounds. Body weight gain, mortality, number of pupae and adults, and their body mass were recorded.

Insecticidal activity of both EOs and pure monoterpenes depended on the age of larvae. The highest activity against young larvae showed *T. occidentalis* EO, followed by *α*-thujone, tansy EO and *β*-thujone. The total mortality caused by these compounds was 65.0, 59.83, 46.88, 45.63%, respectively. The differences between the activity of EO and its major component against the tested insects were not significant. The growth and developmental cycle of younger larvae was affected in opposition to the older larvae, where it was less disturbed and their development was similar to the control.

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P 87. The influence of packaging materials on the composition of the dried dill (*Anethum graveolens* L.) volatiles during storage

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Keywords: dill; volatiles; packaging material; SPME

Dill (*Anethum graveolens* L.) is a popular herb used in many regions, including Baltic countries. Dill is widely used for flavoring foods and beverages due to its pleasant spicy aroma. The aim of this work was to determine the best packaging material for storage of dried dill based on volatile compounds composition.

Dill leaves were cut in slices 0.4±0.1 cm and dried using a microwave-vacuum drier „Musson-1" (OOO „Ingredient", St. Petersburg, Russia). Characteristic parameters of drying program were as follows: number of magnetrons was decreased along the drying process (starting at 4, and then followed by 3, and finally, 2); pressure 12.00-14.63 kPa; drum rotation speed – 6 rpm; product mass per load – 1 kg; drying time – 17 minutes.

Dried dill samples were packed in hermetically sealed monolayer polyethylene, multilayer Multibarrier 60 (MB60) and multilayer aluminium/poliethylene pouches both in vacuum and atmospheric ambiance. Samples were stored at +20 ± 1 C temperature. The quality parameters where analysed before packaging and during the storage for two years. Volatiles from dried dill were extracted using solid phase microextraction (SPME) Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/Car/PDMS) fiber and analysed by gas chromatography-mass spectrometry. Compounds were identified by comparison of their mass spectra with mass spectral libraries (Nist98), and by calculation of linear retention indexes and comparison with literature data.

The main aroma compounds of dill leaves are α-phellandrene and dill ether. Composition of dried dill differs from fresh dill, and significant increase in content of α-phellandrene, β- phellandrene was detected. Results showed that more volatiles were preserved in dill packed in Multibarrier 60 material pouches, whereas, comparing packaging ambiances, more volatiles in vacuum packed samples were determined. Control sample packed in PE pouches showed the lowest results.

The composition of volatile compounds of dried dill is affected both by the packaging material and ambiance in pouches, and results showed that it is very important to choose the most appropriate material for dried dill storage.

Acknowledgment: This research has been prepared within the framework of the ESF Project, Formation of the Research Group in Food Science", Contract Nr. 2009/0232/1DP/1.1.1.2.0/09/APIA/VIAA/122.

P 88. Aromatic compounds of traditional and novel components of chicory coffee

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Keywords: chicory coffee, aroma, traditional and novel components

Chicory coffee is identified with the category of *wellness* beverages. Aroma attributes of chicory coffee result not only from the raw materials used, but most of all from roasting process which leads to considerable changes in chemical composition of the product. The aim of the study was aroma identification of traditional (roasted chicory, barley, sugar beets) and novel (artichoke, hawthorn, lovage) raw materials of chicory coffee. Novel components have high level of chlorogenic acid which may be an aroma precursor of phenols which have spice and smoke flavor [1].

Methods: roasting process in a device by Probat Werke BRZ-2 (Emmericham Rhein), volatiles isolation by SPME/DVB/CAR/PDMS, the identification of volatiles using GC/MS 5975C VL MSD equipped with a HP-5MS capillary column (Agilent Technologies) and NIST05 library.

Results: In the aroma of roasted chicory, barley, ray, sugar beet there were identified 36, 30, 25, 15 compounds respectively. The dominated were pyrazines and phenols which are characteristic for roasted coffee. It can be concluded, that the most aromatic among the traditional materials for coffee substitute is chicory, while the sugar beet is the lowest one. The novel proposed materials are also the source of interesting odorants, particularly roasted artichoke. Among 23 identified volatiles there were: acetaldehyde, furfural, 5-methyl-2-furfural, 2-methyl-furan, 5-methyl-2-furancarboxaldehyde, 2-metoxy-4-(1-propenyl)-phenol, 2-methyl-phenol, 2,5-dimetyl-pyrazine, 3-ethyl-2,5-dimethylpyrazine, 3,5-diethyl-2-methyl-pyrazine, 3,5-diethyl-2-propyl-pyrazine, p-vinylbenzohydrazine. It should be pointed out the occurrence of several pyrazines, and phenols characteristic for coffee aroma. Some identified odorants in roasted hawthorn were following: acetaldehyde, furfural, 2-methyl-5-(1-methylethenyl)-1-one, hexadecanoic acid methyl ester and also 4-heptyl-phenol. In rosted lovage the occurrence of 2-metoxy-4-vinyl-phenol was stated, according to many authors the important odorant in natural coffee as well as other component: furfural, 5-methyl-2-furancarboxaldehyde, 4-mercaptophenol, 6-methyl-2-pyridinamine, 2-methyl-6-(2-propenyl)-phenol. According to the obtained data the novel materials may be a source of some characteristic coffee aroma components.

Acknowledgments: This study was financially supported by the National Centre of Science, Poland, Grant No 2011/01/B/NZ9/01060.

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P 89. Dry and wet seasons they determine the phytochemical profile of essential oils from *Copaifera langsdorffii* Desf. in different vegetation types of the Brazilian Cerrado

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Keywords: terpenes; Cerrado; sazonality; climate changes

The climate change in the atmosphere provides increases of temperature and redistribution of averages of rainfall (1). We can observe a decrease in soil moisture, could lead to increased periods of drought, especial in tropical and savanna biomes (2). The dry and wet seasons from different configurations of the Cerrado physionomical domain were able to promote changes in the essential oil of *Copaifera langsdorffii* Desf. Different ecophysiological conditions of regions and climate change may be important factors for changes the chemical profile of terpenes in the essential oil. The study carried out tries to understand the effect of dry periods (August to September) and rainy (October to February) in the chemical profile of essential oil of *C. langsdorffii*. To this end, the research objectives are: (i) make comparisons in relation to production, yield and essential oil composition between the dry and rainy season, (ii) evaluate the phytochemical profile in different formations of the Cerrado. Analysis by GC-MS oil Copaifera langsdorffii 24 showed the presence of volatile compounds being monoterpenes, sesquiterpenes and oxygenated sesquiterpenes. The cluster analysis by the dissimilarity matrix calculated by the Euclidean distance which method of Square agglomeration was UPGMA gathering areas according to the chemical profile of the essential oil. Germacrene-D, gamma-muuroleno, delta-elemene, alpha-humulene and trans-caryophyllene are substances which varied concentrations. The seasonality effect is most evident at sites of semideciduous forest than in the cerrado sensu. Nevertheless, the production of essential oils correspond an effect of adjustment to the climate, whith increases protection against stress (3).

Acknowledgments: Funding body: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) financial support to the project nº 2010/15585-6.

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P 90. Antimicrobial activity of essential oils against *Paenibacillus larvae*

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Keywords: essential oils; antimicrobial activity; *Paenibacillus larvae*

American foulbrood is a serious bacterial disease that affects *Apis mellifera* colonies; the causative agent is *Paenibacillus larvae* [1]. The aim of the study was to evaluate *in vitro* the antimicrobial activity of 32 essential oils against *P. larvae.* Oils from 21 botanical species were analyzed by gas chromatography (CG and CG/EM). All essential oils were classified according to the composition of their main components in two groups: benzene ring compounds (BRC) and terpene compounds (TC). Minimal inhibitory concentration (MIC) in MYT broth [2] was assessed by the microdilution method. Final serial dilution concentrations of the essential oils ranged between 2,000-12.5 mg/L. The bacterial isolates were collected from different Argentina's region. The chromatographic analysis showed that a 67% of the essential oils contained predominately terpene compounds, while the remaining 33% included mainly compounds with benzene rings. From the TC group, *Cymbopogon citratus* essential oil showed the better antimicrobial activity against *P. larvae* with MIC values between 150 and 250 mg/L. The essential oils from *Aloysia polystachya* and *Mentha spp.* had the lowest inhibitory activity. Among the oils from the BRC group, one of the lowest MIC values was found with cinnamon essential oil (*Cinnamomun zeylanicum*) being between 25 and 50 mg/L; *Origanum vulgare* showed the highest MIC values (350-400 mg/L). Essential oils, especially those with BRC in their composition, presented inhibitory capacity against *P. larvae* strains.

Acknowledgments: This work was supported by UNMDP and CONICET.

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P 91. Chemical composition of leaf and stem oils of *Cupressus tonkinensis* silba from Vietnam

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Keywords: *Cupressus tonkinensis*; essential oil composition; α-pinene; sabinene; cedrol; 13C-NMR

Cupressus tonkinensis was treated for a long time as a synonym of *C. torulosa* D. Don. and it was proposed as new species in 1994.[1] The two species differed by morphological characters and they were distinct in their RAPDs.[2] The aim of this work was to investigate the chemical composition of C. tonkinensis leaf and stem oils from Vietnam.

Leaves and stems of *C. tonkinensis* produce monoterpene-rich oils whose composition is investigated for the first time by combination of chromatographic and spectroscopic techniques. The two samples were analyzed by GC(RI), GC/MS and ^{13}C-NMR. Moreover, the leaf oil sample was also fractionated over silica gel column and all the fractions of chromatography analyzed by GC(RI) and ^{13}C-NMR.

The combination of analytical methods allowed the identification of 47 compounds, accounting for 95.6 and 87.9% of volatile constituents of the essential oils of leaves and stems respectively. Alpha-pinene (25.7%), sabinene (23.3%) and terpinen-4-ol (12.6%) were the major components of the leaf oil that contained also beta-elemol (3.8%). In contrast, alpha-pinene (48.3%) was by far the major component of the stem oil beside myrcene (11.6%) and cedrol (8.7%).

The composition of the leaf oil of *C. tonkinensis* from Vietnam is close to that of the leaf oil from *C. torulosa* cultivated in the Argentinean Patagonia [3] but stem oil differs by its high amount of a-pinene, myrcene and cedrol. Leaf and stem oils of *C. tonkinensis* differ also from the essential oil isolated from the foliage of *C. torulosa* var. *cashmeriana* trees cultivated in Yunnan Province, China. [4]

Acknowledgements: The authors thank the NAFOSTED for financial support (Project 10605.21.09) and Pr. J. Costa (CPN-Lab, University of Corsica) who placed the GC/MS equipment at our disposal.

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P 92. Resolution of diastereoisomeric mixture of secondary bicyclic alcohol obtained from Carenko®

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Keywords: terpenoids; stereochemistry; olfactory compounds

Terpenoids are broad class of compounds which can be used as intermediates for the fragrance industry and chiral synthons in organic synthesis. Their structures in some cases tend to force stereo- and enantioselective course of reactions [1].

Our studies are focused on chemical transformations of (+)-3-carene leading to compounds with potential industrial as olfactory compounds. (+)-3-Carene is a bicyclic hydrocarbon derived from common in Poland Scotch Pine (*Pinus sylvestris* L.). We used it as a starting material in two-step synthesis of bicyclo[4.1.0]heptene derivative in form of pure diastereoisomers and their mixture.

The first step to obtain pure diastereoisomers of designed compound was the chemical acetylation of 1 in acetic anhydride with addition of zinc bromide [2]. Ketone 2, commercially called Carenko® was reduced with $LiAlH_4$ which led to the desired alcohol 3). The separation of diastereoisomers were performed by several recrystallizations in mixture of methanol:water (1:1). The absolute configuration of obtained one diastereoisomer was determinated by X-Ray crystallography and Moshers' esters but the second only by Moshers' esters. The olfactory properties of diastereoisomeric mixture and pure diastereoisomers were also evaluated.

Synthetic and stereochemical details of the applied procedures and olfactory properties of derivatives will be presented.

Acknowledgements: Presented work was supported by Ministry of Science and Higher Education, Grant No N N204 136538 and project „Enterprising PhD student - an investment in the innovative development of the region" part-financed by the European Union within European Social Foundation.

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P 93. Insecticidal and fungicidal activities of herb isolates obtained by different methods

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Keywords: plant protection, supercritical fluid extraction, hydrodistillation, insecticidal activity, fungicidal activity

Many green plants produce biologically active secondary metabolites and can provide valuable sources of natural drugs, natural pesticides and biofertilizers [1, 2]. In the search for alternatives to conventional pesticides, essential oils from aromatic plants have been widely investigated. In particular, terpenes and terpenoids belong to active components of essential oils [3, 4].

The aim of this study was a comparison of pesticidal activity and chemical composition of the isolates from *Pelargonium graveolens*, *Lippia javanica*, *Artemisia afra* and *Eucalyptus grandis* obtained using supercritical fluid extraction by carbon dioxide (SFE) and hydrodistillation. Extracts rich in non-polar components were obtained using SFE at pressure of 28 MPa and temperature of 50 °C. The residual plant material after SFE was subjected to maceration by methanol for 48 hours to isolate polar components. Product of hydrodistillation was pure essential oil. The yields of these isolates were evaluated for each plant material in dependence on used separation methods.

The composition of volatile compounds in the isolates was determined by gas chromatography (GC-MS and GC–FID). The insecticidal activity of isolates was measured on caterpillars of *Spodoptera litoralis* by in terms of contact toxicity (LD50, LD90) and antifeedancy. For antifungal bioassay, standard dilution method for evaluation of inhibition effect on growth of model patogenic and toxinogenic fungi (*Fusarium oxysporum*, *Penicillium expansum* and *Aspergillus fumigatus*) was used.

Strong pesticidal effects were observed for all isolates but significant differences among the particular isolates and among the plants were found. The efficiency of CO_2 extracts was comparable or lower than that of hydrodistillates and higher than the efficiency of macerates. The essentials oils showed the strongest insecticidal and fungicidal activities, however, their yields were 2-4 times lower than the yield of CO_2 extraction.

Acknowledgments: The financial support from the Technology Agency of the Czech Republic via grant TA01010578 is gratefully acknowledged.

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P 94. Association of *Thymbra capitata* essential oil and chitosan (TCCH hydrogel): a putative therapeutic tool for the treatment of vulvovaginal candidosis

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Keywords: Therapeutic tool, vulvovaginal candidosis, chitosan, *Thymbra capitata* essential oil

In this study we tested the anti-*Candida* effect of *Thymbra capitata* essential oil plus chitosan and developed a new therapeutic tool.

The classical checkboard methodology was used to determine the MIC resulting from products association [1]. The incorporation of *T. capitata* essential oil in a chitosan hydrogel using lactic acid as the solvent resulted in the TCCH hydrogel. Its anti-*Candida* activity was studied upon eighteen *Candida* cells, according to the CLSI M27-A3 micromethod [2].

The association of both natural products revealed, in the checkboard technique, an additive effect upon *Candida* and TCCH hydrogel showed to be active upon *Candida* planktonic at concentrations lower than individual compounds. TCCH hydrogel presents an acidic nature (4.3), compatible with the vaginal pH.

Being a new product with an acidic nature compatible with the vaginal environment and presenting a potent effect upon *Candida* cells, TCCH hydrogel could represent a valuable tool for the treatment of vulvovaginal candidosis.

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P 95. Essential oil and extracts from *Callitris intratropica* heartwood, sapwood and bark obtained by hydrodestillation, solvent extraction and headspace solid phase microextraction.

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Keywords: *Callitris intratropica*, essential oil, extract, HS-SPME

The wood and bark of *Callitris intratropica* has been used for a long time in folk medicine by the Australian aborigines [1], as an anti-termite timber [2] and since some years as a source of a now commercially available essential oil with a blue color due to the presence of azulenes [3]. The wood itself has a pleasant warm balsamic odor and the oil has a composition with substances unique to the callitris genus.

In the presented study commercial raw and refined oil, lab destilled oil, solvent extracts from different parts of the wood and headspace solid phase microextracts were analysed by GC-MS-FID. Quantitative (by FID detection) and qualitative analyses (by MS identification) were carried in one instrument with the use of a simple home-made MS-FID splitter. No azulenes were found in the wood itself so that the assumption suggests that the formation of azulenes takes place during the destillation process.

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P 96. Assessment of the antioxidant activity of *Lippia gracilis* Schauer aerial parts essential oil by different methodologies

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Keywords: *Lippia gracilis*; Verbenaceae; antioxidant activity; essential oil

Lippia gracilis Schauer is a Verbenaceae species native to northeastern Brazil. An ethnobotanical survey of some Brazilian medicinal plants showed that this plant, popularly known as *cidreira da serra*, is used in the treatment of several ailments, namely gastrointestinal infections, as well as respiratory and skin problems [1]. In the present study the chemical composition and antioxidant activity of *Lippia gracilis* essential oil was assessed by different methodologies.

The essential oil was isolated by hydrodistillation from *L. gracilis* aerial parts, and analysed by Gas Chromatography and Gas Chromatography-Mass Spectrometry as in [2]. The antioxidant activity was evaluated using three different methods as in [2,3]: free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox equivalent antioxidant capacity (TEAC) or ABTS [2,2'azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] method and the β-carotene bleaching method (coupled oxidation of β-carotene and linoleic acid).

Oxygen-containing monoterpenes (50%) and monoterpene hydrocarbons (46%) dominated *L. gracilis* essential oil. Carvacrol (42%) *p*-cymene (18%), γ-terpinene (17%) and thymol (5%) were the main (≥5%) essential oil components.

L. gracilis essential oil antioxidant ability showed a linear variation with increasing oil concentration for all the assessed methods (DPPH, TEAC and β-carotene bleaching method), showing a half maximal inhibitory concentration (IC50) of 434µg/mL, 625µg/mL, and 388µg/mL, respectively.

Acknowledgments: Partially supported by CNPq, CAPES, FAPEMIG.

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P 97. Antiphytoviral activity of essential oil of two *Eryngium* species

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Keywords: β-caryophyllene; caryophyllene oxide; *Eryngium alpinum*; *E. amethystinum*; satCMV

In this study antiphytoviral activity of essential oils of *Eryngium alpinum* L. and *E. amethystinum* L. from Croatia against satellite associated cucumber mosaic virus (satCMV) was investigated. Water distilled essential oils from aerial parts of plants have been analysed by GC and GC/MS using VF-5ms capillary column. Thirty-two components representing 92.4% of the total oil were identified in the essential oil of *E. alpinum* and thirty-five components representing 93.1% of the total oil of *E. amethystinum*. The oil of *E. alpinum* was characterized by a high concentration of caryophyllene oxide (21.6%), bicyclogermacrene (11.8%) and germacrene D (10.3%) while the main components of *E. amethystinum* oil were *β*-caryophyllene (19.7%) and *α*-pinene (12.3%). Germacrene D, caryophyllene oxide and bicyclogermacrene were also identified as the major compounds in the oil of *E. serbicum*, *E. palmatum* and *E. rosulatum* [1,2]. A comparison (t-test) of the mean number of lesions on the oil-treated *Chenopodium quinoa* plants with the corresponding control showed that both *E. alpinum and E. amethystinum* oils significantly reduced infection with satCMV with antiviral activity rate of 77.8% and 80.5%, respectively. Our previous investigation confirmed that sesquiterpenes-rich essential oils as well as oil components *β*-caryophyllene and caryophyllene oxide inhibit development of local lesions on plants infected with CMV [3-5]. Compared with the oils previously tested and with individuall application of *β*-caryophyllene and caryophyllene oxide, both *Eryngium* oils showed stronger inhibition of local lesions development. We can conclude that combination of *β*-caryophyllene/ caryophyllene oxide with some other oil components resulted with significant antiviral efficiency. Our investigation showed that essential oils isolated from *E. alpinum* and *E. amethystinum* posses strong antiviral activity against satCMV infection by inducing resistance response in the host.

Acknowledgements: This work was supported by the Ministry of Science, Education and Sports of the Republic of Croatia, projects no. 177–1191192–0830 and 006–0000000–3178.

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P 98. Chemical composition and antibacterial activity of *Lychnophora pinaster* Mart. essential oil

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Keywords: *Lychnophora pinaster*; Asteraceae; arnica; antibacterial; essential oil

Several studies have shown the antimicrobial efficacy of essential oils and in the last decades intensive studies were carried out indicating the possibility of their use in the control of foodborne pathogens, contributing to a future reduction in the employment of antibiotics [1,2].

Lychnophora pinaster Mart., is an Asteraceae listed as vulnerable to extinction in Brazil, and locally known as *arnica*. This medicinal plant is commonly used for its analgesic, antiinflammatory, antiprotozoal, antibacterial and antifungal properties. The hydro-alcoholic extract is widely used for the antiinflammatory and anesthetic values [3]. The goal of the present work was to characterize the chemical composition and antibacterial activity of *L. pinaster* essential oil.

The essential oil was isolated by hydrodistillation from *L. pinaster* aerial parts, and analysed by Gas Chromatography and Gas Chromatography-Mass Spectrometry as in [4]. Antibacterial ability of *L. pinaster* essential oil was tested by the agar well method [2] against *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19117, *Escherichia coli* ATCC 11229, *Salmonella choleraesius* ATCC 6539 and *Pseudomonas aeruginosa* ATCC 15442. Several concentrations of the essential oil diluted with dimethylsulfoxide (*DMSO*), to a final volume of 10µL, were assessed (500, 250, 125, 63, 31, 16, 8 and 4µg/mL). The adopted statistical design was in randomized blocks, with 3 repetitions. Data was analysed using Tukey test ($\alpha = 0.05$).

trans-Methyl cinnamate (62%), β-caryophyllene (21%), α-humulene (6%) and β-pinene (5%) were the main (≥5%) essential oil components. Antimicrobial activity was detected only against *Salmonella choleraesius* at 31µg/mL.

Acknowledgments: Partially supported by CNPq, CAPES, FAPEMIG.

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P 99. Chemical composition and repellence activity of *Philodendron imbe* Schott (Cipó imbé) essential oil against *Tetranychus ludeni*

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Keywords: *Philodendron imbe*, Cipó imbé; Araceae; *Tetranychus ludeni*; spider mite; essential oil

Spider mites, such as *Tetranychus ludeni*, are considered significant constraints to vegetable production [1]. Control of these spider mites using synthetic pesticides in not only hazardous to Man but has also the potential for induction of resistance in mites.

The neotropical genus *Philodendron* Schott (Araceae), presents significant landscape interest due to the large number of ornamental species. The bactericidal, insecticidal and antiprotozoal activities against *Trypanosoma cruzi* and *Trichomonas vaginalis* has been encouraged the phytochemical study of species of this genus. The leaf tea is locally used in coastal regions of Brazil as a vermifuge [2]. In the present study the chemical composition and repellence activity of *Philodendron imbe* Schott (*Cipó imbé*) essential oil against *T. ludeni* was assessed.

The essential oil was isolated by hydrodistillation from *Philodendron imbe* roots, and analysed by Gas Chromatography and Gas Chromatography-Mass Spectrometry as in [3]. The level of repellence to spider mites was assessed on tomato leaflets as in [4]. In addition o control experiments (T1: water plus Tween), different concentrations of the essential were evaluated (T2: 0.25%, T3: 0.50%, T4: 0.75%, T5: 1.0% and T6: 1.5%). The distances the mites moved after 20, 40 and 60min were measured. The randomized blocks statistical design was adopted, with 3 repetitions. Data was analysed using Tukey test ($\alpha = 0.05$).

Sesquiterpene hydrocarbons (92%) dominated *P. imbe* essential oil, β-bisabolene (65%), *trans*-α-bergamotene (10%), α-copaene (3%), δ-cadinene (3%), *trans*-β-farnesene (2%) and β-selinene (2%), being the mail components.

The distance the mites moved increased with lowest essential oil concentration used, T3: 6.1mm < T2: 8.3mm < T1: 16.5mm. The shorter distance the mites moved, that is, the repellence activity, was observed with T4: 5.1mm, T5: 4.8mm and T6: 4.2mm essential oil concentrations

Acknowledgments: Partially supported by CNPq, CAPES, FAPEMIG.

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P 100. Essential oil composition and variability of *Salvia nemorosa* L. (Lamiaceae) in the area of Vienna, Austria

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Keywords: essential oil; *Salvia nemorosa*; sabinene; sesquiterpenes; hexadecanoic acid

Studying the aromatic flora of Eastern Austria we report here the composition of the essential oil from leaves, flowers and stems of *Salvia nemorosa*. This plant is an element of the pannonic flora from Eastern Austria. It is growing in semi-dry meadows and on embankments. Plants were collected from five sites in the northern parts of Vienna and along the Danube during the flowering stage and divided in leaves, stems and flowers. These samples were dried in the ambient air and hydrodistilled in a Clevenger type apparatus or extracted by microdistillation. The obtained oils were diluted with hexane and analysed by GC and GC/MS using an apolar Rtx-5 column for the separation. The compounds were identified according to their mass spectra and retention indices (1).

The oil content of all plant parts was below 0.05%. The oil from the flowers was dominated by sabinene (37-44%) followed by germacrene D (9-14%), β-caryophyllene (8-12%), α-thujene (5-8%) and γ-terpinene (2-8%). The leaf oils were rich in β-caryophyllene (14-41%), germacrene D (14-38%) and caryophyllene oxide (5-20%). Hexadecanoic acid (56-60%) was the main compound in the stem volatile fractions.

In sum the plants of all five locations represent the same chemotype but differed from plants collected in Iran with β-caryophyllene, germacrene B and caryophyllene oxide as main oil compounds (2) and plants from Serbia having an oil were hexadecanoic acid, spathulenol and caryophyllene oxide prevailed (3).

Acknowledgements: The research was supported by “Hochschuljubiläums-Stiftung der Stadt Wien“, Project H-02328/2007.

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P 101. Effect of systemic administration of essential oils and main components on honeybee survival

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Keywords: essential oils; survival; *Apis mellifera*

Controlling bee diseases with non contaminant products is a challenge in apicultural research. Essential oils and their main components have been widely studied as alternative treatments for honeybee pathologies [1, 2, 3]. However, there is little information about prolonged systemic administration. The aim of this study was to evaluate, in laboratory assays, the effect of long term consumption of essential oils and main components. Oils were obtained by hydrodistillation from *Laurus nobilis, Cinnamomun zeylanicum, Origanum vulgare, Rosmarinus officinalis* and *Eucalyptus* spp. and were analyzed by gas chromatography. The main components administered were 1,8-cineol, β-myrcene, cinnamic aldehyde, carvacrol and α-phellandrene. Substances were administered *ad libitum* to newly emerged bees at concentrations of 0; 333; 3,333 and 6,666 ppm, on sucrose syrup, throughout 11-18 days. Mortality and substances consumption were measured daily. Survival analysis was performed using Gehan-Breslow test and pairwise multiple comparisons between survival curves (α= 0, 05). Substances consumption was analyzed using one way ANOVA. Bees that received cinnamon oil showed a lower survival than control at concentrations higher than 333 ppm (p-values<0.001). Consumption of cinnamic aldehyde, the main component of this oil (79.3%), also caused lower survival at the same concentrations (p-values<0.001). Eucalyptus oil caused a lower survival rate when it was administered at 6,666 ppm, although 1,8 cineol, its main component (63.5%), was not toxic for bees at any concentration. Carvacrol, a main component of many oregano essential oils, showed toxic effects at 3,333 and 6,666 ppm. Essential oils did not cause differences in consumption rate (p= 0.275) while main components solutions, except for carvacrol, were less consumed than control at the three concentrations. Treatments did not cause dysentery to bees. Our results contribute to understanding the effect of repeated systemic doses of these substances, which is important to design long term pharmacological studies and treatments development.

Acknowledgments The authors wish to thank the collaboration made by María Laura Umpiérrez and the contribution made by Enrique Tkacik who provided *L. nobilis* essential oil.

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P 102. The use of essential oils constituents' effect on shelf life of fresh-cut *Salicornia ramosissim*a and *Sarcocornia perennis*

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Keywords: Fresh-cut salads, halophytes, quality, antioxidants, eugenol, citral.

The halophyte plants *Salicornia ramosíssima* and *Sarcocornia perennis* have been exploited in gourmet cuisine combined with fresh salads as substitute of salt due to their nutritional value. The objective of this work was to evaluate the ability of the essential oil constituent's citral and eugenol on the preservation of the quality of these halophyte plants through shelf life at two temperatures.

The fresh tips of the plants (7-8 cm long) were washed in tap water and, after removal of excess water, immersed in solutions of 0.025% citral (w/v), 0.025% (w/v) eugenol, or just distilled water (control), during 2 minutes. The samples were prepared for storing in polyethylene foam packages, covered with low density polyethylene film of 15 µm thickness and put in refrigeration rooms at 1, and 6 °C and relative humidity of ≈90%. At 0, 7 and 14 days samples were removed and quality measurements were taken: color, weight loss, electrolyte leakage, antioxidant activity, phenolic compounds, bacteria, yeasts and molds. A taste panel was also performed.

Results showed that temperature was the main factor affecting storage ability of fresh-cut salicornia and sarcocornia, being 1ºC better temperature. General quality parameters were slightly better preserved through 14 days at 1 ºC when the fresh-cut halophytes were treated with 0.0025% citral or eugenol. Taste panel performed well through shelf life, being some parameters scored higher in citral and eugenol treated fresh-cut halophyte plants than control.

Acknowledgements: This research was partially supported by the Project: PTDC/AGR-ALI/121085/2010-INNCOAT - Innovative edible coatings to improve storage of small fruit and fresh-cut.

P 103. Volatiles components from the leaves of *Centaurea vlachorum* Hartvig (Asteraceae), growing wild in Albania

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Keywords: *Centaurea vlachorum*, Asteraceae, essential oil, GC-MS.

The genus *Centaurea* is a polymorphous genus of the family Asteraceae and includes grassy plants, from annual to perennial, rarely suffruticose. The aerial parts of several species of *Centaurea* are used in popular medicines in many countries as antibacterial, antimicrobical, ipoglycemic, cytotoxic and phytotoxic agents.

Centaurea vlachorum Hartvig (Asteraceae) is a new species discovered lately in Albania. It was found at the altitudes between 1600-2000m above sea level, in the Lura Lakes region. There are no reports in the literature about the chemical analyses of this plant.

For the present study, the leaves of the plant were collected during its flowering season, early July, dried in darkness till a constant weight, grinded and used as such for hydro distillation using a Clevenger type apparatus according to standard procedures. The volatile components were analyzed by Gas Chromatography-Mass Spectrometry. Ôhe major compounds found in essential oil were: benzaldehyde (21.0%), spathulenol (7.9%) and caryophyllene oxide (10.0%),

P 104. Chemical characterization and biological activity of *Margotia gummifera* essential oil

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Keywords: *Margotia gummifera*; essential oils; antifungal activity; anti-inflammatory activity; cytotoxicity

This study was designed to elucidate the composition, cytotoxicity, antifungal and anti-inflammatory activities of the essential oil of *Margotia gummifera* (Desf.) Lange. Ripe umbels with mature seeds were submitted to water distillation in a Clevenger-type apparatus and the oils were analysed by GC and GC-MS, as previously reported [1]. The oil was characterized by high contents of monoterpene hydrocarbons, being the main compounds myrcene (20.4%) and sabinene (22.5%). The antifungal activity (MIC and MLC) was evaluated against dermatophyte strains, using the macrodilution method according to Clinical and Laboratory Standards Institute. *Microsporum canis* FF1 and *Trichophyton rubrum* CECT 2794 are the more sensitive strains. Assessment of macrophages viability was performed using the MTT assay. The results showed that the oil is not cytotoxic at concentrations ranging from 0.32 to 1.25 µL/mL. This study also evaluated the anti-inflammatory properties of *M. gummifera* oil, through the measurement of the pro-inflammatory mediator. After cells activation with lipopolysaccharide (LPS) for 24 h, nitrite production increased to162.8% above control, while in the presence of the oil concentrations 0.32 µl/ml, 0.64 µl/ml and 1.25 µl/ml, nitrite production was reduced to 140.7%, 139.9% and 128.8% of the control, respectively. Furthermore, we also addressed the NO scavenging activity of *M. gummifera* using SNAP (S-nitroso-N-acetylpenicillamine) as NO donor and we observed that the oil exhibits a slight scavenging activity at the concentration 0.64 µl/ml.

These results indicate that the biological activity of *Margotia gummifera* essential oil should be explored and suggest its potential as a natural source of new anti-inflammatory drugs.

Acknowledgements: Funding: FCT (Fundação para a Ciência e a Tecnologia) project CEF/POCI2010/FEDER

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P 105. Effect of citral in rat mesenteric artery: evidences of the involvement of calcium channels

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Keywords: calcium channels, citral, vasorelaxant effect, rats

Citral is a monoterpene found in essential oils of various herbs with activity on the cardiovascular system. This study aimed to evaluate the vasorelaxant effect of the citral in rings of rat superior mesenteric artery.

Male wistar rats (200 – 300 g) were euthanized by exsanguination under anesthesia and superior mesenteric artery was removed and cut in rings (1-2 mm). These rings were then mounted in organ baths containing Tyrode's solution at 37 ºC and gassed with carbogen. For isometric tension recordings, each ring was fixed in a force transducer connected to an acquisition system. All procedures described in this work were approved by the Ethical Committee on Animal Experiments from UFS under protocol number 37/2009.

In rings pre contracted with phenylephrine (10 µM), citral (10^{-7} - 10^{-2} M) was able to induce relaxations (pD2 = 2.52 ± 0.10; Emax = 103.4 ± 10.2%; n = 6) that was not affected after removal of the endothelium (pD2 = 2.34 ± 0.15; Emax = 107.2 ± 4.3%; n = 6) or in rings without endothelium pre-contacted with KCl 80 mM (pD2 = 2.04 ± 0.12; Emax= 101.3 ± 7.1%; n = 6). In a calcium-free solution, citral (3 x 10^{-4} and 10^{-3} M) was able significantly ($p < 0.05$) to inhibit the $CaCl_2$-induced contractions (10^{-5} – 10^{-2} M) up to 88.6 ± 3.1%. Furthermore, citral (3 x 10^{-4} M) was not capable to induce an additional effect on maximal relaxation of nifedipine (10 µM), a L type voltage-operated calcium channel blocker.

These results demonstrate that citral induce vasorelaxation of mesenteric artery possibly caused by the inhibition of the Ca^{2+} influx through voltage-operated Ca^{2+} channels.

Acknowledgments: CNPq/FAPITEC, Brazil.

P 106. *In vitro* antimicrobial activity and cytotoxic potential of North-West Romanian propolis

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Keywords: antimicrobial; cytotoxic; propolis phenolics; flavonoids

Propolis is a honeybee product demonstrated with complex therapeutic effects [1,2,3]. Objective: the aim of the study was to evaluate antimicrobial activity of ethanolic extract of North-West Romanian propolis samples against pathogens of veterinary significance (*Escherichia coli, Staphylococcoccus aureus, Pseudomonas aeruginosa, Malassezia pachydermatis*) and to investigate the cytotoxic potential. Methods:

Propolis samples phenolic profile was characterized by spectrophotometric methods. The antimicrobial efficacy was assessed by agar diffusion and broth microdilution method, while the cytotoxic potential was estimated considering propolis biocompatibility on human fibroblasts cell culture (cell line HFL-1) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and evaluating the cell morphology and attachment level.

Propolis samples, with total phenolics of 38,02% ±2,34% as determined by Folin Ciocalteu method, presented high amounts of total flavonoids (9±0,3%), where 1,74-9,22% belong to the group of flavones/flavonols and 1,96-4,01% were flavanones/dihydroflavonols. Based on the bacterial growth inhibition diameter zone (25-26mm) and values determined as MICs and MBCs (0,125-2%v/v) an important antimicrobial activity was suggested for all tested propolis samples, with the most intense effect against Gram positive strains. MTT test data indicated concentration dependence of propolis-induced effect, some dilutions stimulated cell viability, while higher concentrations had moderate expressed cytotoxicity.

The propolis samples taken in this study presented the typical poplar composition profile with flavonoids and phenolic acids as main biological active compounds. Chromatographic analysis confirmed the data obtained spectrophotometrically. Propolis phenolic profile and biological activity evaluation facilitated the selection of samples with antimicrobial activity and moderate or absent cytotoxic potential.

Acknowledgments: We wish to thank Dr. Olga Soritau and Dr. Virag Piroska from the Laboratory of the Oncology Institute "Ion Chiricuță" Cluj-Napoca, Romania for technical support during cytotoxicity screening.

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P 107. Vasorelaxant effect induced by geraniol in rat mesenteric artery

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Keywords: geraniol, essential oils, vasorelaxant effect, rat mesenteric artery

Geraniol is a monoterpene found in essential oils of various herbs with activity on the cardiovascular system [1]. The objective of the present study was to evaluate the vasorelaxant effect induced by geraniol in rat mesenteric artery.

Male Wistar rats (200 – 300g) were euthanized by exsanguination under anesthesia and superior mesenteric artery was removed and cut in rings (1-2 mm), which were mounted in organ baths containing 10 mL of Tyrode´s solution at 37°C and gassed with carbogen. For isometric tension recordings, each ring was fixed in a force transducer connected to an acquisition system.

In mesenteric artery rings with functional endothelium pre-contracted with 10 µM of phenylephrine (control), geraniol (10^{-8} – 10^{-2} M) was able to induce relaxation in a concentration-dependent manner (E_{max} = 110 ± 5%; n = 6) which was not attenuated after removal of endothelium (E_{max} = 108 ± 6 %; n = 4) or after 1 mM of tetraethylammonium, a non-selective blocker of K^+ channels (E_{max} = 109,2 ± 3%; n = 6). In endothelium-denuded rings pre-contracted with KCl 80 mM, geraniol produced relaxation that was significantly ($p<0.05$) higher than those obtained in rings with functional endothelium pre-contracted with phenylephrine (E_{max} = 142 ± 14%; n = 6). Futhermore, the incubation with 10^{-4}, 3 x 10^{-4} or 10^{-3} of geraniol was able to antagonize the tonic contractions induced by $CaCl_2$ (10^{-7} – 3 x 10^{-2}) and phasic contractions induced by phenylephine in without calcium solution. CONCLU

Taken together, these results suggest that geraniol produces a vasorelaxant effect by an endothelium-independent mechanism in rat mesenteric artery. This effect appears to be due to an inhibition of the Ca^{2+} influx through voltage-operated Ca^{2+} channels associated to an inhibition of the Ca^{2+} release through phenylephrine sensitive intracellular Ca^{2+}stores.

Acknowledgments: CNPq/FAPITEC, Brazil.

P 108. Counter-current chromatography as a tool to identify minor oxygenated sesquiterpenes in *Baccharis dracunculifolia* volatile oil.

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Keywords: *Baccharis dracunculifolia*, CCC, GC/MS, oxygenated sesquiterpenes.

Baccharis dracunculifolia (Asteraceae) is an aromatic native plant of Brazil, Paraguay, Uruguay and Argentine. The essential oil of *B. dracunculifolia* leaves has a special odor that attracts bees [1] and also is used in the cosmetic and perfumery industries. Many of the minor compounds are co-responsible by the bouquet. Their presence can add value to the product. The goal of our investigation was to use of counter current chromatography (CCC) as a tool to obtain fractions enriched of oxygenated sesquiterpene.

Essential oil of *B. dracunculifolia* leaves was extracted by hydrodistillation in a modified Clevenger system. A CCC , model Quattro HSCCC, was used. The biphasic solvent system was hexan-methanol-water (5:4:1)[2]. Isocratic elution was conducted in a tail-to-head manner at 850 rpm and flow rate 2.0 mL/min. 500mg of volatile oil sample was dissolved in upper phase/lower phase. Fractions of 1.5mL was collected and analyzed by TLC and GC/MS.

The TLC analysis of the fractions showed that the major sesquiterpene, nerolidol, eluted after fraction 12. The applied CCC conditions afforded nerolidol in high purity (92.5 %). Spathulenol eluted after nerolidol and was obtained fraction with 50.2% purity. The minor oxygenated sesquiterpenes co-eluted with spathulenol and was identified as a group of three cadinols.

CCC technique has many advantages over traditional soli-liquid chromatography, for example: isolation in semi-preparative scale, good mass recovery and compounds are obtained in high purity. This purification was used in order to isolate fractions enriched in cadinols, trace alcohols present in *B. dracunculifolia* essential oil.

Acknowledgments: PETROBRAS, UNICAMP.

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P 109. Evaluation of chemical composition and *in vitro* antiproliferative activity of essential oils of different parts of *Aldama linearifolia* Chodat

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Keywords: *Aldama linearifolia*; *Viguiera linearifolia*; antiproliferative activity; essential oil

Aldama linearifolia synonym of the *Viguiera linearifolia* (Asteracea) was perennial herbs provided with thickened underground system and potentially aromatic resins. The objective of this study was to analyze the chemical composition and evaluate *in vitro* the antiproliferative activity of the essential oils (EO´s) from the different parts of *A. linearifolia*. Plants were collected at flowering stage in Ponta Porã - MS, Brazil and separated into roots, xylopodia, stems and leaves. The essential oils (EO´s) were obtained by hydrodistillation in Clevenger system using fresh plant and analyzed by GC-MS. The activity was performed according to protocols recommended by the *National Cancer Institute* (NCI) for nine human tumor cell lines: U251 (CNS), MCF-7 (breast), NCI-ADR/RES (multi-drug resistant ovarian), 786-0 (kidney), NCI-H460 (lung), PC-03 (prostate), OVCAR-03 (ovarian), HT-29 (colon), K-562 (leukemia), and HaCat (strain normal cells). The higher yield of EO was observed in roots and xylopodia (0.23 and 0.14%) whereas the stem and leaves had low yields (0.07 and 0.05%). EO´s of roots and xilopodia showed only monoterpenes: a-pinene (77 and 66%), b- pinene (7.4 and 8.5%) and 3-carene (15.6 and 17%). The leaves presented several monoterpenes (90%) and the main were D-limonene (17%) and b-pinene (10.3%). The stems OE presented large amount of sesquiterpenes (88%), and the main were bicyclogermacrene (30%), germacrene D (12%) and spathulenol (11%). The antiproliferative activity *in vitro* showed that the four oils are active. From the curves of cell growth as a function of concentration of the samples were calculated TGI (*Total Growth Inhibition*). The EO´s of the stems and leaves were selectivity for the strains of the resistant ovary (27.84 $\mu g.mL^{-1}$) and prostate (34.36 $\mu g.mL^{-1}$) and the OE´s of the roots and xilopodia showed cytotoxicity with TGI < 100 $\mu g.mL^{-1}$. The toxicity the EO´s strain against normal cells showed that the roots EO was less cytocide (TGI > 250$\mu g.mL^{-1}$).

P 110. Composition and biological activities of *Seseli rigidum* Waldst. & Kit. fruit essential oil

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Keywords: *Seseli rigidum* Waldst. & Kit.; essential oil; antioxidant activity; antimicrobial activity; cholinesterase inhibition

Seseli rigidum Waldst. & Kit. is an endemic Serbian plant species known as “devesilje”, indicating its ability to cure nine diseases. Composition of *S. rigidum* fruit essential oil was determined by GC and GC-MS [1]. Antioxidant activity was estimated as free radical scavenging capacity towards DPPH and ABTS, and total reducing power assay [2]. The antimicrobial activity was investigated using micro well-dilution method against Gram (+) bacteria: (*Staphylococcus aureus*, *Bacillus cereus*), Gram (-) bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and fungal strains (*Candida albicans*, *Aspergillus niger*) [3]. For anticholinesterase activity, modified Ellman’s assay was utilized [4].

A total of 36 components were identified representing 94.4% of *S. rigidum* fruit oil. The most abundant compounds in the oil were *α*-pinene (37.8%) and sabinene (13.5%). All three antioxidant assays revealed weak antioxidant capacity of *S. rigidum* fruit essential oil in contrast with significant activity against all examined bacterial and fungal strains, especially against Gram (+) bacteria *S. aureus*. Inhibition of pooled human serum cholinesterase and horse serum cholinesterase was significant, referring to neostigmine bromide as standard, mainly due to dominance of *α*-pinene, as an evaluated cholinesterase inhibitor in the same experiments.

Composition of *S. rigidum* fruit essential oil corresponds to the data reported for *Seseli* species from Serbia, regarding the main component, but there are significant differences in the presence and distribution of other components. Remarkable anti-cholinesterase activity of essential oil, opens promising prospective in prevention of neurodegenerative diseases.

Acknowledgements: The research was supported by the Serbian Ministry of Education and Science (Grant no. 172047).

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P 111. Composition of the volatile oils of *E. dilatatum* Lam. and *E. pandanifolium* Cham. & Schltdl.

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Keywords: Essential oil, *Eryngium dilatatum*, *Eryngium pandanifolium*

Eryngium L. is probably the most extensive and taxonomically complex genus of the Apiaceae family, including about 250 species distributed all around the world. We report now on the composition of the essential oil of two species growing wild in Portugal, *E. dilatatum* and *E. pandanifolium.*The first grows in dry and stony fields of the Iberian Peninsula and North-Africa, the latter, with a broader distribution, prefers well-drained soils and sunny places, being frequent in the region of “Baixo Mondego”, in the center of Portugal.

The compositions of the oils islated by water distillation were established by GC and GC-MS according a methodology previously reported [1]. The oil of *E. dilatatum* is mainly composed by sesquiterpene hydrocarbons (35.6%) and oxygen containing sesquiterpenes (23.6%), being *Z*-α-chrysanthenyl acetate (11.1%), germacrene D (10.3%), α-pinene (9.2%), bicyclogermacrene (8.1%), spathulenol (5.9%) and *E*-caryophyllene (4.5%) the major components. Differently, the oil of *E. pandanifolium* is predominantly composed by monoterpene hydrocarbons (41.8%); α-Pinene (20.8%), β-elemene (10.6%) and limonene (5.8%) are the most representative components.

Acknowledgements: Authors are grateful to Fundação para a Ciência e Tecnologia (FCT), POCI 2010/FEDER for financial support.

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P 112. Chemical composition, biosynthesis sites and evaluation of *in vitro* antiproliferative activity of essential oils of *Aldama filifolia* (Asteraceae)

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Keywords: anatomy; human tumor cell lines; monoterpenes; secretory canals; vegetative organs

Phytochemical studies have shown the pharmacological potential of *Viguiera* (*lato senso*) species [1,2]. This study aimed to analyze the chemical composition, to indicate the sites of biosynthesis and to evaluate the antiproliferative activity of the essential oils (EO´s) of *Aldama filifolia* (= *Viguiera filifolia*). Plants were collected in Alto Paraíso de Goiás, GO, Brazil and separated into roots, xylopodia, stems and leaves. The extraction was carried out by hydrodistillation in Clevenger using fresh plant material, for three hours. The analyses were performed by gas chromatography with mass selective detector (GC-MS). Anatomical analyses were performed using usual histological techniques. Tests for *in vitro* antiproliferative activity were performed according to protocols recommended by the *National Cancer Institute* (NCI), for nine human tumor cell lines: U251 (CNS), MCF-7 (breast), NCI-ADR/RES (multi-drug resistant ovarian), 786-0 (kidney), NCI-H460 (lung), PC-03 (prostate), OVCAR-03 (ovarian), HT-29 (colon), K-562 (leukemia), and a strain normal cells (HaCat). From the curves of cell growth as a function of concentration of the samples were calculated TGI (Total Growth Inhibition - effective concentration for total inhibition of cell growth). The EO's yield (% w/w) was 1.29 in leaves, 0.22 in roots, 0.12 in xylopodia and 0.09 in aerial stems. The major compounds were Myrcene in leaves (86.0%) and aerial stems (73.1%) and α-Pinene in roots (70.01%) and xylopodia (69.18%). EO's were found into secretory canals consisted of simple or multisseriate epithelium and variable diameter, in all analyzed organs. The results of studies on antiproliferative activity *in vitro* showed that only underground EO's are actives. The EO's of the roots were more selective for CNS and ovarian with TGI's values of 94.62 and 55.64 $\mu g/mL^{-1}$, respectively. The EO's of the xylopodia was active for other lines, especially leukemia and prostate, with TGI's values of 0.27 and 34.74 $\mu g.mL^{-1}$.

Acknowledgments: We thank to The National Council for Scientific and Technological Development (CNPq) for research grants awarded to B.A.G. and V.L.G.R. and São Paulo Council for Research (FAPESP) – (Thematic Project Proc. nº 2010/51454-3 and Proc. nº 2010/01931-0), for the financial support and grant for the second author.

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P 113. Chemical composition and pharmacological activities of essential oils of *Lavandula* spp.

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Keywords: *L. stoechas* subsp. *luisieri*; *L. viridis*; Acute toxicity; Analgesic effect; Anti-inflammatory activity

Lavandula spp. belong to the family *Lamiatae* and some species are often used in popular medicine and have been used for centuries in a large number of medical applications and in aromatherapy. Although similar ethnobotanical properties of *Lavandula* spp., its essential oils, general chemical composition and therapeutic applications differ from different species.

Lavandula stoechas L. subsps. *luisieri* (Rozeira) Rozeira and *L. viridis* L'Hér are endemic to the Iberian Peninsula, widespread in the South of Portugal, namely in Southern Alentejo and Algarve. The aim of our study was evaluate the chemical composition and toxicological and pharmacological activities of leaves essential oils of spontaneous plants of *L. stoechas* L. subsps. *luisieri* (Alentejo) and *L. viridis* (Algarve). The essential oils of these wild plants, collected in spring, were obtained by hydrodistillation in a *Clevenger*-type apparatus and its chemical composition was evaluated by GC/FID. The acute toxicity of essential oils was evaluated "*in vitro"* using brine shrimp (LC_{50}) and "*in vivo"* using *Swiss* mice (DL_{50}). The analgesic and anti-inflammatory pharmacological properties of *L. stoechas* subsp. *luisieri* essential oil were evaluated in mouse or rats by the *Amour-Smith* and carrageen-induced paw edema tests, respectively.

Results showed important differences in chemical composition of essential oils from two species analyzed either to diversity and proportion of its constituents. The essentials oils showed citotoxicity against *Artemia salina* and a DL_{50} higher than 2000 mg/kg for mice. The analgesic and anti-inflammatory activities of essential oils were exhibit for the doses of 100 and 200 mg/kg.

These essential oils from *Lavandula* spp. showed important biological properties and studies will continue in order to clarify its hepatotoxicity and nephrotoxicity and to evaluate its potential use for pharmacological and nutritional applications.

P 114. Chemical composition and antibacterial activity of five chemotypes of *Chamomilla recutita* (L.) Rauschert cultivated in Europe, India and South Africa

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Keywords: *Chamomilla recutita*; chemotype; antibacterial activity; chemical composition

Chamomilla recutita (L.) Rauschert exists according to the literature from 4 to 6 chemotypes [1,2]. As the chamazulene type is not mentioned there, it could be concluded that there are probably 7 chemotypes.[3] All chemotypes are characterized by their different chemical composition. In continuation of our work on chemical composition and antimicrobial activities of essential oils we selected the following chamomile ct oils: Type A from Hungary (24.1% α-bisabolol oxide A), Type B from South Africa (12.1% α-bisabolol oxide B), Type C from Hungary (11.5% α-bisabolol), Type D from India (5.7% α-bisabolol , 6.0% α-bisabolol oxide A, 5.9% α-bisabolol oxide B) Type F from Hungary (11.5% α-bisabolol), 23.2% chamazulene). The essential oils were analysed using GC/FID and GC/MS on polar and apolar columns.

Antimicrobial activity of the essential oils were tested against one Gram-positive (*Staphylococcus aureus*, ATCC 6538) and three Gram-negative bacteria (*Escherichia coli*, ATCC 25922; *Salmonella abony*, ATCC 6017; *Pseudomonas aeruginosa*, ATCC 27853) as well as one yeast (*Candida albicans*, ATCC 10231) by using serial broth dilution [3] method. It could be verified, that the essential oils possess medium antimicrobial activity against Gram-positive *Staphylococcus aureus*, and Gram-negative *Escherichia coli*, *Salmonella abony* but less against *Pseudomonas aeruginosa*. The highest activity was achieved by the yeast *Candida albicans*. This shows clearly the high value of chamomile oils in medicine as well as in cosmetics.

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P 115. Antioxidant, antimicrobial and toxicological properties of *Schinus molle* L. essential oils

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Keywords: *S. molle*; essential oils; antioxidant activity; antimicrobial activity; brine shrimp mortality test

Schinus molle L. is commonly known as pink pepper or American pepper, of *Anacardiaceae* family, from subtropical regions of South America, introduced and naturalized in South Europe, including Portugal. In folk medicine, plant extracts and essential oil has related as having antibacterial, antiviral, antifungal, anti-inflammatory, antitumoral, antispasmodic, analgesic and antidepressive properties.

The aim of present study was to evaluate the chemical composition and biological activities of essential oil extracted from leaves and fruits of *S. molle*. For this purpose, the essential oils were analyzed by gas chromatography (GC/FID) and antioxidant properties were evaluated by the free radical DPPH and by system â-carotene/linoleic acid methods. The antimicrobial activities were screened against pathogenic bacteria and fungi and food spoiling fungi by the disc diffusion assay and minimal inhibitory concentration (MIC) was determined for sensitive strains. Toxicity of essential oils were carried out by the brine shrimp mortality test (EC_{50}) and acute lethal dose (DL_{50}) determination by oral administration in *Swiss* mice. The major components in leaf essential oil were alfa-phellandrene, beta-phellandrene and limonene, while myrcene, alfa-phellandrene and 1,8-cineole are the main components in the fruit essential oil. The essential oils of leaf and fruit of *S. molle* showed antioxidant activity through the two mechanisms: the ability to capture free radicals and protection of lipid peroxidation. These oils exhibited also a broad microbial activity spectrum, against pathogenic bacteria Gram-positive and Gram-negative and *Candida* spp. The fruit essential oil showed high cytotoxicity against *Artemia salina*.

Essential oils of leaves and fruits of *S. molle* showed significant antioxidant and microbial properties, so the studies continue to clarify more in deep its toxicity, including hepatotoxicity and nephrotoxicity, and to evaluate its medicinal or nutraceutical potential.

P 116. Chemical composition and phytotoxic effects of *Origanum vulgare* L. essential oil against *Portulaca oleracea* L. and *Conyza canadensis* (L.) Cronq.

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Keywords: *Origanum vulgare*, essential oil, herbicidal activity, GC, GC/MS, seed germination

The chemical composition of the essential oil from a commercial sample of *Origanum vulgare* L. was determined by GC and GC/MS. A total of 23 compounds accounting for the 99.25% of the total oil were identified. The monoterpene hydrocarbons fraction constituted 50.54% of the oil, while the oxygenated monoterpenes reached 48.65%. No sesquiterpene compounds were found. The main compounds were carvacrol (29.16%), *p*-cymene (27.58%), limonene (13.63%) and thymol (12.06%).

Different chemotypes of *O. vulgare*, as carvacrol [1, 2 y 3], thymol, caryophyllene, sabinene, γ-terpinene and β-cubenene [4 y 5] have been described. Terpinen-4-ol and γ-terpinene were the main compounds of *O. vulgare* essential oil from Chile [6]. The essential oil here analyzed is carvacrol chemotype. This compound is usually found in samples of *O. vulgare* from Brasil [3]. Great variances have been detected in the composition of *O. vulgare* from different origins.

The herbicidal potential of the essential oil was tested *in vitro* against *Portulaca oleracea* L. and *Conyza canadensis* (L.) Cronq., two important weeds in Mediterranean crops. The higher concentrations applied (0.5 y 1 µl/ml) were very effective on both weeds, but *C. canadensis* showed more sensibility. Its germination was completely inhibited, while *P. oleracea* germination was blocked completely only at the concentration of 1 µl/ml, being reduced 71.6% at 0.5 µl/ml. The lower doses (0.125 y 0.25 µl/ml) showed no effect on *P. oleracea* germination but inhibited *C. canadensis* germination 64.2 and 92.6% respectively.

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P 117. Classification of oregano (*Origanum vulgare* L.) genotypes grown in Latvia

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Keywords: oregano, volatiles, genotype, morphological parameters

Oregano (*Origanum vulgare L*.) is a perennial herb, belonging to plant family *Lamiaceae*, which grow in Latvia both wild and cultivated. A number of studies showed that oregano is very variable in chemical composition of essential oils depending on geographical origin, vegetative stage and used treatment. The aim of current research was to classify oregano genotypes based on the volatile composition and morphological parameters.

Ten genotypes of oregano were analysed in the current study. Flowers and leaves were collected separately; air dried and stored in sealed bags until analysis. Morphological parameters were determined according to standard methods. Volatiles from dried oregano were extracted using headspace autosampler and analyzed by gas chromatography-mass spectrometry. Compounds were identified by comparison of their mass spectra with mass spectral libraries (Nist98), and by calculation of linear retention indexes and comparison with literature data. The data obtained from the analysis of the oregano were analyzed by means of multivariate analysis; employing hierarchical cluster analysis. The method used was linkage between-groups. The distances between samples were calculated using square Euclidean distances. As a pre-treatment of data was carried transform values of variables (average zero and standard deviation 1) called Z scores. The dendrogram similarity scales that are generated by the SPSS program range from zero (greater similarity) to 25 (lower similarity). A linear correlation analysis was performed in order to determine relationship between morphological parameters and volatile compounds.

The studied oregano genotypes differ both in quantitative and qualitative content of aroma compounds. Also the dendrograms obtained by hierarchical cluster analysis showed that the genotypes of oregano are quite heterogeneous. Totally 35 volatile compounds were identified in Latvian oregano samples. Sabinene, caryophillene, germacrene D and Z-β-ocimene are the major aroma forming compounds. Mainly weak correlations between volatile compounds and morphological parameters were detected. Moderate correlation (r= - 0.51) between oregano flower color and total peak area of identified aroma compounds were observed, meaning that more volatiles are in plants with more pink flowers instead of dark violet.

P 118. Chemical composition, leishmanicidal, cytotoxic and hemolytic activity of the essential oil from leaves of *Cymbopogon winterianus* Jowitt ex Bor.

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Keywords: *Cymbopogon winterianus*; cytotoxic; hemolytic; leishmanicidal

Cymbopogon winterianus (Poaceae) is usually known as citronella grass and is used in folk medicine as analgesic, anxiolytic and anticonvulsant. This study aimed to evaluate the toxic activity of essential oil (EO) from leaves of *C. winterianus* against promastigotes forms of *Leishmania amazonensis* as well as its cytotoxic activity to macrophages and its hemolytic potential on human erythrocytes. The EO was obtained by hydrodistillation and then analyzed by GC and GC/MS. All bioassays were carried out at concentrations of 400; 200; 100; 50; 25; 12,5; 6,25 e 3,12 mg/L of the essential oil. The leishmanicidal activity was evaluated *in vitro* using 1 x 10^6 promastigotes per well in 96-well plates with the EO and then counted in a Neubauer haemocytometer at 24, 48 and 72 h [1]. In the cytotoxicity assay, murine peritoneal macrophages were incubated for 48 h at the tested concentrations, then MTT was added and the results were analyzed in a spectrophotometer [2]. The hemolytic activity was investigated using erythrocytes (human O^+) and cell lysis was determined spectrophotometrically [3]. The leaves of *C. winterianus* provided colorless oil, with yield of 1.3%. Among the identified constituents, monoterpenes were 75.8% and sesquiterpenes 19.0%. The major constituents were citronellal (26.5%), geraniol (16.2%) and elemol (14.5%). In the leishmanicidal test, the oil causes 100% of inhibitory effect at the concentration of 400 mg/L and the IC_{50} value was 89 mg/L after 72h of exposure. The EO caused low toxicity against the macrophages with a CC_{50} value of 376 mg/L. The toxic action of oil on erythrocytes was also low, with 14.2%, 11% and 6.7% of hemolysis at concentrations of 400, 200 and 100 mg/L respectively and 0% of hemolysis in other concentrations. The specie exhibited promising activity as a leishmanicide, with high selectivity for promastigote forms of *L. amazonensis* and with low toxicity to erythrocytes and macrophages.

Acknowledgments: The authors are grateful to CNPq/BIONORTE, FAPEMA and CAPES for their financial support.

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P 119. Phytochemical screening and antinociceptive activity of *Lippia microphylla* CHAM. (Verbenaceae) leaf essential oil in rodents

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Keywords: *Lippia microphylla*; Monoterpenes; Camphor; (*E*)-Caryophyllene; Pain

Lippia microphylla Cham. (Verbenaceae) is a medicinal plant known in Brazilian Northeast as 'alecrim-de-tabuleiro' and it is a plant used popularly to treat heart disease, inflammatory and painful disorders. However, there is little information about *L. microphylla*'s biological properties. Thus, we investigated the antinociceptive effect of the essential oil obtained from the leaves of *L. microphylla* (LEO) in mice. LEO was obtained by hydrodistillation in a Clevenger-type apparatus using 1200 g of dried leaves. The identification of the components was made through comparison of substance mass spectrum with the database of the GC-MS, literature and retention index. Male mice (26-30g) were pretreated with LEO (25, 50 or 100 mg/kg, orally route, *per os*, p.o.), morphine (5 mg/kg, i.p.) or vehicle (distilled water + Tween 80 0.2%), before acetic acid- (0.85%) (writhing reflex) or formalin (20 µl of 1%)-induced nociception tests. The motor coordination was also evaluated using Rota rod (8 rpm, 180 s). Experimental protocols and procedures were approved by the Universidade Federal de Sergipe Animal Care and Use Committee (CEPA/UFS # 26/09). The obtained data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test. The phytochemical analysis of LEO demonstrated the presence of camphor (28.698%), (E)-caryophyllene (11.47%), bicyclogermacrene (10.22%), camphene (9.45%) and borneol (7.64) and as the main compounds. LEO pre-treatments inhibited writhing ($p<0.001$) in the acetic acid test, 25 (17.0 ± 1.5), 50 (15.5 ± 1.9) or 100 (10.1 ± 1.8) mg/Kg, respectively, when compared to control group (28.5 ± 2.9). In the formalin test first phase only the doses of 25 (42.2 ± 5.9), 50 (41.3 ± 4.9) or 100 (35.2 ± 3.4) mg/Kg had promoted a reduction in the time spend licking the paw with $p<0.01$ or $p<0.001$, when compared to control group (64.7 ± 4.3). In addition, in the second phase all doses reduce the time ($p<0.001$) at doses 25 (72.6 ± 8.6), 50 (41.8 ± 5.0) or 100 (27.2 ± 4.1) mg/Kg, respectively, when compared to control group (136.5 ± 8.9). Such results were unlikely to be provoked by motor abnormality (data not shown). Our results suggest that *L. microphylla* leaf essential oil modulates inflammatory and central nociception and might represent important tool for management and/or treatment of painful conditions.

Acknowledgments: Financial Support by FAPITEC/SE/Brazil, CNPQ/Brazil, CAPES/Brazil.

P 120. Evaluation of the cytotoxicity of an essential oil obtained from leaf of *Xylopia laevigata*

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Keywords: *Xylopia laevigata*, citotoxicity, phytochemical analysis

Investigations have explored that natural products have been source of most active ingredients of medicines. For that reason, it is necessary to evaluate the toxicity of natural products, to guarantee the secure use. *Xylopia laevigata* unknown to scientists have been use for years by Brazilian people to cure diseases, such as kidney problems and inflammations. In this behalf, the proposal of this study was to investigate the citotoxicity of an essential oil obtained from leaf *Xylopia laevigata* (EOX).

EOX was extracted by hydrodistillation of the fresh leaves of *X. laevigata*. The components of the EOX were identified by comparison of substance mass spectrum with the database of the GC-MS, literature and retention index. For the cytotoxicity assay, L929 fibroblast cell lines were plated in 96-well plates and the essential oil was added in serial dilutions for 24 hours. After that, the viability of fibroblast cells was quantified by neutral red (NR) assay. The values were obtained of three independents tests.

The phytochemical analysis of EOX showed the presence of g-muurolene (17.78%), δ-cadinene (12.23%), bicyclogermacrene (7.77%), α-copaene (7.17%), germacrene D (6.54%) and (E)-caryophyllene (5.87%) as the main compounds. It was observed that EOX at 100 µg/mL and 200 µg/mL decreased 40% and 80% of the cell viability, respectively. Therefore, the value of IC50 was 125.0 µg/mL to fibroblast cell line.

Research with essential oils of plants whose similar components described in EOX showed cytotoxic activity against tumor cells, such as germacrene D and bicyclogermacrene [1]. It is suggested that the reduction of the cell viability observed can be associated the presence of these compounds in EOX. New studies are required for elucidation of the mechanisms of EOX cytotoxic activity.

Acknowledgments: Financial support by CNPq, FAPITEC/SE.

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P 121. Residues of fungicides in citrus essential oils from Brazil

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Keywords: fungicides residues; citrus essential oils; essential oils from Brazil; carbaryl

Brazil is one of the largest exporters of essential oils (EO), and *circa* 95% of the exported volume is due to citrus oils [1]. The country is also one of the largest consumer of fungicides. Residues of organoclorides and organophosphorides pesticides have been found in Brazilian citrus oils in amouts well above those allowed by *Codex Alimentarius* standards [2]. Continuing a sistematic investigation on contaminants from Brazilian citrus oils, the objective of this work was to develop a method for the detection and quantification of fungicide residues, namely carbaryl, delta-cyhalothrin, dithianon, prochloraz, tebuconazole and thiabendazole in citrus oils. The oils were passed through solid phase extraction column (C18) and after elution and concentration, the samples were analyzed by gas chromatography coupled to mass spectrometry using a DB-5 (30m X 0.25mm X 0.25 µm) capillary column. Helium was used as carrier gas at 1.0mL/min. Mass detector was operated in selective ion monitoring (SIM) in electronic ionization mode at 70eV. Calibration curves were built for each fungicide with standards. The limits of detection (LOD) and quantification (LOQ) were, respectively, 6.25 mg/L and 15,6 mg/L for carbaryl, 0.61 mg/L and 1.53 mg/L for delta-cyhalothrin, 1.33 mg/L and 3.32 mg/L for dithianon, 0.94 mg/L and 2.36 mg/L for prochloraz, 0.60 mg/L and 1.51 mg/L for tebuconazole and 2.21 mg/L and 5.53 mg/L for thiabendazole. Fifteen samples of commercial oils from different producers were analyzed. In one sample of lime oil (OE1) were found residues of carbaryl (18.0 mg/L), dithianon (7.9 mg/L) and tebuconazole (6.81 mg/L). A second sample of lime oil (OE10) was contaminated with 16,1 mg/L of carbaryl. Delta-cyhalothrin was detected in sample OE2 (orange oil) in 3.87 mg/L. In these oils, the residues found are well above those stated in *Codex Alimentarius*.

Acknowledgments: Conselho Nacional de Desenvolvimento Tecnológico (CNPq).

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P 122. Hydrodistillation *vs* steam distillation coupled to Clevenger *vs* Dering essential oil separators in antioxydant molecules recovery from aromatic plants

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Keywords: Aromatic plants; natural antioxidants; steam distillation; hydrodistillation; Dering apparatus; Clavenger apparatus

Essential oils were extracts obtained by hydrodistillation or steam distillation of aromatic plants combined to an essential oil separator, either the Clavenger apparatus, very popular in West Europe, nor the Dering apparatus, widely used in Eastern Europa countries. In otherhand, apart the odorous fraction, aromatic plants appeared as a valuable source for natural antioxidant molecules. If such components could be obtained from essential oils, extraction cake which represented up to 95% of the raw distillated material was reported as a potential complementary source for natural antioxidants. In the framework of the AROMATIC Program, we have applied to Medieval Aromatic Plants (aka forgotten ones) the agroreffinery concept based on a valorization of the entire plant by sequential extractions of molecules of interest while not penalizing the subsequent valorization of residual by-products as biosourced molecules [1]. This strategic approach was recently applied to colsfoot (*Tussilago farfara*) originated from Europe (France, Lithuania) or Asia (China) [2]. In the present paper, we reported the cross-evaluation of two extraction process (HydroDistillation (HD) and Steam Distillation (SD)) combined with two essential oil separators (Clavenger Apparatus (CA) and Dering Apparatus (DA) on both essential oils composition and antioxidant molecules recovery from extraction cake. Results are expressed as comparison of HD-CA, HD-DA, SD-CA and SD-DA for i) volatiles chromatographic profiles of essential oil by GC-FID and GC-MS; ii) antioxidant activities of methanolic extracts of corresponding extraction cakes evaluated by measuring radical scavenging activity with DPPH assay. If GC profiles are similar in the four cases, extraction yields of essential oils were higher for HD than SD while for antioxidant fractions opposite was reported. Regarding DA vs CA, if the first one allow to minimize the essential oils extraction time while the second make easier their collection, their impact on the antioxidant fraction recovery were limited [3].

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[3] T M Zhao, INP Doctorate thesis (2013, in progress)

P 123. Crushing finger device and flash aroma dispenser: two innovative tools for native oils analysis

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Keywords: Aromatic plants; native oils; artificial crushing finger device; flash aroma dispenser

Essential oil plants are aromatic crops used in perfumery, cosmetic and food industry which extracts were obtained by hydrodistillation or steam distillation. But such degrading methods generated artefacts due mainly to in situ chemical reactions (hydrolysis) and consequentatively the obtained extract's odor could differ from initial plant ones. Access to the naturally emitted odorants was possible by crushing between fingers a couple of leaves or flowers followed by immediate sniffing of the generated volatiles. But the poor reproducibility of such manual method did not allow to apply it for an instrumental analysis of emitted volatiles. In the framework of the AROMATIC Program, we developped/adapted devices in order to analyze native volatile compounds emitted just after instrumental crushing of leaves, i.e. Artificial Crushing Finger device (ACF) and Flash Aroma Dispenser (FAD) respectively based on a mechanical and a gas flash pressure crushing. By explosing storage vesicles located on leaves, ACF and FAD, coupled with either a Tenax trap or SPME system, allowed to perform a rapid evaluation of native oil content with only a couple of leaves. First developped and based on a modified artificial mouth, AFD allowed to instrumentaly mimic the total crushing of leaves between fingers but appeared for fragile leaves to be too destructive [1]. FAD is based on a whipped cream siphoon in which a gas (NO2 or CO2) flash pressure increase, allowed to reach to a partial explosure of storage vesicles and consequentatively appeared to be a more respective method. In the present paper, we reported the comparison of volatiles chromatographic profiles of essential oil from our model plant, balsamite (*Tanacetum balsamita*), obtained by hydrodistillation with the ones obtained with crushed entire fresh leaves processed by ACF or FAD for which emitted volatiles were concentrated by SPME. Microscopic observations of processed leaves allowed to compare ACF and FAD efficiency regarding explosure of vesicles or secretory hairs and destruction of leave's structure [2]

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P 124. Phytotoxicity and antimicrobial activity of essential oils in plant products treatment

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Keywords: Essential oil; Seed treatment; Shelf-life; Organic farming; Model system; Pathogens

Essential oils (EOs) cause disturbance of cell membranes leading to cell death. This effect is used in antimicrobial treating, but plant products treatment causes tissue damage and irreversible degradation of the treated material. One of the main problems during treatment with EOs is keeping vitality and structure of fresh products (such as fruits, vegetables and seeds). Products should not be exposed to EO in high concentration for long time.

EOs of oregano (*Origanum vulgare*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*) and lemon grass (*Cymbopogon citratus*) were tested for antimicrobial activity on poppy and wheat seeds, potato, carrot, cucumber, strawberry, mung beans, apple and lemon. Products were treated by different methods using EOs vapours. Every treatment shows decrease of antimicrobial contamination and, in higher or equal concentrations of EOs, phytotoxicity detectable by browning of fruits and vegetables and by reduction in seeds germinationin.

Generally, the EOs from oregano and cinnamon have strongest antimicrobial effect, but they have strongest negative effect on plant material also. Clove EO applied on seeds still has required antimicrobial effect with less damage on plant materials in comparison with both previous EO. Lowest effect in antimicrobial activity and phytotoxicity shows lemon grass EO. For example, oregano EO inhibits growth of *Alternaria alternata* on carrot roots in 16 $\mu l.l^{-1}$ and cause tissue damage in 32 $\mu l.l^{-1}$.

Antimicrobial activity of EOs is well-known, but has been compared with phytotoxicity in literature only rarely [1]. As our results demonstrate phytotoxicity of EOs has to be taken into account in model systems.

Acknowledgements: This presentation is supported by University internal grant agency - CIGA 20122008 – Plant products treatment with essential oil vapours under different physical conditions.

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P 125. Poppy volatiles and their role in attracting poppy root weevil

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Keywords: *Papaver rhoeas*; *Papaver somniferum*; *Stenocarus ruficornis*; distillation

The Czech Republic is the world´s largest producer of low morphine food grade poppy (*Papaver somniferum*) with yearly average of 30 000 ha of harvested area. Poppy root weevil (*Stenocarus ruficornis)* is one of its major pests causing large losses of young plants. The adult beetles overwinter in the soil or plant debris and become active in the spring. They search for poppy plants, mate, feed and place eggs almost exclusively on different *Papaver* species [1]. The heaviest damage is done by larvae feeding on the roots. Therefore, we have decided to investigate, if some of the volatile compounds emitted by *Papaver* spp. are able to attract the beetle.

Fresh aerial plant material of autumn- and spring-sown low morphine poppy and corn poppy plants were distilled in clevenger apparatus and the volatiles were captured into layer of hexane due to low yield. GCxGC-MS and GC-EAD analyses revealed that the main components were phytol, fatty acids and green leaf volatiles. Among them, 3Z-hexen-1-ol, 3Z-hexenyl acetate and other compounds were identified as active by EAD when using *S. ruficornis* antenna.

The results could be used for development of signalization traps and hence for effective application of insecticides.

P 126. Chemotypes of *Eugenia uniflora* (Myrtaceae) from Rio de Janeiro, Brazil

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Keywords: *Eugenia uniflora*; essential oil; sesquiterpene chemotypes; antinociceptive activity

Eugenia uniflora L. (Myrtaceae), native of Brazil and known as Surinam cherry, is of great interest to food and cosmetic Brazilian industries. In this study, we evaluated the existence of *E. uniflora* chemotypes from the essential oils obtained from the leaves of some previously investigated material, related to genetic variability [1] and collected at Grumari, Rio de Janeiro, RJ. GC-MS data were analyzed using Principal Component Analysis (PCA) and resulted in four different groups (A-D). Group A, consisting of nine specimens, showed a major peak with IRL equal to 1516. After NMR analysis, two co-eluting isomers were identified as 3-atractylone and furanoeudesmene [2]. The group B (eight specimens) showed a major peak with IRL 1774. Analysis by NMR resulted in the structure of the sesquiterpene 6-beta-acetoxy-5alfa-H-guaian-1(10),3-diene. The four representatives of the group C showed a chromatographic profile with two major components, IRL 1674 and 1787, which were identified by NMR as selina-1,3,7 (11)-trien-8-one and its oxide, respectively. In group D (nine specimens) two major peaks were observed. The first IRL is in accordance with group A. The second (IRL 1870) is an epicurzerenone isomer. Analysis of variance (One-way ANOVA) complemented with Dunnett´s test showed that all treatments, at the dose tested (200 mg / kg), were effective in antinociceptive activity using albino mice (Swiss) 2, reducing the number of writhings compared with the control group (Tween 20/EtOH/H2O 1:1:10, * $p<0.05$). Due to popular usage, the National Health Surveillance Brazilian Agency - ANVISA - regulated the use of cherry tree leaves in teas in the 5th edition of the Brazilian Pharmacopoeia (2010). Through its essential oil, *E. uniflora* is regulated by the presence of curzerene isomers (IRL 1845). However, it is demonstrated in this work that this species has different sesquiterpenes chemotypes and that other chemical markers should be used for its characterization, that are possibly related to its biological activity.

Aknowledgements: CNPq, CAPES, FAPERJ.

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P 127. Biotransformation of geraniol by *Penicillium oxalicum* and *Aspergillus niger*

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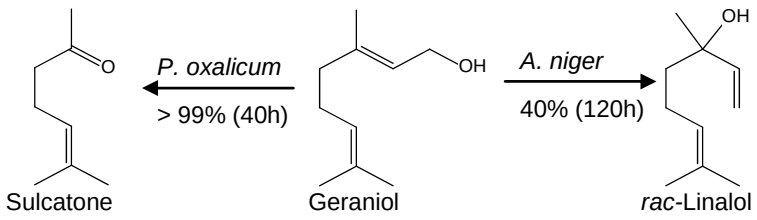
Keywords: biotransformation; geraniol; *Penicillium oxalicum; Aspergillus niger*

This study sought to evaluate the bioconversion of geraniol by the fungi *Penicillium oxalicum,* isolated from the marine alga *Caulerpa* sp*,* and *Aspergillus niger* obtained from *Hancornia speciosa* Gomes leaves. Geraniol (50 mg dissolved in 400 µL of DMSO) was added in 250 mL Erlenmeyer flasks containing mycelium of the fungus previously grown for three days in 100 mL of malt extract (2%). The mixtures were incubated in a shaker (150 rpm) at 32 ºC, and the reaction progress was monitored periodically by removing aliquots for GC-MS (Shimadzu, QP-5050A model) analysis*.* Identification of reaction products was carried out by comparison of the observed *spectra* with spectra from the literature[1] and confirmed by co-injection of authentic standards. An oxidative process was observed in the biotransformation of geraniol by *P. oxalicum,* producing pure sulcatone (Figure 1). In the reaction, all the substrate has been converted into sulcatone in just 40 hours, showing a high catalytic activity of the fungal enzymes. Demeyttenare & Pooter (1996) reported a similar reaction using *Penicillium italicum*, but with a lower yield of sulcatone (69-76%) and a longer reaction time (4 days). In the biotransformation of geraniol by *A. niger* there was an isomerization of the geraniol to form linalool. The conversion rate was 17% in 120 hours. After this period, there were no significant changes in the chromatographic profile. This study has demonstrated that the fungus *P. oxalicum*, through its associated enzymes, is an efficient biocatalyst for conversion of geraniol to sulcatone with a high purity (99% as checked with GC) and a molar yield of >99% during a short period of 40 hours. Biotransformations with *A. niger* converted geraniol into (+/-)-linalool (40% after 120 h).

Figure 1. Bioconversion of geraniol by *P. oxalicum* and *A. niger*



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P 128. Biological activity of essential oil from leaves of *Tetradenia riparia* Hochstetter. Codd. (Lamiaceae) grown in western Amazon, Brazil

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Keywords: Myrrh; essential oil; biological activity; antileishmanial activity; *Trypanosome cruzi* activity; neoplasic activity

T. riparia Hochst.Codd. is a species from Lamiaceae family that have been used in traditional medicine in several places around the world [1,2,3,4]. In Brazil, this exotic species was the introduced firstly as an ornamental plant [5]. Its volatile oil was already reported [6]. Although its current use as a medicinal herb there is a need to know all about its pharmacological properties as well the search for new botanicals inhibitors to provide a competitive alternative to current drugs. The aim of this study was to evaluate some biological activities of essential oil obtained from fresh leaves of *T. riparia*. The results showed the oil had no cytotoxicity property, without inflammatory activity based on carrageenan-induced pleurisy on live animals, and protein leakage. However the anti-inflammatory activity in mouse paw edema showed 56.69%, and 95.98% at a dose of 1 mg/well and 99.22% at a dose of 10 mg/well, presenting the production of nitric oxide *in vitro*. *T. riparia* oil also presented a significant antileishmania activity to *Leishmania amazonensis* promastigotes. *In vitro* this oil was assayed at concentrations 5.0, 10.0, 20.0, 40.0, 80.0, 120.0, 160.0 and 320.0 µg/mL for 24h. Promastigotes viability was analyzed and the IC_{50} was 0.47 mg/mL. The evaluation of the antineoplastic activity of essential oil of myrrh was performed *in vitro* using seven tumor cell lines. The results demonstrated that the essential oil of myrrh was able to inhibit cell growth in five of the seven strains studied (SP2 / 0 with IC50 =86.60%; Neuro-2a with CI50 = 86.60%; P3653 with IC50 = 74.60%; BW with IC50 = 82.00% and Ehrlich carcinoma with CI50=68.48%). Leishmanicidal activity was demonstrated by MTT colorimetric assay. This oil showed potent toxic effects on epimastigote forms of *Trypanosoma cruzi,* with values of IC_{50} = 100% at 0.6 mg/mL. The biological activity potential of the oil of myrrh showed a wide spectrum and therefore studies in this line of research should be continued.

P 129. Influence of storage time and temperature on the chemical composition of *Hyptis pectinata* (L.) Poit essential oil

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Keywords: *Hyptis pectinata*; medicinal and aromatic plant; volatile oil; postharvest; chemical constituents

The aim of this work was to evaluate the influence of storage and temperature on the chemical composition of *Hyptis pectinata* L. Poit essential oil. The effect of storage time of the essential oil of *H. pectinata* was evaluated (0, 15, 30, 60, 90, 120, 150, 180, 240, 300 and 360 days) at two temperatures [room (±27°C) and freezer (-20°C) temperature]. The essential oil was obtained from dry leaves by hydrodistillation using a Clevenger apparatus. Qualitative analysis of the chemical composition of the essential oils was performed using a gas chromatograph coupled to a mass spectrometer (GC-MS). A quantitative analysis of the essential oil components was conducted in a gas chromatograph equipped with a flame ionization detector (FID). Comparing the compounds in the essential oil of *H. pectinata*, β-caryophyllene was notable due to its high concentration, decreasing from 44.88% to 42.91% when stored at room temperature for 360 days and increasing from 44.88% to 46.15% when stored in freezer for 300 days. In addition, caryophyllene oxide was also found to be a significant compound, increasing from 15.54% to 21.82% when stored at room temperature for 360 days and from 15.54% to 18.15% when stores in freezer for 300 days. The storage of essential oil in freezer resulted in minor instability of the major constituents. The essential oil of *H. pectinata* may be stored at -20°C for 10 months.

Acknowledgments: We thank FAPITEC/SE, CNPq and CAPES for their financial support.

P 130. Essential oils from *Hypericum undulatum* Schousboe ex Willd

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Keywords: essential oils; leaves; flowers; ripened seed capsules; *n*-nonane; sesquiterpenes.

The constituents of the essential oils (EO) of fresh leaves, stems, ripened seed capsules and flowers of *Hypericum undulatum* (Clusiaceae) obtained by hydro-distillation were identified by GC-MS and quantified by GC. A different EO composition profile was recorded for each one of the different organs. The highest yield of total EO expressed in terms of percentage by biomass dry weight was obtained from leaves (0.9%), followed by ripened seed capsules (0.8%), flowers (0.6%) and stems (0.3%). The most complex composition profile was that of flowers (97 compounds) followed by ripened seed capsules, leaves and stems with 85, 83 and 47 compounds, respectively. The *n*-alkanes group, from which *n*-nonane was the dominant compound corresponded to 84%, 42%, 37% and 24% of the EO of stems, flowers, ripened seed capsules, and leaves, respectively, being the major group of EO constituents in the *H. undulatum* organs with the exception for leaves where the sesquiterpene hydrocarbons group predominated (59%). A complete series of *n*-alkanes from C_{22} to C_{29} was identified in leaves whereas in stems the only long-chain *n*-alkanes detected were *n*-heptacosane and *n*-nonacosane. Although the EO from leaves contained a broad range of sesquiterpene hydrocarbons, the dominant compound was the *n*-nonane (21.6%). The absolute content of this *n*-alkane corresponded to around 0.2% of the dried biomass of leaves and around 0.3% of the dried biomass of stems, flowers and ripened seed capsules of *H. undulatum*. The second major group of compounds in the EO of leaves was that of alkanes while in EO of stems, flowers and ripened seed capsules was that of sesquiterpene hydrocarbons which represented around 8%, 34% and 23%, respectively.

Acknowledgements: This work was sponsored by EU (FSE/FEDER) and Portuguese Government (FCT) through the Project PTDC/AGR-AAM/70418/2006 (*Hypericum* Biotech).

P 131. Identification of volatile constituents in freeze-dried powder bark of mature fruit *Syzygium malaccense* Merr. & Perry using selective techniques SPME and GC-MS.

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Keywords: Myrtaceae; *Syzygium malaccense*; SPME; GC-MS; essential oil.

The family Myrtaceae is represented by approximately 140 genera, which together has more than 3,000 species that are distributed in tropical and subtropical regions of the Midwest and Southeast regions of Brazil, reaching beyond the limits of the country to reach the land of Uruguay, Argentina and Paraguay. The fruits of *S. malaccense* (jambo) have economic importance, being consumed raw or as candies, ice cream and refreshments, and they are used as flavoring in alcoholic distillates. The literature reports that studies of the volatile components carried out with whole ripe fruit of the genus *Syzygium*, using the hydrodistillation showed the presence of monoterpenes, sesquiterpenes and other volatiles compounds like alcohols, aldehydes and esters [1,2]. The objective of this study was to identify the volatile chemical components in freeze-dried powder of the bark of mature fruits of *S. malacenses* using polydimethylsiloxane fiber SPME and GC-MS techniques. The mature fruits were purchased at supermarkets in the metropolitan region of Rio de Janeiro (Brazil) and their peels were removed, frozem and then lyiphilized before SPME-GC-MS analysis. The mass spectra obtained were compared with the NIST mass spectra library [3] and data from literature [4], showing a profile of monoterpene, sesquiterpenes, esters, aldehydes and alcohols which the major constituents are (*E*)-3-hexen-1-ol acetate, phenyl-ethyl alcohol, *D,L*-limonene (racemic), *β*-caryophylene, *α*-humulene and *β*-selinene. Thus, it is concluded that the use of SPME techinique on lyophilized powder of the jambo peel is suitable for the identification of its volatile constituents and provides similar results those described in the literature.

Acknowledgments: To IFRJ by financial and institutional support.

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P 132. Scents from Brazilian Cerrado: The essential oil from *Porophyllum angustissimum*

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Keywords: *Porophyllum angustissimum*; essential oil; Cerrado; Brazilian flora; myrcene; Astereaceae

Cerrado (savannah) is the second largest Brazilian bioma, but the first in number of endemic species and the most threaten by anthropic pressure. A huge amount of non investigated aromatic species make the Cerrado a very promising source for flavour and fragrance applications. Considering this, Embrapa has started a research project to study the aromatic species from this bioma in order to propose sustainable alternatives for their commercial use. *Porophyllum angustissimum* Gardner (Asteraceae) is a herbaceous plant endemic to the Brazilian Cerrado flora, presenting a strong scent [1]. It has brown inflorescences, leaves slightly blue, and occurs on a disperse and small population. Plant material was collected from six individuals in the Ecological Reserve of the Brazilian Institute of Geography and Statistics (IBGE) in Brasilia, on April 2012. Vouchers were deposited on Embrapa Genetic Resources and Biotechnology herbarium (CEN 2419). The dryed 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 6890GC equipped with a FID and a 5973N MSD. For separation of the oil components a DB-5 (30m X 0.25mm X 0.25 µm) capillary column was used, with either helium (for MS) or hydrogen (for FID) as carrier gas (at 1.0mL/min). Column temperature rised from 60ºC to 240ºC at 3ºC/min. Mass detector was operated in electronic ionization mode at 70eV. For identification, both mass spectra and retention indices were used [2]. The essential oil was obtained in 0.2% yield. The major compounds present were myrcene (40.6%), (*E*)-2-dodecenal (37.5%) and limonene (3.4%).

Acknowledgment: Conselho Nacional de Desenvolvimento Tecnológico (CNPq).

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P 133. A preliminary study of limonene concentration from essential orange oil (*Citrus sinensis*) by using a new distiller prototype.

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Keywords: Prototype, Limonene, wiped film reboiler, surface response

Nowadays, the production of natural essential oils is being intensified due to the wide industrial interest. Orange oil is a complex mixture composed of hydrocarbon terpenes, oxygenated compounds, pigments, waxes, resins and flavonoids. One compound of commercial interest has been Limonene obtained from citrus oil. Limonene is widely used in cosmetics, pharmaceuticals, food products, it is also used as a botanical insecticide. Limonene is oxidized easily and can be distilled without decomposition, however at elevated temperatures it can be degradated. In this sense, it is important to study the beneficial techniques of separation and concentration that allow obtaining more valuable products such as Limonene by using low temperatures. An ICL-04WR laboratory system with wiped film reboiler and reflux column (InCon Processing LLC, Batavia IL, USA) for sensitive materials was used in order to concentrate Limonene. In order to evaluate the temperature conditions, the evaporator temperature was evaluated ranged from 50 to 120ºC, the pressure system was ranged from 1.7-10 torr and feed rate was 10 mL/min. Looking at the response surface of the factor evaporator temperature and pressure it is observed that Limonene content increase when temperature increase and the pressure decrease. The equation for Limonene content from the two processing variables was: $\%Limonene = 89.038-1.87982*P^2+0.0947551*P*T+0.132182*P^3-5.24555*10^{-6}*T^3$ (R^2=80.11). These preliminary results demonstrate the potential of this new distiller prototype as an alternative distillation process for thermally sensitive compounds.

P 134. Antifungal activity of essential oils and isolated compounds

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Keywords: *Drimys angustifolia*; essential oils; myristicin; cyclocolorenone; antifungal assay.

Natural products are fundamental source of chemicals with diverse structures and biological properties, thus providing an important alternative for new antimicrobial agents [1]. This work aim the isolation, characterization and antifungal evaluation of essential oils (EOs) from *Drimys angustifolia* [2] and isolated compounds.

Leaf and branch EOs from *D. angustifolia* were obtained by hydrodistillation under nitrogen atmosphere using a Clevenger type apparatus. The oils were characterized by means of gas chromatography (GC-FID) and gas chromatography coupled to mass spectrometry (GC-MS). The sesquiterpenes cyclocolorenone and bicyclogermacrene, and the arylpropanoid myristicin were isolated from the leaf essential oil by column chromatography on silicagel. Pure hexane and the binary mixture (hexane:dichloromethane) were used as eluents. Drimenol was obtained from the branch essential oil using crystallization in cold hexane. EOs and isolated compounds were assayed against 10 different types of filamentous fungi and yeast with their Minimum Inhibitory Concentration (MIC) being expressed in ppm.

The EOs were active against all the fungi tested, with CIM ranging from 31.25 to 1,000 ppm. The most active myristicin and cyclocolorenone exhibited MIC values of 15.6-500 ppm and 62.5-1,000 ppm, respectively, being the activity of the former consistent to the literature [3]. Bicyclogermacrene was inactive against 6 of the assayed fungi. *E. flocosum*, *C. neoformans* and *M. gypseum* were the most sensitive microorganisms.

The EOs from *D. angustoflia* and isolated compounds exhibited promising antifungal activity. Myristicin and cyclocolorenone embody structural features enabling to access semi-synthetic derivatives for further antifungal screening.

Acknowledgments: FURB, UNIVALI, FAPESC, INCT catalysis.

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P 135. Antidermatophytic and antileishmanial activities of essential oil from *Lippia gracilis* genotypes

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Keywords: *Lippia sidoides*; *Trichophyton rubrum*; *Leishmania chagasi*; thymol; carvacrol

The aim of this work was to evaluate the antidermatophytic and antileishmanial activities of essential oil from *Lippia gracilis* genotypes. The leaves of three *L. gracilis* genotypes, including LGRA-106, LGRA-109, and LGRA-110 were collected from the Active Germplasm Bank located in the “Campus Rural da UFS” Research Farm at the São Cristovao County, Sergipe State, Brazil. The essential oils were obtained by hydrodistillation and their constituents were characterized by gas chromatography coupled to mass spectrometry (GC/MS). The susceptibility of *Trichophyton. rubrum* strains, MYA3108 and *TruMDR2* to essential oils from LGRA-106 and LGRA-109 was determined by serial microdilution method. The leishmanicidal activity of essential oil from LGRA-106 and LGRA-110 was assayed on promastigotes of *Leishmania chagasi* and cell viability was determined by MTT method. The oxygenated monoterpene thymol was the main component of the essential oil from LGRA-106 (59.26%) while carvacrol was more abundant in LGRA-109 and LGRA-110 (54.56 and 48.92%, respectively). The *L. gracilis* essential oils evaluated in this study presented antifungal and leishmanicidal activities. However, LGRA-106 essential oil exhibited the best fungicidal activity at a concentration of 46.87 µg/mL. Both, LGRA-106 and 110 essential oils had similar antileishmanial effect, showing IC_{50} of 96.34 nL/mL and 86.23 nL/mL, respectively. The results obtained in this study suggest that essential oil of *L. gracilis* is a potential source of thymol and carvacrol and a promising antimicrobial agent, mainly fungicidal agent, since the concentrations that completely eliminate the fungi tested, were comparable to those observed for the common antifungal drug fluconazole.

Acknowledgments: The authors thank FAPITEC/SE, CNPq and CAPES for their financial support of this work.

P 136. Chemical analysis of *Hypochaeris maculata* ssp. *pelivanovicii* (Velen.) Hayek essential oil

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Keywords: *Hypochaeris maculata* ssp. *pelivanovicii*, Asteraceae, essential oil

Cat ear, the common name for a number of annual and perennial herbs from the genus *Hypochaeris* (Asteraceae, Cichorieae), are in many areas considered a weed. Phytochemical data related to the genus are scarce, including a small scale investigation on sesquiterpen lactones (germacranolides, guaianolides and eudesmanolides) [1] and on flavonoids [2].

The above ground parts of Balkan endemics *Hypochaeris maculata* ssp. *pelivanovicii* were collected in July 2007, from highland meadows (natural populations), on Mountain Stara Planina (East Serbia). The composition of hydrodistilled essential oil was investigated by means of GC and a GC-MS analysis.

The analysis resulted in the identification of one hundred twenty five components, accounting for 89.1% of the total oil. Main identified constituents were hexadecanoic acid (14.0%), heneicosane (10.9%), nonacosane (10.4%) and tricosane (7.2%). Fatty acid derived compounds were predominant compound class in the oil (70%), while terpenoids comprised only 8.9% of the compounds detected.

To the best of authors' knowledge, this would be the first report on essential oil chemistry of genus *Hypochaeris* in general.

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P 137. Chemical composition and biological activities of ten different species of the Asteraceae family from Nepal

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Keywords: Asteraceae, bioactivities, chemodiversity, essential oil

There are more than 22750 species of plants belonging to the Asteraceae family world wide. Within the flavor and fragrance industry, Asteraceae family is considered to be a major volatile oil source for commercial usage. In our study ten species from Nepal were analyzed for leaf essential oil. The species included: *Artemisia dubia, Artemisia vulgaris, Matricaria chamomilla, Blumea lacera, Eupatorium adenophorum, Chrysenthemum cinerifolium, Parthenium hysterophorus, Xanthium strumarium, Ageratum conyzoides,* and *Artemisia indica*. Essential oil compositions were determined using an Agilent 6890 GC coupled with Agilent 5973 mass selective detector. The chemical composition for each essential oil were compared within the genera and family. Further analysis of biological activity was accomplished via various bio-assays including: cytotoxicity, anti-microbial, larvicidal, brine shrimp lethality, nematicidal activity, and insecticidal activity.Chemical compositional analysis showed taxonomic variation as well as geographical and climatic influences on the essential oil chemotype among the species. Biological testing showed wide variation in activity.Through our research, deeper understanding of chemodiversity and species differentiation within the Asteraceae family has been developed. In addition, bio-assay results have shown potential medicinal value of the species. Out of those oils *Matricaria chamomilla* and *Artemisia indica* has shown strong evidence to be therapeutically important essential oils for parasites and microbiological organisms. *Artemisia vulgaris* and *Parthenium hysterophorus* has shown strong evidence for allelopathy and insecticidal activities. Overall, this study has been a strong evidence for Nepal as a promising source of natural products.

Acknowledgments: We would like to express our gratitude to Tilak Gautam for identification of plants and to Dr. Bernhard Vogler for technical assistance.

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P 138. Analysis of sandalwood essential oil by GC-MS with ionic liquid stationary phases

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Keywords: *Santalum* spp.; Santalols; Farnesols; GC-MS; Ionic liquid stationary phases

Commercial sandalwood essential oils are obtained by hydrodistillation of the heartwood of 20-year-old trees mainly from three *Santalum* species (family Santalaceae): a) *Santalum album* L., also known as 'East Indian', (Indonesia, Sri Lanka, Northern Western Australia), b) *S. austrocaledonicum* Vieill., (New Caledonian and Vanuatu archipelagos), c) *S. spicatum* (R. Br.) A. DC, (Southwestern Australia).

The Indian type is the most considered in perfumery because of its organoleptic properties. Quality control (QC) of *S. album* and *S. spicatum* essential oils are nowadays regulated with international norms [1,2] requirimg a primary free alcohol content, expressed as santalol, not lower than 90%, with (*Z*)-α-santalol ranging from 41 to 55% and (*Z*)-β-santalol from16 to 24%. Analysis of sandalwood essential oil has recently been reviewed by Baldovini et al [3].

Regulatory rules require that (*E*,*E*)-α-farnesol, a minor sandalwood component suspected to be a fragrance allergen, is quantified; unfortunately, this alcohols cannot be base-line separated in a single GC run, since it co-elutes with (*Z*)-β-santalol on non-polar columns, and with (*Z*)-α-santalol on polar columns. Multidimensional GC methods including GCxGC and MDGC have therefore successfully been developed [4 – 6].

New perspectives have recently been opened up in GC by the introduction of ionic liquids as stationary phases because of their selectivity towards specific chemical classes that are completely different from those of the conventional phases.

This communication reports the results of a study dealing with the simultaneous separation of farnesol and santalol isomers in a single GC run and (*E*,*E*)-α-farnesol quantitation in a set of sandalwood essential oils from different species by GC-MS with an ionic liquid stationary phase column (Supelco – IL 60)

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P 139. Control of the pinewood nematode *Bursaphelenchus xylophilus* by essential oils and extracts obtained from plants: a review.

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Keywords: *Bursaphelenchus xylophilus*; essential oils; extracts; nematicides; plants

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a serious threat to forest ecosystems at a global scale. The nematode has become a major quarantine problem due to its capability to completely destroy *Pinus* spp. trees, with great damage to the wood industry. Controlling the nematode inside a living tree is quite difficult, the techniques used being often ineffective and quite expensive. In the coming years, most chemicals used to control nematodes will be banned and replaced by safer and environmentally friendly products. As so, chemicals naturally produced by plants will play an important role in controlling diseases such as pine wilt. Plants, particularly aromatic ones, are commonly used due to the chemical properties of their secondary metabolites. Among these, essential oils and/or extracts are highly employed and are being tested as possible control of some organisms, like nematodes. Recent publications have evaluated essential oils derived from different plant species as natural nematicides [1; 2], anti-bacterial [3], anti-fungal [4] as well as insecticidal [5]. Concerning control of the PWN, a significant amount of information on plants tested, results obtained and employed techniques, is available. Our revision has extensively gathered this information, making it easier to search, read and use. It may become useful information for future studies on the subject, since it will be possible to check the plants already tested. Although numbers aren´t definitive, so far, tested plants are distributed amongst 148 families. The extracts or essential oils of plants belonging to the Asteraceae, Lamiaceae and Euphorbiaceae families show promising results on controlling the pinewood nematode.

Acknowledgments: Studies partially funded by FCT, under research contracts PTDC/AGR-CFL/117026/2010 and PTDC/AGR-CFL/120184/2010.

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P 140. Volatiles from the edible mushroom *Lepista nuda*: comparison between wild and commercial samples

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Keywords: *Lepista nuda*; mushrooms; volatiles; linalool; pulegone

Lepista nuda (Bull. ex Fr.) Cooke, sometimes given the common name ´´blewit", is a *Basidiomycete*, from the *Tricholomataceae, Agaricales*. It is asaprotrophic species; growing alone, gregariously, or in clusters in organic debris, in woods or in urban settings. The fruity flavour makes it an excellent edible mushroom and it is commercially grown in Britain, France and the Netherlands [1].

Wild mushroom species showed to be less energetic than the commercial ones, with higher contents of protein and lower fat concentrations [2]. Mushrooms volatile compounds are less known although they play an important role for the flavour and organoleptic properties.

Wild *L. nuda* (Blewit) fruit bodies were collected from populations at two different habitats in Northeast Portugal: oak forest (OF) and pine forest (PF), and were also obtained from a commercial source (CS). Volatiles were isolated by distillation-extraction and analyzed by Gas Chromatography and Gas Chromatography-Mass Spectrometry as in [3].

In total, twenty two components were identified in each of the three samples, attaining from 84-94% of the volatile fraction. Differences between volatiles from wild growing and commercial samples were mainly quantitative. Linalool (17-26%), pulegone (12-14%) and limonene (10-11%) were the three major components in all samples. The main difference was observed in the percentage of 2-pentyl furan, present in small amount in the wild growing mushrooms (2%, OF and 5%, PF) and in considerable amount in the commercial sample (15%), where it was the second major component.

Eight-carbon (C8) volatiles are ubiquitous among fungi and characteristic of the fungal aroma, 1-octen-3-ol being the most abundant [4]. In the present study, 1-octen-3-ol was detected in small amounts in all samples (traces-2%) and the major components, linalool, limonene and pulegone, are not common in other mushroom volatiles.

Acknowledgments: Supported by FCT, COMPETE/QREN/UE project PTDC/AGR-ALI/110062/2009

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P 141. The way to ensure high quality aroma of decontaminated spices

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Keywords: spices, aroma, key odorants, microencapsulated preparations

Spices become contaminated microbiologically during harvesting, drying and storage. The addition of contaminated spices to foodstuffs may be dangerous for the health of consumers. For this reason several methods of spices decontamination are used. All known and used methods of decontamination lead to a greater or lesser losses of aroma of leafy spices. The aim was provide high quality leafy aroma of decontaminated spices.

Materials: marjoram, thyme, microencapsulated powders of linalool, sabinene hydrate, thymol.

Methods: steam decontamination of spices at the Institute of Agricultural and Food Biotechnology in Poznan. Steam distillation: in a Deryng' apparatus. 1 g sample with 0.5 ml o-xylene. Internal standard was tetradecane. Distillation was run for 3 h. GC/MS: 5975C VL MSD with HP-5MS (30 m x 0.25 mm x 0.25 µm) (Agilent Technologies). Library NIST05. GC/O-AEDA: HP 5890 with an inlet splitter and a smelling port, a DB-5 (30 m x 0.53 mm x 0.25 µm). Separated fractions were smelled in successive double dilutions of analyzed spice distillates, until the last detectable aroma disappeared. SPME/PDMS: samples 100 mg, vials 10 ml, addition of 2.5 ml deionized water and 300 µl methanol. Extraction temperature 50ºC, time 15 min. OAV: concentration of compounds / OT (odor threshold). Profile sensory analysis: 10 experts, 8 attributes for marjoram, 6 for thyme. Obtained data were interpreted by Principal Component Analysis. Preparing spices with microencapsulated powders of key odorants: mixer Mesko R-1000.

Results: Volatile aromatic compounds were identified in spices. Quantitative composition of these compounds was determined before and after spices decontamination. The GC/O analysis and the determination of odor activity values (OAV) showed that the primary compounds responsible for the aroma in analyzed spices were hydrate cis-sabinene and linalool in marjoram and thymol in thyme. It was found that the applied steam decontamination method resulted in relatively high losses of aroma. In order to supplement these losses of aroma compounds caused by decontamination, attempts were made to supplement their key odorants in the form of microencapsulated preparations. Results of chromatographic and sensory analyses of such prepared spices made it possible to state that an adequate addition of microencapsulated preparations of main odorants to decontaminated spices eliminated differences between decontaminated and control spices.

P 142. Effect of the speed of the drying air on the quality of essential oil from *Aristolochia cymbifera* Mart. & Zucc.

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Keywords: Medicinal plants, Chemical composition, Content of essential oil, Extraction

The species *Aristolochia cymbifera* Mart. & Zucc., popularly known as “jarrinha”, ´´milhomem, or ´´cassau. This species is an herbaceous perennial vine native to Brazil that is characteristically vigorous and better adapted to hot environments.[3] According to these authors, *A. cymbifera* contains mono/diterpenes and sesquiterpenoids in the leaves, stems, and roots. In folk medicine, *A. cymbifera* is used for various problems and is considered to be a diuretic, antiseptic, and antispasmodic.[3]

The object of the present study was to evaluate the effect of three drying air speeds on the content and the chemical composition of essential oil from *A. cymbifera* Mart. & Zucc. The drying was conducted in a fixed-layer dryer manufactured from #16 metal sheets. The drying chamber measured 0.60 x 0.60 x 0.60 m, for a total volume of 0.216 m3, and contained a plate with 25 % perforation placed at a height of 0.33 m. The fan was of the centrifugal type, driven by a three-phase motor with a power of 1.5 HP and rotation at 1,720 rpm. Each dryer was composed of six swinging temperature sensors and four electrical resistors of 1,500 ohms, for a total of 6,000 ohms. The system also featured an automatic controller that managed the system and stored the data generated. The system was set to 34.7±1.5 °C with controlled air speeds of 0.5, 1.0, and 2.0 m·s-1. The essential oil was extracted by hydrodistillation. The chemical analyses were performed on a gas chromatography apparatus coupled to a Shimadzu QP5050A quadrupole mass spectrometer (GC-MS) (Kyoto, Japan).

Increasing the speed from 0.5 m·s-1 to 2 m·s-1 reduced the drying time from 22 to 16 h. The speed of the drying air did not influence the content of essential oil extracted from *A. cymbifera* leaves. The minor constituents germacrene, hex-2-enal, viridiflorol, and cedrol were influenced by the drying process. The major constituents, mainly spathulenol, were not influenced by the drying air speed.

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P 143. Chemical synthesis and microbial transformation of biologically active derivatives of β-damascone

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Keywords: damascone; biotransformation; lactones; natural compounds; norisoprenoids

Terpene lactones are a large group of compounds with noteworthy biological and therapeutic activities [1,2]. They occur in plants and microorganisms. Some of them were obtained also in the chemical synthesis. Recently we have published the synthesis of isoprenoid lactones, which exhibit interesting antifeedant activity [3]. Here we report the results of chemical synthesis of lactones from cyclic natural ketone – β-damascone. We expect that they will show antifungal and antifeedant activity and interesting odoriferous properties [4]. Our expectations arise from the fact that β-damascone itself possesses important valuable odour and has been identified as component of tea aroma. It possesses fruity, roselike odour and is used in perfume compositions. Besides, it is tested as potential insecticidal agent [5,6,7]. In our studies we focused on the chemical synthesis of lactone derivatives of β-damascone and microbial transformations of obtained products using fungal cultures.

Acknowledgements: This Project was financed by European Union from the European Regional Development Found Grant No. POIG.01.03.01-00-158/09-03.

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P 144. Effects of the essential oil of *Alpinia zerumbet* leaves in muscle activity in spastic gastrocnemius of patients with stroke

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Keywords: Electromyography; stroke; spastic muscle

Stroke occurs due to a disturbance in focal cerebral function, secondary to obstruction or bleeding blood vessels[1]. One of the sequels is the hemiplegia, which reaches the lower limb spasticity associated involving Extenders[2,3]. To investigate the effects of the essential oils of Alpinia zerumbet leaves in muscle activity in the medial and lateral gastrocnemius of patients with stroke using electromyography. This is a prospective and analytical study of clinical trial I, realized with six patients with stroke associated with hemiparesis. The spastic gastrocnemius muscle activity of each subject was assessed before and after application of the essential oil of *A. zerumbet,* compared by electromyography. The treatment was realized with 0,10 mL/kg dose of the oil associated to 10 applications of physiotherapy. The analysis of the gastrocnemius muscle activity before and after treatment showed a significant improvement in muscle recruitment ($p < 0.0087$) in the medial portion in all patients, while the lateral portion of the muscle showed a slight recruitment was not statistically significant ($p < 0.0511$). Treatment with essential oil of *A. zerumbet* associated with physiotherapy improved the motor activity in spastic gastrocnemius in stroke patients.

Acknowledgments: We thank the Hebron Pharmaceuticals LTDA for the financial support of research.

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P 145. Chemical composition and biological activity of essential oil of *Coffea arabica* L. In the larvae of *Aedes aegypti* and *Artemia salina*

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Keywords: Dengue; toxicity; volatile compounds

Aedes aegypti L. is the vector responsible for transmitting the viral infection (dengue)[1]. The most effective proven method for disease prevention has been by vector control by chemical or biological means[2], but chemical insecticides resistance has already been observed in some insect populations in the Brazil[3]. In this regard, we have investigated the effectiveness of compounds extracted from plants in the control of development of *A. aegypti* larvae stages of development and that are free of toxicity to the environment. This work aimed study the chemical composition, toxicity and larvicidal activity of essential oil of *C. arabica*, in the search of new methods to control dengue.

The essential oil of *C. arabica* was extracted by hydrodistillation and submitted quantitative analysis of chemical constituents that was performed by gas chromatography flame ionization (FID) using a Shimadzu GC-17A. Bioassays followed proposed standards by the WHO[4], using dosages of 1000, 500, 250, 125, 62.5 mg/mL to calculate the lethal concentration (CL_{50}) compared with negative and positive control (distilled water with temephos). The evaluation of toxicity in *Artemia saline* was performed according methodology by McLaughlin[5]. The calculation of the CL50% was estimated using the probit analysis method (Minitab sorftware, version 15), with 95% confidence interval.

The components α-furfuryl alcohol (24.22%), 5-metilfurfural (10.15%) and furfural (21.9%) were characterized as majoritary n the essential oil of *C. arabica*. The essential oil not showed larvicidal effect on *A. aegypti*, causing mortality at a concentration of 1000 ppm after 48 h of exposure, different of studies with caffeine, which showed results with concentrations of 200 and 500 µg/mL[6]. The bioassay toxicity in *A. salina* was found an CL_{50} of 358 mg / mL, indicating that low toxicity oil. In conclusion, the essential oil of *C. arabica* not proved to be an effective larvicidal to *A. aegypti*, but it has low toxicity.

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P 146. GC/MS analysis of essential oils from the flowers of some *Cirsium* species

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Keywords: *Cirsium*, volatile oils

The aim of this study was the phytochemical investigation of essential oils from *Cirsium pannoicum*, *Cirsium decussatum* Janka., *Cirsium ligulare* Boiss., *Cirsium heterophyllum* L. (Chill.), *Cirsium eriophorum* L. (Scop.) , *Cirsium hispanicum* (Lam.) Lag, *Cirsium rivulare* (Jacq.) All., *Cirsium erisithales* (Jacq.) All., *Cirsium acaule* (L.) Scop., *Cirsium ferox* (L.) CD flowers by GC/MS method.

In this study, air dried and powdered flowers were used for essential oil distillation, which was carried out in the Deryng apparatus using the method presented in the Polish Pharmacopoeia (6^{th} ed.). GC-MS analysis was performed with a Thermo-Finnigan (USA) GCQ GC-MS spectrometer, using the electron impact (E) ionization mode 70 eV. Full-scan mass spectra were recorded in the range *m*/*z* 35–500 a.m.u. The identification of individual compounds was based on the comparison of mass spectra obtained from the samples with those of authentic standards, those of the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) libraries, those of our own library, and related literature [1]. The compounds were also identified by the comparison of GC retention indices and retention times.

Forty one constituents were identified in the essential oils obtained from the flowers of *Cirsium* species. They were qualitatively and quantitative examined.

Volatile oils contained: thymol, lauric and tetradecanoic acids. Thymol was the predominant constituent identified in flowers of *C. rivulare* (24,5%) *C. pannoicum (8,8%), C decussatum* (5,2%), *C. ligulare* (2,4%). Lauric acid is a predominant constituents in *C. pannoicum* (17,3%), C. *rivulare* (11,1%), C. *hispanicum* (6,8%), *C eriophorum* (6,2%).

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P 147. β-Myrcene biotransformation by *Pseudomonas* sp. M1

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Keywords: β-myrcene; *Pseudomonas*; biotransformation

There are two ways to produce compounds in biotechnology: de novo synthesis and biotransformation. The advantage of biotransformation, in comparison with chemical synthesis, is the reduced environmental impact, either by decreasing the amount of wastes or by their easier elimination [1]. Due to the proved important biological activities of some volatile terpenes, the biotransformation of lead compounds within this group can raise new commercially valued products for industry with enhanced biological activities. β-Myrcene, with validated analgesic and antimicrobial activities, besides its importance as intermediate for the production of a wide variety of flavourings and beverages [2], appears as a paramount target. As part of a broader research project towards the metabolic engineering of β-myrcene pathway of *Pseudomonas* sp. M1, in this work we report the preliminary results on de biotransformation of β-Myrcene by the parental strain of *Pseudomonas* sp. M1.

Pseudomonas sp. M1 was grown in liquid mineral medium supplemented with β-Myrcene as sole carbon source. Samples for metabolites isolation and identification were collected along the exponential growth phase. Bacteria were separated from the culture medium and the biotransformation volatile compounds were isolated by distillation-extraction and analyzed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) as in [3]. The crude volatiles, dissolved in pentane, were extracted with aliquots of a 5% sodium bicarbonate solution (acid fraction) prior to being pre-fractionated by liquid-solid chromatography on a silica gel column. Elution was performed with distilled *n*-pentane (hydrocarbon fraction) followed by dichloromethane (oxygen-containing fraction). *E*-Myrcenol and *E*-Myrcenal were two of the main components of the oxygen-containing fraction whereas *E*-Myrcenoic acid occurred in trace amounts in the acid fraction. The occurrence of these compounds in the volatiles isolated from *Pseudomonas* sp. M1 cultures strongly support the suggested catabolic pathway of β-Myrcene by this strain [4].

Acknoewledgements: This work is funded by FCT through project ref. PTDC/EBB-BIO/104980/2008.

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P 148. Greenhouse production of pineapple [*Ananas comosus* (L.) Merr.] in S. Miguel (Azores, Portugal): production of volatiles from traditional growing beds and their effect on soil microorganisms

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Keywords: *Ananas comosus*, leiva, *Pittosporum undulatum*, *Cryptomeria japonica*, volatiles, antibacterial

Producing 1483 t in 2010 [1], Portugal ranks 13th among the world's pineapple exporters [2]. Traditional pineapple cultivation [Protected Designation of Origin] in the Azores used a substrate composed of the topsoils from uncultivated fields, locally known as *leiva*, and mulch from branches of incense (*Pittosporum undulatum* Vent.) and *Cryptomeria japonica* (Lf) D. Don. Decline in *leiva* soils has stimulated the search for alternative substrates [3,4]. As part of this search, this work reports on the composition of volatiles from the components of the traditional substrate and their effects on soil microorganisms.

The volatiles were isolated by hydrodistillation, and analysed by GC and GC-MS [5]. The antibacterial properties of the volatiles were studied using common soil bacteria and evaluated by the agar diffusion method [6].

Leiva is a complex mixture of bryophytes, lichens and mosses, as well as various herbaceous species, thus presenting a high diversity of volatile compounds. The main volatile from *leiva* was hinesene (15-26%). *P. undulatum* essential oil was dominated by limonene (62%). *C. japonica* samples essential oil was dominated by α-pinene (28%) and phyllocladene (7-16%). Agar disk diffusion tests showed that the three types of essential oils inhibited Gram+ and Gram– bacteria. *Leiva* essential oil showed antimicrobial effects against a larger number of bacterial species (6) than *P. undulatum* (4) and *C. japonica* (4) essential oils. However, when observed, the inhibition of the essential oils from *P. undulatum* and *C japonica* was higher than that of the *leiva*.

Understanding the effect of volatiles from traditional pineapple substrates on the rhizospheric microbial community can provide insight into their plant-substrate-microbial interactions; and may become a useful tool to promote pineapple growth and quality.

Acknowledgments: Partially supported by INOVA-4/2010, PROCONVERGÊNCIA – EU/FEDER e Secretaria Regional da Ciência, Tecnologia e Equipamentos

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P 149. Antibacterial activity of *Eucalyptus* essential oils against pathogenic bacterial strains

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Keywords: *Eucaliptus*; essential oils; antibacterial activity; infections.

Essential oils (EOs) of several plant species have been used in the pharmaceutical, cosmetics, food, industries and medicinal purposes [1,2]. Also, the antimicrobial properties of EOs have been recognized for many years [3,4]. In the present study *in vitro* antimicrobial activity of 15 plant species EOs of *Eucalyptus* have been evaluated, using disk diffusion bioassay, in triplicate [5,6] and minimum inhibitory concentration (MIC) [7]. Four reference bacteria were tested: *Pseudomonas aeruginosa* (ATCC10145), *Escherichia coli* (CECT434), *Staphylococcus aureus* (CECT976) and *Listeria monocytogenes* (ATCC15313). Gram-positive bacteria revealed higher sensitivity, when compared with the Gram-negative. Among the Gram-positive, the EOs from 5 *Eucalyptus* species (*E. bosistoana*, *E. botryoides*, *E. camaldulensis*, *E. cinerea* and *E. citriodora*) showed a total inhibition. This study reveals higher broad-spectrum antibacterial activity against Gram-positive in EOs than antibiotics (gentamicin and ciprofloxacin). *L. monocytogenes* was the most sensitive bacteria and *P. aeruginosa* was resistant to all EOs. These three bacteria were tested against dilutions (50, 25, 15, 5 and 1 % v/v) of the pure EOs to determine the MIC. The dilutions of 50 and 25% presented the same inhibition areas as the pure EOs, while the 15% dilution revealed inhibition for the three bacteria tested with *S. aureus* showing higher values (10.3-30mm). *E. coli* and *L. monocytogenes* revealed no sensitivity to the 5% dilution. The dilution of 1% did not exert sensitivity in any of the tested bacteria. Our results showed that these EOs could be useful as alternative tool to control pathogenic bacteria with natural compounds.

Acknowledgements: To the Centro de Biotecnologia Vegetal, Instituto de Biotecnologia e Bioengenharia (Uni. Lisboa, Faculdade de Ciências) and the FCT financial support (Carla Dias-SFRH/BGCT/33354/2008 and Maria do Carmo Vasconcelos-SFRH/BPD/70310/2010).

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P 150. Antimicrobial activity of essential oils from squash pumpkin (*Cucurbita pepo*) seeds

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Keywords**:** *Cucurbita pepo* seeds; Essential oils; Industrial by-products; Antimicrobial activity

The nutritive value of proteins, fatty acids and vitamins from pumpkin seed oils has been previously well described [1,2]. In this study we aim to evaluate the antibacterial activity of residues from the industrial processing (squash pumpkin seeds essential oils) in order to be used as sources of beneficial compounds evaluating the presence of antibacterial agents. The seed samples were provided from a large Portuguese company (Douromel - Fábrica de Confeitaria, Lda.) that processes fruits. In the present study *in vitro* antimicrobial activity of EOs from *Cucurbita pepo* seeds against two pathogenic bacteria (*Staphylococcus aureus* - CECT976 and *Listeria monocytogenes* - ATCC15313), using disk diffusion bioassay, in triplicate [3,4] and minimum inhibitory concentration (MIC) was determined [5]. The seeds were freeze-dried and the essential oils extraction was performed by hydro-distillation [6]. The pure EO and a diluted sample (120 mg/ml in dimethyl sulfoxide) were tested. The results showed that the pumpkin seeds EOs had an inhibitory effect on the growth of *S. aureus*, while *L. monocytogenes* revealed to be resistant to the EO tested. There were no significant differences between the pure (9 mm) and the diluted (10 mm) EO inhibitory effect on the growth of this bacteria. The *S. aureus* was also tested against dilutions (50, 25, 15, 5 and 1 % v/v) of the pure extracts to determine the MIC. However the dilutions did not exert sensitivity in the tested bacteria. These results reveal that the pumpkin seeds essential oils are potentially a good source of antibacterial agents against pathogenic bacteria.

Acknowledgements**:** To the company Douromel (Confitaria Lda.) for the kind supply of the pumpkin residues samples. The authors also acknowledge the financial support provided by QREN (Quadro de Referência Estratégico Nacional) to the project Nutridouro "*Healthy crystalised fruits and jams with a traditional flavour"* (I&DT in Co-Promoção Nº 13111).

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P 151. Chemical characterization and biological evaluation of essential oils from *Plectranthus* species grown in Portugal

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Keywords: *Plectranthus grandidentatus*, *Plectranthus porcatus*, *Plectranthus venteri*, essential oils

Plectranthus is a Lamiaceae genus with interesting potential in drug discovery due to the bioactivities of its constituents and to the ethnopharmacological uses, namely, in Southern Africa [1, 2]. The essential oils (EOs) constituents of *Plectranthus* spp. are poorly studied. Following our research on *Plectranthus*, now we present a study of the *P. grandidentatus*, *P. porcatus* and *P. venteri* [3] EOs (grown in Lisbon from cuttings provided by the Kirstenbosch Nat. Bot. Gardens, South Africa).

Unlike *P. venteri* oil, the chemical composition of *P. grandidentatus* and *P. porcatus* EOs is varied and complex. Camphor is the most abundant monoterpene (52.8 %) of *P. venteri* together with elemol (5.1%), and sandaracopimarinal (6.7%). β-Caryophyllene (16.3%), caryophyllene oxide (5.2%), α-humulene (6.5%) and 6,7-dehydroroyleanone (6.3%) were some of the compounds of *P. grandidentatus,* while γ-terpinene (8.2%), 4-terpineol (7.6%), δ-cadinene (6.6%), caryophyllene oxide (5.7%), cineol (5.9%) and α-thujene (5.4%) were detected in *P. porcatus* EO. *P. grandidentatus* EO diverges from the one described for the plant collected in South Africa [4]. Diterpenes have rarely been identified on *Plectranthus* oils, being 6,7-dehydroroyleanone the only isolated from the oil of *P. madagascariensis* [5]. This diterpene was now identified in *P. grandidentatus* EO*,* while sarandaracopimarinal was identified in *P. venteri* EO for the first time.

The EOs showed low antimicrobial activity against *M. smegmatis, S. aureus, E. coli, P. aeruginosa* and *E. faecalis* (MIC 62.50-125 $\mu gml^{-1)}$. C*. albicans* growth inhibition by *P. venteri* EO was higher (44.4%) than *P. grandidentatus* EO (27.1%) at 250 μgmL^{-1}. However, the activity of the tested components was lower than EOs, being sandaracopamarinal the only showing some activity (20.18 % at 200 µM).

Acknowledgments**:** Grateful to E van Jaarsveld, T Vasconcelos and A Duarte. Supported by FCT-MCES (iMed.UL, SFRH/BD/19250/2004), ANPCyT, PICT 0608, PEst-OE/QUI/UI0612/2011.

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P 152. Composition and antibacterial activity of the essential oil of Peruvian *Dalea strobilacea* Barneby

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Keywords: *Dalea strobilacea* Barneby; Fabaceae; essential oil composition; GC and GC-MS; antibacterial activity

The genus *Dalea*, is highly diversified in the northern Peruvian Andes with some of these species being used as medicines [1]. Our research program is focused on the evaluation of the popular use of medicinal plants of the Chilean and Peruvian altiplano [2-5]. In this work we selected *Dalea strobilacea* Barneby (hierba de chil [6]), because residents use it to reduce gastrointestinal smooth muscle spasm and digestive disorders. Its infusion is highly prized as breakfast tea for its mild flavour that replaces the lemon verbena.

Regarding the chemical composition of essential oils (EOs) from genus *Dalea*, data are scarce in the literature. Moreover, no reports have been published about the chemical composition of the EO from *D. strobilacea*, collected in the region of Cajamarca, Perú. Therefore, we decided to carry out a study to determine its composition and also to explore its antibacterial capacity.

The composition of the EO from *D. strobilacea*, obtained by hydrodistillation of the aerial parts, was analyzed by GC and GC-MS, showing that β-phellandrene is the most abundant monoterpene (43.5%), together with α-pinene (17.7%).

The *D. strobilacea* oil was tested against two Gram-negative and two Gram-positive bacteria. We observed a selective effect on Gram-positive bacteria, with MIC values of 8.7 and 10.7 μg/mL as compared to the MIC value required to inhibit Gram-negative bacteria (59.5 μγ/mL). A similar profile was observed in the effects caused by the antibiotic Vancomycin, namely low MIC values (2 and 5 μg/mL) in Gram-positive as compared to MIC value in Gram-negative bacteria (15.4 μg/mL). This antibacterial activity may be related to the chemical proportion of the main compounds, β-phellandrene, α-pinene, as well as the minor components also present (β-pinene, myrcene and limonene).

Acknowledgments: Thanks to APEAC and A. C. Figueiredo, J. G. Barroso, L. G. Pedro. This study was supported by DI-UNAP, Conicyt-MEC 80100002 and PEst-OE/QUI/UI0612/2011.

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P 153. Chemical composition and potential antioxidant activity of essential oil and different solvent extracts of discarded tobacco leaves

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Keywords**:** discarded tobacco leaves, essential oil, solvent extracts, antioxidant activity

Every year in China, almost 20% of tobacco resources are discarded as processing waste. The discarded tobacco leaves not only pollute the environment, but also cause a big waste. In fact, there are abundant bioactive compounds in discarded tobacco leaves [1]. This study is designed to investigate the chemical composition and potential antioxidant activity of the essential oil and different solvent extracts of discarded tobacco leaves.

Essential oil was obtained by hydrodistillation (HD) and its composition was analyzed by GC and GC–MS. Antioxidant activity of essential oil and different solvent extracts (Petroleum ether, diethyl ether, dichloromethane, ethyl acetate and ethanol) were determined by two different systems, the DPPH free radical scavenging and the FRAP (Ferric Reducing Antioxidant Power).

GC and GC–MS analyses were resulted in the detection of 55 compounds in essential oil, representing 81.2% of the oil. Major components of the oil were neophytadiene (33.03%), β-damascenone (4.72%), methyl linoleate (1.86%), methyl linolenate (1.83%), megastigmatrienone (1.58%), β-damascone (1.54%) and solanone (0.22%). Results of the DPPH assay gives an IC_{50} range value of (2.07–9.88) mg/mL for all the samples studied. BHA (butylated hydroxyanisole) and ascorbic acid were used for comparison purposes. In all systems, ethyl acetate extract exhibited excellent activity potential than those of other extracts (Petroleum ether, diethyl ether, dichloromethane, and ethanol) and the oil. As expected, amount of total phenolics was very high in this extract (163.47±2.119g GAE mg/g extract). A positive correlation was observed between the antioxidant activity potential and total phenolic levels of the extracts

These results indicated that the essential oil and solvent extracts of discarded tobacco leaves had potential antioxidant activity, the discarded tobacco leaves could be considered as a potential resource of natural antioxidant.

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P 154. Comparative chemical study of *Uvariodendron angustifolium* (Engl. & Diels) R. E. Fries essential oils, a medicinal plant in benin

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Keywords: *Uvariodendron angustifolium*; essential oils; citral; methyl eugenol

Uvariodendron angustifolium, syn. *Uvaria angustifolia* (Annonaceae) is a West African forests tree which can reach 15-40 ft high [1]. This species, which is found in the south of Benin, is used in traditional medicine to treat rheumatics and gastric ailments, paludism, or for flavouring local dishes (leaves) [2]. The only chemical investigations on *Uvariodendron* genus concern two species: *U. connivens*, which is characterized by phenylpropene compounds in its seeds (elemicin, cinnamaldehyde and 3,4,5-trimethoxy cinnamyl alcohol) [3] and *U. calophyllum*, which furnished, by hydrodistillation of its wood, stem bark and roots, essential oils rich in sesquiterpenes (mainly (E)-β-caryophyllene and α-santalene) [4].

The present work reports a comparative chemical study of volatile components obtained by hydrodistillation of leaves, stems, bark and roots of *Uvariodendron angustifolium* collected in Ketou (Benin). The essential oils, obtained with yields comprised between 0.12 and 0.66%, were analyzed by GC/FID and GC/MS on two capillary columns of different polarities (HP-5 and Carbowax 20M). A great variability of the chemical composition was observed depending on the plant material part treated. The essential oils from leaves and stems were constituted by high levels of neral (29.7-34.2%) and geranial (42.7-46.5%) while methyl eugenol was the major component of the volatile extracts from bark (68.3%) and roots (85.3%). These chemical characteristics justify the use of leaves as food flavouring.

Acknowledgments**:** The authors wish to thank the University of Abomey-Calavi Scientific Council members for their agreement for this work and the Government of Benin Republic for its financial support.

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P 155. Mycorrhiza biotechnology in the cultivation of essential oil yielding traditional african medicinal plants.

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Keywords**:** Sustainable, symbiosis, soil biota, mineral supplementation

The cultivation of quality essential oil yielding Traditional African Medicinal (TAM) plants in Africa is threatened by low levels of minerals in local soils, combined with environmental stressors such as water and salt stress. Mycorrhiza is the symbiotic association between plant roots and specific species of soil borne fungi, and it can play a large part in the cycling of mineral nutrients from the soil into the plant, as well as protecting the host plant against stress, both environmental and cultural. The relationship of the fungi and host can take a variety of forms and can have a range of effects upon the growth and development of the host plant. The most commonly utilized mycorrhizal relationship type is arbuscular mycorrhiza (AM). AM is used in the cultivation of many important horticultural crops, and has been shown to have an effect upon the growth, development and yield of both ornamental and medicinal plant species. There is a large amount of information available on the use of AM in an ornamental horticultural situation, and so this paper will outline the current perspectives on the use of AM in the cultivation of essential oil yielding Traditional Medicinal plants, with a specific focus on the use of AM in an African setting (based on soil and environmental qualities that are relevant to Africa). This paper will summarise the history of traditional medicine and its cultivation/ harvesting in the unique African setting, specifically pertaining to nutrient applications and the potential of mycorrhiza to decrease reliance on alternate forms of nutrient supplementation. To further illustrate the potential of AM in the cultivation of TAM in Africa, the paper will also discuss a selection of TAM plants and review the potential for the use of AM in their cultivation in Africa.

P 156. Gamma irradiation in food preservation and health promotion – influence in the aroma compounds of spices

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Keywords: Spices; Clove; Volatile compounds; Gamma Irradiation; HSPME-GC-MS

History illustrates how spices have played a prevalent part in man's life and death. Due to their strong scent, mankind have used spices to fulfil the absence of hygiene habits, for embalming and for religious purposes. However, the most important role of spices was their ability to heal and perpetuate life, through their use as aphrodisiac, in dieting and as medicines. In fact, at 1500s, when the "Spice Wars" occurred between the Portuguese and the Dutch and later the Dutch and the English, the reason was their medicinal importance, because spices were considered as a miracle cure for the plagues. A list of cures may justify why many of them are exactly the same spices which commonly are found in spice racks for our everyday cooking. For over 2000 years, clove, *Syzygium aromaticum,* was consumed in different parts of Asia and later transported to Europe. Cloves are the dried aromatic flower buds of the evergreen trees, highly flavored and renowned for their health benefits (antiseptic, bactericide and fungicide) and nutritional content. The spice industry is faced with the problem of reducing microbial contamination derived from the techniques used in the post-harvest steps. Gamma irradiation is becoming a food preserving methodology, reducing also the incidence of foodborne disease. Depending on the energy, the irradiation process is capable of extending the shelf life of food, reducing spoilage, inhibiting sprouting and destroying insects. However, the irradiation treatment can induce chemical changes in food [1, 2]. The aim of this work is to investigate the effect of gamma irradiation in the chemical composition of spices. Beginning with the aroma compounds, using the free solvent HS-SPME-GC-MS, the effect of ^{60}Co irradiation, 5.0, 10.0, 20.0 and 30.0 kGy, in the volatile chemical profile of clove are being studied. Preliminary results show some quantitative differences, but also some qualitative differences in terpenoid composition.

Acknowledgments: The authors acknowledge financial support from Brazilian CNPq, CAPES (Renato Duarte - 201998/2011-9) and to IPEN-CNEN/SP-Brazil and Portuguese FCT (João Lopes - FCT/SFRH/BD/40564/2007; José Santana - SFRH/BNDE/51585/2011 and Inês Nunes - SFRH/BD/33809/2009). Authors also are grateful to Hikari (Brazil) for the samples supply.

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P 157. Bio-valorization of Azorean *Cryptomeria japonica* essential oils

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Keywords**:** *Cryptomeria japonica*; Essential oils; GC and GC-MS; Antimicrobial activity

Cryptomeria japonica D. Don wood production is of great economic importance in Azores Archipelago (Portugal), having a high value as a building material, furniture and also as an ornamental tree. Every year Azorean *C. japonica* wood industry generates a large amount of residues (bark, wood and aerial parts) with 30% of the wood wastes derived only from sawmills wood processing [1].

Essential oils (Eos) from aerial parts, bark and heartwood of *C. japonica* from Azores were analyzed by GC and GC-MS. The main compounds found in aerial parts were α-pinene (9.6-29.5%), (+)-phyllocladene (3.5-26.6%), *ent*-kaur-16-ene (0.2-20.6%), sabinene (0.5-19.9%) and limonene (1.4-11.5%). Heartwood oils were characterized by a high content of cubebol (2.7-39.9%) and *epi*-cubebol (4.1-26.9%), absent in aerial parts. Elemol and eudesmol isomers were found in all aerial parts and heartwood oils, while (+)-phyllocladene was not present in heartwoods. Bark oils were composed of dehydroferruginol and ferruginol diterpenes and a wide variety of sesquiterpenes (*δ*-cadinene, *α*-muurolene, *epi*-zonaren, cubenol, T-muurolol, *β*-eudesmol, *γ*-eudesmol and hedycariol). Azorean *C. japonica* oils exhibited significant chemical differences comparatively to native plants from Asia [2].

From the several fungi that affect many plant species, *Botrytis cinerea,* was inhibited by all Eos (MIC 100 µg/Ml) tested with fungistatic activity, while *Fusarium* spp. And *Cryphonectria parasitica* showed resistance towards all oils tested. Furthermore, all the Eos exhibited moderate activity against the wood surface contaminant *Trichoderma harzianum* and the human pathogenic bacterias *Streptococcus aureus* and *S. faecium* (MIC 100-200 µg/Ml). The Eos of leaves (MIC 25-50 µg/Ml) were more active than those of the heartwood (MIC 50-200 µg/Ml) against multidrug-resistant *Mycobacterium tuberculosis*. These results illustrate the potential bio-valorization of the wastes produced by *C. japonica* wood industry, until now only seen as disposable.

Acknowledgments**:** Collaborative work was performed under the auspices of the CYTED, PIBATUB, INETI and project Pest-OE/QUI/UI0612/2011. The authors thank Engineer J. Belerique, of the DRRF for providing the C. japonica and the facilities for this study.

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P 158. Floral scents of *Adansonia*: chemical characterization and role in the pollination process

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Keywords**:** Baobab; *Adansonia*; scent; pollination; Madagascar

The genus *Adansonia* (baobab, Malvaceae family) includes eight species among them seven were identified in Madagascar and six are endemic and belong to the two sections Brevitubae and Longitubae [1]. They are emblematic in the island, nevertheless more knowledge is necessary regarding their productive biology; among the different parameters involved in the pollination process, the mechanism of scent release by flowers is of importance [2]. The aim of this study was : (i) the identification of the volatile compounds released by the flowers of the different botanical species and (ii) elucidation of their role in the flowers/pollinating agents interaction.

The study was performed on individual trees of the six species endemic in Madagascar during their flowering period.

Two complementary methods were used to catch these volatile compounds: solvent extraction (using 3M engineered fluid HFE-7200, ethoxy-nonafluorobutane) and solid phase microextraction (using ´´Mono Trap´´ disks). Their identification was performed by GC/FID and GC/MS. A great chemical variability was observed according the botanical section. The scents released by flowers of the species belonging to the Longitubae section were dominated by aromatic compounds (mainly 2-phenylacetonitrile) while aliphatic compounds (mainly heptadec-8-ene) characterized the Brevitubae group.

The chemical characteristics of the scents related to the Longitubae section were previously described as possessing entomophilous properties [3]. Other studies on the floral morphology, the nectar and the pollinating agents are in progress.

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P 159. Antifungal and anti-inflammatory potential of *Lavandula stoecha*s and *Thymus herba-barona* essential oils

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Keywords: *Lavandula stoechas*; *Tymus herba-barona*; essential oil; antifungal; anti-inflammatory; toxicity

This study reports the chemical composition and antifungal activity of *Lavandula stoechas* and *Thymus herba-barona* essential oils against fungi responsible for human infections and food contamination. Also the anti-inflammatory potential and toxicity of the oils on a macrophage cell line is reported.

L. stoechas essential oil was rich in fenchone (38.0%) and camphor (28.1%) whereas *T. herba barona* oil showed high amounts of two phenols, carvacrol (54.4%) and thymol (30.2%). The latter was the most active oil against the tested fungi but evidenced high toxicity on macrophages. *L. stoechas* was active against dermatophyte strains and showed potential anti-inflammatory activity, by inhibiting the production of NO in LPS-stimulated macrophages, at concentrations from 0.16 to 0.64µl/ml. The oil did not affect cells viability up to 0.32µl/ml.

These results support the use of *L. stoechas* in the development of phytopharmaceuticals for the management of dermatophytosis and/or inflammatory-related diseases. Regarding *T. herba-barona*, it can be used as a preservative in storage products, due to its ability to inhibit *Aspergilllus* growth.

Acknowledgments: This work was funded through national funds from FCT – Fundação para a Ciência e a Tecnologia under the project CEF/POCI2010/FEDER and a PhD fellowship to Mónica R. Zuzarte (SFRH/BD/40218/2007).

P 160. Essential oil of *Ocimum americanum* L. and its application in perfumery

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Keywords: *Ocimum americanum*; estoraque; essential oil; perfumery

Studies on biodiversity prospection are extremely important for the cosmetic industry and Brazil has a great potential in this field, due to its huge diversity of native and adapted species [1]. Based on previous works of aromatic species prospection in the Amazon region, the aim of this study was to investigate the potential use of essential oil obtained from aerial parts of the endemic methyl cinnamate chemotype of *Ocimum americanum* (popularly known in north of Brazil as Estoraque) for the fragrance industry. Production of Estoraque was done following an organic agricultural model (IBD organic certification) in the community of Campo Limpo - Santo Antonio Tauá, state of Pará. The steam distillation of aerial parts of Estoraque yielded 0.15-0.25% of essential oil that was analyzed by gas chromatography-mass spectrometry (GCMS). The major compounds were: *E*-methyl cinnamate (42.0%), carvone (9.3%) and *trans*-caryophyllene (9.1%). This study reports the industrial scale production, phenological aspects, toxicology and olfactory significance of methyl cinnamate chemotype of *O. americanum,* revealing an innovative ingredient for perfumery use.

Acknowledgements**:** The authors would like to thank Veronica Kato (Natura Perfumer) and Givaudan.

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P 161. Microalgae: Promising source in the production of carotenoids and essential oils

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Keywords: *Haematococcus pluvialis*, *Chlamydomonas* sp., fatty acids, carotenoids.

Microalgae are a potential source of biologically active compounds, namely carotenoids and fatty acid, showing higher efficiency than those obtained from traditional crops. Regarding this they can be considered as functional “ingredients”, justifying the recent increased interest in their commercial, pharmaceutical, environmental and/or agri-food industries applicability. This work aimed to evaluate and determine the total lipid content and fatty acid profile, as well as to quantify the amount of carotenoids, in two species of microalgae *Haematococcus pluvialis* Flotow (Hp), *Chlamydomonas sp*. Ehrenberg (Csp), both belonging to the phylum *Chlorophyta* [1] Their growth was monitored and assessed in two different culture media. Solvent extraction technique was used for lipid content and gas chromatography, spectrophotometry UV-vis and mass spectrometry (MS) to evaluate the profile of methyl esters of fatty acids and carotenoids, respectively [2, 3]. The percentages of total lipid for Hp and Csp were 12,05%, 8,33% and 23,82% and 14,65% in the two media, respectively. The profile of fatty acids proved to be variable, both in size of the hydrocarbon chains and for the position of the unsaturated carbon atom, conditioned by the culture media. The evaluation of the carotenoids content for both species, showed similar variability. Hp (H2) presented higher content of this bioproduct, 28.4% and 39% in both media. Our results were similar to those obtained in other studies, with both species, which have shown the increasing importance of these microorganisms in the production of essential compounds, with specific physiological functions [4].

Acknowledgements**:** Abílio, Sobral, PhD – Departamento de Química (FCTUC). Jorge Varejão, Professor Adjunto - ESAC.

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P 162. Understanding the ecological role of volatiles in the host-plant resistance: the case of the portuguese sweet potato (*Ipomoea batatas*) from Aljezur

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Keywords: *Ipomoea batatas*; Lira; Protected Geographical Indication; volatiles; GC; GC-MS

A major constraint to sweet potato production worldwide is the sweet potato weevil, *Cylas* spp.. Although there are no records of this species in Europe, interceptions of this pest should be considered due the worldwide trade of sweet potato. The development of insect-resistant sweet potato lines is seen as a viable component in the integrated management of this and other pests. Qualitative and quantitative analysis of the host-plant volatiles has been one of the approaches for successful identification of resistance in germplasm resources [1].

Sweet potato, *Ipomoea batatas* (L.), is an important food crop and it is an alternative source of carbohydrates, taking the fourth place after rice, corn and cassava. The tuberous roots and the green tops constitute a important source of vitamins and minerals [2,3].

In Portugal, *Lira* variety is cultivated in Aljezur – a Protected Geographical Indication (PGI) area [4]. Phytosanitary problems have been rarely detected. Only sporadic attacks of red spider mite (*Panonychus ulmi*) and some fungi have occurred, which normally do not involve treatments, since them haven´t represented any economic loss for farmers [5].

In order to understand the potential resistance of this Portuguese variety, the volatile composition of all plant is being studied. The volatiles were isolated by hydrodistillation and analyzed by GC and GC-MS as in [6]. Different chemical profiles were obtained for the aerial parts and storage roots. β-Caryophyllene and germacrene are the main compounds of the volatiles from the aerial parts, while hexadecanoic acid is the major component of storage roots. α-Humulene, a sesquiterpene known to be repellent to insects [7] is also present in the aerial parts and storage roots. Additional studies are needed in order to elucidate the role of sweet potato volatile composition, mainly the sesquiterpene fraction, in the resistance of plant against pests.

Acknowledgements: ISN, JFL and JCS acknowledge FCT (SFRH/BD/33809/2009; SFRH/BD/40564/2007; SFRH/BDE/51535/2011) and Associação de Produtores de Batata Doce de Aljezur.

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P 163. Phytochemical and genetic diversity in *Mentha cervina* (L.) Opiz based on essential oils profile and ISSRs markers fingerprinting

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Keywords: *Mentha cervina*; Essential oils; Genetic diversity; ISSRs; Conservation genetics

Mentha cervina L. is an aromatic plant traditionally used in Portugal to flavour food dishes and for its medicinal properties. In the last decade, the growth of commercial demands, the excessive harvesting from the wild and the unfavourable conservation status of their habitat, has shrunk the natural resource of *M. cervina* to a narrow distribution [1], leading to the classification of this species as Near Threatened in the IUCN Red List of Threatened Species [2].

In this study, phytochemical and genetic variation was studied to evaluate the level and distribution of diversity in *M. cervina* populations in order to provide guidelines for the conservation and sustainable use of this medicinal species. Gas Chromatography and Gas Chromatography–Mass Spectrometry was used to reveal the essential oil profile and Inter-simple sequence repeats (ISSRs) markers fingerprinting to assess the population's genetic structure and diversity.

M. cervina essential oils showed high uniformity(S_{corr}≥0.95%), with pulegone has the major essential oils compound in all of the populations (68-83%) collected at full flowering, in different growing conditions (51-82%), and for all the developmental stages studied (47-82%). The *M*. *cervina* populations exhibited a relatively low genetic diversity (*PPB* = 14.3-64.6%, H_e = 0.051-0.222, *I* = 0.076-0.332), with high structuring between them (G_{ST} = 0.51). However, the genetic diversity at species level was relatively high (*PPB* = 97.7%; H_e= 0.320).

The low genetic diversity found within these populations seems to result from genetic drift and inbreeding that lead to diminished fitness in this species, which may be observed in the rather unusual uniformity of the essential oil composition. From a conservation perspective, the low genetic and phytochemical diversity observed is symptomatic and a signal that ecological management of *M. cervina* habitats is necessary to prevent the consequent decline in population size that could increase the risk of extinction due to demographic and environmental stochasticity.

Acknowledgments**:** Leandra Rodrigues is grateful to the FCT for the grant SFRH/BD/38143/2008.

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P 164. Anti-candidal activity of 7-hydroxycalamenene isolated from *Croton cajucara*

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Keywords: 7-hydroxycalamenene; *Croton cajucara*; red sacaca; essential oil; anti-Candidal activity

The leaves and bark from *Croton cajucara* Benth. (family Euphorbiaceae), a shrub from the Amazon, have been used locally used in folk medicine to treat diabetes, malaria, gastrointestinal and liver disorders [1]. A chemotype of this species was found, with an essential oil rich in 7-hydroxycalamenene [2]. 7-hydroxycalamenene is a hydroxylated sesquiterpene of molecular weight 218 found in *Heritiera ornithocephala* [3], *Eremophila drummondii* [4], *Heteroscyphus planus* [5], *Tilia europea*, *Morus alba* [6], *Ulmus thomasii* [7] and other elm species, and methanolic and dichloromethanic extracts of *Bazzania trilobata*. This substance is reported to have antifungal activity against *Botrytis cinerea*, *Cladosporium cucumerinum*, *Phythophthora infestans*, *Pyricularia oryzae* and *Septoria tritici* [8]. During our studies with *C. cajucara* essential oil, we isolated 7-hydroxycalamenene by silicagel column chromatography. The pure compound (+98% by GC) was tested against some Candida species. Minimum inhibitory concentration (MIC) was evaluated in triplicate according standard method from the Clinical and Laboratory Standards Institute (CLSI) [9]. The calculated MIC's were 39,06 µg/mL was found to *C. albicans* (ATCC10231), *C. dubliniensis* e *C. albicans* (CaA), of 78,125 µg/mL to *C. albicans* (Cab) e *C. parapsilosis* and *C. albicans* (CaB) > 2500 µg/mL. From these data, it was observed 7-hydroxycalamenene is a compound with good activity against these *Candida* species.

P 165. Antimicrobial activity of essential oils from *Lippia alba* (Miller) N.E. Brown

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Keywords**:** *Lippia alba*; essential oils; antimicrobial activity

Lippia alba (Miller) N.E. Brown (Verbenaceae) is an aromatic plant known in Brazil as "Erva-cidreira-do-campo", and that is native to southern Texas in the United States, Mexico, Caribbean, Central America, and South America [1]. This specie occurs in practically all regions of Brazil and has a large importance in brazilian folk medicine, with its use widely distributed in the country. *L. alba* is locally used as sudorific, expectorant, on the treatment of colds and flu, antidysenteric, stimulant, antireumatic and against hypertension. Its use in popular medicine can be explained, partly, by the bioactive volatile constituents. However, this specie is characterized by variability in morphology and in the chemical composition of the essential oil. Secondary metabolics as limonene, carvone, citral, myrcene, are often found in essential oils of the plant [2]. The present study evaluated the antimicrobial potential of 6 different chemotypes of essential oils (EOs) of *L. alba*, it was analyzing the inhibition of the activity of these oils against pathogenic microorganisms and his virulence factors, like peptidase activity. During the studies with *L. alba* essential oils, were made tests against bacteria, yeasts and filamentous fungi, like *Escherichia coli*, *Candida albicans* and *Trichophyton rubrum*. The minimum inhibitory concentration was based on protocols CLSI [3] and the results showed MIC of 312 µg/mL against *C. albicans* (ATCC10231) and 70 µg/mL to *T. rubrum*. To determining a possible target of antifungal activity, it was performed assays of proteolytic activity inhibition, through to the method described by Buroker-Kilgore and Wang [4]. The results showed that *L. alba* EOs presented proteolytic activity inhibitions to *C. albicans* secreted proteases, mainly serine and aspartic classes. The results show a great antifungal activity of *L. alba* EOs, suggesting that more researches are required to considerate the EOs like alternatives for a new and effective antimicrobial therapy.

P 166. Inhibition of extracellular peptidases of *Rhizopus oryzae* by *Melissa officinalis* essential oil.

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Keywords**:** *Melissa officinalis*; essential oil; *Rhizopus oryzae*; extracellular peptidases.

Lemon balm (*Melissa officinalis* L., Lamiaceae) is a well-known herb used to give fragrance to different food and beverage products. It has also been used as a medicinal plant for treatment of headaches, gastrointestinal disorders, nervousness, and rheumatism [1]. The essential oil is a well-known antibacterial and antifungal agent. Mucormycosis is a life-threatening fungal infection of worldwide distribution and increasing importance affecting mainly immunocompromised hosts. It is caused by fungi of the Mucorales order involved in human or animal diseases. *Rhizopus* spp. usually causes infections of sinuses and rhinocerebral structures, cutaneous tissue or lungs. In the latter infections, the fungi generally disseminates in almost half of all cases. Pathological hallmarks of mucormycosis are vascular invasion with hyphae, thrombosis, infarction, and necrosis of tissue, which lytic enzymes produced by *Rhizopus* spp. are thought to be directly or indirectly involved [2]. In addition to the lack of potent, well-tolerated antifungals, the poor prognosis can clearly be attributed to the exceptionally aggressive behavior of the fungus in the human host. In this study, we evaluated the antifungal and anti-peptidase activities of *M*. *officinalis* essential oils (EO) against *R*. *oryzae*. Firstly, the susceptibility of *R. oryzae* to the EO was tested by microdilution, based on protocol M38-A2 [3]. Spores were diluted in RPMI 1640 containing *L*-glutamine and buffered to pH 7.0 with 0.165 M MOPS, and the MIC obtained for *R. oryzae* was 625 µg/mL. To determining a possible target of this antifungal activity, it was performed assays of proteolytic activity inhibition, through the method described by Buroker-Kilgore and Wang [4]. Cell-free supernatants of *R. oryzae*, grown in RPMI medium, were incubated with bovine serum albumin (BSA) 0.1 mg/mL as proteic substrate and some pHs buffers. The preliminary results showed that the extracellular peptidases were able of hydrolizate the proteic substrate in pHs 3 and 12, and their proteolytic inhibitions were obtained with EO concentration 0.1 µL/mL, suggesting a possible target that justify the antifungal activity this essential oil.

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P 167. Chemical composition of essential oils of *Ocimum gratissimum* L (Lamiaceae) from Limpopo province, South Africa: A comparative analysis of leaf and stem oil

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Keywords**:** *O. grattisimum* L; Lamiceae; essential oil; South Africa

Ocimum gratissimum L is a perennial herb or shrub with medicinal properties [1,2]. It is found in tropical countries including Limpopo Province, South Africa [2]. Our interest arose because the aromatic smell of crushed leaves and stem is used by locals for warding off mosquitoes [3]. Fresh leaves and stem were collected (flowering period, June-July) from Ha-Mutsha. The essential oils were extracted using hydrodistillation and analyzed by GC-MS. The percentage yield of the essential oils were 0.78% and 0.14% (w/w) for leaf and stem oils, respectively. Both leaf and stem oils have a high content of limonene, neral and geranial (Table 1). A closer look reveals that the non-oxygenated mornoterpene o-cymene (3.36%), eugenol (2.37%), germacrene-D (2.88%) and piperitenone (19.04%) are only found in the leaf oil and d-3-carene (1.73%), a-terpinolene (1.27%) and a-copaene (1.24%) are only found in the stem oil. The components that are only found in the stem oil occurs in lesser amounts. The oil is characterized by the absence of thymol, ethyl cinnamate and linalool which have been reported in similar plant species [4].

Table 1. Chemical composition of *O. gratissimum* L leaf and stem oils from Ha-Mutsha

Compound	Leaf oil	Stem oil
Δ-3-Carene		1.73
o-cymene	3.36	
Limonene	24.80	36.11
cis β-Ocimene	6.15	19.81
trans β-Ocimene	3.64	1.44
α-Terpinolene		1.27
Nerol	2.60	1.13
Neral	14.39	17.16
Geraniol	1.98	1.26
Geranial	15.21	16.71
Piperitenone	19.04	
Eugenol	2.37	
α-copaene		1.24
β-Caryophyllene	3.59	2.14
Germacrene-D	2.88	

Acknowledgements: Prof Magwa ML (Forthare University) for assistance with GC-MS. Mr Paulus Sebothoma (National Botanical Institute, Pretoria) for plant identification. National Research Foundation for financial assistance. The study was conducted while the authors were still at the University of Venda.

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P 168. Effect of essential oil of orange peel on germination, growth and production of soybean

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Keywords**:** *Glycine max*; photosynthesis; allelopathy; mineral oil

Many essential oils have bioherbicide effect. Their effects on germination and growth occur in very low doses. However, there are commercial formulations in which essential oils are used to replace mineral oil in the agriculture. Thus, the essential oil may be used as alternative to petroleum oils. Therefore, the objective of this study was to evaluate the inhibition effect of a commercial formulation containing 5% essential oil of orange peel on the germination, growth and yield of soybeans. Growth, photosynthesis rate and yield were evaluated in greenhouse condition with nine treatments (commercial mineral oil Assist (Basf), Nimbus (Syngenta) to 0.5% with 2 and 3 applications, and commercial essential oil of orange peel Orobor (Oro Agri) at 0.1 and 0.4% with 2 and 3 applications). Germination test was evaluated with 200 seeds in germitest paper at 25^0C for 7 days [1] at the concentration of 0, 2.5, 5, 7.5 and 10% of commercial essential oil of orange peel.

Essencial oil of orange peel affected all the germination variables but did not affect the biometric variables, photosynthesis and yield. These results suggest that essential oil of orange peel presented as a potential alternative to replace mineral oil in agriculture.

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P 169. Phytochemical constituents of leaf oil of *Lantana camara* L (Verbernaceae) from Limpopo province, South Africa

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Keywords: *Lantana camara* L; Verbernaceae; essential oil; limonene; ß-caryophyllene; South Africa

We have recently completed phytochemical investigation of *L. camara L*, a much-branched shrub that is widespread in summer to winter [1]. Its purplish berries are eaten, mostly by young people [2]. Our interest in its leaf oil constituents arose mainly because the leaves are used traditionally for treatment of eye injuries. Fresh leaves were collected from University of Venda campus. The oil, with percentage yield of 0.06% (w/w), was extracted using hydrodistillation and analyzed by GC-MS. The cyclic mornoterpene hydrocarbon limonene is the major component of the oil comprising 46.74% of the total oil while the bicyclic sesquiterpene ß-caryophyllene content of the oil is 17.14%. Six other essential oil constituents appear in lesser amounts: α-curcumene (5.36%), *p*-cymene (4.21%), ß-elemene (4.47%), α-humulene (4.52%), *cis*-ß-ocimene (4.73%) and *trans*-ß-ocimene (6.64%). A comparison with the constituents of various essential oils of *L. camara L* with different origin will also be given.

Acknowledgements**:** Prof Magwa ML (Forthare University) for assistance with GC-MS analysis. Mr Tshisikhawe MP (University of Venda) for plant identification. National Research Foundation for financial assistance. The study was conducted while the authors were still at the University of Venda.

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P 170. Phytotoxic activity of essential oils from three *Copaifera* L. species

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Keywords**:** Phytotoxic; *Copaifera duckei*; *C. martii*; *C. reticulate*; inhibitory effects.

The rich and diversified Amazonian flora represents an excellent resource for new chemical structures. In this work, the chemical composition of the essential oils from leaves and stems of *Copaifera duckei* Dwyer, *C. martii* Hayne and *C. reticulata* Ducke (Leguminosae – Caesalpinioideae) were characterized. The phytotoxic activity these essential oils have on seed germination, and development of root and hypocotyl of the pasture weeds *Mimosa pudica* L. (malicia) and *Senna obtusifolia* (L.) H. S. Irwin & Barneby (mata-pasto) was analyzed. Inhibitory effects were more intense on root development and less intense on seed germination. *Mimosa pudica* tended to be more sensitive to phytotoxic effects than *S. obtusifolia*. Leaf oils presented a greater potential to inhibit root and hypocotyl development, while stem preferentially inhibited seed germination, although in some cases these differences were not statistically significant. The oils of leaves had a greater number of constituents when compared to those of stem, especially from *C. martii* which could justify the differences observed in the intensity of the phytotoxic effect between stems and leaves. Among the constituents identified, only d-cadinene and linalool had been previously related to phytotoxic activity.

P 171. Effect of different culture media on the essential oil composition of *in vitro* grown *Thymus caespititius*

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Keywords: *Thymus caespititius*; essential oils; chemotypes; culture media

Thymus caespititius Brot. (Lamiaceae) is an endemic species of the NW Iberian Peninsula and of the Azores and Madeira archipelagos. These plants possess essential oils rich in thymol-, carvacrol-, á-terpineol-, sabinene- or carvacrol/thymol [1]. Given the interest on this species' essential oil*, in vitro* cultures of selected *T. caespititius* chemotypes were established. In the present study, the effect of different culture media on the essential oil composition of *in vitro* grown *T. caespititius*, was assessed.

Five *T. caespititius* genotypes corresponding to three distinct chemotypes, carvacrol (C), carvacrol/thymol (CT1 and CT2) and sabinene/carvacrol (SC1 and SC2) were used. The essential oils were isolated by hydrodistillation and analyzed by GC and GC-MS as in [2].

In vitro cultures of *T. caespititius* were established as in [3]. Six months following culture initiation, the explants were transferred to MS medium [4] with reduced growth regulators for shoot elongation. Prior to the determination of the volatiles composition, the cultures were kept, for at least three subcultures, in each of the two culture media studied, MS and SH [5].

The different nutrient composition of the two media altered quantitatively the composition of the essential oils of the C and CT plantlets. Carvacrol, thymol, carvacryl acetate and thymyl acetate were the main components of the essential oils from MS grown plantlets. In the essential oils from the plantlets grown on SH medium a decrease of the relative amount of the acetate compounds and an increased of γ-terpinene and *p*-cymene was observed. These components are biosynthetically related to carvacrol and thymol, which synthesis proceeds from γ-terpinene to *p*-cymene.

No differences were observed between the SC plantlets essential oils grown in MS and SH medium. Sabinene was the main component identified in these oils, followed by carvacrol.

Acknowledgements**:** Partially funded by FCT, under PTDC/AGR-GPL/101334/2008. Marta D. Mendes is grateful to FCT for the PhD grant SFRH/BD/60244/2009.

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P 172. Essential oil composition from twenty six *Eucalyptus taxa* from Mata Experimental do Escaroupim (Portugal)

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Keywords: Myrtaceae; *Eucalyptus* spp.; essential oils; cluster analysis; GC GC-MS; Escaroupim

Eucalyptus is one of the world's most important and most widely planted genera. This economically important Myrtaceae genus includes more than 700 species [1]. *Eucalyptus* are an important source of timber, pulpwood and for the production of essential oils, used for medicinal and pharmaceutical purposes [2]. To a minor extent, some are used as ornamental trees. The Mata Experimental do Escaroupim (MEE), located in Salvaterra de Magos, Portugal, includes an *arboretum*, harbouring more than 125 different species of this genus [3]. In continuation of our work on sixteen *Eucalyptus* species [4], and integrated in a study that aims at evaluating essential oils as nematoxics, we herewith report on the composition of essential oils isolated from additional ten *Eucalyptus* taxa grown in MEE.

The essential oils were isolated by hydrodistillation, analysed by GC and GC-MS and the percentage composition of the essential oils was used to determine the relationship between different samples by cluster analysis as in [4]. Cluster analysis including previously [4], and presently studied essential oils samples showed two poorly correlated clusters (S_{corr}<0.04). Cluster I that included twenty four out of the twenty six taxa showed several subclusters. Cluster Ia, with twenty two samples from eighteen species (*E. bosistoana*, *E. botryoides*, *E. camaldulensis*, *E. cinerea*, *E. cordieri*, *E. ficifolia*, *E. gigantea*, *E. globulus*, *E. hemiphloia*, *E. macarthurii*, *E. pauciflora*, *E. piperita*, *E. polyanthemos*, *E. radiata*, *E. saligna*, *E. smithii*, *E. urophylla* and *E. viminalis*) and Cluster Ib (two samples of *E. citriodora*) were also poorly correlated (S_{corr}<0.10). All oils from Cluster Ia showed variable amounts α-pinene (1-94%), 1,8-cineole (1-83%) and limonene (1-41%), without citronellal, whereas Cluster Ib was citronellal rich (36-47%), with lower α-pinene (1%), 1,8-cineole (0.1-11%) and limonene (0.2-0.3%) contents. Cluster II which included only the two *E. dives* samples was characterized by a high piperitone content (40-55%), which was absent to 0.5% in the other taxa.

Acknowledgments: Jorge Faria is grateful to the FCT for the PhD grant SFRH/BD/43738/2008. This study was partially funded by FCT, under research contract PTDC/AGR-CFL/117026/2010.

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P 173. The phytotoxic effects of the secondary metabolites myrthenal, carvone and carvacrol

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Keywords: myrthenal; carvone; carvacrol; phytotoxicity; microtubules; *Arabidopsis*

Myrthenal, carvone and carvacrol are natural compounds commonly found in essentials oils of plants of the families Apiaceae and Laminaceae [1, 2, 3]. These secondary metabolites have been found to possess antibacterial and fungicide activity [3, 4], but their phytotoxic potential has been poorly investigated. Therefore the phytotoxicity of carvone, carvacrol and myrthenal was tested on *Arabidopsis thaliana* (L.) Col-0, a model plant broadly used in phytotoxic studies [5]. *Arabidopsis* seedlings were treated with 0, 50, 100, 200, 400, 800 and 1200 µM of carvone, carvacrol or myrthenal and root length was measured 15 days after allelochemical exposition. A strong inhibition and highly significant effect were detected on the development and growth of *A. thaliana* roots with very low IC_{50} (compound concentration required for 50% inhibition). Root thickness and presence of root hairs were analyzed under a magnifier and a left-handed growth, which suggests microtubule alteration [6, 7], was observed in the treated roots. Immunofluorescence assays were done to confirm microtubule malformations after carvone, carvacrol and myrthenal treatments. The results suggest the different mode of action of these metabolites and confirm the multiple targets on the mode of action of natural compounds.

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P 174. *Ammoides verticillata* and *Thymus ciliatus* oils from Algeria: chemical composition, antimicrobial and antioxidant activities

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Keywords: *Ammoides*, *Thymus*, Algeria, essential oils, biological activities

Ammoides verticillata (Apiaceae) is an Algerian endemic species which flowers are rich in essential oils [1]. A great variability on the chemical composition of the essential oils has been reported depending on the part of the plant as well as on the developmental stage [2,3]. The chemical composition and antimicrobial activity of *Thymus ciliatus* (Desf.) Benth. ssp. *eu-ciliatus* Maire essential oil from Algeria was reported by Bousmaha-Marroki et al [4]. Carvacrol dominated the oil which may explain the good antimicrobial activity detected. In the present work, the chemical composition, antioxidant and antimicrobial activities of the essential oils of those two species collected in Algeria were studied. The essential oils were isolated from the aerial parts by hydrodistillation, and analyzed by Gas Chromatography and Gas Chromatography-Mass Spectrometry as in [5].

Cumin alcohol (44%), *p*-cymene (18%), limonene (14%), thymol (11%) and g-terpinene (7%) dominated in the *A. verticillata* oil; whereas carvacrol (71%) predominated in the *T. ciliatus* oil.

The antibacterial activity of the essential oils of *A. verticillata* and *T. ciliatus* was determined by agar diffusion against *Escherichia coli*, *Salmonella enterica* serovar Thyphimurium, methicillin-resistant *Staphylococcus aureus* and *Listeria monocytogenes*. The tested bacteria were more susceptible to the essential oil of *T. ciliatus*.

A. vericillata oil was the most effective for scavenging the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS); and peroxyl radicals. The same oil was also the best for prevent lipid peroxidation when measured through the TBARS (thiobarbituric acid reactive species).

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P 175. Essential oil variation of *Lippia origanoides* with occurrence in the Carajás national forest, PA, Brazil

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Keywords: *Lippia organoides*, essential oil variation, circadian activity

The harvest time can be an important aspect in the production of essential oils. The interactions of environmental conditions that occur throughout the day can influence directly or indirectly the processes of secondary metabolism that result in quantitative and qualitative variations in the essential oils [1]. *Lippia origanoides* Kunth is a small shrub growing up to 3 m, with different aromatic characteristics and some known chemotypes. The species occurs in countries of Central and South America, especially in the Brazilian Amazon. It is used for culinary and medicinal purposes. The aim of this study was to evaluate the circadian activity of the plant, based on the analysis of its leaf essential oil. The leaf samples were collected between 9 h and 18 h, during 3 days (triplicate) in the Carajás National Forest, state of Pará, Brazil, September 2011. The leaf samples were hydrodistilled using Clevenger apparatus and the oils analyzed by GC and GC-MS, both equipped with DB-5ms capillary column (30 m x 0.25 mm; 0.25 um film thickness), temperature programmed to 60-240 °C (3 °C/min) and carrier gas helium and nitrogen, respectively. The leaf oil yields were as follows: 9 h (3.1%), 12 h (2.8%), 15 h (2.8%) and 18 h (2.5%). The main constituents (over 3%) identified at 9 h were *p*-cymene (27.0%), carvacrol (11.8%), (*E*)-methyl cinnamate (8.3%), methyl thymol ether (5.2%), α-pinene (4.6%), (*E*)-nerolidol (3.9%) and 1,8-cineol (3.5%); at 12 h were (*E*)-methyl cinnamate (26.5%), (*E*)-nerolidol (16.9%), 1,8-cineol (9.9%), α-pinene (5.0%) and *p*-cymene (4.0%); at 15 h were (*E*)-nerolidol (14.7%), 1,8-cineol (12.3%), (*E*)-methyl cinnamate (10.8%),*p*-cymene (9.1%), α-pinene (6.9%) and α-phellandrene (5.0%); and at 18 h were 1,8-cineol (17.6%), *p*-cymene (9.1%), (*E*)-nerolidol (6.5%), α-pinene (4.7%), methyl thymol ether (4.7%), (*E*)-caryophyllene (3.9%) and α-copaene (3.4%). In addition, the main components (over 3%) found in the oil of fine stems were (*E*)-nerolidol (25.8%), 1,8-cineol (13.3%), (*E*)-caryophyllene (9.3%), α-copaene (7.4%) and α-pinene (3.6%), while in the oil of flowers were 1,8-cineol (25.1%), (*E*)-caryophyllene (12.1%), α-copaene (5.1%) and α-pinene (4.5%). The constituents with greater variation during the day were *p*-cymene, (E)-methyl cinnamate, 1,8-cineol and (*E*)-nerolidol, specially the carvacrol which has a maximum value at 9 h. The study of seasonal activity in *L. origanoides* is ongoing.

P 176. Detection of shiikuwasha juice adulteration by calamondin juice using volatile components

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Keywords: Citrus volatiles; essential oil; γ-terpinene; adulteration; Shiikuwasha; Calamondin

Shiikuwasha (*Citrus depressa* Hayata) has been a very popular fruitlet as a flavor enhancer in Okinawa, Japan, and contains large amount of nobiletin reported to show anti-tumor activities [1] in the edible part of this citrus [2]. Therefore, market of Shiikuwasha products has grown rapidly by today's health-conscious consumers. Although Shiikuwasha farmers could not keep up with the supply of this fruit, Shiikuwasha juice adulterated with Calamondin (*Citrus madurensis* LOUR.) juice produced in Taiwan and Philippines is widely commercialized. The objective of this work was to investigate the volatile compounds to detect simply the Shiikuwasha juice adulterated with Calamondin juice.

Shiikuwasha juice, calamondin juice and 10 commercial shiikuwasha juice was used for the experiment. One milliliter of shiikuwasha juice was placed in a vial. The vial solution was held at 40℃, and solid phase microextraction (SPME) fiber was introduced into the headspace of vial and kept for 20 minutes. Heating of GC column-oven was stopped and a column head was dipped into liquid nitrogen to collect the volatiles in splitless mode. Then, the SPME fiber was introduced into the injector of GC and kept there for 7 minutes with cryofocusing. Headspace (HS) gas was analyzed by GC and GC/MS.

Chromatograms with high resolution were obtained by HS-SPME-cryofocusing, and 39 aromatic components were identified or presumed. Calamondin contained a slight amount of γ-terpinene (1.7%: composition rate) as compared with shiikuwasha (17.3%), it is supposed γ-terpinene detected shiikuwasha juice adulterated with calamondin juice. We obtained γ-Terpinene ratio (γ-terpinene peak area/total peak area). γ-Terpinene ratio was with a 0.07-0.11 % range for 3 commercial juices, and these juices were doubt adulterations with calamondin juice.

γ-Terpinene was a useful volatile marker component to detect shiikuwasha juice adulterated with calamondin juice.

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P 177. Antifungal activity of some essential oils on pathogenic fungi isolated from medicinal plants

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Keywords: essential oils; medicinal plants; antifungal activity

Microbiological quality of the medicinal plants very often is not good enough to fulfill official criteria (standards) because of the presence of various pathogenic fungi. On the other side, aromatic plants presents significant source of essential oils with various microbiological activities. In this study, the antifungal activity of four widely used essential oils (Rosemary, Oregano, Tea tree and Eucalyptus oil) were determined against eight pathogenic fungi: *Aspergillus flavus, A. niger, Penicillium sp., Alternaria alternata, Chaetomium sp., Gliocladium roseum*, *Trichotecium roseum* and *Phomopsis* sp. All tested fungi were previously isolated and identified in our laboratory [1] from medicinal plants (*Mentha piperita*, *Urticaria dioica*, *Calendula officinalis*) that very often could have unsufficient microbiological quality. Essential oils were commercially obtained (Frey + Lau, Germany). The microdilution method was used to establish minimal inhibitory concentrations (MIC) [2]. Standard antimycotic fluconazole was used as a positive control.

The most abundant compounds were limonene (89.9%), carvacrol (75.8%), terpinen-4-ol (40.7%) and 1,8-cineole (45.3%) detected in Eucalyptus, Oregano, Tee tree and Rosemary oil, respectively. All selected oils exhibited antifungal activity against tested pathogenic fungi. Oregano oil showed the highest antifungal activity, followed by Tea tree oil, Rosemary and Eucalyptus oil. Oregano oil inhibited the complete growth of different fungi at the concentration of 0.15 and 0.3µl/ml. Tea tree oil completely inhibited the growth of *A. niger*, *T. roseum* and *Phomopsis sp*. at the 1.25 µl/ml, while *A. flavus* was most resistant to this oil. The lowest MIC for Rosemary oil was noticed against *Phomopsis sp*. and *A. alternata* (2.5 µl/ml), while *A. flavus* and *Penicillium sp*. were the most resistant. Among these oils, Eucalyptus oil was the least active with MIC values ranged from 5-15 µl/ml. According to our results, essential oils tested may be useful as alternative antifungal agents.

Acknowledgments: The authors acknowledge their gratitude to the Ministry of Education and Science of Serbia for the financial support (Project No. 46013).

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P 178. Volatile components of *Lamium amplexicaule* and *Lamium purpureum* growing wild in Huntsville, Alabama

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Keywords: essential oil composition, α-pinene, β-pinene, germacrene D, 1-octen-3-ol

The overall goal of the project is to test the hypothesis that *Lamium* species use phytotoxic allelochemicals in interplant competition. Both *Lamium amplexicaule* and *Lamium purpureum* are non-native perennial weeds, originally from Eurasia [1]. We hypothesize that these two *Lamium* species use chemicals to compete with other plant species [2,3].

Several samples of each plant species were collected from Huntsville, Alabama. The fresh aerial parts were hydrodistilled/extracted for four hours using a Lickens-Nickerson apparatus with continuous extraction with dichloromethane to give the volatile fractions. The volatiles were analyzed using gas chromatography – mass spectrometry (GC-MS) [4].

The volatile fraction *of Lamium amplexicaule* was composed largely of germacrene D (33%), α-pinene (16%), β-pinene (11%), α-thujene (7%), (*Z*)-β-ocimene (4%), 1-octen-3-ol (4%), and germacrene B (4%). *Lamium purpureum* volatile, on the other hand, was dominated by β-pinene (16%), α-pinene (15%), 1-octen-3-ol (15%), germacrene D (15%), β-ylangene (4%), β-elemene (4%), and (*E*)-phytol (4%).

The high concentrations of monoterpene and sesquiterpene hydrocarbons are probably responsible for the allelopathic effects of the *Lamium* species [5].

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P 179. Essential oil of peppermint grown in the presence of methanolic leaf extract *Leonurus sibiricus* L.

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Keywords**:** essential oil; *Mentha piperita*; terpenes

Peppermint (*Mentha piperita* L.) is an important and commonly used as flavoring agent world-wide. Leaves of Mentha species are used as condiments, and essential oils of these plants, are used as flavorings for foods and beverages, fragrances, and fungicides or insecticides in many pharmaceutical and industrial products [1, 2]. This work evaluated the allelopathic activity of different concentrations of a methanolic extract of *Leonurus sibiricus* L. leaves in the chemical compositions of volatile oil of *M. piperita*. The plants were cultivated using complete Hoagland and Arnon [3] n^{o} 2 nutrient solution. The control (0 mg L^{-1}) contained only the complete nutrient solution in the absence of the methanolic extract from *L. sibiricus* leaves; the treatments were 25, 50 and 100 mg L^{-1} of methanolic extract of *L. sibiricus* leaves in complete nutrient solution. Aerial part of *M. piperita* were collected at 77, 94, 108 and 120 days after transplanting (DAT) and subjected to hydrodistillation in a Clevenger-type apparatus. The composition of oils was analyzed in a gas chromatograph (GC/MS). Principal Component Analysis (PCA) was applied to substances, limonene, pulegone, menthone, menthofuran, menthol and menthyl acetate. The results revealed that at the beginning of the developmental cycle plants *M. piperita* showed a highest correlation with precursors of the metabolic pathway, such as limonene, pulegone and menthone, regardless of the presence leaf extract of *L. sibiricus*. However, with the development the plants the *M. piperita* showed a higher correlation with menthol when these plants were cultivated with 25 mg L^{-1} of extract. Thus, the leaf extracts of *L. sibiricus* influenced in chemical compositions of volatile oil of *M. piperita*, because low concentrations of the extract ensured the quality of essential oil.

Acknowledgments**:** Financial support CNPq.

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P 180. Composition and antimicrobial activity of the essential oil of *Hyssopus seravschanicus* growing wild in Tajikistan

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Keywords: *Hyssopus seravschanicus*; essential oil composition; antimicrobial activity; *cis*-pinocamphone

The volatile compounds were extracted from *Hyssopus seravschanicus* Pazij, a perennial, branched semi-shrub that grows wildly near the high ridge mountainous regions of Varzob, Northern Dushanbe in Tajikistan at an altitude approximately 2500 meters above sea level. The samples were analyzed using gas chromatography-mass spectrometry (GC-MS) in order to study the composition of the essential oil of hyssop. Eighty-seven chemical components of the essential oil were found and characterized representing approximately 95.4% of the oil. The most abundant components were cis-pinocamphone (57.0-88.9%), β-pinene (0.4-6.0%), 1,8-cineole (1.8-3.6%), camphor (0.5-4.0%), and spathulenol (0.1-5.0%). An antimicrobial screening was performed on five essential oil samples against Gram-positive bacteria, Bacillus cereus (ATCC No. 14579) and Staphylococcus aureus (ATCC No. 29213) as well as Gram-negative bacteria, Pseudomonas aeruginosa (ATCC No. 27853) and Escherichia coli (ATCC No. 10798). The essential oil showed notable antimicrobial activity against Gram positive *Bacillus cereus* and *Staphylococcus aureus*. cis-Pinocamphone was the most abundant chemical component in this study and in most others. It has been reported that cis-pinocamphone has antimicrobial effects which can be attributed to the low molecular weight and lipophilic constituents of the oil. In this way, the essential oil can alter the cellular composition of bacteria and influence normal cell growth. As a result, the essential oil of hyssop can be cultivated more abundantly and utilized for its antimicrobial activity.

P 181. Neutralizing effects of two *Nectandra* species essential oils and extracts against *Bothrops neuwiedi* snake venom

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Keywords**:** *Nectandra* sp; snake venom; anti-snake venom; oil composition; chemotaxonomy

Venomous snakebite remains an important medical problem in both developing and developed countries. Through serum therapy, neutralization of the systemic toxic effects is usually reached, but that of local tissue injury is not. *B. neuwiedi diporus* (yarará chica) is the species responsible for the major proportion of the viper accidents that occur in Argentina. Its venom is a complex mixture of hemorrhagic, clot-forming, anticoagulant, proteolytic, neurotoxic and edema-inducing toxins, among others. Although bothropic antiserum therapy has been shown to be effective against the systemic effects of envenomation, hitherto no antidote exists to combat the local damage.

The present study evaluated the potential hemolytic and anti-hemorrhagic effect of the aqueous and alcoholic extracts, and essential oil obtained from *Nectandra angustifolia* and *N. megapotamica* against *B. neuwiedi diporus* snake venom. Additionally, and in order to better characterize the chemical taxonomy of the population studied, we report the chemical composition, including the enantiomeric distribution of different mono and sesquiterpenes, of both *Nectandra* species essential oils. Venom was analyzed by SDS-PAGE [1]. Neutralization of *B. neuwiedi* venom enzymes by plant extracts and essential oils was measured using an indirect hemolytic assay on agarose-erythrocyte–egg yolk gel plate to define the minimum indirect hemolytic dose. Neutralization of coagulant activity was also evaluated. The components of the oil were analyzed as previously reported [2]. Comparison of fragmentation patterns in the mass spectra with those stored on the GC-MS database was also performed. Enantiomeric ratios of selected oil components were obtained by multidimensional GC using the experimental conditions previously described [2]. From these investigations, it may be concluded that, even when oils were active, the ethanol extract of *N. angustifolia* leaves showed a promising venom inhibition effect against *B. neuwiedi*.

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P 182. Potential use in cosmetic formulations and antimicrobial activity of essential oil of *Ocotea pulchella* (Nees) Mez (Lauraceae) and *Xylopia aromatica* (Lam.) Annonaceae

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Keywords**:** essential oil, antimicrobial activity, pharmaceutical and cosmetic formulations

The aroma and fragrance industry is a billion-dollar world market which grows annually. Essential oils comprise the majority of compounds used by these industries. Each component of an essential oil has a characteristic profile and each aroma and flavor is one combination of its representatives´ profiles. The essential oils have been showing effective in the control of several microorganisms (Santos *et al*.,2008). The main objective of this work was to evaluate the antimicrobial activity and the technological characteristics of the oil in cosmetics formulations. Althought, the chemical composition will be performed can be verified anothers study of this subject by some authors (Botega et al. 1993. Potenza, 1999a, 2006, Pontes et al. 2007and Raggi, 2008). The aerial parts of the plant were collected in the proximities of the city of Divinópolis - Minas Gerais State, area that prevails ecosystems of cerrado. Essential oil of leaves was extracted by hydrodestillation (Clevenger apparatus). Aliquots of those oils were submitted the tests of antimicrobial activity being used *Brain* Heart Infusion Agar (BHI) medium for *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosas* and *Saboraund* medium for *Cândida albicans*. The tested formulations were no-ionic cream-base and antimicrobial ointment. The obtained oil of *Ocotea pulchella* had antimicrobial activity against *C. albicans*, *B. cereus* and P*. aeruginosa*. The essential oil of *Xylopia aromatica* it was active against *S. aureus* and *C. albicans*. The essential oil of Ocotea *pulchella* it was classified as a transparent liquid to milky appearance. For *Xylopia aromatica* the essential oil shows a limpid and slightly astringency liquid. There was verified compatibility of the oils with the different ones tested formulations. These partial results stimulate searches for new compounds with potential cosmetics and pharmaceutical use.

Acknowledgement – FAPEMIG, UNIFENAS/ Divinópolis- MG, IFMG/ Bambuí

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P 183. Antimicrobial activity of the essential oil extracted from *Thymus vulgaris, Ocimum basilicum* (Lamiaceae) and *Piper aduncum* (Piperaceae)

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Keywords: Essential oil, antimicrobial activity, Food-borne illness

Food-borne illness is a major international problem and an important cause of reduced economic growth. The contamination of the food supply with the pathogens and its persistence, growth, multiplication and/or toxin production has emerged as an important public health. *Piper aduncum* (Piperaceae), *Thymus vulgaris* and *Ocimum basilicum* (Lamiaceae) are seasoning products obtained from plants widely used in foods. Previous study were performed of the effect of essential oil composition and bioactivity of these plants related to toxicological and antimicrobial action (Sousa et al., 2008; Dandlen et al., 2011; Silva et al., 2012). However, there are few study of the synergism between essential oils on the antimicrobial activity against microorganism of the food-borne illness. The aim of this study was to evaluate *in vitro* activity of the essential oil extracted from *Thymus vulgaris*, *Ocimum basilicum* and *Piper aduncum* against *Salmonella enteretidis, Staphylococcus aureus* and *Escherichia coli*. The essential oil of this plants was extracted by hydrodestillation (Clevenger apparatus) about 2 hour and was analyzed by GC and GC/MS (SHIMADZU). The oils were mixed in different proportions and the antimicrobial activity were performed with isolated oils and the mixed oils from different proportions using 10µL of the extracts in Agar Muller Hinton medium with 10^8 UFC mL^{-1} of each microorganism. The major components of the essential oil were tymol, carvracol, o-cimeno, γ-terpineno and linalol (*Thymus vulgaris*). Linalol, 1,8-cineol and eugenol (*Ocimum basilicum*) and linalol, (E) nerolidol and cariofilene oxyde (*Piper aduncum*). The essentials oil can be able to inhibit growth of microorganism caused of food-borne illness. The essential oil from this plants showed antimicrobial activity against *E. coli, Salmonella enteritidis* e *S. aureus.* The essential oil of *T. vulgaris* was the most active. Concerning the mixed essential oil were verified antagonist effect in some mixture and synergistic effect in others. The results of this study showed that these seasoning plants have an important activity isolated and/or in mixture, could be source for the discovery of new antimicrobial products.

Acknowledgement – FAPEMIG, UFLA/ Lavras- MG and IFMG/ Bambuí

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P 184. The use of vibrational spectroscopy to rapidly assess the quality of tea tree oil (*Melaleuca alternifolia*)

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Keywords: Tea tree oil, GC/MS, spectroscopy, NIR, MIR, correlation

Tea tree oil is a native Australian essential oil obtained mainly from the leaves of *Melaleuca alternifolia* (Myrtaceae). The oil is a common ingredient in many commercial detergents, cosmetics and herbal remedies. Tea tree oil is also incorporated as active ingredient in various topical formulations used to treat skin infections. The quality of the oil is usually determined by GC/MS based on the major constituents (terpinen-4-ol, γ-terpinene, α-terpinene, α-terpineol, terpinolene, limonene and 1,8-cineole). This analytical method is however time consuming and expensive. The need to identify a fast, accurate alternative method for routine analysis is thus important.

The study was aimed at investigating the possibility of using vibrational spectroscopy in combination with chemometric data analyses as alternative tool for the quality assessment of tea tree oil.

Samples of tea tree oil (n = 60) obtained from different suppliers and natural populations were analysed using GC/MS as reference method. For spectroscopy analyses, Fourier transform NIR spectra of each oil was recorded on a NIRFlex N500 liquid cell spectrometer. The oil spectra were collected in the transmittance mode between the wavenumbers 10,000 and 4000 cm^{-1}. Fourier transform MIR spectra of each oil was recorded in the wavenumber range 4000–550 cm^{-1} on an alpha-P Bruker spectrometer. The spectral data combined with the reference data were analysed using chemometric analysis software (Simca P+, version 12.0).

The partial least squares (PLS) regression method was used to develop calibration models with good correlation coefficients (R^2) based on both MIR and NIR spectra for terpinen-4-ol (MIR = 0.91; NIR = 0.78), terpinolene (MIR = 0.90; NIR = 0.80), γ-terpinene (MIR = 0.90; NIR = 0.89), limonene (MIR = 0.89; NIR = 0.88) and a-terpineol (MIR = 0.85; NIR = 0.90). 1,8-Cineole had the highest R^2 of greater than 0.96 for MIR and NIR. Generally, the error parameters (RMSEE and RMSEP) after external validation (n = 18) were low (< 3%). The lower RMSEE and RMSEP values recorded demonstrated accurate predictions of compounds investigated.

The results have shown the potential of MIR and NIR spectroscopy as alternative methods to GC/MS for quality assessment of tea tree oil.

Acknowledgements**:** The financial support of the National Research Foundation (South Africa) and Tshwane University of Technology is gratefully acknowledged.

P 185. The phytotoxic activity of the secondary metabolite eugenol

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Keywords: Eugenol; phytotoxicity; microtubules; *Arabidopsis*; essential oil; weed management

Eugenol is a natural allylbenzenic compound commonly found in essential oils of plants of the families Lauraceae, Mirtaceae and Miristicaceae [1]. Although the antiseptic, anesthetic and hepatotoxic activity of eugenol has been already demonstrated [2, 3, 4], its phytotoxic activity has been poorly investigated. The phytotoxicity of this essential oil was analyzed on *Arabidopsis thaliana* (L.) Col-0 seedlings at 50, 100, 200, 400, 800 and 1200 µM eugenol concentrations. The results showed a strong growth inhibition with the IC_{50} (the concentration required for 50% inhibition) at 246 µM. The root structure, thickness and presence of root hairs was studied under a magnifier. Eugenol-treated roots showed left-handed growth, indicative of microtubule alteration [5, 6], which was confirmed by immunofluorescence. Ultra-structural analysis was performed by electron microscopy on 7 and 14 days eugenol-treated roots.The strong root growth inhibition, the alteration of microtubules and the changes at cellular level confirmed the high phytotoxicity of eugenol, a strong candidate for weed management.

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P 186. Evaluation anti-*Rhizopus oryzae* activity and synergistic effect of *Hyptis pectinata* essential oil and its bioactive compounds

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Keywords**:** *Hyptis pectinata*; calamusenone; calamusenone oxidized analogous; dehydroaromadendrene; *Rhizopus oryzae*

In the last few years, the number of cases of zygomycosis has increased, especially among immunocompromised patients, although several authors have also reported infections in patients with unknown underlying conditions. Zygomycetes are a heterogeneous group of fungi with a wide antifungal susceptibility profile. Amphotericin B is the agent of choice to treat zygomycosis. However, its toxicity remains a problem and therefore alternative therapies [1]. Previous works described antimicrobial activity of $CHCl_3$ extract and also essential oil (EO) from leaves of *H. pectinata*, and only pyrones, and pectinolides A-C (1-3) and H, were isolated of $CHCl_3$ extract, and their anti-staphylococcal activity was confirmed [2, 3, 4, 5]. No antimicrobial investigation with EO compounds was made to elucidate its bioactivity. So the anti-*R. oryzae* activity of SAM002 genotype of *H. pectinata* EO was evaluated and several bioactive compounds were isolated and tested. The TLC and bioautography showed that three substances were active. After purification of the three substances and GC-MS analysis, they were identified as calamusenone, calamusenone oxidized analogous (COA), and dehydroaromadendrene (DHAD) [5]. The minimal inhibitory concentration (MIC) and minimal microbicidal concentration (MMC) of all these substances were also determined, confirming that *H. pectinata* EO and its purified substances exhibited fungicidal activity with MICs ranging from 375 to 1250 µg/mL [6]. In combination with amphotericin B (AMB) plus essential oil or calamusenone presented additive activity although a synergistic effect was detected when AMB was combined with the COA e DHAD [7]. Once the therapeutic dose of AMB used against zygomycetes is extremely toxic, our results demonstrate that both the oil and its bioactive are promising candidates when it associated with AMB, minimizing side effects in patients with zygomycosis.

Acknowledgement**:** CNPq, CAPES e FAPERJ.

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P 187. Volatile compounds are involved in the phytotoxicity of *Vicia faba* used as green manure

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Keywords: Faba bean; essential oil; in vitro volatile assay; bioactivity; weed control

Cover crops grown as green manure are keystone in sustainable management of agricultural systems. Some of the crops used traditionally as green manure are known to possess allelopathic activity so they can provide natural weed control, in addition to their environmental services such as soil conservation and fertility building. Faba bean (*Vicia faba* L.) is a legume crop usually included in rotations with maize in temperate areas. Our previous *in vitro*, greenhouse and field studies revealed strong phytotoxic effects of *V. faba* on some problematic weeds (Álvarez-Iglesias *et al*. submitted). Nonetheless, references about the bioactivity of the volatiles emitted by faba bean on other plants are absent in literature. The aim of our study was to determine if the volatile compounds from complete blossom aerial parts of *V. faba*, as used for green manure, could take part in the observed global phytotoxic effects, as well as to determine the composition of its essential oil.

Biological activity of fresh blossom aerial parts of *V. faba* was evaluated against several species by placing fresh material at a dose of 2 g dw L^{-1} air in sealed containers together with seeds and seedlings of some target species [1]. Seedling growth of the model species *Lactuca sativa* and the weeds *Amaranthus retroflexus* and *Digitaria sanguinalis* was reduced over 50 %; moreover, germination of *D. sanguinalis* was strongly inhibited.

In order to elucidate which compounds were potentially responsible of these effects, the essential oil from blossom aerial parts of *V. faba* was obtained by continuous water distillation / solvent extraction using a Likens-Nickerson type apparatus [2]. Analysis was carried out by combination of gas chromatography (GC) and gas chromatography-mass spectroscopy (GC/MS). Aliphatic compounds and oxygenated monoterpenes represented the main fractions. Several compounds with known biological activities, such as terpinen-4-ol, geraniol, and linalool were identified. These compounds, acting individually or by synergistic interactions [3], could be partially responsible of the phytotoxic activity of *V. faba* against weeds observed in our previous investigations.

Acknowledgements**:** Thanks are due to Fundação para a Ciência e Tecnologia (FCT), POCI 2010/FEDER for financial support, as well as to the FPU grant from the Spanish Ministry of Education AP2008-03101.

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P 188. Fast-RP-HPLC/PDA analysis of oxygen heterocyclic compounds in citrus essential oils

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Keywords: coumarins, furocoumarins, fast-RP-HPLC, qualitative and quantitative analysis.

Oxygen heterocyclic components present in the non volatile fraction of cold pressed *Citrus* essential oils are mainly represented by coumarins, psoralens and polymethoxylated flavones.

An important role in the characterization of *Citrus* oils has been attributed to these components. In fact, qualitative and quantitative composition of the fraction is characteristic of each oil. Moreover, many pharmacological and toxicological activities have been demonstrated for most of them. These components exhibit strong absorption in the ultraviolet region (λ_{max} 315 nm).

Due to their non volatile nature, liquid chromatography has been considered the ideal technique for their analysis. Most of the methods found in literature propose the RP-HPLC analysis of oxygen heterocyclic compounds in an analysis time higher than 40 minutes [1,2]. The aim of this work is to reduce the total analysis time under 15 minutes.

One of the two methods investigated in this work allows a total baseline separation and a correct quantification of all the oxygen heterocyclic compounds within 10 minutes with an Ascentis Express C18 column (50 x 4.6 mm x 2.7 um). The second one permits a good baseline separation of coumarins, furocoumarins and polymethoxyflavones within a 3 minutes analysis time, with the same column and under the same chromatographic conditions.

Both the two methods present numerous advantages: first, the total analysis time is greatly reduced thus allowing the characterization of a larger number of sample in the same day. Moreover, a conventional HPLC instrumentation can be used along with a lower amount of mobile phase with respect to conventional analysis.

Acknowledgments**:** The Project was funded by the “Italian Ministry for the University and Research (MIUR)” within the National Operative Project “Hi-Life Health Products from the industry of foods”. Project ID: PON01_01499.

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P 189. Exploiting the alternative selectivity of a novel medium-polar ionic liquid stationary phase in the gas chromatographic analysis of essential oils

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Keywords: ionic liquid stationary phase, terpene compounds, peppermint essential oil

Recently a new class of compounds has received increasing attention for their exploitation as GC stationary phases, namely room temperature ionic liquids (ILs), that have recently demonstrated great suitability in the analysis of essential oils [1] being capable of unravelling coelutions that could arise on the most widely used columns in such an application. Essential oils represent a valuable product, widely employed in several fields such as the flavor, fragrance and food industries. The volatile fraction of essential oils is usually rather complex, since it can be composed of hundreds of compounds, mainly monoterpenes, sesquiterpenes and their oxygenated counterparts, which are difficulty separated in a single GC run. For such a reason, it is common, both for qualitative and quantitative purposes, to analyze essential oils on both apolar and polar columns [2].

In this work a novel IL GC column, recently commercially available, and possessing an overall polarity close to that of polyethylene glycol phase [3], has been evaluated towards the separation of terpene compounds, in terms of peak symmetry and resolution of target volatile analytes and, furthermore, it has been applied to the analysis of a peppermint essential oil; the results have been compared to those attained on the polyethylene glycol phase demonstrating the great suitability of this novel IL GC stationary phase in this kind of application, since the overall separation performance and, thus, selectivity of the IL column was better than that obtained on the most widely used polar column.

Acknowledgements: The Project was funded by the “Italian Ministry for the University and Research (MIUR)” within the National Operative Project “Hi-Life Health Products from the industry of foods”. Project ID: PON01_01499.

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P 190. Biological investigations by rapid TLC methods on essential oils from *Hua gabonii* grown in Gabon

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Keywords: *Hua gabonii*, essential oils, sulfide compounds, HPTLC, DPPH method, acetylcholinesterase essay

The plant *Hua gabonii* Pierre ex De Willd. (Huaceae), also called “garlic tree” for the characteristic garlic aroma of different parts of the plant, is widespread in dense tropical and humid forest, especially in Gabon, Cameroon and Congo [1]. In Cameroon, the seeds are used with other spices for the flavoring of various local dishes as alternative to common garlic (*Allium sativum*) [2]; in Gabon, the bark is used in alimentary for the same properties; seeds and bark are also traditionally utilized as infusions against colds or in fumigation against rheumatism and headaches [3].

Leaves, barks and roots collected on a specimen of *Hua gabonii* grown in Franceville (Gabon) were submitted to hydrodistillation to give essential oils with 0.21%, 0.37% and 0.39 % yields respectively. All samples presented characteristic odors reminiscent of sulfide compounds.

These essential oils were screened by rapid TLC according to two different methods: a bioautographic enzyme assay for the screening of acetylcholinesterase inhibition [4], involved in Alzeimer’s disease, as well as a DPPH assay, for their antiradical properties evaluation.

The three samples showed some faint acetylcholinesterase (Rf = 0.52, 0.73) and DPPH (Rf = 0.71) active zones.

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P 191. Use of computer simulation in steam distillation of essential oils – novel design strategies for enhanced isolation of volatile plant extractives

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Keywords: bubble point, essential oils, simulation, stripping.

Computer simulation is widely used in all the different areas the chemical industry such as refining and petrochemicals, pharmaceuticals, agrochemicals, and specialty chemicals. However its application in small to medium scale industries such as steam distillation of essential oils is limited. This limitation of the use of computer simulators or absence of it in the distillation the essential oils is mainly due to the fact that that this industry often operates as a cottage industry with expertise transferred as an art as well as limited resources such computing resources and expertise in advanced process design technology. The advent of the computer (simulation) applications in the design and analysis of chemical industry can also be applied to essential oils production. This application will result in optimized, cleaner and more economic operations. The work in this paper seeks to outline a methodical approach and tools that can be used based on Aspen Hysys computer simulation platform. The results are an improved process that results in a seventh of the energy use and a corresponding reduction in equipment size. This equipment includes condensers and all other equipment downstream to the packed bed or still to include piping. The reduction in hydraulic loading of the process units means a reduction in capital cost as well as operational costs. Though the reduction is not linear for all process parameters, the utilities consumption are reduced by about seven times that of the current process units. One of the key findings from this work is steam distillation units as currently operated operate at the lower end of the process window. That uses more energy and more water in the distillate. The increased aqueous fraction in the distillate most likely increases the solubility of the polar components. This changes the quality of the final oil product. If these polar components make up the top notes constituents, then they are lost in the aqueous fraction. The work improves the process by eliminating such losses and improving the utilities consumption and capital outlay.

P 192. On the study of some essential oils of Lauraceae family from Vietnam

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Keywords: Lauraceae, Essential oils, Terpenoids

We have developed a systematic approach at the analysis of volatile compounds of Vietnamese [1]. The essential oils obtained by hydrodistillation from seven plants of Lauraceae family were analysed by GC and GC/MS. The leaf oil of *Phoebe angustifolia* Meisn., had spathulenol (17.0%), 1,2-Benzenedicarboxylic acid (13.0%) and sabinene (6.4%)in abundance; while the fruit consist mainly of 1,2-Benzenedicarboxylic acid (15.0%) and spathulenol/ cis-α-bisabolene (ca. 5.1%). The main constituents of *Machilus velutina* Champ. ex Benth., were (*E*)-β-ocimene (9.5%), (*Z*)-β-ocimene (8.2%), bicycloelemene (7.1%), germacrene D (6.8%) and *allo*-ocimene (6.4%). However, (*E*)-β-ocimene (85.6%) was the most singly abundant component of *Neolitsea polycarpa* Liou.

The main compounds of *Cinnamomum sericans* Hance, were the sesquiterpenes spathulenol (14.5%), caryophyllene oxide (9.3%), α-pinene (9.3%), sabinene (8.0%) and β-caryophyllene (7.1%). The monoterpenes- *ρ*-cymene (15.6%), limonene (13.9%) and α-phellandrene (9.2%)- were the dominant class of compounds in *Cinnamomum durifolium* Kosterm. *Cinnamomum magnificum* Kosterm., was devoid of monoterpenes, while the major sesquiterpens were bicyclogermacrene (33.9%), β-caryophyllene (25.5%), bicycloelemene (7.2%) and caryophyllene oxide (7.5%). β-Caryophyllene (35.9%), caryophyllene oxide (12.6%) and spathulenol (5.2%) were the dominant compounds of Reinw. ex Blume. The low content of (*E*)-cinnamaldehyde in the *Cinnamomum* species is typical for majority of other species already reported from Vietnam [2-3].

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P 193. Chemotaxonomy of essential oils of some *Cassia* species from Nigeria

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Keywords**:** Fabaceae, Essential oils; Terpenoids; Chemical markers

In continuation of our study on the chemical composition of the volatile oils obtained from the genus *Cassia* growing in Nigeria [1], we report herein the compounds identified from four *Cassia* species (Fabaceae family). The oils were by obtained hydrodistillation of the air-dried leaves samples and then analyzed by GC and GC/MS. The main compounds of *Cassia lutea* L. were hexahydrofarnesylacetone (20.0-22.5%), *(E)*-geranyl acetone (11.5-11.8%), farnesylacetone (4.8-5.8%), *(E)*-2-decenal (4.3-4.5%). There are significant amounts of *(Z)*-nerolidol (2.6-2.9%), *(E)*-α-ionone (2.1 and 2.5%) and 6-methyl-5-hepten-2-one (2.4-2.8%). The oil of *Cassia ferruginea* (Schrader) DC consisted mainly of farnesylacetone (4.3%), 1-octen-3-ol (5.5%), pentadecanal (6.1%), phytol (5.8%), *(E)*-α-ionone (9.5%) and *(E)*-geranyl acetone (13.7%). We have identified *(E)*-geranyl acetone (5.8%), 1-octen-3-ol (5.8%), linalool (7.8%), *iso*-italicene (15.4%) and *(E)*-β-damascenone (11.0%) as the abundant constituents in *Cassia siemens* L. On the other hand, the oxygenated compounds dominated in the oil of *Cassia occidentalis* L. These are *(E)*-geranyl acetone (8.0%), hexahydrofarnesylacetone (24.0%) and *(E)*-phytol acetate (40.7%). All the studied oil samples contained *(E)*-β-ionone (1.1-3.7%), *(E)*-geranyl acetone (5.8-13.7%), *(E)*-β-damascenone (0.2-11.0%), hexahydrofarnesylacetone (1.2-22.5%) and farnesylacetone (2.8-5.8%). In addition, the oil samples except *C. siemens* had phytol (0.4-5.8%). These compounds may serves as chemical markers of Nigerian grown *Cassia* species.

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P 194. Yield and composition of the essential oil of *Origanum vulgare* in function of the different types of fertilizer, systems and seasons of planting

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Keywords: *Origanum vulgare* L., aromatic plant, fertilization, greenhouse, seasons

Origanum vulgare L. better known as marjoram or oregano is a herbaceous plant belonging to the family Lamiaceae. It is native to the Euro-Siberian and Irano-Siberian, which determines long-range chemical and morphological diversity. The content and composition of essential oil of herbs like oregano, depends on different factors such as climate, geographical origin, harvest season, fertilizer and mineral nutrition can significantly affect the production and oil quality. The objective of the work was to analyze the production of biomass, content, yield and composition of the essential oil of oregano under different systems and seasons of planting. The experimental design used in both experiments was a randomized block design in a factorial 2 x 2 x 2 factorial with five replications, two tillage systems (greenhouse and field), two types of fertilizers (mineral and organic) and two growing seasons (autumn and spring). The field plots and greenhouse were three rows with three meters in length and the useful plot of each replicate consisting of seven plants in the central line. The row spacings were 40 cm and 35 cm between plants. The essential oil was extracted by hydrodistillation in a modified Clevenger apparatus. The chemical composition was analyzed by GC/MS. The essential oil presented the same compounds for all treatments; however, the relative proportion of some chemical constituents was altered according to the treatment. Carvacrol, terpinene, ortho-cymene were the major constituents. Both the seasons and the plantation in the field had favored greater essential oil yield and production of carvacrol. The biomass production was better at the hot and humid season. At the season spring/summer, the relatives percentages of carvacrol and ortocimeno had been lesser in the field. But, for terpinene, the percentage was bigger in the field using chemical or organic fertilizer. At the season autumn winter, the relative percentage of the orto-cimene component was bigger in the field, but for carvacrol and gamma-terpinene the percentages had been bigger in the greenhouse.

Acknowledgements: FAPEMIG and CNPq for the support to this work.

P 195. Tomato aroma volatiles measured by PTR-MS following artificial chewing

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Keywords**:** Tomato; PTR-MS; SPME-GC-MS; flavor

The availability of rapid and accurate methods to assess food flavor is of utmost importance to support quality control measures.

The perception of food flavor and aroma is a complicated process since flavor compounds are released from the food matrix during eating and then transported to the receptors in the mouth and nose. *In vivo* measurements of volatile patterns are therefore most desirable since they reflect the volatile profile reaching the olfactory receptors and therefore they relate better with sensory perception. However, due to the high variability generally observed in oral physiology parameters, flavor release kinetics and sensitivity to flavor stimuli, *in vivo* measurements are not suitable as standard and repeatable method.

The aim of this work was to develop a fast and reliable system to study the volatile aroma profile as close as possible to human perception, using tomatoes as model fruit.

The volatiles emitted by fruit of nine tomato varieties (at ripe stage) were analyzed by using two solvent-free headspace methods: solid-phase micro extraction/gas chromatography-mass spectrometry (SPME/GC-MS) and proton transfer reaction / mass spectronomy (PTR-MS) coupled with an artificial chewing device. In addition, volatiles were analysed from human mouth following natural chewing.

Parallel with the chemical analysis, a quantitative descriptive analysis (QDA) by trained panellists was carried out to qualitatively characterize the flavor of the nine tomato varieties.

Volatile patterns measused after artificial chewing showed good correlation with patterns produced during chewing in the human mouth. Multivariate statistical analysis (principle component, cluster and partial last square regression) of the PTR-MS results allow an unambiguous fingerprinting separation between different tomato varieties. A good correlation with scored sensory parameters, particularly for “tomato fragrance” and “tomato flavour” was shown.

In this study, it is shown that PTR-MS coupled with an artificial chewing device is a suitable method to monitor at high sensitivity the emission of volatiles determining the tomato aroma profile.

P 196. Conformational analysis of the predominant antistaphylococcal sesquiterpene lactones from *Inula helenium* L. root essential oil

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Keywords: alantolactone; isoalantolactone; diplophyllin; *Inula helenium* L. (Asteraceae); conformational analysis

It was recently shown that eudesmane-type sesquiterpene lactones: alantolactone (**1**), isoalantolactone (**2**) and diplophyllin (**3**), the dominant constituents of *Inula helenium* L. (Asteraceae) root essential oil, possess a very potent antistaphylococcal activity, with obvious membrane-damaging effects [1]. Although **1-3** are isomers without any essential structural difference, the activity of diplophyllin seems to be significantly higher than that of the other two isomers [1]. Alongside with the presence of some specific functional groups, three-dimensional structure (conformation) can play a significant role when speaking of biological/pharmacological activity of a certain compound [2]. Having this in mind, we decided to compute (MM+ and MMFF94 molecular mechanics and AM1 and PM3 semi-empirical methods; semi/empirical computations were performed with Polak-Ribiere (conjugate gradient) minimization method and with an energy convergence criterion of 0.01 kJ/mol) energetically favorable conformations of the dominant biologically active *I. helenium* oil constituents, and to seek for possible differences between them. Energetically favorable conformations of **1**-**3** were additionally described in the terms of selected 2D and 3D molecular descriptors (topological indices, conformational, molecular surface and partitioning descriptors). According to the obtained results, alantolactone and isoalantolactone mainly adopt “U”-shaped conformations (>99%, calculated at 298 K; “closed” geometries), with the carbonyl moiety being rather sterically hindered from one side. Contrary to that, in diplophyllin, the cyclohexene and lactone rings are mutually oriented in such a way as to give the overall “S”-shape to the molecule (“open” geometry). These conformational differences (that may influence optimal placement of **1**-**3** in the binding region of the target biomolecule(s)) could be, at least partially, responsible for the higher observed antistaphylococcal activity of diplophyllin in comparison to the other two isomeric lactones.

Acknowledgments**.** This work was funded by the Ministry of Education and Science of Serbia (Project 172061). PB was supported by UNESCO-L´OREAL National Fellowships Programme for Young Women in Life Sciences.

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P 197. What is nanjamonjagoke (*Takakia* sp.)? Taxonomical position of *T. lepidozioides* and *T. ceratophylla*

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Keywords: liverwort; *Takakia*; sesquiterpene lactones; coumarin; chemosystematics

Takakia lepidozioides was first discovered in Japan. Hattori and Inoue [1] classified it into the Marchantiophyta (liverwort) although Bryologists confused to where this plant should be classified; to algae, bryophyte or fern. In 1979 we concluded that *T. lepidozioides* could be classified into liverwort because of the presence of sesquiterpenoe lactone, diplophyllolide [2]. However, since the forms of sporophytes of *T. ceratophylla* later discovered was similar to the moss, *Andereaobryum macrosporum, Takakia* species was reclassified into the moss [3].To reconfirm the above new discussion, a large amount of *Takakia lepidozioides* (1.3g) was analyzed by TLC, GC/MS and NMR to identify the presence of diplophyllolides, coumarin (major), the strong scent of this species, dihydrocoumarin, 1,4-benzoquinone, hydroquinone, dihydrobenzofurane,eudesm-4,11-dine and alpha-asarone (**9**). These compounds, especially, sesquiterpene eudesmanes, are one of the most important chemical markers of liverworts. In conclusion, *T. lepidozioides* is chemically more close to the Marchantiophyta than Bryophyta. *T. ceratphylla* shows the chemical character both of liverwort and moss because it produces anastreptene (**10**), a significant chemical indicator of many liverworts and hopane-type triterpenoids of mosses.

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P 198. Biotransformation of *p*-cymene by *Neurospora crassa* and *Aspergillus niger*

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Keywords: biotransformation; *p*-cymene; *Neurospora crassa*; *Aspergillus niger*

In the continuing studies on the microbial transformation of terpenoids and aromatic compounds[1,2], we report the biotransformation of *p*-cymene (**1**) , a naturally occurring aromatic organic compound and a constituent of the essential oil of cumin and thyme, by 2 kinds of *Neurospora carassa* strain F40 and F42 and *Aspergillus niger* TBUYN-2.*N. crassa* was cultivated in the czapek-pepton medium at 30°C for 3 days. After full growth of microorganisms **1** was added to the cultured medium of microorganisms everyday at ca. 20mg in the 200ml cultured medium to 29 days. Totally 12.48g (2.08%) of substrate was added to the 6 flask. The ether extract of medium is 373.5mg (85.5%), ethyl acetate extract of medium 20.1mg (4.6%), ether extract of mycelium 21.4mg (4.9%) and ethyl acetate of mycelium 21.8 (5.0%). Each cultured broth was filtrated, and the resulting broth and the mycelium were extracted with ether, and then with ethyl acetate. These extracts were chromatographed on silica gel and each fraction was applied to GC-MS. Compounds shown in Fig.1 are identified by GC-MS with comparison of authentic compounds.The identification of each metabolite and the metabolic pathway of **1** will be discussed.

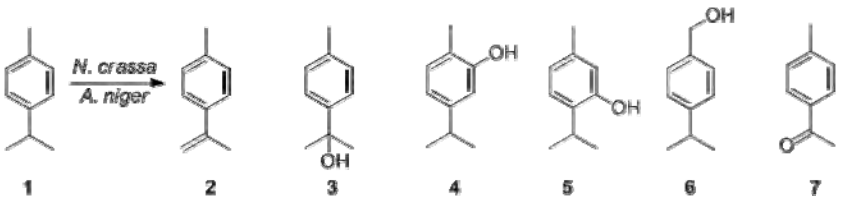

Fig.1. The biotransformation of **1** by *N. crassa* and *A. niger* TBUYN-2.

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P 199. Chemical composition and antibacterial activity of the essential oils isolated from leaves and fruits of *Peucedanum austriacum* (Jacq.) W. D. J. Koch

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Keywords: *Peucedanum austriacum* (Jacq.) W. D. J. Koch; Umbelliferae, essential oil composition; *β*-phellandrene; caryophyllene oxide; antibacterial activity

Chemical composition and antibacterial activity of the essential oils isolated from fruits and leaves of *Peucedanum austriacum* (Jacq.) W. D. J. Koch were determined in order to define their medical potential. An analyses of leaves and fruits essential oils by GC and GC/MS, resulted in identification of 141 different components, representing 93.6% and 96.1% of the total oils. The most abundant class of compounds in fruits essential oil were monoterpenoids (64.0%), while in the leaves essential oil were sesquiterpenoids (82.0%). The major contributors of fruits essential oil were *β*-phellandrene (45.2%) and *α*-pinene (10.1%). Major constituents of leaves essential oil were caryophyllene oxide (23.1%), germacrene D (12.2%) and (*E*)-caryophyllene (10.2%). Minimal inhibitory (MIC) and minimal bactericidal concentrations (MBC) were determined by performing broth microdilution assay against two Gram-positive bacteria (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and three Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella typhimurium* ATCC 14028). In the antibacterial activity assay, both samples showed moderate activity against *Bacillus subtilis* and *Staphylococcus aureus* with inhibitory effect at 0.625 and 1.25 mg mL^{-1}, weak activity against *Pseudomonas aeruginosa* with inhibitory effect at 5.00 mg mL^{-1} and no effect on the growth of bacteria *Escherichia coli* and *Salmonella typhimurium*. The results showed that essential oils composition of *P. austriacum* leaves and fruits differed considerably. Tested oils were proven to be selective antibacterial agents.

Acknowledgements: The authors are grateful to the Serbian Ministry of Education and Science Ministry for financial support (Project 172047).

P 200. The composition of *Hypericum umbellatum* A. Kern. essential oil from Serbia

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Keywords: *Hypericum umbellatum*, Hypericaceae, essential oil composition

Hypericum L. (Guttiferae/Clusiaceae/Hypericaceae) is a genus represented by more than 400 species, widespread in warm-temperate areas throughout the world, as well as on the Balkan Peninsula [1]. Plants of the genus *Hypericum* have traditionally been used as highly esteemed medicinal plants particularly *H. perforatum* (St. John's wort) [2]. The aim of this study was to perform a detailed analysis of the *H. umbellatum* essential oil originating from Serbia, and establish chemotaxonomic correlation within the members of the section *Drosocarpium* Spach (*H. umbellatum* is a member of this section). The essential oil of fresh aerial parts of *H. umbellatum* obtained by hydrodistillation was analyzed by GC and GC/MS. One hundred and twenty-six identified compounds accounted for 93.9% of the total oil. The main oil components were: germacrene D (6.1%), (*E*)-nerolidol (4.4%), n-nonane (4.0%), (*E*)-caryophyllene (3.0%) and caryophyllene oxide (3.0%). Dominant class of compounds - terpenoids (75.9%), was unevenly distributed between mono- and sesquiterpenoids (14.9% and 59.2%, respectively). Monoterpenoids were mostly made of hydrocarbons (12.3 %), opposed to sesquiterpenoids made of relatively comparable amounts of hydrocarbons (27.3%) and oxygenated derivatives (31.9%). Non-terpenoid compounds (17.0%) consisted mainly of n-alkanes (9.6%). To the best of our knowledge the essential oil of *H. umbellatum* has not been studied previously. The results of this study showed that chemical composition of *H. umbellatum* oil is in agreement with previous reports on the species belonging to the section *Drosocarpium* Spach.

Acknowledgments*:* This work was funded by the Ministry of Education and Science of Serbia (Project No. 172047).

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P 201. Enantioselective GC-MS analysis of aroma components of essential oils and hydrosols extracted from rosemary (*Rosmarinus officinalis*)

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Keywords: optical isomer; enantioselective GC-MS; essential oil; hydrosol; rosemary; principal component analysis

When essential oils are extracted by hydrodistillation from plants, hydrosols in the form of aqueous solution are obtained as byproducts. While essential oils are often used for food production as well as aromatherapy, utilization of hydrosols is under way because of the absence of the data about their chemical composition. In this study, essential oils and hydrosols were separated from rosemary harvested in several seasons, and chemical composition of aroma components in the two fractions was analyzed by GC-MS. Optical isomer composition of some aroma components was also analyzed.

Leaves of rosemary were collected every two months from February 2010 to February 2011. Leaves were soon subjected to hydrodistillation after harvesting. Essential oils and hydrosols were analyzed using GC-MS. For the analysis of the aroma component compositions, DB-1MS column was used. Optical isomer composition was determined using MEGA-DEX DET-beta column, which can separate optical isomers of aroma components. Results were analyzed by using principal component analysis.

The main components of the rosemary essential oil were eucalyptol and alpha-pinene. On the other hand, camphor, borneol and eucalyptol were predominant in the hydrosols. Classification of aroma components based on chemical groups revealed that essential oils contained the high amount of monoterpene hydrocarbons but hydrosols not. There is no difference in the ratio of enantiomer for optically active aroma components between essential oils and hydrosols. Principal component analysis indicated some seasonal variations of aroma component compositions in both essential oils and hydrosols.

The main aroma components of rosemary essential oils and hydrosols were different with each other, suggesting the different biological functions of the two fractions. Similar results were obtained for the optical isomer composition of some aroma components between essential oils and hydrosols.

P 202. Synergistic antimicrobial activities of the combination essential oils of *Cymbopogon citratus* and *Alpinia galanga*

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Keywords: Synergistic antimicrobial activity; Essential oils*; Cymbopogon citratus; Alpinia galanga*

To evaluate antimicrobial activities of the single and combined essential oils of *Cymbopogon citratus* and *Alpinia galanga* against four pathogenic microorganisms: *Staphylococcus aureus* ATCC6538, *Pseudomonas aeruginosa* ATCC9027*, Streptococcus bovis* DMST18769 and *Candida albicans* ATCC10231.

Fresh leaves of *C. citratus* and rhizome of *A.galanga* were submitted to hydrodistillation in a Clevenger-type apparatus for 2 hours [1]. Qualitative analyses were conducted with Finnigan Trace GC ultra equipped with Finnigan DSQ Quadrupole detector [2, 3]. Minimum inhibitory concentration (MIC) of essential oils was determined by broth microdilution method [4, 5, 6].

The light yellow essential oils of *C. citratus* (lemongrass) and *A.galanga* (galanga) were obtained in yields of 0.24% and 0.11%, respectively, based on dried extracted material. Nineteen compounds were identified in lemongrass oil sample that included geranial (16.4%), neral (10.2%) and myrcene (7.67%) as major components. Thirty-six compounds were identified in galanga oil. The major constituents were 1,8-cineole (68.18%), limonene (2.5%), terpinen-4-ol (2.23%), á-terpineol (1.16%), and á-pinene (1.1%). The MIC of lemongrass oil and galanga oil were observed against *S. aureus* (0.5% and 4% v/v), *P. aeruginosa* (40%v/v and >40%v/v), *S. bovis* (0.25%v/v and 0.5%v/v) and *C. albicans* (0.25%v/v and 0.5%v/v). The combination profiles of lemongrass oil with galanga oil (ratios 3:7, 5:5, and 7:3) were tested against four pathogenic microorganisms. Synergy was best noted for only one ratio (ratio 7:3).

Results of the present investigation provide evidence that the utilization of two essential oils combination could be assessed for synergistic antimicrobial activity in order to reduce their minimum effective dose.

Acknowledgements**:** National Research Council of Thailand (NRCT) is acknowledged for financial support.

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P 203. Larvicidal activity of Apiaceae essential oils against *Anopheles atroparvus* Van Thiel (Diptera, Culicidae) a former european malaria vector

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Keywords: bio-pesticides; fennel; parsley; cumin; phenylpropanoids; mosquito

In the context of research for new bio-pesticides, extracts from plants have received special attention and volatile constituents of essential oils (EOs) are among the candidates with higher potential [1]. *Anopheles atroparvus* is a European mosquito with proved transmission capacity for malaria parasite, which, in a scenario of climate change, can raise concerns of malaria re-emergence in regions actually not affected by this disease [2]. EOs of three well-known Apiaceae species, wild fennel (*Foeniculum vulgare* Miller subsp. *vulgare* var. *vulgare*), parsley (*Petroselinum crispum* (Miller) A.W. Hill) and cumin (*Cuminum cyminum* L.), were included in this study as potential sources of bio-insecticides and characterized by gas chromatography (GC) and mass spectrometry (GC-MS). Mosquito larvicidal assays were conducted against third-instar larvae of *An. atroparvus*. Lethal concentrations causing 50 and 90% of mortality after 24 hours of exposure were determined by log-Probit regression. Typical constituents of the fennel fruits EO, estragole and *trans*-anethole, were also evaluated. The wild fennel fruits EO was mainly constituted by estragole (65%), fenchone (16%), phellandrenes (10%) and pinenes (5%). A high content of phenylpropanoids, myristicin (32%), apiole (16%) and 1-allyl-2,3,4,5-tetramethoxybenzene (9%), and a considerable amount of pinenes (30%) were found in parsley fruits EO. Cumin fruits EO was rich in cumin aldehyde (39%), *p*-mentha-1,4-dien-7-al (10%) and several monoterpenes hydrocarbons. Both parsley and cumin EOs were more effective against *An. atroparvus* third-instar larvae (LC50= 13 ppm/ LC90= 28 ppm and LC50= 17 ppm/LC90= 43 ppm, respectively) than fennel fruits EO (LC50= 61 ppm/LC90= 107 ppm). The larvicidal activity of fennel EO was, in some extend, justified by its high estragole and low *trans*-anethole contents (65% and 2%, respectively), since the former was less active (estragole LC50= 56 ppm and trans-anethole LC50= 17 ppm).

Acknowledgments**:** This work was financially supported by the FCT (Fundação para a Ciência e Tecnologia) with a PhD grant SFRH/BD/66041/2009 and through the CITAB research center.

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P 204. Antimycoplasmatic evaluation of essential oils, isolated compounds and semi-synthetic indoles

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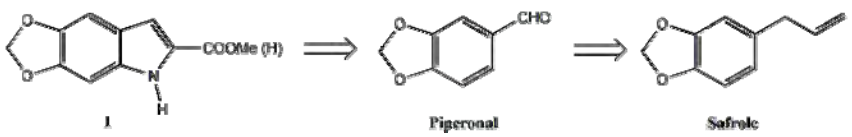
Keywords: *Mycoplasma mycoides*; essential oils; myristicin; sesquiterpenes; indoles; safrole.

Mycoplasmas are obligately parasitic bacteria with no cell wall causing diseases in a wide range of hosts, including humans [1]. In the search for new antimicrobial agents, phytochemicals represent a potential source for such class of compounds [2], therefore several natural products and two semi-synthetic indoles derived from safrole were assayed against *Mycoplasma mycoides* subsp. *capri*.

Essential oils (EOs) from *Drimys angustifolia* (leaf and branch), *Aloysia gratissima* (leaf) and *Melaleuca alternifolia* (leaf) were obtained by hydrodistillation under nitrogen atmosphere using a Clevenger type apparatus. The oils were characterized by means of gas chromatography (GC-FID) and gas chromatography coupled to mass spectrometry (GC-MS). The sesquiterpenes cyclocolorenone and bicyclogermacrene, and the arylpropanoid myristicin were isolated from leaf essential oil of *D. angustifolia* by column chromatography on silicagel. Pure hexane and the binary mixture (hexane:dichloromethane) were used as eluents. Drimenol was obtained from branch essential oil of *D. angustifolia* using crystallization in cold hexane. Semi-synthetic indoles (1) were obtained using piperonal, a safrole derivative, as starting material. EOs, isolated natural products and semi-synthetic índoles were assayed against *M. mycoides* using the broth microdilution method.

The tested samples showed activity against *M. mycoides* with Minimum Inhibitory Concentration (MIC) values of 50 mg mL^{-1}. The most active sample was the indole esther 1, which showed MIC value of 17.5 mg mL^{-1}.

EOs of several plant species as well as their isolated compounds showed weak activity against *M. mycoides*. The most promising compound, the semi-synthetic índole esther, may be suitable for chemical transformations in order to obtain more active molecular structures.



Acknowledgments: FURB, UNIVALI, FAPESC, Vegetal Brasil, INCT Catalysis.

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P 205. The essential oil of *Eryngium duriae* subsp. *juresianum* inhibits IL-1β-induced NF-KB AND MAPK activation and INOS expression in human chondrocytes

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Keywords: *Eryngium duriae*; Anti-inflammatory properties; Osteoathritis; IL-1β; MAPK

Osteoarthritis (OA) is characterized by cartilage and joint destruction. The nuclear factor-kappaB (NF-kB) and mitogen-activated protein kinase (MAPK) pathways mediate the increased expression of inflammatory factors and matrix metalloproteinases. Nitric Oxide (NO) produced by the inducible NO synthase (iNOS) is increased in OA chondrocytes and is involved in inhibition of matrix synthesis and induction of matrix degradation, leading to cartilage loss and joint destruction. IL-1β is a major pro-inflammatory and pro-catabolic cytokine that is increased in OA and activates the NF-kB and MAPK pathways and iNOS expression [1,2].

This work reports on the ability of the essential oil of *Eryngium duriae* subsp. *juresianum* (M. Lainz) (Ej) to decrease IL-1β-induced responses in human chondrocytes.

Knee cartilage samples were obtained from multi-organ donors (n=8) at the University Hospital of Coimbra. The essential oil of the aerial parts of *Ej* was diluted in DMSO. Final concentrations of 5 to 200 mg/mL were added to chondrocyte cultures 30 min before of IL-1β (10 ng/mL) for 5 min, 30 min or 18 h. The MTT reduction assay was used to rule out cytotoxic effects. NO levels were measured by the Griess reaction. Protein levels of iNOS, IkB-α and the phosphorylated forms of IkB-α and of the MAPK, p38, JNK and ERK1/2, were assessed by western blot. Results were considered statistically significant for $P<0.05$.

Ej significantly decreased IL-1-induced NO and iNOS levels in a concentration-dependent manner. The greatest inhibition of IL-1-induced NO (38.4 ± 8.4%, $P<0.001$) and iNOS protein (53.1 ± 6.3%, $P<0.001$) levels was achieved with a concentration of 25 mg/mL. 200 mg/mL were required for maximal inhibition of IκB-α (70.9 ± 5.6%, $P<0.001$), JNK (36.4 ± 0.6%, $P<0.001$), p38 (57.1 ± 4.4%, $P<0.001$) and ERK1/2 (24.4 ± 3.3%, $P<0.001$) phosphorylation.

These results suggest that by acting on various signalling pathways, *Ej* elicits the synergistic inhibition of iNOS expression and show the potential of *Ej* as a promising source of compounds with anti-inflammatory and anti-catabolic properties that may be useful as anti-OA drugs.

Acknowledgments: This work was supported by grants PTDC/EME-PME/103578/2008 and PTDC/EME-TME/113039/2009 from FCT.

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P 206. Composition and antioxidant activity of essential oil from Portuguese myrtle (*Myrtus comunis* L.) through the vegetative cycle

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Keywords: *Myrtus communis* L.; essential oil; Antioxidant activity; Folin-Ciocalteu; TEAC; ORAC.

Myrtus communis L., commonly known as myrtle, possesses a set of qualities that make it very interesting for the pharmaceutical, nutraceutical and cosmetic industries. Previous studies have shown that some differences arise in the composition of the essential oil and in the plant extract due to the different stages of the vegetative cycle of the plant and also due to different geographical locations.

In this work a time perspective is also investigated. Thus, Portuguese Myrtle (*Myrtus communis* L.) is being studied over a period of three years covering the main stages of the vegetative cycle of the plant, and the chemical composition of the essential oil was determined. The oil, extracted from leaves and berries separately, was obtained by Clevenger distillation and analysed by GC and GC/MS. The results show that the major components are limonene+1,8-cineole, myrtenyl acetate, a-pinene and linalool, and, over the three-year period, a decrease in the composition of the most volatile compounds was observed.

The aqueous phase obtained from the Clevenger distillation was extracted with diisopropylether obtaining what is hereby designated as the Liquid Phase extract. The extracts were subject to a determination of antioxidant activity using three different methods: the Folin-Cicolteau, the TEAC and the ORAC methods. The results obtained for the first year of collection showed that the leaves at the flowering stage and the ripe fruit presented better antioxidant activity, thus the study of the remaining two years was focused on them. The antioxidant activity varies between 1404- 2052 µmoltrolox/g for ORAC, 4554-5457 µmoltrolox/g for TEAC and 843 – 1831 µmol GAE/g for Folin-Cicolteau, with a wider range of variability on the leaves than in the fruits. In addition, there seems to be a similar trend in the Folin-Cicolteau and TEAC methods differing from the ORAC method.

The fruit antioxidant activity was similar in the three years; however, the leaves presented higher activity in the first year.

P 207. Lab-on-a-chip: a technique for detection of antibacterial effect of essential oils on outer membrane proteins

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Keywords: Essential oil, *Erwinia* sp., *Pseudomonas aeruginosa*, capillary electrophoresis, chip technology, outer membrane protein

The structural changes of outer membrane protein (OMP) composition may have an effect on the adhesive ability and pathogenic properties of bacteria. Previously, it has been demonstrated that some factors, e.g. antibiotics or iron restriction, can change the bacterial OMP composition [1, 2]. The aim of our study was to examine how the essential oils of thyme, cinnamon bark and clove modify the OMP composition of the plant pathogen *Erwinia* and the human pathogen *Pseudomonas aeruginosa* strains. In case of thyme oil, we wanted to analyse which components might play a significant role in the changes induced.

The essential oils were obtained from a Hungarian pharmacy. Chemical composition of the oils was controlled with GC and GC-MS. The minimum inhibitory concentration (MIC) values of the oils were determined by a modified tube dilution method. 0.5 and 2 x MIC concentrations of the oils were administered to the culture and incubated for 60 min. OMP preparation was performed according to ref [3]. Measurements were performed in the Protein 230 Plus LabChip Kits of the 2100 Bioanalyzer System of Agilent.

Thymol was the main component of the thyme oil (49.9%), eugenol (83.7%) of clove oil and trans-cinnamic aldehyde (73.2%) of cinnamon bark oil. Thymol as the main component of thyme oil caused major changes in the protein composition of *Erwinia*. Cinnamon and clove oil also influenced the OMP composition of *Pseudomonas* strains. After the treatment, the protein peak with molecular weight 42.7 kDa and 79.4 kDa disappeared in case of cinnamon oil and clove, respectively.

Quantitative changes in the protein profile may contribute to the explanation of antibacterial effect of thyme, cinnamon bark and clove essential oils on pathogenic *Erwinia* and *Pseudomonas* strains. The structure of proteins considerably reduced will be analysed with MALDI-TOF/MS.

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P 208. Transfer of volatiles from oregano or caraway essential oils into cow's milk

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Keywords**:** aroma; caraway; milk; oregano

Essential oils have a number of applications as a feed additive in ruminant production of milk and meat (1). The objective of this experiment was to study the transfer of volatile compounds, mainly terpenes, from oregano and caraway essential oils into cow's milk. During feeding and rumination the animals lungs and intestines are both exposed to volatile compounds present in the feed (2). In order to study the differences between respiratory and gastrointestinal exposure Holstein cows equipped with a duodenum cannula were used in two setups with two animals recieving each treatment. In the first the animals were placed in a controlled environment to inhale vapours of the essential oils for 9 hours. In the second essential oils diluted in deodorised sesame oil was injected through the cannula over a period of 9 hours, with two different levels being tested. Milk was collected prior to and immediately after treatment, as well as the following morning. Commercially available essential oils from *Origanum vulgare* plants and *Carum carvi* seeds were used. The aroma profile of essential oils and milk was analysed using purge-and-trap coupled with GC/MS.

The results show that milk contains a number of terpenes naturally at very low levels. When the animal is exposed to essential oils several terpenes, present in the essential oils, increase or appear in the milk, suggesting that the aroma compounds are absorbed through the lungs as well as the intestine. It also indicates that the absorption, and subsequent transfer from blood into milk, is very fast. In addition to the terpenes two esters were identified that increased in the milk after exposure, despite the essential oils contained no or insignificant amounts of them. This indicates that these esters are more readily absorbed or synthesised within the animal following exposure to essential oils. Little or none of the increased amounts of aroma compounds, terpenes and esters, could be found in the milk one day after exposure.

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P 209. Flavor active compounds from minor fruits grown in Uruguay

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Keywords: flavor, Uruguayan native fruits, isoprenoids

The perception of fruits flavor and aroma is the result of a multitude of interactions between a large number of chemical compounds and sensory receptors. In the case of isoprenoids, their role in the aroma of exotic fruits is a determining factor in their acceptation by consumers. Aroma compounds are present in fruits in free volatile form but also as non-volatile precursors such as glycosides

Considering the complexity associated with the composition of the different aromatic fractions (free and glycosidated) present in exotic fruits, the development of chromatographic techniques make available not only to separate these components, but also to provide information about the main biological activity to be considered: their aroma description [1]. Gas chromatography coupled to specific detectors as mass spectrometry and olfactometry, are able to respond to this challenge [2].

In this work we present the results obtained in the developing of the required methodology to make possible to evaluate the aromatic potential represented by the glycosylated isoprenoids present in native Uruguayan fruits. The fruits used as a model of study were: *Acca sellowiana* (Berg.) Burret (Myrtaceae, “guayabo del país”), *Psidium cattleianum* Sab. (Myrtaceae, “arazá”) and *Butia capitata* (Mart.) Becc. (Arecaceae, “butiá”). The results will be applied to develop new strategies in order to generate original elements of quality and authenticity for the fruits consumption and transformation.

Acknowledgments**.** This research was financially supported by the Agencia Nacional de Investigación e Innocacion (ALI_1_2011_1_2545) and CSIC-UdelaR, Uruguay (Group Project 656).

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P 210. Mathematical Modeling of Supercritical CO_2 extraction of volatile oils from pennyroyal (*Mentha pulegium*)

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Keywords: Supercritical extraction, volatile oils, aromatic plants, mass transfer models.

The modelling of the experimental data of supercritical extraction of volatile oils from aromatic plants is very important, since it can be used as a tool for the design, improvement and scale up of these processes from laboratory to pilot and industrial scales. A discussion of the modelling of our data on supercritical fluid extraction of volatile oil from pennyroyal was carried out. The most successful models in this scientific area describe the experimental process by using differential mass balances for the fluid and solid phases, as shown with the modelling studies carried out using the model proposed by Reverchon. This model was used successfully to describe our data on pennyroyal [1] and other aromatic plants [2]. Another successful model with two directly adjustable parameters, which are the external mass transfer coefficient, k_f, and the internal mass transfer coefficient, k_s, was developed by Sovová [3]. A development of this model, considering the extended Lack's plug flow model for vegetable oil extraction, was reported by Sovová [4], using one adjustable parameter, the external mass transfer coefficient, k_s. The yields of volatile oil from pennyroyal as function of extraction time described by both models [3,4] for different particle sizes are presented in Figures 1 and 2, respectively.

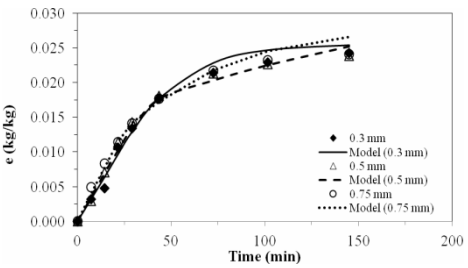

Fig. 1. Pennyroyal essential oil yield for diferent mean particle size and a CO2 fow rate of 4.7 x 10-4 $kg.s^{-1}$. Continuous curves from model [3], ks = 0.89 x 108 $m.s^{-1}$ and kf = 1.89 x 106 $m.s^{-1}$

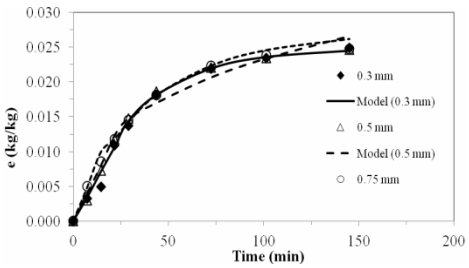

Fig. 2. Pennyroyal essential oil yield for diferent mean particle size and a CO_2 fow rate of 4.7 x 10^{-4} $kg.s^{-1}$. Continuous curves from model [4], k_s = 1.50 x 10^8 $m.s^{-1}$

The experimental results obtained at three different particles sizes using two or only one adjustable parameter, provided a fairly good agreement between the model curves and the experimental data, as shown in Figures 1 and 2, which evidence the capability of these models.

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P 211. Extraction of Volatile Oils from Aromatic Plant with Supercritical Carbon Dioxide

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Keywords: Supercritical extraction, hydrodistillation, volatile oils, aromatic plants

In recent years supercritical fluid extraction (SFE) has been used to obtain oils from aromatic plants, as an alternative to the traditional techniques, namely, hydrodistillation (HD) and steam distillation (SD).

The aim of this communication is to discuss an overall view of the supercritical extraction of volatile oils of the aromatic plants, rosemary (*Rosmarinus officinalis*) [1], pennyroyal (*Mentha pulegium*) [2], fennel (*Foeniculum vulgaris*) [3], coriander (*Coriandum sativum*) [4], savory (*Satureja fruticosa*) [5], winter savory (*Satureja montana*) [6], cotton lavender (*Santolina chamaecyparisus*) [7] and thyme (*Thymus vulgaris*) [8] carried out in our laboratories.

This separation technique was performed using a flow apparatus, provided with a 1L-extraction vessel and two separators of 0.27 L each. The oil obtained by supercritical extraction is actually designated as volatile oil to differentiate it from essential oil, which, by definition, is mainly produced by HD and SD.

Different conditions of pressure (90 and 100 bar), temperature (40 and 50 ºC), mean particle sizes (0.4, 0.6 and 0.8 mm) and CO_2 flow rate (0.8, 1.1 and 1.3 kg/h) were studied in order to understand the influence of these parameters on the composition and yield of the oils. The results were compared with those obtained for the essential oil isolated by HD. The volatile and essential oils were analysed by GC and GC-Ms.

One major difference between those types of oils respect to the absence of waxes in the essential oils. Another important difference is the higher concentration of thymoquinone in volatile oils from thyme (*Thymus vulgaris*) and savoury (*Satureja montana*). In the latter aromatic plant the content in volatile oil can reach a value 15 times higher (SFE- 3%) than the essential oil (HD-0.2%). It is noteworthy that thymoquinone has shown to have important antioxidant and anti-cancer activities.

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P 212. Chemical composition and trypanocidal activity evaluation of essential oils from *Hedychium coronarium* against *Trypanosoma brucei* strains

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Keywords: *Hedychium coronarium*, Caryophyllene oxide, 1,8-cineole, *Trypanosoma brucei*, Sleeping sickness.

Trypanosoma brucei is the etiologic agent of the sleeping sickness transmitted by flies of Glossina genus, known as tsetse flies. The search of new trypanocidal compounds is important to develop new drugs more effective and with less adverse effects than pentamidine. In this work, the trypanocidal activity of essential oils from the leaves and rhizomes of Hedychium coronarium was investigated using T. brucei procyclic strains. The essential oils were prepared by hydrodistillation (4 h) in a Clevenger-type apparatus and the compositions established by gas chromatography and gas chromatography-mass spectroscopy analysis. Caryophyllene oxide and 1,8-cineole are, respectively, the major constituents of the oils from the leaves and rhizomes. Procyclic forms of T. brucei (427 and 29-13 strains) were treated with essential oils to determine the cytotoxicity index (CI50), using the colorimetric method of MTT. Both essential oils revealed a weak activity (CI50>100 µg.ml-1) when compared to that of pentamidine (CI50 = 2.19 µg.ml-1), used as control. However, when testing individually the major constituents of the oils, caryophyllene oxide showed a promising activity on both T. brucei strains (T. brucei 427, CI50=65.77 µg.ml-1 and T. brucei 29-13, CI50=24.53 µg.ml-1).

Acknowledgements: Authors are grateful to Fundação para a Ciência e Tecnologia (FCT), POCI 2010/FEDER and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support.

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P 213. Chemical composition and immunomodulatory activity of the essential oil of *Melampodium divaricatum* (RICH. IN PERS.) DC (Asteraceae) and its major compound

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Keywords: *Melampodium divaricatum*, essential oil, nitric oxide, hydrogen peroxide, *E*-Caryophyllene

*Melampodium divaricatum (*Rich. In Pers.*)* DC *(*Asteraceae*)* is a native plant of South America, popularly known as falsa-calendula and commonly used as diaphoretic and diuretics in the treatment of leucorrhoea. The main goal of the present study was the evaluation of the immunomodulatory activity of essential oil of *M. divaricatum* by determining nitric oxide (NO) and hydrogen peroxide (H_2O_2) released in Swiss mice peritoneal macrophages cells. E-Caryophyllene (56.0%), germacrene D (12.7%) and bicyclogermacrene (9.2%) were found to be the major constituents. NO production was inhibited at 80% and 42% on the concentrations of 0.075 µl.ml-1 and 0.04 µl.ml-1, respectively. Inhibition process is dose dependent. The production of H_2O_2 resulting from PMA stimulation was also strongly inhibited in the presence of essential oil of M. divaricatum, but not in a dose dependent way. E-Caryophyllene, the major constituent of the oil, did not affect significantly NO and H_2O_2 release. Therefore, results confirm that the immunomodulatory activity of essential oil of *M. divaricatum* can be due to minor constituents or to the complex of the substances. The dose-dependent inhibition of macrophage NO suggests immunomodulatory and / or anti-inflammatory potential to be confirmed in further mechanistic and in vivo studies.

Acknowledgements: Authors are grateful to Fundação para a Ciência e Tecnologia (FCT) and POCI 2010/FEDER for financial support.

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P 214. Extraction and identification of volatile compounds from mature fruit *Averrhoa bilimbi* L. by solid phase microextraction (SPME) and GC-MS

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Keywords**:** *Averrhoa bilimbi*; solid phase microextraction; volatile compounds.

The fruit *Averrhoa bilimbi* L. belonging to the family of Oxalidaceae and is popularly known as biri-biri, gooseberry from China and bilimbi, among others, being useful in the food industry [1]. In Asia it is widely used in traditional medicine for various ills cure as it has anti-bacterial pharmacological properties [2] and have been also used in diabetes treatment [3]. Volatile compounds were investigated with hydrodistillation followed by solvent extraction [4]. The bilimbi, we had studied was collected in the northwestern of State of Rio de Janeiro (Brazil), near the city of St. Anthony of Padua and transported under refrigeration to the Embrapa's Laboratory of Liquid Chromatography where the analysis took place. The aim of our study was to perform the extraction and identification of volatile constituents of fresh ripe fruit bilimbi using solid phase microextraction (SPME) with polydimethylsiloxane fiber and GC-MS techiniques. We found that under analytical conditions used the chromatographic profile revealed only the region of monoterpene hydrocarbons, ethers and esters and some of these have not cited in previous studies. The mass spectra were compared with those of literature and NIST mass spectra library, had been identified 25 substances which six are the majority, among them the monoterpene limonene and substances: ethyl acetate; ethoxyethane, butyl acetate, methyl butanoate, ethyl butanoate . The result of this work shows that it is possible to study the bilimbi volatile compounds by SPME minimizing time of analysis, sample size and possible losses caused by the technique of solvent extraction.

Acknowledgments: To IFRJ by financial and institutional support

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P 215. Chemical composition and analgesic effect of the essential oil of *Origanum vulgare* L.

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Keywords: Volatile compounds; oregano; nociceptive

Essential oils are compounds produced by secondary metabolism of plants and used for their pharmacological activities and aromatic value[1]. *Origanum vulgare* L., known as oregano or wild marjoram, is an aromatic plant widely used in cookery. Infusions of the leaves are recommended against liver diseases; digestive, spasmolitic and respiratory disorders[2]. The essential oil this plant is known as an interesting source of antimicrobial compounds to be applied in food conservation and some of its activities related to the compounds carvacrol and thymol[3]. The aim of this work was to investigate the chemical composition and the antioxidant and analgesic activities of essential oil of *O. vulgare* L.

The essential oil of dried leaves from *O. vulgare* was obtained by hydrodistillation on a Clevenger-type apparatus for approximately 3h. The Chemical and physical properties the essencial oil was analyzed for Gas chromatography-mass spectrometry (GC-MS) and gas chromatography (GC-FID)[4]. The antioxidant activity of extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay system[5]. For the abdominal writhings tests, the writhings were induced by the intraperitoneal administration of acetic acid 0,6 %, in male albino Swiss mice (20-25 g) in 100, 200 mg/kg dosage, that was intraperitoneal administered, thirty minutes before performing the experiment in six-animal groups[6].

The major compounds of essential oil of *O. vulgare* were Terpinen-4-ol (35.73%), g-terpinene (15.58%), thymol (9.01%) and a-terpinene (8.39%). The oil did not show antioxidant activity, but exhibited an analgesic effect in the doses tested with inhibition of 100% in the number of writhings compared with the saline control group (34.83 $\pm$1.94) with $p < 0.001$.

The essential oil of *Origanum vulgare* showed be not only a similar literature composition[3], but also a potential analgesic.

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P 216. Comparative GS-MS volatile profile of different origyn chrism samples

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Keywords**:** Great Chrism, Nard Chrism, Greek Orthodox Chrism, GC-MS

There are several types of chrysm used in the different orthodox religious practice. The most important is the Great and Holy Chrism because of the complexity of the chemical composition and religious significations. The preparatory rites began on the morning of Great and Holy Monday, 9th of April, and continued through Great and Holy Wednesday. Clergy stirred the Chrism — a mixture of dozens of oils, herbs, fragrances — continuously, day and night, as Scripture was read. In this study we intended to compare four different Chrism types from four different origin and give some explanations for that.[1]

The volatile oil profile was evaluated by GC-MS using headspace injection at 85^{0}C, for 15 min, ZB-5MS 50 m x 0,32 mm x 0,25 microm capillary column respectively helium as carrier gas. The separated compounds were identified using an MS spectra library. The quantitative determination was performed by normalization.[2]

The volatile compounds found in chrism samples varied from sample to sample. Thus, in the case of Great Chrism sample, 32 compounds were separated the main ones being alpha-pinene, 1,8-cineol and beta-linalool. Instead, from the Nard Chrism (Israel) sample, 35 compounds were separated from which 32 identified, the major ones being: diphenyl ether, benzyl acetate, phenylethyl alcohol, alpha-terpineol, limonene. From the Greek Orthodox Chrism sample, 36 compounds were identified from the total of 38 separated and beta-linalool, cyclohexane isothiocyanate, as well as limonene being found in high concentrations. The Orthodox Chrism sample from Arad-Gai was characterized by high contents of phenylethyl alcohol, beta-linalool, benzyl acetate and alpha-terpineol.

The difference between chemical composition of the studied chrism samples can be explain by using different herbs or the local variation in composition of the used herbs.

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P 217. Analysis on floral scent of 9 *Syringa* species and varieties by ATD-GC/MS

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Keywords: *Syringa*; Floral scent; ATD-GC/MS

Syringa is an important aromatic woody flower, its dry flowers are traditionally used in tea and spices in China[1]. This study is to determine the volatile components and emission pattern of Syringa floral scent. The floral volatiles of *Syringa chinensis*, *S. protolaciniata*, *S. oblata, S. oblata* var. *Giraldii*, *S. oblata* var. *plena*, *S. vulgaris* ´Mrs Harry Bickle´, *S. vulgaris* ´Bright Centennial´, *S. vulgaris* ´White Spires´, and *S. vulgaris* ´Pres Lincoln´ were collected in vivo by dynamic headspace, and then were identified by ATD-GC/MS[2]. The results showed that except *S. chinensis* (29) and *S. vulgaris* ´Pres Lincoln´ (38), more than 50 compounds were detected in floral volatiles of other 7 Syringa plants, and the *S. oblata* var. *Giraldii* emitted the most compounds with 61. Among the volatile components of all Syringa studied, terpenoids showed the highest relative amount with more than 50% of total release amount, even arrived at 88% in *S. vulgaris* ´Bright Centennial´. And the relative amount of â-ocimene was over 70% in *S. protolaciniata, S. oblata* var. *Giraldii*, and *S. vulgaris* ´Bright Centennial´. Benzenoids and their derivatives were also important volatile componets, whose relative amounts varied from 8%-22%, benzaldehyde with 6%-18% of total release amount was the most abundant benzenoids for those 6 Syringa plants except *S. oblata* and its varieties. Aldehydes, alcohols, esters, and fatty hydrocarbons were all detected in all Syringa, but their release amounts were below 8%. And ketones (1.8%) were only detected in *S. oblata* var. *Giraldii*. In addition, 4 cultivars of *S. vulgaris* were chosen to evaluate the aromatic degrees of the floral scents through smelling by persons. The flowers of *S. vulgaris* ´White Spires´ emitted the richest fragrance, followed by *S. vulgaris* ´Mrs Harry Bickle´ and *S. vulgaris* ´Bright Centennial´, and the floral fragrance of *S. vulgaris* ´Pres Lincoln´ was too low to be smelled. Moreover, á-pinene and alcohols, especially 2-ethyl-1-hexanol, were considered to contribute mostly to aromatic difference of these 4 Syringa cultivars. The results provided basis for revealing the main components and their role in aromatic degree, as well as the intraspecific and interspecific difference of floral scent in Syringa.

Acknowledgments: The work was supported by National Natural Science Foundation of China

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P 218. Changes in the essential oil composition and their biological activities during the maturation of *Schinus terebinthifolius* fruits

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Keywords*:* *Schinus terebinthifolius*; fruit maturation; essential oil composition; GC-MS; antioxidant; antibacterial

Schinus terebinthifolius commonly known as Brazilian pepper tree is a potential producers of essential oils which have numerous biological activities such as antibacterial, antifungal, antiviral and antitumoral, among others. The chemical composition of the essential oil of the fruits and leaves of *S. terebinthifolius* has been the subject of numerous investigations, and monoterpene hydrocarbons namely á-phellandrene and â-phellandrene were the prominent compounds. Usually the essential oil is isolated from the leaves and/or the mature fruits. The influence of the stage of maturation of the fruits on the chemical composition of the essential oil of *S. terebinthifolius* has not been reported yet. The aim of the present contribution were to (a) analyze the chemical composition of the essential oils collected at three stages of fruit maturation, and (b) to evaluate the antioxidant and antibacterial activities of the three different oils.

Essentials oils were isolated by hydrodistillation from the air dried fruits collected at three stages of maturation (immature: green; intermediate: green-red and mature: fully red). The oils were dried over anhydrous sodium sulphate and analyzed by gas chromatography-masse spectrometry. At the same time, the essential oils were evaluated for their antiradical property against the DPPH and ABTS radicals and their antibacterial activity.

From the fruits collected at different stages of maturity, a pale yellowish oils with pungent and pepper like aroma were obtained in a yields of 4.6, 4.6 and 4.5% (w/w on dry weight basis), for immature, intermediate and mature fruits, respectively. In all oil samples, Germacrene-D (57.8-59.2%), elixene (9.5**-**14.5%), terpinolene (7.4-9.08%) and ä-cadinene (3.27-3.9%) were the main components. The oil samples were unable to reduce the DPPH and ABTS radicals, while they found to possess good antibacterial activity against *Enterococcus faecalis, Enterococcus faecium* and *staphylococcus aureus.* Among the oils samples, those from the intermediate stage of maturation were found to be the most efficient.

The results obtained herein indicate that *S. terebinthifolius* could be considered as a potential source of volatile oils which could serve not only as flavour agents but also as antiseptic supplements preventing deterioration of foodstuff and beverage products and pharmaceuticals.

P 219. Method to study the repellent, irritant and toxic effects of essential oils on *Bemisia tabaci* for a combination with insect proof net

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Keywords: behavior; whitefly; tomato; insect proof net; natural product; pest management

Bemisia tabaci, vector of the tomato leaf curl virus, is a serious problem in tomato production in many parts of the world. In tropical countries, the use of netting to protect horticultural crops is an effective and sustainable tool against Lepidoptera but not against small pests on the contrary of the Mediterranean region [1]. A previous study showed the efficiency of a repellent net impregnated with alphacypermethrin against the whitefly [2]. But needs to reduce the use of pesticide and resistance in population bring up the issue of finding a natural alternative to alphacypermethrin. The objective of this study was to evaluate the repellent, irritant and toxic effects of 10 essential oils on *B. tabaci* adults in laboratory. The repellent effect due to volatiles compounds was evaluated thanks to a still-air olfactometer. It was expressed as a distance to the essential oil support [3]. The irritant effect which is the contact effect with essential oil was evaluated thanks to a choice test in tube and was illustrated by a different cross rate through an essential oil treated net and a solvent treated net [2]. The toxicity effect was evaluated thanks to a no-choice assay in tube. The toxicity was quantified by the mortality after 24h of *B. tabaci* which crossed an essential oil treated net. Nets were dipped in essential oils at concentrations of 0.01%, 0.1% and 1%. Responses varied according to oil type and dose. The three effects were dose dependent. If one effect showed for an oil it did not mean there was another effect. For example an essential oil can be irritant but not repellent so we could expect that the repellent mechanism are different than the irritant or toxic effect. Several essential oils or a combination of them could be an alternative to alphacypermethrin.

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P 220. Synergy between the main compounds of savory and thyme essential oils on control of some plant pathogens

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Keywords: Bacteria, fungi, pathogen, savory, synergy, thyme.

Among the interesting features of the application of essential oils in post-harvest of plant products, it is significant that their effectiveness in controlling pathogens may be due to a synergy between their components [1]. So the aim of this study was to evaluate the synergy between the main compounds of savory (*Satureja montana*) and thyme (*Thymus vulgaris*) essential oils against two fungal pathogens (*Fusarium fujikuroi* and *Penicillium expansum*) and a bacterial pathogen (*Pseudomonas syringae* pv. *actinidiae*) isolated from diseased plant tissues (from rice cv. Galileo, apples cv. Golden Delicious, and actinidia cv. Hayward, respectively). After a GC-MS analysis, the main components of the savory essential oil resulted to be carvacrol and *p*-cymene (45.1% and 16.1%), while for the thyme essential oil the main components were thymol and α-pinene (53.7% and 19.8%). Different mixtures of the two main components of both essential oils were tested *in vitro* for their inhibition of pathogen growing using the *agar diffusion* technique [2]. From the obtained results it was possible to highlight the synergy between carvacrol and *p*-cymene, since *F. fujikuroi* and *P. syringae* pv. *actinidiae* inhibition due to the application of the mixtures at 75% -25% and 50% -50% of carvacrol and *p*-cymene was statistically higher than that shown by both compounds applied alone at 100%. Nevertheless, the application of thymol at 100% showed an inhibition statistically higher than the mixtures of thymol and α-pinene, that behaviour confirmed the thymol-based antimicrobial activity of thyme essential oil. *In vitro* analysis was efficient for screening the potential of essential oils application on plant pathogen control, but *in vivo* trials under commercial conditions are necessary to achieve a better response for the agricultural sector.

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P 221. Antifungal activity of savory essential oil by fumigation in apple storage.

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Keywords: Apple, *Botrytis cinerea*, fumigation, *Penicillium expansum*, savory.

Application of essential oils in post-harvest of apples has been reported as an alternative method to control fungal pathogens such as *Botrytis cinerea* and *Penicillum expansum* [1]. Besides application by spraying or dipping, the antimicrobial activity of essential oils in the vapour phase makes them possible fumigants within the structures of fruit storage [2]. So the aim of this study was to evaluate the antifungal activity of savory (*Satureja montana*) essential oil as a fumigant in storage of apples cv. Golden Delicious against *B. cinerea* and *P. expansum*. The apples were disinfected, punctured with a sterile plastic tip at the equatorial region, and inoculated by spraying a suspension of $1x10^5$ conidia ml^{-1} of each pathogen. Inoculated apples were stored separately in hermetic cabinets (60x75x75cm) for 30 days: *a.* with 50 ml of a 10% emulsion of savory essential oil (10% essential oil, 88% sterilized water and 2% Tween20); *b.* with 50 ml of a 10% emulsion of savory essential oil (10% essential oil, 88% sterilized water and 2% Tween20) gelified with agar-agar (15g per liter); *c.* alone as a control. After storage, apples were brought out and kept 7 days in *shelf-life* at room temperature. From the obtained results it was possible to observe that the diameter of the fungal rot on the apples stored with the essential oil emulsion was statistically lower than the control. The emulsion of savory essential oil was lightly less effective on pathogen control than the gelified one, but better results were found against *B cinerea* than against *P. expansum*, even after *shelf-life*. A better comprehension of the fungicidal and fungistatic activity of essential oils could define their real efficacy against post-harvest pathogens of fruit and their possible commercial use.

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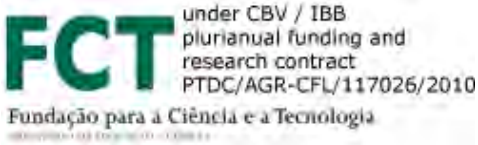

The generous obligingness of the following entities is gratefully acknowledged: