



# Book of Abstracts



**50<sup>TH</sup>**  
**INTERNATIONAL**  
**SYMPOSIUM ON**  
**ESSENTIAL OILS**

September 9<sup>th</sup> – 12<sup>th</sup>,  
Vienna, Austria



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# Welcome

On behalf of the Organizing Committee it is our great pleasure to welcome you to Vienna to celebrate the 50th Anniversary of the International Symposium on Essential Oils (ISEO 2019)!

Vienna's history started around 4,000 years ago. Due to its position next to the Danube between the extension of the foothills of the Alps (Wienerwald) in the Southwest and the Hungarian lowlands in the Southeast it has always been a strategically perfect settlement and trading area. Vienna's old name Vindobona derives from the Celtic language. The Romans built a military camp at the territory of today's city center during the 1st century and their famous emperor Marcus Aurelius died here in 180 A.D. Later on Vienna became the capital of the Babenberg dynasty and subsequently of the Austrian Habsburgs. Walking around, especially in the first district, you can find archaeological excavations and magnificent buildings everywhere.

In 2019 Vienna topped for the 10th year in a row the worldwide Mercer Quality of Living Survey. It is well known for its high quality tap water that directly is being brought to the capital from the mountains by two water conduits with a length of 95 and 183 km, respectively, with no pumps involved. Austria's capital is the only metropolis worldwide with noteworthy viniculture within the city limits and almost 43% of the city are green space. It is a multicultural melting point in the middle of Europe and its inhabitants are said to have "a heart of gold".

ISEO's history started 50 years ago. In 1969, in the course of a symposium of the Society of Medicinal Plants Research in Leiden (The Netherlands), four researchers founded a workshop to discuss their common research interests. The main agenda was to find a way to optimize the analysis of volatile compounds by means of an analytic technique which, at that time, was still in its infancy. Since then this meeting was repeated annually with increasing participants, which exceeded the number of 100 participants in 1982 in Wurzburg (Germany) for the first time. Till 1984 ISEO was exclusively organized by academia but in 1985 the symposium was hosted by Dragoco and Haarmann & Reimer (now Symrise) in Holzminden/Neuhaus (Germany). The entry of the essential oil industry brought another interesting perception to the meetings and it has become an integral part of ISEO.

In the meantime the interest in this symposium has literally spread worldwide, to all continents. 50 years ago its founders probably never would have thought of such a long-standing success! It provides an excellent possibility for researchers from academia and industry to exchange their experience and to discuss their results and findings in the field of essential oils and volatile compounds.

This year ISEO takes place in Vienna for the second time (after 1996) and the organizers wish all participants a highly exciting and fruitful symposium as well as many unforgettable memories of a very pleasant stay. Thank you for joining this meeting!

**Johannes Novak and Iris Stappen**

Presidents of the ISEO 2019 Organizing Committee

## Permanent Scientific Committee

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Nicolas Baldovini (Nice, France)  
Hüsnu Can Baser (Eskisehir, Turkey)  
Carlo Bicchi (Turin, Italy)  
Humberto Bizzo (Rio de Janeiro, Brazil)  
Alain Chaintreau (Geneva, Switzerland)  
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## Event Management Universität Wien

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## Location

The venue will take place at the University of Veterinary Medicine, Vienna, Veterinärplatz 1, A-1210 Wien, Austria.

## ISEO Medal of Honour

The first promoters of the ISEO Medal were Giovanni Dugo and Luigi Mondello, the Chairmen of the 35th International Symposium on Essential Oils organized in Giardini Naxos (Italy) in 2004. The idea of the ISEO Medal came up once again in 2017 when Humberto Bizzo proposed to implement the award/medal for lifetime achievements in 'essential oil research' during the Permanent Scientific Committee meeting on the occasion of 48th ISEO symposium in Pecs (Hungary).

At the ISEO in Nis (Serbia) we decided that the 50th anniversary of the ISEO symposia would be the best time to present the medal. With the help of the members of the Permanent Scientific Committee and an artist from Messina, as well as the Merck company, the official sponsor of the medal, the ISEO Medal of Honor was created.

By an unanimous decision of the all members of the ISEO Permanent Scientific Committee, Professor Jan Karlsen (Oslo, Norway) was elected to be first recipient of the ISEO Medal of Honor. We planned to present him the medal during ISEO 2019 after his lecture on a 50-years-survey on ISEO meetings (<https://dergipark.org.tr/nveo/issue/46268/581291>). Life, however, wrote a different scenario, and the information about Prof. Karlsen's sudden death shocked us. We have lost a great scientist and friend. He was one of the founding fathers of ISEO, as well as a valuable and highly respected member of the ISEO symposia. ISEO meant very much to him, not only the science involved and the development of the different subjects, but the people he met there - his old friends, and also the younger generation of scientists, of whom he was most impressed and proud. His obituary can be found at <https://dergipark.org.tr/pub/nveo/issue/47884/605073>. We all decided to appreciate Prof. Jan Karlsen's life work and humanity posthumously and, in this particular year, present the ISEO Medal to Prof. Karlsen's wife Inger Krogvig during the ceremony on September 8th, 2019.



On behalf of the ISEO Permanent Scientific Committee

*Agnieszka Ludwiczuk*

# Young Scientists Fellowship

The Organizing Committee is very grateful to the Executive Committee of the International Federation of Essential Oils and Aroma Trade (IFEAT), who gave financial support to 20 selected young scientists for the participation in this symposium. This fellowship comprises the reimbursement of the registration fee and the awardees will receive a certificat.

Since we had 50 submissions this year and we are celebrating ISEO's 50th anniversary, ISEO 2019 Organizing Committee decided to expand this fellowship to another five young scientists.

Therefore twenty-five contributions were accepted after an intense evaluation and selection procedure, seven of which were chosen for an oral presentation and eighteen for a poster presentation. These young researchers are now able to contribute their enthusiasm to the essential oils and natural volatiles community. They will also have the possibility to share their expertise and develop new ideas. This year's awardees are:

## Oral Presentation:

**Francesca Capetti** (University of Turin, Italy)  
**Stephanie de Rapper** (University of the Witwatersrand, South Africa)  
**Mohammad-Taghi Ebadi** (Tarbiat Modares University, Iran)  
**Leila Gimenes** (Agronomic Institute of Campinas, Brasilia)  
**Francesca Maggio** (University of Teramo, Italy)  
**Dilek Türkmen** (Hatay Mustafa Kemal University, Turkey)  
**Carmen M. Sinche Ambrosio** (University of São Paulo, Brasilia)

## Poster Presentations:

**Nurunajah Ab. Ghani** (Atta-ur-Rahman Institute for Natural Product Discovery, Malaysia)  
**Jelena Aksic** (University of Nis, Serbia)  
**Ina Aneva** (Bulgarian Academy of Science, Bulgaria)  
**Aurélien Cuchet** (Albert Vieille SAS; Analytical Science Institute, France)  
**Juan Henríquez** (CENIVAM, Columbia)  
**Maja Ivic** (Faculty of Technology Novi Sad, Serbia)  
**Ana Miltojevic** (University of Nis, Serbia)  
**Marko Mladenovic** (University of Nis, Serbia)  
**Javzmaa Namshir** (Mongolian Academy of Sciences, Mongolia)  
**Milan Nešic** (University of Nis, Serbia)  
**Milica Nikolic** (University of Nis, Serbia)  
**Cristian Andrés Oliveros** (Universidad Industrial de Santander, Columbia)  
**Angela Pawlowski** (Instituto Federal Farroupiha, Campus Santo Ângelo, Brasilia)  
**Bartłomiej Piasecki** (Medical University of Lublin, Poland)  
**Milica Stevanovic** (University of Nis, Serbia)  
**Nikola Stojanovic** (University of Nis, Serbia)  
**Ayaka Uehara** (Université Côte d'Azur, France)  
**Hongxia Zhang** (Kunming Institute of Botany, China)

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# Scientific program

## Sunday, September 8<sup>th</sup>

14.00		Registration	Building CA
15.00-18.00	WS	<b>Philip Wenig</b> (Lablicate GmbH, Germany) <i>Analysis of chromatographic fingerprints - Workshop on Openchrom, an open source software for chromatography and mass spectrometry</i>	Building FA Lecture Hall A
18.00-20.00		Welcome Reception	Building CA
19:00-19:45	Concert	Marc Aurel Quartet	Building CA Banquet Hall
20.00-20.45	WL	<b>Honorary Lecture on the occasion of the 50<sup>th</sup> ISEO</b> <b>Carlo Bicchi</b> (University of Torino, Italy) <i>Essential oils: Are these traditional/national products the “seed” of a multitask future discipline</i>	Building CA Banquet Hall
20.45-21.15		Award of the ISEO Medal of Honor	

## Monday, September 9<sup>th</sup>

8.00-9.00		Registration	Building CA
9.00-9.30		Opening Ceremony	Building CA Banquet Hall
<b>SESSION I</b> 9.30-10.30		<b>Industrial Aspects of Essential Oils I</b> Chairs: Daniel Joulain, Gerhard Buchbauer	Building CA Banquet Hall
9.30-10.10	PL1	<b>Eva Heuberger</b> (Forum Essenzia, Germany) <i>Essential oils: From marketing to psychopharmacology</i>	
10.10-10.30	OP1	<b>Antonello Paparella</b> (University of Teramo, Italy) <i>Essential oils application in food environments: A potential weapon to fight microbial adhesion</i>	
10:30-10.55		Coffee break	
<b>SESSION II</b> 10.55-12.50		<b>Industrial Aspects of Essential Oils II</b> Chairs: Alain Chaintreau, Patrizia Rubiolo	Building CA Banquet Hall
10.55-11.40	PL2	<b>Michel Meneuvrier</b> (Société Anonyme des Plantes Aromatiques du Diois (SAPAD) – Robertet, France) <i>The industrial production of essential oils: From theory to reality</i>	
11.40-12.10	IS1	<b>Alastair Hitchen, Wladyslaw Brud</b> (IFEAT, United Kingdom) <i>Industrial uses versus scientific research on essential oils</i>	

12.10-12.30	OP2	<b>Daniel Joulain</b> (SCBZ Conseil, France) <i>The sesquiterpene hydrocarbons of patchouli and their contribution to the odour of the essential oil</i>	
12.30-12.50	OP3	<b>Stephen Johnson</b> (The Aromatic Plant Research Center, USA) <i>Chemistry and industry implications of Boswellia occulta, a new species of frankincense from Somaliland</i>	
12.50-14.15		Lunch	Building DA Canteen (Mensa)
<b>SESSION III</b> 14.15-16.30		<b>Essential Oil Activity</b> Chairs: Ana Cristina Figueiredo, Agnieszka Ludwiczuk	Building CA Banquet Hall
14.15-15.00	PL3	<b>Sandy Van Vuuren</b> (University of the Witwatersrand, South Africa) <i>Antimicrobial activities of essential oils: Lessons from the past and recommendations for the future</i>	
15.00-15.30	IS2	<b>Nurhayat Tabanca</b> (United States Department of Agriculture, Agricultural Research Service, Subtropical Horticulture Research Station (USDA-ARS-SHRS), USA) <i>Tea tree essential oil: A source of potential new attractants for Mediterranean fruit fly</i>	
15.30-15.50	OP4	<b>ZhiZhi Du</b> (Kunming Institute of Botany, China) <i>Bioassay-guided investigation of Adenosma buchneroides essential oil as mosquito repellent against Aedes albopictus</i>	
15.50-16.10	OP5	<b>Isiaka Ogunwande</b> (Lotto College, Nigeria) <i>Chemical compositions and larvicidal activity of essential oil from the leaf and fruits of Manglietia dandyi (Gagnep.) Dandy growing in Vietnam</i>	
16.10-16.30	OP6	<b>Adam Feyaerts</b> (Katholieke Universiteit Leuven, Belgium) <i>Essential oils: A largely unexplored source of potentially novel medicines</i>	
16.30-18.00		<b>POSTER SESSION I (odd numbers) &amp; Coffee break</b>	Building CA

## Tuesday, September 10<sup>th</sup>

<b>SESSION IV</b> 8.45-10.30		<b>Essential Oil Chemistry</b> Chairs: Györgyi Horváth, Nicolas Baldovini	Building CA Banquet Hall
8:45-9.30	PL4	<b>Niko Radulović</b> (University of Niš, Serbia) <i>Essential oils are a gold mine for an organic/medical chemist</i>	
9.30-9.50	OP7	<b>Daniel Strub</b> (Wrocław University of Science and Technology, Poland) <i>Combinatorial synthesis of fragrant volatiles from natural raw materials - a sustainable approach towards discovery of new odorants</i>	
9.50-10.10	OP8	<b>Yoshinori Asakawa</b> (Tokushima Bunri University, Japan) <i>Volatile components of several South American liverworts and their biological activity</i>	
10.10-10.30	OP9	<b>Nicolas Lebouvier</b> (University of New Caledonia, New Caledonia) <i>Chemosystematic significance of essential oils obtained from characteristic taxonomic groups of the New Caledonian flora</i>	

10.30-10.55		<i>Coffee break</i>	
<b>SESSION V</b> 10.55-12.40		<b>Analytical Aspects of Essential Oils</b> Chairs: Niko Radulović, Carlo Bicchi	Building CA Banquet Hall
10.55-11.40	PL5	<b>Ivana Bonaccorsi</b> (University of Messina, Italy) <i>Carbon Isotope ratios of volatiles as a powerful parameter for genuineness assessment of essential oils</i>	
11.40-12.00	OP10	<b>Agnieszka Ludwiczuk</b> (Medical University of Lublin, Poland) <i>Essential oils of Marchantia polymorpha L. from Serbian populations</i>	
12.00-12.20	OP11	<b>Elena Stashenko</b> (Universidad Industrial de Santander, Columbia) <i>Comprehensive study of volatile secondary metabolites from Verbenaceae plants grown in Colombia</i>	
12.20-12.40	YS1	<b>Leila Gimenes</b> (Agronomic Institute of Campinas, Brasilia) <i>Chemical composition of essential oils from Lippia alba genotypes analyzed by GC-MS and Flow-Modulated Comprehensive Two Dimensional Gas Chromatography</i>	
12.40-14.05		<i>Lunch</i>	Building DA Canteen (Mensa)
<b>SESSION VI</b> 14.05-16.30		<b>Young Scientists: The Future of Essential Oil Research</b> Chairs: Humberto Bizzo, Daniel Strub	Building CA Banquet Hall
14.05-14.25	YS2	<b>Francesca Capetti</b> (University of Turin, Italy) <i>In vitro permeation kinetics, skin layers distribution and emission in the surrounding environment of biologically active Melaleuca alternifolia (tea tree) essential oil components from topic formulations</i>	
14.25-14.45	YS3	<b>Ebadi Mohammad</b> (Tarbiat Modares University, Iran) <i>Packaging methods and storage duration affect essential oil content and composition of hyssop (Hyssopus officinalis L.)</i>	
14.45-15.05	YS4	<b>Stefanie De Rapper</b> (University of the Witwatersrand, South Africa) <i>Therapeutic blending: The use of essential oils in combination against pathogens of the respiratory tract</i>	
15.05-15.25	YS5	<b>Dilek Türkmen</b> (Hatay Mustafa Kemal University, Turkey) <i>Volatile compounds of some raw pistachio nut varieties with solid phase micro-extraction technique</i>	
15.25-15.45	YS6	<b>Francesca Maggio</b> (University of Teramo, Italy) <i>Sub-lethal concentrations of Origanum vulgare essential oil exert inhibitory activity on Listeria monocytogenes strains in different environmental conditions</i>	
15.45-16.30	PD	<b>Alain Chaintreau, Daniel Joulain, Carlo Bicchi</b> (FFJ) <i>The survival of essential oils calls for a revolution!</i>	

16.30-18.00	<b>POSTER SESSION II (even numbers) &amp; Coffee break</b>	Building CA
20.00	<i>Symposium Dinner at „Weingut Klager“</i> <a href="http://www.weingutklager.at/weingutklager/home.html">http://www.weingutklager.at/weingutklager/home.html</a>	„Weingut Klager“ Stammersdorferstr. 14, 1210 Vienna

## Wednesday, September 11<sup>th</sup>

<b>SESSION VII</b> 8.45-10.10	<b>Essential Oils in Animal Health and Welfare</b> Chairs: Chlodwig Franz, Hüsni Can Baser	Building CA Banquet Hall
8.45-9.30	PL6 <b>Johanna Fink-Gremmels</b> (University of Utrecht, The Netherlands) <i>Essential oils in veterinary medicine and animal nutrition</i>	
9.30-9.50	OP12 <b>Klaus Teichman</b> (BIOMIN Research Center, Austria) <i>Impact of a phytogetic feed additive on inflammation markers and microbiota composition of weaned piglets as assessed by NGS</i>	
9.50-10.10	YS7 <b>Carmen Sinche</b> (University of São Paulo, Brasil) <i>Effect of a microencapsulated citrus essential oil on in vitro fermentation kinetics of pig gut microbiota</i>	
10.10-10.35	<i>Coffee break</i>	
<b>SESSION VIII</b> 10.35-12.40	<b>Essential Oils in Plant Biology</b> Chairs: Yoshinori Asakawa, Eva Zámbořiné-Németh	Building CA Banquet Hall
10.35-11.20	PL7 <b>Massimo Maffei</b> (University of Torino, Italy) <i>Plant volatiles: The language of plants</i>	
11.20-11.40	OP13 <b>Marie-Laure Fauconnier</b> (Laboratory of Chemistry of Natural Molecules, Belgium) <i>Molecular biophysics: A novel integrative approach to investigate the bioherbicide mode of action of essential oils</i>	
11.40-12.10	<i>Closing Ceremony</i>	Building CA Banquet Hall
12.15	<i>Excursion</i> Departure: 12.30	Main Entrance in front of CA-Building

### Key of Abbreviations:

WS	workshop
WL	welcome lecture
PL	plenary lecture
IS	invited speaker
OP	oral presentation
YS	young scientist presentation
PD	panel discussion
PP	poster presentation
YS PP	young scientist poster presentation

# Poster Presentations

7 odd numbers = Session I

8 even numbers = Session II

YS PP01	A rare santolina-type monoterpene ketone acetate found in Georgian <i>Artemisia</i> sp. essential oil	<a href="#">Milica G. Nikolić</a>
YS PP02	A synthetic approach to the identification of (iso)bornyl esters in the essential oil of feverfew ( <i>Tanacetum parthenium</i> L.)	<a href="#">Milica Stevanović</a>
YS PP03	Antimicrobiological properties of essential oils obtained from selected <i>Mentha</i> species against <i>Helicobacter pylori</i>	<a href="#">Bartłomiej Piasecki</a>
YS PP04	Authentication of the naturalness of wintergreen ( <i>Gaultheria</i> genus) essential oils by gas chromatography, isotope ratio mass spectrometry and radiocarbon assessment	<a href="#">Aurélien Cuchet</a>
YS PP05	Common conifer tree`s essential oils in Mongolia: chemical profile, antioxidant and antimicrobial properties	<a href="#">Javzmaa Namshir</a>
YS PP06	A comparative study on the essential oil composition of <i>Clinopodium vulgare</i> L. and <i>Micromeria dalmatica</i> Benth.	<a href="#">Ina Aneva</a>
YS PP07	Effects of immortelle essential oil on macrophage NO production	<a href="#">Jelena Aksic</a>
YS PP08	Gender-related differences in the xenobiotic metabolism of two <i>Choisya ternata</i> essential-oil constituents, methyl and isopropyl N-methylantranilates	<a href="#">Ana Miltojevic</a>
YS PP09	Hyssop essential oil improves quality of cooked pork sausages	<a href="#">Maja Ivic</a>
YS PP10	Identification of new 3-phenylpropyl esters from the essential oil of <i>Pleurospermum austriacum</i> (L.) Hoffm. (Apiaceae) through the preparation of a synthetic library of isomeric hexanoates	<a href="#">Marko Mladenovic</a>
YS PP11	Lemon balm ( <i>Melissa officinalis</i> L., Lamiaceae) essential oil prevents spontaneous and induced rat ileum contractions	<a href="#">Nikola Stojanovic</a>
YS PP12	Odor-active compounds of yuzu ( <i>Citrus junos</i> Sieb. ex Tanaka) peel oil	<a href="#">Ayaka Uehara</a>
YS PP13	Phytotoxic effect of different essential oils on <i>Eragrostis plana</i> germination	<a href="#">Ângela Pawlowski</a>
YS PP14	Potent odorants contributing to the characteristic flavor of <i>Panax Notoginseng</i> Flower Buds	<a href="#">Hongxia Zhang</a>
YS PP15	Structure elucidation of new monoterpene dimers from the essential oil of French marigold ( <i>Tagetes patula</i> L.)	<a href="#">Milan Nešić</a>
YS PP16	Study of patchouli essential oil chemical composition as a function of part of the plant, distillation time and enzymatic treatment	<a href="#">Cristian Andrés Oliveros</a>
YS PP17	Trans-methyl cinnamate: Marker compound of stressed <i>Conocephalum conicum</i>	<a href="#">Nurunajah Ab. Ghani</a>
YS PP18	Use of essential oils and natural extracts of plants that grow in Santander as antioxidants in a cosmetic product.	<a href="#">Juan Henríquez</a>
PP19	A comparative study of the chemical and aroma profile of <i>Rosa damascena</i> essential oil from Saudi Arabia and Bulgaria: An effect of the technology and geographic origin	<a href="#">Daniela Nedeltcheva-Antonova</a>
PP20	Amazon & Madagascar: Two tropical regions for essential oils production and opportunities for species diversification	<a href="#">Jennifer Bufalo</a>
PP21	Analysis of essential oils residues in egg albumen and yolk	<a href="#">Federico Cozzi</a>
PP22	Antibacterial activity and essential oil composition of <i>Calendula arvensis</i>	<a href="#">Hüseyin Servi</a>
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# Book of Abstracts

## WS Analysis of chromatographic fingerprints - Workshop on OpenChrom, an Open Source Soft- ware for Chromatography and Mass Spectrometry

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### Abstract

OpenChrom® is an open source software for chromatography, spectrometry and spectroscopy. Data from different systems can be imported and analyzed, hence it's a vendor independent software. Moreover, it runs under Windows, macOS and Linux. So the user has the choice to work wherever he wants, independent of the system. Generally, it supports to handle GC/MS, GC/FID, HPLC-UV/VIS, FTIR, PCR and NMR data. Originally, OpenChrom® was designed to analyze data from analytical pyrolysis, called Py-GC/MS. Thus, it has a strong focus on chromatography and nominal mass spectrometry. In the course of time, additional requirements have been added. More features are already in the pipeline. Its strength is to handle GC/MS and GC/FID measurements. Methods for peak detection, integration, identification, quantitation and reporting are supported. Using internal (ISTD) and external standards (ESTD) for quantitation purposes is supported as well. Additional filter help to optimize the measurements and classifier calculate key values of the chromatographic data and help to point out problems like shifted retention times or degraded columns. Did you know that it is easy to detect and identify peaks in a GC/MS file and to transfer these peaks to its corresponding GC/FID measurement for quantitation purposes. In summary, the modularity of OpenChrom® allows to recombine the contained functionality for many different purposes. Both working in target screening (TS) and non-target screening (NTS) modus is possible. Therefore, the platform can be utilized for quality control purposes or for the analysis of chromatographic fingerprints.

A typical workflow consists of the following steps:

- Load chromatogram [MSD]
- Filter and optimize the data (optionally)
- Detect and integrate peaks
- Identify peaks (NIST-DB, user specific databases)
- Transfer peaks to chromatogram [FID]
- Set internal standard(s)
- Quantify peaks
- Report results

All these steps can be applied manually. It's recommended to run the analysis of the data a few times by hand. But OpenChrom® also supports process methods and batch processes. The aforementioned steps can be automated and customized in a very flexible way.

Extensions can be easily added. Lablicate GmbH offers a commercial extension called ChromIdent. This tool allows to add chromatographic peaks as a whole to a database. While adding chromatographic peaks to the database, typical peak patterns are detected and automatically extracted into a composite peak table. This peak table is used for identification purposes of unknown samples. By using the peak patterns, it possible to tell apart complex mixtures and their composition.

## **WL Essential Oils: Are these traditional/national products the “seed” of a multitask future discipline? A tribute to Jan Karlsen**

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### **Abstract**

This lecture is based on a number of lively discussions I had with Ian Karlsen on the future of essential oils (and thereby of ISEO) in 2006 when he spent some weeks in our laboratory in Turin to study with us some endemic samples of juniper essential oil of Norwegian mountain.

The volatile fraction of a plant is an important biosensor diagnostic of the changes taking in its metabolism. In chemical terms, it is a mixture of compounds that can be sampled because of their ability to be vaporised both spontaneously and with suitable sampling conditions or techniques. Essential oils are isolated through steam- or hydro-distillation (with the exception of citrus fruits), a technique providing “true” samples of a rather homogenous composition in terms of molecular mass and polarity of their components. This characteristic is sometimes underestimated but it put essential oils and their chemistry in a nodal crossbreed of the thread agronomy, biology, botany, and biochemistry and their applications in a countless fields in particular in the food, cosmetic and pharmaceutical fields. This widespread need of different knowledge had induced Ian (and myself) to consider “essential oils” not as products but as a multidisciplinary entity involving several research fields to be collected under the above magic word. Our original idea would have had to include synergistically industry, regulatory organizations and universities with their technologists, lawyers, biologists and toxicologists, pharmacologists and medical doctors, agronomists and botanist and analytical and synthetic chemists. Maybe Jan’s idea was only a dream not feasible, or it arrived too early, and/or things are changed too fast in the opposite direction, but in a future vision of ISEO, it merits at least to be illustrated and discussed.

## PL1 Essential oils: From marketing to psychopharmacology

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**Keywords:** administration route, autonomic nervous system, affective state, behavior, cognition

### Abstract

In humans, odors are the most powerful cues to memory among all sensory stimuli. They may readily bring back memories of events that lie long in the past. Odors also tap directly into our emotions and affects. Thus, compared to cues from other sensory domains, such as vision or hearing, olfactory stimuli elicit memories that are older, more vivid and more emotional. Consequently, marketing experts have begun to incorporate odors to enhance consumer experience. In my talk, I will explain the key concepts of scent marketing and olfactory branding, how they are related to the principles of aromachology, and give some examples of recent research in this field.

Of course, the exploitation of the affective and mnemonic properties of odorants and particularly essential oils is not limited to marketing. In a more health-related context, their administration may enhance mood states and alter (cognitive) behavior, autonomic nervous system functioning and even brain activation. However, the existing literature suggests that one major moderator of their effectiveness is the route of administration, i.e., whether they are presented via the sense of smell or exclusively absorbed through the skin. In the second part of my talk, I will thus elaborate on the differences in effectiveness of odorants on human behavior, physiology and affective state as a function of administration. In addition, I will discuss which mechanisms of action might be involved.

## OP1 Essential oils application in food environments: A potential weapon to fight microbial adhesion

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**Keywords:** essential oils, biofilm, Salmonella, sublethal concentration, lettuce

### Abstract

Beside the common uses of essential oils in perfumery, cosmetics and pharmacy, nowadays the food industry is moving towards the exploitation of these natural compounds as flavouring agents and, more recently, as potential food bio-preservatives. The antimicrobial activity of essential oils (EOs) is well known [1,2] but interesting effects can be achieved also at sub-lethal concentrations, when EOs can modify microbial physiology, for example by stimulating the production of enzymes such as cellulose, amylase, and pectinase in fungi [3].

With regards to the application in food products, EOs are usually active at low concentrations but have a strong impact on the sensory characteristics of the product. Thus, to decrease the quantities to be applied and to limit food exposure to EOs, still maintaining the advantages of their application, a pre-treatment of the food product could be hypothesized.

In particular, we evaluated the effectiveness of a pre-treatment with *Cinnamomum zeylanicum* EO (CzEO) in lettuce. One hour of exposure of lettuce to CzEO at 5 µL/mL concentration was effective in reducing the adhesion of *Salmonella enterica* during refrigerated storage, with a significant effect up to 120 h of storage ( $p < 0.05$ ). The EO also inhibited polyphenol oxidase activity and preserved lettuce colour. CzEO was selected among eight different EOs, because it was the most effective against five *S. enterica* serovars at low concentrations (Minimal Inhibitory Concentrations of 1.25-1.87 µL/mL), also able to modify growth dynamics. The results are of great interest for the food industry as vegetables are increasingly involved in foodborne outbreaks.

Sublethal concentrations of essential oils could also be used to impair biofilm formation in food environments. In particular, *Origanum vulgare* EO (OvEO) was effective in reducing the biofilm-forming capability of *Pseudomonas fluorescens*. By confocal laser scanning microscopy of *P. fluorescens* after 48 h of incubation at 10°C, we demonstrated that OvEO concentrations from 1.25 to 5.0 µL/mL were able to dramatically hamper biofilm formation and thickness, reducing the number of attached cells, damaging or killing them. In presence of 5.0 µL/mL EO, the cells lost great part of their ability to adhere and aggregate [4].

In the light of these considerations, EOs demonstrate great potential as antimicrobials in the food industry, although some aspects still need to be further investigated. Nevertheless, the antimicrobial activity exerted on pathogens at low concentrations, the capability to reduce microbial adhesion, biofilm production, and to remove biofilm, also suggest the exploitation of essential oils as surface sanitizers in food production environments.

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## **PL2 The industrial production of essential oils: From theory to reality**

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### **Abstract**

I have seen and read so many brochures or worse, pharmaceutical dossiers, presenting the distillation of essential oils as if it happened in a lab or like in the old times.

The production of essential oils is mainly the fact of growers or collectors. They are using three main technologies: hydrodistillation, steam-distillation and expression.

Since two decades lots of improvements happened, the distillation process follows the GACP rules and the focus lies on saving energy, water and time.

In this lecture the improvements that have been made in the steam distillation will be presented.



## IS1 Industrial uses versus scientific research on essential oils

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### Abstract

Essential oils qualified as Natural Complex Substances due their composition and diversity are a group of most difficult ingredients used in the Industry - Flavours, Fragrances, Cosmetics, Pharmaceuticals, Aromatherapy and many others. Considering market conditions, quality, reproducibility, properties, and safety of essential oils used in consumer products are these factors which affect sales but also position of manufacturer and very important legislation. Relations between above mentioned factors and research on essential oils are discussed.

## OP2 The sesquiterpene hydrocarbons of patchouli and their contribution to the odour of the essential oil

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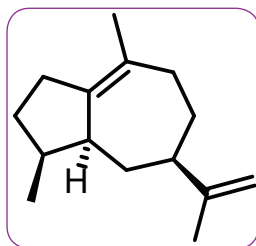
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**Keywords:** patchouli, *pogostemoin cablin*, bulnesene

### Abstract

To date, twenty-eight sesquiterpene hydrocarbons have been identified in patchouli essential oil. Additionally three were elucidated with reasonable certainty [1,2]. On both qualitative and quantitative standpoints, many of them do not correspond to the reality of their distribution in the unprocessed plant material. Based on unpublished original data, an updated status of this knowledge is presented, with a particular attention to  $\alpha$ -bulnesene (syn.  $\delta$ -guaiene).



In considering the long lasting controversy about the odour contribution in patchouli essential oil of pure patchoulol w/o sesquiterpene hydrocarbons, some results are presented and discussed.

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## OP3 Chemistry and industry implications of *Boswellia occulta*, a new species of frankincense from Somaliland

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**Keywords:** frankincense, olibanum, *Boswellia occulta*, *Boswellia carteri*, methoxydecane

### Abstract

Frankincense is an aromatic oleoresin derived from trees in the genus *Boswellia*. It is frequently distilled into essential oil for perfumery and, more recently, to supply an explosion of interest in aromatherapy and complementary medicine in the United States. *Boswellia sacra* syn. *carteri* and *Boswellia frereana* are two of the most popular species, and are harvested in Somaliland and Puntland. Although the essential oils of these species are normally dominated by terpenes, alkyl methyl ethers have recently been detected in many commercial essential oils. Further investigation uncovered a previously undescribed species of *Boswellia* growing in the Sanaag region of Somaliland it was named *Boswellia occulta*, and resin samples taken directly from individual trees showed high levels of alkyl methyl ethers as well as the diterpene alcohol serratol and sesquiterpene alcohol 4,10-di-*epi*-guaiol. This chemistry is dramatically different from other *Boswellia*, which are typically dominated by terpenes or aliphatic esters methoxyalkanes have previously only been described from arthropods and microbes. Analysis of commercial samples of essential oil sold as pure *Boswellia carteri* found the majority to be contaminated with alkyl methyl ethers, indicating that the resin distilled was a mixture of *B. carteri* and *B. occulta*. Additionally, the majority of certified organic samples contained *B. occulta* essential oil, showing that organic certification has in this case been ineffective at preventing biological contamination of a wild-harvested species. This also suggests that organic certification alone is insufficient to guarantee sustainable harvesting of a wild species, as it is unable to distinguish source trees.

## PL3 Antimicrobial activities of essential oils: Lessons from the past and recommendations for the future

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### Abstract

This year ISEO celebrates 50 years of essential oil research. On this marked occasion we should take cognisance that the therapeutic use of essential oils, specifically relating to infectious diseases, is one of the most prominent focus areas of research. While investigations have come a long way since the simple disc diffusion assay, it is worth reiterating areas of concern regarding methods to study the antimicrobial activity.

Aromatherapy is the back-bone for the practical application of essential oils, and scientific investigations should take this into consideration. The blending of oils is common practise and some examples of commercially relevant combinations will be demonstrated with an overview of synergistic, antagonistic, additive and non-interactive interactions provided. Some combination examples will be given from an African perspective where aromatic medicinal plants are combined for increased efficacy. When investigating essential oils, investigators should not forget the very important aspect of olfactometric properties. Combined with antimicrobial properties, several examples will be shown from both commercial oils and indigenous South African counterparts on how essential oils show promising economic potential for the bothersome condition of bromodosis. The phytochemical analysis of essential oils is critical in any tandem biological study. Using a model where gas chromatography coupled to mass spectrometry is correlated with antimicrobial activity, multivariate tools are able to identify biomarkers providing a more in-depth overview when examining large data sets.

While pure essential oil antimicrobial studies are important, the future should look to how we can incorporate these into formulations making them stable antimicrobial entities. Some examples will demonstrate the move in this direction. There is no doubt that with the onslaught of antimicrobial resistance and popularity, essential oils hold promise for future infectious diseases.

## IS2 Tea tree essential oil: A source of potential new attractants for Mediterranean fruit fly

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**Keywords:** *Melaleuca alternifolia*, tea tree essential oil, invasive species, mediterranean fruit fly, medflies

### Abstract

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: *Tephritidae*), is one of the most important agricultural pests worldwide due to the large number of fruit and vegetable crops vulnerable to attack. Current management practices often rely on the application of pesticides, which may have a negative impact on the environment and non-target organisms (e.g. beneficial insects). Consequently, there is a need for more environmentally friendly pest management strategies.

Plant essential oils are rich sources of terpenoids, many of which function as natural insect attractants, repellents, or other behavior-modifying kairomones. In short-range laboratory bioassays, we observed that male *C. capitata* are highly attracted to the essential oil from tea tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel, Myrtaceae). Therefore, we used thin-layer chromatography (TLC) to separate tea tree oil into multiple fractions, and then assayed each fraction to determine if it contained active constituents responsible for attraction. Of the five initial TLC fractions, two were found to be bioactive. The chemical composition of tea tree essential oil and subfractions were analyzed by gas chromatography–flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Additionally, electroantennography (EAG) was used to quantify male olfactory response to each of the fractions. This study identified potential new attractants that may aid in the development of environmentally sound management strategies for Mediterranean fruit fly.

## OP4 Bioassay-guided investigation of *Adenosma buchneroides* essential oil as mosquito repellent against *Aedes albopictus*

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**Keywords:** *Adenosma buchneroides*, ethnobotanical survey, mosquito repellent, carvacrol, structure-activity relationship

### Abstract

Mosquitoes can spread a variety of mosquito-borne diseases. The use of repellents is a significant strategy of preventing mosquito-borne diseases, but nowadays the widely used synthetic repellent, DEET (*N,N*-diethyl-3-methylbenzamide), has been reported increasing mosquito resistance, toxicity effects on skin, nervous and immune systems for humans. Due to these disadvantages, many customers prefer to use alternatives such as repellents of natural origin. Hence, research effort is necessary to continue to identify new mosquito repellents as alternatives to DEET, especially botanical repellents to meet the public demand for more natural products.

Prior to the arrival of synthetic chemicals, utilization of plant-derived materials to repel or kill mosquitoes has occurred for centuries. Local knowledge and practices of indigenous people demonstrated the extensive information available associated with native plants with such potential usages. But only few of these traditional knowledge and usages have been investigated systematically to determine the efficacy and the chemical constituents responsible for the activity.

*Adenosma buchneroides* has been used as an insect repellent by the AiNi people in southwest of China for a long time, but this traditional knowledge and usage has not been investigated systematically to determine the efficacy and the chemical constituents responsible for the activity. In the mosquito repellent bioassay, the essential oil of the plant showed strong repellent activity with minimum effective dosage (MED) of  $0.019 \pm 0.007$  mg/cm<sup>2</sup>, compared to reference standard DEET ( $0.031 \pm 0.014$  mg/cm<sup>2</sup>). Systematic bioassay-guided fractionation of the essential oil was conducted to isolate the active compounds. Carvacrol, carvacrol methyl ether and a new fragrant compound, adenosmin A, were found to be the active compounds responsible for the mosquito repellent activity, with MEDs in the range of 0.011–0.125 mg/cm<sup>2</sup>. In addition, to discover and develop more and stronger natural mosquito repellents, structure-activity relationship, larvicidal activity and cytotoxicity of the active repellent compounds were also investigated. An investigation on SAR of carvacrol analogues led to the discovery of three analogues with further lower MEDs (0.002–0.009 mg/cm<sup>2</sup>) than that of DEET, and other three compounds with similar MEDs (0.029–0.039 mg/cm<sup>2</sup>) to that of DEET. Larvicidal bioassay showed that carvacrol was the best larvicide among the tested active repellent compounds (LD<sub>50</sub> of 24.8 ppm). The essential oil and repellent compounds against seven mammalian cell lines revealed low or no cytotoxicity. Scientific evidences reported here validate the plant's traditional use as insect repellent and imply promising application of the essential oil and carvacrol analogues as mosquito repellents.

## OP5 Chemical compositions and larvicidal activity of essential oil from the leaf and fruits of *Manglietia dandyi* (Gagnep.) Dandy growing in Vietnam

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**Keywords:** *Manglietia dandyi*, essential oil, sesquiterpenes, larvicidal activity.

### Abstract

In continuation of an extensive study on the chemical constituents and biological actions of essential oils from Vietnamese flora [1-3], we report herein the results of our investigation on *Manglietia dandyi* (Gagnep.) Dandy. The constituents of the oils were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The yields of the oils were 0.22% and 0.31% (v/w, leaf and fruit, respectively), calculated on a dry weight basis. The main constituents of the leaf oils were (*E*)-nerolidol (18.4%) and  $\alpha$ -selinene (11.0%) while the fruit oils contained abundance of  $\beta$ -caryophyllene (27.7%), d-cadinene (13.7%),  $\alpha$ -humulene (13.2%) and  $\alpha$ -copaene (11.6%). The essential oils good larvicidal activities against larvae of *Aedes albopictus* with LC<sub>50</sub> values of 12.00 mg/mL and 8.21 mg/mL, respectively, at 24 h and 48 h.

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## OP6 Essential oils: A largely unexplored source of potentially novel medicines

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**Keywords:** drug discovery, medicine, essential oil, essential oil component, data mining

### Abstract

Plant extracts, including essential oils, have been used therapeutically for millennia. It has been extensively shown that essential oils and their components have a wide range of biological activities. Randomized controlled clinical trials have supported effectiveness in specific therapeutic indications for some of them, but only few essential oils and their components have been approved as drugs. In 2015 a shared Nobel Prize was awarded to Dr. Youyou Tu for her research on plant extracts for the development of a novel anti-malaria medicine. Although a relatively large number of essential oils and their components is available as a dietary supplement for animals and humans, they are considered products used primarily in complementary and alternative medicine.

In recent decades plant extracts, have been avoided in drug discovery, in part due to (potential) technical problems in high-throughput screening. Recent insights and advancements, however, have largely addressed these problems. Moreover, is ignoring essential oils and their components still justified, especially with our current understanding of drug discovery?

To answer this question, we studied select physicochemical parameters of a large representative set of 175 commercially available (certified organic) essential oils. In a novel approach, we demonstrated that essential oils are composed of components that typically meet the current requirements of medicinal chemistry for good drug candidates. Based on our results, it seems that their therapeutic potential is currently vastly under-used. Therefore, we recommend that their drug-like potential should be explored more vigorously using modern drug discovery methods.



## PL4 Essential oils are a gold mine for an organic/ medicinal chemist

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**Keywords:** essential oil, organic chemistry, medicinal chemistry

### Abstract

Organic chemistry has its roots in the chemistry of natural products, where simple isolation techniques, such as distillation with water steam, enabled accessibility of materials for analysis and a starting point for synthetic work. Many fundamental concepts were conceived through the classical analysis of what we today call essential oils and their components. The volatility of these substances derived from natural sources was of paramount importance since it led to the development of sophisticated analytical techniques such as gas chromatography, and later on its coupling with mass spectrometry. Once regarded as metabolism dead waste due to at that time unapparent function, plant volatiles, collectively but not completely accurately termed essential oils, remain the subject of work of many scientists. However, today it is primarily a multidisciplinary field.

Essential oils represent complex mixtures of usually several hundreds of constituents of widely varying relative content coming from different biosynthetic and/or artefactual background. Although studies are mostly centered on major constituents, on many occasions it has been demonstrated that minor volatiles may be of equal if not greater importance in a biological sense, especially an olfactory one. Serving plants as a means of protection against pathogens and predators, as well as both internal communication and with its surroundings, and assuming many more roles, it is logical to expect the onset of significant biological effects connected with essential-oil constituents. Since never alone these compounds exert synergistic (biological) properties when acting in unison with other volatile or non-volatile compounds. This is where medicinal chemists also find a fertile land and many essential oils and single constituents have their place in the pharmacopeias of many nations, while their mechanism of action is being elucidated by medicinal chemists and pharmacologists alike.

In this lecture, a series of examples illustrating the recent interplay between organic and medicinal chemistry, and the research on essential oils/plant volatiles will be presented. The examples will not be limited to the chemistry directly involved in the compositional analyses or the pharmacology of genuine essential-oil constituents but will cover the possible derivatives of these compounds coming from their possible or current application. These examples have a purpose of stimulating further similar research and to show that essential oils provide a practically endless opportunity for both chemical and biological studies.

## OP7 Combinatorial synthesis of fragrant volatiles from natural raw materials - a sustainable approach towards discovery of new odorants

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### Abstract

The fragrance industry is based on delivering natural and synthetic commodities. Production of most types of these commodities often strains the environment and may pose possible health hazards such as the case of the high demand for deer musk secretions resulting in a decrease in the deer population. Six out of seven of deer musk species are listed as endangered. Synthetic odorants are mostly prepared from raw materials derived from fossil fuels and generally use toxic chemicals. The industry addresses the above issues by using several approaches such as process intensification by turning from batch to flow production, implementation of biotechnological processes, and the use of safer, alternative reagents. The other problem involves prohibition of certain health hazard-related ingredients such as geranyl nitrile (lemon scent), which was a popular citral replacer for alkaline formulations. Preventing health hazards is one of the reasons why there is still a need for a search for new, more substantive odorants. The most benign tools for this search are *in-silico* methods, but unlike medicinal chemistry, rational odorant design suffers from a large number of limitations. The most popular strategy in the field of fragrance chemistry is a slow, time- and resource-consuming classical organic synthesis of a large number of volatile compounds or a serendipitous discovery. We addressed these disadvantages by implementing a combinatorial approach for screening compounds bearing a phenylpropane skeleton and alkoxyimine moiety that have interesting odoriferous properties. Seventeen small combinatorial libraries were prepared on a milligram scale and the sensory potential of each compound was evaluated using gas chromatography-olfactometry. Twenty compounds (out of 163) were selected as promising odorants, synthesized on a gram scale and fully characterized. Their scents belong to the several olfactive families: aromatic (spicy, sweet and cinnamon notes), fruity (lime, red currant and grape notes), floral (tuberose, narcissus, violet), green and mossy. The details of the approach and the potential of the new compounds in functional products will be presented.

### ACKNOWLEDGEMENT

This work was supported by the project "Synthesis of new fragrances from raw materials of a natural origin with application in perfumery, cosmetics and household chemistry" (SYNFRA) grant no. LIDER/4/0099/L-7/15/NCBR/2016, which is financed by the National Centre for Research and Development within the LIDER Programme.

## OP8 Volatile components of several South American liverworts and their biological activity

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### Abstract

Liverworts are rich sources of biologically active terpenoids and aromatic compounds having antimicrobial, antiviral, antifungal, antioxidant, muscle relaxant, tubulin polymerization inhibitory and antitrypanosomal activity [1-3]. In the southern hemisphere, 51 endemic genera of bryophytes have been known, however, only a few % of liverworts in this region have phytochemically been studied. Thus Chilean and the other South American liverworts become an interesting potential source of new molecules, molecular skeletons and related applications in pharmaceutical, pesticide and the other fields.

The aim of this study is to increase the phytochemical knowledge of South American liverworts, especially from those of the southern tip of the continent, due to the uniqueness of its poorly known liverwort flora. Sixteen and 22 species of liverworts were collected in Chilean and Peruvian territory, respectively, and their ether extracts were analyzed spectroscopically. Within the new results from the analyzed liverworts, we highlight the presence of the brown algae sex pheromones, (E)-ectocarpene (1) and dictyopterene (2) in *Anastrophylopsis involutifolium*, together with a new sesquiterpene aldehyde, 6,7-secoeudesm-7(11)-en-6-al (3). These pheromones have been reported by our group [1c] from the Tahitian and Greek liverworts, *Chandonanthus hirtellus* and *Fossombronina angulosa*. The Plagiochila species are rich sources of 2,3-secoaroma-dendrane sesquiterpenoids the precursor of which is an enantiomeric bicyclogermacrene found in almost all of the Plagiochila species analyzed. The biological activity of the isolated compounds from the present liverwort species and their chemosystematics will be discussed.

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## OP9 Chemosystematic significance of essential oils obtained from characteristic taxonomic groups of the New Caledonian flora

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**Keywords:** essential oils, chemodiversity, new caledonia, gymnosperms, *Myodocarpaceae*

### Abstract

The archipelago of New Caledonia is a French overseas collectivity of the south Pacific that is renowned worldwide as a hot spot of biodiversity. Its vascular flora is characterized by a large number of species (3371 species for 19 000 km<sup>2</sup> of land area) but especially by its high endemism at the species level (77.8% for the flowering plants) [1]. The characteristics of New Caledonia's flora have largely resulted from the complex geological history of the archipelago and its isolation in the southwestern Pacific Ocean.

Secondary metabolites especially those from essential oils can be useful chemosystematic markers to distinguish plants at different taxonomy levels. In order to deepen the knowledge on this specific flora, the essential oil compositions of several taxonomic groups have been analyzed by GC-FID and GC-MS and discussed in light of their chemosystematic significance. A classification of different species is proposed by comparing the structures of the major secondary metabolites according to their biosynthetic pathways.

We report analytical results obtained for three distinctive taxonomic groups: 1) gymnosperms which is a primitive group 2) Myodocarpus, an endemic genera of the Myodocarpaceae family and 3) Myrtaceae, the most represented family in New Caledonia which has been subjected to an intense speciation within several genera. Volatile compounds composition of essential oils show specific distribution patterns depending on taxonomic group and provide valued evidence about the diversity and evolutionary relationships of these species.

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## PL5 Carbon isotope ratios of volatiles as a powerful parameter for genuineness assessment of essential oils

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**Keywords:**  $d^{13}C$  data evaluation, essential oils, citrus, Helichrysum

### Abstract

The most successful applications of IRMS on cold pressed citrus peel, neroli, petitgrain, and other essential oils will be described along with few other applications on food flavours. For example, the case of GC-C-IRMS applied for a direct comparison of isotope ratios determined in several secondary metabolites of Mexican and Persian Lime, with myrcene as internal standard, will provide a good glimpse of how the values of  $d^{13}C_{Myrcene}$  enable to distinguish between the botanic species eliminating the geographic influences. Similar approaches applied to authenticate natural flavours will be given.

In addition a newly developed method for  $d^{13}C$  data evaluation of natural compounds will be presented. This method, based on Comprehensive Isotopic Data Evaluation (CIDE), consists of the analysis of the entire volatile's carbon isotope ratio, obtained by integrating the whole gas chromatogram (volatile bulk) determined by GC-C-IRMS and by plotting this value vs. the  $d^{13}C$  of a selected pure volatile compound ( $y$  compound). The results allow the formation of clusters from samples of identical nature based on their origins on a bi-dimensional separation plot. Few examples of how this analytical approach works to distinguish the geographic origin of food ingredients, the botanical source, and eventually the differentiation of natural and synthetic flavourings will be given to appreciate CIDE's capability to unveil possible frauds and assess the origin of food ingredients.

## OP10 Essential oils of *Marchantia polymorpha* L. from Serbian populations

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**Keywords:** liverworts, *Marchantia polymorpha*, subspecies, essential oils composition

### Abstract

Liverworts are a rich source of a diverse array of specialized metabolites, especially terpenoids, that play an important role in bryophyte-environment interactions and are potentially useful for pharmaceutical applications. The genus *Marchantia* (Marchantiaceae) is one of the largest in the order Marchantiales, and its geographical range is worldwide. The most characteristic species belonging to this genus is *Marchantia polymorpha* L. Our previous studies have shown that cyclopropanecuparenol is the most characteristic volatile compound for this species. This cuparane-type sesquiterpene alcohol was absent in other species belonging to the genus *Marchantia* [1,2].

The aim of our studies was the chemical analysis of essential oils (EOs) hydrodistilled from three subspecies of *M. polymorpha* namely: *M. polymorpha* subsp. *montivagans*, *M. polymorpha* subsp. *polymorpha*, and *M. polymorpha* subsp. *ruderalis*. Plant material was collected in June and August 2017 from four different localities in Serbia. Liverworts were dried at room temperature, then grinded to a fine powder and hydrodistilled for 3 h, using a Clevenger-type apparatus. The yield of EOs was ranged from 0.18 to 0.82%. All EOs were analyzed by GC-MS and compounds were identified using a computer supported spectral libraries and mass spectra of reference compounds together with the comparison of measured retention indices with reference compounds and published data.

GC-MS fingerprint analysis of EOs obtained from the Serbian populations of *M. polymorpha* indicated considerable variation in the composition of the volatile components. The chemical composition of specimens collected in Topčider and Petnica, and identified as *M. polymorpha* subsp. *ruderalis* is very similar to that reported previously for specimens collected in Japan and Europe. The most characteristic component of these specimens is cyclopropanecuparenol, as well as other sesquiterpenoids of cuparane-, chamigrane- and thujopsane-type. However, the chemical composition of two other *M. polymorpha* subspecies are completely different. The presence of cuparanes, chamigranes and thujopsanes has not been confirmed. 5-Guaia-11-ol, cyclomytlayan-15-ol, viridiflorol,  $\alpha$ -gurjunene, and  $\alpha$ -selinene are the most characteristic components identified in *M. polymorpha* subsp. *montivagans* collected in Golija and *M. polymorpha* subsp. *polymorpha* collected in Ladevac.

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## OP11 Comprehensive study of volatile secondary metabolites from Verbenaceae plants grown in Colombia

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**Keywords:** *Lippia alba*, *origanoides*, ORAC

### Abstract

Colombia's large biological diversity involves plant metabolite chemical variety, revealed by the chromatographic analysis of essential oils and flower scents. CENIVAM is a network of research groups that for the past 15 years has been carrying out a bioprospection study of aromatic plants found in Colombia. It aimed to characterizing their volatile secondary metabolites and exploring their potential sustainable uses. This has implied vegetal material recollection, its botanical identification, the use of various extraction techniques (solvent extraction, hydrodistillation, static and dynamic headspace, solid-phase microextraction) on the whole plant or some of its parts, followed by physico-chemical and detailed chemical secondary metabolite characterization (GC, GC-MS, GCxGC, HPLC, LC-MS). These analyses permitted the identification and quantification of typically over 90% of the constituents of essential oils and flower fragrances. Plant material of *Lippia alba* and *L. origanoides* from different Colombian regions were found to correspond to four different chemotypes of each species. Sesquiterpene hydrocarbons were the main compound family found in *L. americana* and *L. dulcis* oils; monoterpene hydrocarbons were the distinctive components of *L. micromera* and the phellandrene-rich *L. origanoides* chemotype oils. The oils of the two other *L. origanoides* chemotypes and of *L. graveolens* were rich in oxygenated phenylpropanoids. The oils from the four *L. alba* chemotypes studied were mostly composed of oxygenated monoterpenes. The essential oils of *L. alba* (carvone), *L. alba* (citral), *L. alba* (carvone+citral), *L. alba* (myrcenone), *L. origanoides* (carvacrol), *L. origanoides* (thymol), *L. origanoides* (phellandrene), *L. citriodora*, *L. micromera*, *L. americana*, *L. graveolens*, and *L. dulcis*, exhibited higher antioxidant capacity than BHT and  $\alpha$ -tocopherol in the ORAC assay. This makes them very good candidates to become ingredients of final products in substitution of synthetic antioxidants. The detection of thymol and carvacrol as main essential oil constituents as well as pinocembrin, naringenin, quercetin, and luteolin in mg/g amounts in extracts of various *L. origanoides* chemotypes support the recognition of this species as a promising source of bioactive substances. A computational model of *L. origanoides* essential oil batch fractional distillation at reduced pressure is being developed to facilitate scale up of its standardization.

## YS1 Chemical composition of essential oils from *Lippia alba* genotypes analyzed by GC-MS and flow-modulated comprehensive two dimensional gas chromatography

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### Abstract

*Lippia alba* (Mill) N.E. Brown is a shrub of the Verbenaceae family. This plant is widely used in popular medicine due its different properties such as sedative, antidepressant and analgesic [1]. However, as an aromatic plant, its essential oil has attracted great attention in food (flavouring), cosmetic (fragrances) and pharmaceutical (aromatherapy) industries [2]. Some of the terpenoids usually found in this plant constitute as fragrance and flavor components common in plenty industrial products, as for example the monoterpene linalool, are widely used in perfumes. Due to the economic importance of this specie, studies in plant breeding program were conducted at the Agronomic Institute of Campinas (IAC) through crossing different genotypes from the Active Germplasm Bank (AGB), aiming for obtaining new chemotypes which might have an industrial value. Hence, the study and knowledge of the chemical composition of these new materials allows the discrimination of accesses and consequently can act as a guide for the genetic breeding programs.

Thus, the present study aimed to evaluate and characterize the chemical composition of the essential oils of four *L. alba* genotypes (IAC X2, IAC X2M1, IAC X6M9 and IAC X6MIA). In a specific way, this work aimed to compare the conventional Gas Chromatography-Mass Spectrometry (GC-MS) with the impact of a Comprehensive Two-Dimensional Gas Chromatography (GCxGC) technique as for enhancing the separation and detection of the analytes. For this, each essential oil was obtained by hydrodistillation of the dried leaves using a Clevenger apparatus, which yielded 0.42% (IAC X2), 0.38% (IAC X2M1), 0.30% (IAC X6M9) and 1.03% (IAC X6MIA) of each oil, respectively. The samples were stored in the dark at 4°C until analysis. Significant differences regarding the full chemical profile were observed among the genotypes, although monoterpenes were identified as the major constituents in all samples. Therefore, myrcene (24.9%) and linalool (22.7%) were identified as the major constituents of IAC X2. IAC X2M1, however, showed exclusively myrcene (56.6%) as its major constituent, while citral (58.6%) and myrcene (27.8%) were observed to IAC X6M9. At last, IAC X6MIA indicated linalool (61.3%) and myrcene (19.7%) as its major constituents. In addition, a total of 55 compounds could be detected by GC-MS. The GCxGC-QMS analysis, on the other hand, allowed separating and detecting 175 compounds. In conclusion, in this study, besides a screening of the constituents from new genotypes of *L. alba*, we inferred the applicability that these new crossing might represent to the development of new flavors and fragrances. Moreover, we highlighted and showed the high sensibility and resolution of the GCxGC-QMS technique as an important tool in metabolomics studies of volatiles.

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### ACKNOWLEDGEMENT

Fapesp (2018/26066-1)



## YS2 ***In vitro* permeation kinetics, skin layers distribution and emission in the surrounding environment of biologically active *Melaleuca alternifolia* (Tea Tree) essential oil components from topic formulations**

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**Keywords:** *Melaleuca alternifolia*, essential oil, head space solid phase microextraction, *in vitro* skin permeation, skin layers distribution

### Abstract

*Melaleuca alternifolia* essential oil (Tea Tree Oil, TTO) is widely employed both as such and as ingredient in skin care products for its several well-known biological activities. The European Scientific Committee on Consumer Products constantly promotes the collection of information on both skin distribution and systemic exposure to TTO components after the application of topic formulations. In this context, our study aimed to obtain quantitative data on the percutaneous absorption and skin layers distribution of seven TTO major components (i.e.  $\alpha$ -pinene,  $\beta$ -pinene,  $\gamma$ -terpinene, 1,8-cineole,  $\alpha$ -terpinene, 4-terpineol,  $\alpha$ -terpineol) when applying a 5% TTO cream.

The study also focused on the evaluation of the TTO major components' release in the surrounding environment due to their high volatility. The permeation study was performed *in vitro* on full thickness pig ear skin samples using both conventional and *ad hoc* modified static glass Franz diffusion cells to monitor the compounds permeation and environmental release, respectively. The amount of formulation to apply was chosen to be representative for the in-use condition (finite dose regime). The applied formulation (before and after the study), the collected receptor fluid and the individual skin layers (*stratum corneum*, epidermis and dermis) - previously separated - were quantitatively analyzed by a fully-automatized and solvent-free analytical method employing Headspace Solid Phase Microextraction (HS-SPME) in combination with Gas Chromatography coupled with Mass Spectrometry (GC-MS). Method validation showed satisfying values of sensitivity, repeatability and intermediate precision with percent relative standard deviation (%RSD) below 15%. External calibration curves were built through both simple HS-SPME-GC-MS and HS-SPME-GC-MS in Multiple Head Space Extraction mode (MHE) to quantify TTO markers in the receptor fluid and in skin layers and formulation respectively. At the end of the permeation study, the applied formulation was almost free from TTO components. Skin layers overall contained less than 1% of each TTO component. Only the oxygenated terpenes: 4-terpineol,  $\alpha$ -terpineol and 1,8-cineole, significantly permeated through the skin membrane (around 50% and 12% of the total for 4-terpineol/  $\alpha$ -terpineol and 1,8-cineole, respectively), while hydrocarbons were found at trace level in the receptor fluid. As expected from the TTO markers volatile nature, a substantial fraction of total amount of each TTO component applied on the skin at the beginning of the study was released in the surrounding environment. To the best of the authors' knowledge, in this work for the first time 1) an *ad hoc* modified static Franz cells was used to quantify the loss of TTO components by evaporation and 2) a complete solvent-free method was employed to quantify and extract seven major, volatile TTO components from skin layers.

## YS3 Packaging methods and storage duration affect essential oil content and composition of hyssop (*Hyssopus officinalis* L.)

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### Abstract

Medicinal and aromatic plants (MAPs) are often stored for long periods before use, in order to preparing various types of products from them. MAPs undergo many physical, chemical, and microbiological changes during storage. Unsuitable packaging and storage can induce alterations in the chemical composition of their active substance which may affect the flavor and fragrance properties of the herbal product, with a negative impact on the industrial value and consumer satisfaction. Therefore, determining a suitable packaging method to maintain higher concentrations of active substances during storage is very important.

Changes in essential oil (EO) content and composition of hyssop leaves at different packaging methods (packaged with air, nitrogen, and under vacuum) and during storage period (0, 3 and 6 months) were determined. All the samples were hydro-distilled every three months during storage for EO content evaluation. EO composition was determined by gas chromatography and gas chromatography–mass spectrometry. The results showed that by extending the storage period in all packaging methods, EO content was decreased, which was not significant in samples packed with nitrogen gas and air. In air and nitrogen packaging, with increasing storage time, the amount of *cis* and *trans*-pinocamphone was reduced. The highest amount of *cis*-pinocamphone was observed in vacuum packaging (51.4% and 55.3% after 3 and 6 months, respectively) and the lowest amount (47.6%) was determined in packaging with air after six months of storage. In general, the results of hyssop EOs analysis had valuable findings due to the type of packaging and the storage time. Changes in EO components in packaging methods during storage may be a useful marker for the indication of storage duration.

## YS4 Therapeutic blending: The use of essential oils in combination against pathogens of the respiratory tract

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### Abstract

Essential oil blends are believed to work synergistically to heal the mind, body and senses. Aromatherapy, a form of complementary therapy, uses essential oils as therapeutic agents to treat several diseases including respiratory tract infections. At least 48 essential oils can be identified as being recommended for respiratory use, with over 300 recommended essential oil combinations. However, very few scientific studies have been conducted on their combined efficacy. This study looks at the antimicrobial activity of essential oils in combination, as recommended for respiratory use.

The minimum inhibitory concentration (MIC) assay was used incorporating fractional inhibitory concentration (FIC) calculations as well as isobolograms to demonstrate interactive profiles. Chemometrics methods (PCA and OPLS-DA) coupled to gas chromatography-mass spectrometry were used to analyze the chemical composition and correlation with antimicrobial activity. Of the 387 essential oil combinations investigated, 80% were noteworthy in antimicrobial activity (MIC value of 1 mg/ml or less) and 25% were synergistic when blended in 1:1 combinations. One of the most synergistic combinations identified was the oil *Abies balsamea* (Linn.) Miller (fir) in combination with *Myrtus communis* Linn. (myrtle) against *Streptococcus pneumoniae* ( $\Sigma$ FIC of 0.03). Antagonism was noted, with the most antagonistic blend identified as *Syzygium aromaticum* (Linn.) Merr. & Perry (clove) in combination with *Ocimum basilicum* Linn. (basil) against the pathogen *Cryptococcus neoformans* ( $\Sigma$ FIC of 74.67). The S-plot constructed from the discriminant model (OPLS-DA) obtained made it possible to detect the connection between chemistry and antimicrobial activity of essential oils. Biomarkers identified as indicated by the S-plot were 1,8-cineole,  $\alpha$ -pinene, estragol, eugenol, furanogermacrene, limonene, linalool, menthone, *p*-cymene, terpinen-4-ol and thymol. These biomarkers were recognized as compounds that may be associated with good activity against respiratory pathogens. Chemometrics may be adopted as a useful tool to identify compounds responsible for antimicrobial activity. This study on essential oils used to treat respiratory tract infections provides valuable data and serves as a therapeutic guide for aromatherapists when treating these infections.

## YS5 Volatile compounds of some raw pistachio nut varieties with solid phase microextraction technique

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**Keywords:** Pistachio varieties, SPME, volatile compounds

### Abstract

Pistachio nut (*Pistachia vera* L.) is one of the most popular fruits all over the world in terms of its nutritional and health benefits. Due to fat content and unsaturated fatty acids content pistachio kernels are good source for human nutrition. According to reports by FAO, Turkey is the third largest pistachio producer after Iran and the USA with 170,000 tonnes in 2016. In this study, it was investigated the volatile compounds (VCs) of three raw pistachio nut (*Pistachia vera* L.) varieties 'Siirt', 'Uzun' and 'Kırmızı' from 'Siirt', 'Mardin' and 'Gaziantep' provinces in Turkey, respectively. The VCs were analyzed by using solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS). A total of 43 VCs including alcohols (13), terpenes (11), aldehydes (6), phenyls and phenols (6), ketones (3), esters(2), acids (1) and alkenes (1) were identified in the head space of pistachio nut samples. According to the chemical groups, the main VCs identified in pistachio nuts were terpenes (approximately %55-83), followed by phenyls and phenols (3-16%), alcohols (3-15%), aldehydes (4-6%). The majority of VCs identified in the head space of 'Siirt', 'Uzun' and 'Kırmızı' pistachio nuts fruits were constituted limonene (approximately 62%, 17% and 11%, respectively),  $\alpha$ -terpinolene (19%, 41% and 34%), ethanol (6% for 'Siirt' only),  $\alpha$ -pinene and p-cymene (10% and 5% for 'Uzun' variety only), 1,8-menthadien-4-ol, 3,7-dimethyl-1,3,6-octatriene and anethole (7%, 6% and 5% for 'Kırmızı' variety only). These VCs accounted for approximately 87%, 73% and 63% of total VCs identified in 'Siirt', 'Uzun' and 'Kırmızı' Pistachio nut fruits, respectively. In conclusion, the profiles and relative proportions of VCs were affected by the variety. Due to the high limonene and also ethanol proportions, 'Siirt' variety was separated from the other varieties. All the Pistachio nut varieties are a good source in terms of terpene abundance, especially limonene and  $\alpha$ -terpinolene.

## YS6 Sub-lethal concentrations of *Origanum vulgare* essential oil exert inhibitory activity on *Listeria monocytogenes* strains in different environmental conditions

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**Keywords:** essential oil, food, biopreservative, *Listeria monocytogenes*

### Abstract

In food systems, mild preserving strategies include the combination of treatments that minimize the sensory impact on the product and at the same time increase food safety and stability. In this respect, essential oils (EOs) are excellent biopreservatives, in particular for their antimicrobial activity, due to their action on cell membranes, disturbing cell structures and impairing enzymatic systems [1]. Many EOs possess strong antibacterial activity against spoiling and pathogenic bacteria [2]. *Listeria monocytogenes* is an important food pathogen that primarily affects sensitive populations such as pregnant women, elderly and immunocompromised, with high mortality rate. It is common in Refrigerated Processed Foods with Extended Durability (RePFEDs).

The aim of this study was to evaluate the effects of *Origanum vulgare* EO at sub-lethal concentrations on the growth of different *L. monocytogenes* strains, also modifying NaCl content and the carbon source in the culture medium, thus simulating conditions encountered in food products. The growth dynamics of *L. monocytogenes* ATCC 7644 and two strains isolated from smoked salmon were investigated by automated turbidimetry, combining sub-lethal quantities of EO with different NaCl quantities. Our results showed that minimal concentrations of EO (1.25 and 0.156  $\mu\text{l/ml}$ ) combined with NaCl (1.0- 2.5%) extended the lag phase of type and wild strains, in comparison to single treatments. Furthermore, treatments with NaCl alone at high concentration (8%) were less effective than the combination of the two stresses at sub-lethal concentrations, causing only a smaller extension of the lag phase. ATCC 7644 was also exposed to 1.25  $\mu\text{l/ml}$  of *O. vulgare* EO for 1 hour, then the EO was removed, and the cells were incubated at 37°C for 48 hours in different culture media. The pre-treatment still affected growth dynamics but the growth value reduction and the lag phase extension were modified differently, depending on the carbon source present in the culture media (e.g. glucose, lactose, etc). Moreover, confocal microscopy revealed that the pre-treatment also caused an aggregation of ATCC 7644 cells, likely due to increase in hydrophobicity or to a stress response.

In conclusion, it is known that the combination of different hurdles allows the reduction of the concentrations used, boosting the effectiveness of the treatment. However, to optimize food treatments by means of essential oils, it is fundamental to understand their effect on microbial physiology. *O. vulgare* EO in very small quantities was able to strongly affect the growth performance and the behaviour of *L. monocytogenes* in culture media reproducing environmental conditions that are common in food products.

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## PD The survival of essential oils calls for a revolution!

Alain Chaintreau<sup>1,\*</sup>, Daniel Joulain, Carlo Bicchi

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### Abstract

Since centuries, the essential oils market has progressively grown up in a context of traditional practices from plant cultivation up to their use in final consumer products. A first revolution occurred in the 60s' due to the arrival of chromatographic and spectrometric tools for their analysis. In spite of optimistic economic forecasts for the forthcoming years, many severe challenges must be addressed. Ethical and environmental rules are emerging at the plant production and essential oil manufacture stages. The research stage is unfortunately characterized by a lack of knowledge of good scientific practices. Less publications but with a much higher level would be incomparably more useful than the current multitude of unreliable papers. In terms of bioactive properties, Many teams look for bioactive beneficial properties while deliberately ignoring the undesirable effects, in spite of a growing regulation when used in the F&F domain. Last be not least, the recent appearance of alternatives to essential oils now is a significant threat strongly competing with the production of certain traditional essential oils. The whole chain of essential oils has to be adapted to face all these challenges and to give an answer to all expectations of consumers and authorities. This is not less than a revolution compared to the current situation!

## PL6 Essential oils in veterinary medicine and animal nutrition

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**Keywords:** antibiotic activity, target attainment, mode of action, encapsulation, animal health and welfare

### Abstract

Essential oils have been used since ancient times in the treatment of various diseases in humans and animals, as food additives to improve taste and shelf-life and as repellent and mild biocidal agents against insects and plagues. Despite this apparently common use, the use of medicinal plants in animals was until recently restricted to individual therapeutic applications based on traditional (ethno-) veterinary medicine, following often the applications and indications in human health care.

In the last decade, however, an unprecedented interest in phytogetic substances emerged, driven by the increasing concerns about the global increase of antimicrobial resistance. In particular, the demand to reduce the use of antibiotics in farm animals, particularly for non-therapeutic applications such as growth promotion, stimulated the search for natural (alternative) strategies to improve animal health and welfare. Subsequently, research focussed initially on a comparison of the antibiotic activity of powerful substances such as thymol, carvacrol, cinnamaldehyde, allicin and related compounds. Many of these plant-derived substances proved to be effective also against antibiotic-resistant strains due to a multi-target mechanism of action affecting the integrity of the bacterial cell membrane, bacterial metabolic traits, adhesion, quorum sensing and virulence. However, many of these investigations were limited to *in vitro* assays, but failed to show the same efficacy in *in vivo* trials. Stability of volatile compounds, kinetic parameters and lack of target attainment readily explain these differences in clinical outcome. Subsequently, objectives were re-defined, and a research strategy was implemented in animal health care involving the following considerations:

- Re-assessment of MIC/MBC properties of EOs and related plant metabolites in combination with kinetic data (target attainment)
- Re-assessment of the overall biological properties (mode of action) of plant-remedies, with a clear focus on pre-systemic effects (including effects on intestinal integrity, the intestinal microbiome, antioxidant and direct and indirect anti-inflammatory properties).
- Standardized production (including the use of hairy-root cultures) to allow a high standardization and a reliable availability also for the farm-animal sector.
- Coating and encapsulation technologies to increase stability und allow a safe and easy use under practical conditions, including the oral application with drinking water and feed.

This novel approach based on inherited knowledge, is now considered as one of the most promising strategies to improve and support animal health and welfare and reduce the negative impact of food-producing animals on the environment.

## OP12 Impact of a phytogenic feed additive on inflammation markers and microbiota composition of weaned piglets as assessed by NGS

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**Keywords:** phytogenic, growth promotion, inflammation, microbiota, next-generation sequencing

### Abstract

Current efforts in reducing the over-use of antibiotics in animal production drive the demand for alternative feed additives. These should offer similar benefits while lacking undesirable effects, e.g. promotion of microbial resistance. It is hypothesised, that the observed growth promotion, when feeding subtherapeutic levels of antibiotics, is mediated partly by their inflammation-restricting properties and partly by effects on the gut microbiota composition. Phytogenics are plant-derived feed additives, often produced from traditional medicinal plants or spices with anti-inflammatory activities. In this study, the impact of a phytogenic feed additive (based on essential oils) on inflammatory signals was studied *in vitro* and *in vivo*, while the microbial diversity and composition was additionally assessed *in vivo*.

First evidence for anti-inflammatory and anti-oxidative effects was obtained from cell culture studies with IPEC-J2. Subsequently, *in vivo* studies with weaned piglets were conducted for 21 days. Piglets received either a basal diet alone or a basal diet additionally supplemented with the phytogenic feed additive. Blood samples were analysed for markers of inflammation (acute phase proteins; APP), while fecal samples were used for characterization of the gut microbiota by Next-generation Sequencing (NGS) of the 16S rRNA gene (Illumina MiSeq).

NF- $\kappa$ B translocation and expression of related genes were down-regulated when transiently transfected and TNF- $\alpha$  stimulated IPEC-J2 were incubated *in vitro* with an extract of the phytogenic product. Additionally, less inflammatory IL-6 was released by the cells. Phytogenic-fed weaned piglets had significantly lower levels of the serum acute-phase-protein PigMAP ( $p < 0.05$ ) than the control group. Statistically significant differences in the microbial composition between diets were found after eight days of trial duration, but not at the start or the end of the experiment. *Bacteroides* and *Desulfovibrio* were reduced in the phytogenics group ( $p < 0.05$ ), compared to the control.

The findings support the hypothesis that restriction of inflammatory processes is one key mechanism exerted by a growth-promoting phytogenic feed additive and provide insight into its effects on the gut microbiota. NGS technologies offer novel opportunities to study the consequences of nutritional intervention strategies on diversity and characteristics of the gut microbiota.



## YS7 Effect of a microencapsulated citrus essential oil on *in vitro* fermentation kinetics of pig gut microbiota

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**Keywords:** citrus essential oil, microencapsulation, pig gut microbiota, SCFA, ETEC

### Abstract

Essential oils (EOs) have emerged as a potential alternative to using antibiotics in pig breeding due to their antimicrobial properties. Citrus EOs, common by-products of food industry, can be a feasible alternative for this purpose due to their huge availability on the global market. Thus, the effect of a microencapsulated citrus EO, Brazilian Orange Terpenes (BOT), on *in vitro* fermentation kinetics of pig gut microbiota was evaluated. The encapsulation efficiency by spray-drying was 61.2% with particle size of  $2.77 \pm 0.03 \mu\text{m}$ . Limonene (78.65%) was detected as the major compound in BOT by GC-MS. Initially, the antibacterial activity of the microencapsulated BOT (MBOT) and the raw BOT was evaluated by microdilution method on an enterotoxigenic *Escherichia coli* (ETEC) strain and on *Lactobacillus rhamnosus* ATCC 7469. Tests on minimal inhibitory and bactericidal concentrations (MIC, MBC) indicated that both MBOT and raw BOT presented a selective antibacterial activity, with higher activity on ETEC than *L. rhamnosus*. Furthermore, MBOT presented higher activity on ETEC (MIC = 3.5 mg microcapsules/mL equivalent to 0.46 mg BOT/mL) than the raw BOT (MIC = 1.85 mg/mL). Subsequently, the effect of MBOT and raw BOT (at MIC and 2MIC) on *in vitro* fermentation kinetics and metabolites of ileal and colonic pig microbiota was evaluated by inoculating anaerobic media bottles with fresh digesta from both gut sections. Both MBOT and raw BOT reduced the bacterial diversity and altered significantly the ileal and colonic bacterial community composition ( $p < 0.05$ ). The fermentation time (0, 12, 24, 36, 48, 72h) also influenced compositional shifts, and differences in the relative abundance of bacterial taxa were observed with MBOT compared to the raw BOT and the control. Also, MBOT stimulated the production of short chain fatty acids in colon fermentation. Therefore, microencapsulation could enhance the antibacterial efficiency of BOT and the application of EO in animal feed could prove to be a feasible practice. Besides the shifts on the bacterial composition by the delivered EO, the wall material of microcapsules (chitosan/modified starch) could act as prebiotic for colonic bacteria.

### ACKNOWLEDGEMENT

The authors are grateful to CIENCIACTIVA for Carmen M.S. Ambrosio Ph.D. scholarship (Contract 278-2015-FONDECYT) from the “Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica” (CONCYTEC - Peru).

## PL7 Plant volatiles: The language of plants

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### Abstract

Plants evolved the ability to communicate each other with scents long before humans discovered the possibility of using distillates and extracts to improve their quality of life and appealing. The volatilome tree shows different branches each one from different metabolic pathways leading to thousands different molecules, each of them like words written in the poem of nature. Based on physiological, biochemical and molecular studies we are now aware of the scented language of plants, although we are still unable to decipher its intricate meaning. Plants react to stress and communicate within the same plant and among other plants according to its presence and intensity by modulating volatilome pathways by acting on its biodiversity and content.

The aim of this lecture is to provide evidence of plant-plant communication in response to both biotic (virus, bacteria, herbivores, animals, humans) and abiotic (temperature, water availability, light quality and intensity, salinity) stress. I will present evidence of volatile emission in plants producing secretory structures and plant apparently unable to produce volatiles (like the Lime bean) but strongly emitting scents when under biotic attack, by stressing the real meaning of volatiles in the biosphere. The plant volatilome will be presented in its complexity (chemical and molecular) as a tool to withstand the plant surrounding environment and to improve fitness.

## OP13 Molecular biophysics: A novel integrative approach to investigate the bioherbicide mode of action of essential oils

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**Keywords:** essential oils, bioherbicide, action mode, biophysics

### Abstract

Essential oils (EOs) are used in an increasingly number of sectors like medicine, cosmetics, food industry and more recently agronomy. In agronomy, EOs are used as bio-pesticides for their insecticidal, antifungal or bactericidal effects but also as bio-herbicides. Owing to the current attraction for natural products, a better understanding of their mode of biological action for new and optimal applications is of importance. Indeed most of the studies on EOs in agronomic applications are mostly descriptive, the action mode being superficially or not investigated at all. Nevertheless, it has been shown that EOs antimicrobial activity, quite well described in the literature, is at least partly due to their interaction with the plasma membrane. They notably change the lipid composition, thus altering fluidity, leading to various effects which can induce cell lysis, apoptosis or necrosis. Citronellol, a major compound of lemongrass EO, was notably shown to have antifungal activities by changing the membrane composition and inhibiting cell growth. Cinnamaldehyde (cinnamon EO) has been reported to have a broad spectrum of antibacterial activity, notably by affecting cell morphology, membrane integrity, permeability and composition.

Our three laboratories are currently working on the development of a bioherbicide made from *Cinnamomum zeylanicum* Blume (cinnamon) and *Cymbogognon winterianus* Jowitt (lemongrass) EOs. We have shown that the application of the whole EOs and their major individual compounds on the leaves and cotyledons of *A. thaliana* appears to be promising: when applied on cotyledons or leaves, EOs induce damages that are as important as those observed for commercial herbicides.

Since EOs are small amphiphilic molecules, they can cross the mesh of cell wall and interact directly with the plant plasma membrane (PPM). Modifying the lipid organization could lead to crucial cellular effects, notably on protein functions. We used a unique and original combination of *in silico* (molecular dynamics simulations) and *in vitro* (Langmuir monolayers, isothermal calorimetry, fluorescence and infrared spectroscopies) biophysical approaches, previously developed to study structure-function relationships of molecules of biological interest (pharmacological drugs, proteins, peptides, surfactants ...) to investigate the interaction of EOs or their individual compounds with bio-mimetic plant plasma membranes to better understand the structure- activity relationships in the context of their bioherbicide activity.

## YS PP01 A rare santolina-type monoterpene ketone acetate found in Georgian *Artemisia* sp. essential oil

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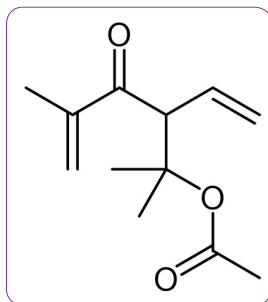
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### Abstract

*Artemisia* L. (Asteraceae) is a genus of small herbs and shrubs, which comprises over 500 species. Although essential oils of *Artemisia* taxa have been extensively studied, cases of “unidentified” constituents detected in their essential oils are still quite frequent in the literature [1]. We came across a sample of ca. 50 g of fresh aboveground parts of a highly fragrant Asteraceae taxon collected during the summer period from Tbilisi region, Georgia. Since the plant material did not contain any mature inflorescence (only buds), the botanical identification of the species was not possible with certainty, but it was obvious that it belonged to the genus *Artemisia*. Due to the highly aromatic nature of the plant material and rather unusual smell for *Artemisia* sp., we decided to investigate the hydrodistilled essential oil of this taxon. The plant material yielded a light green intensely fragrant essential oil (ca. 0.5%, w/w), and subsequent GC and GC-MS analyses enabled the identification of more than 120 constituents.

The major constituents were found to be 1,8-cineole (28.0%), santolinyl acetate (13.3%), camphor (9.8%) and an unknown compound, which eluted in the RI range of oxygenated monoterpenes (RI on a DB-5 MS column was 1239), that represented 8.0% of the total detected GC peaks. Next, we subjected the obtained essential oil to chromatography on a silica column, which resulted in one fraction enriched with the compound in question. Direct analysis of <sup>1</sup>H- and <sup>13</sup>C-NMR (at 400 MHz, in CDCl<sub>3</sub>), along with selective <sup>1</sup>H-<sup>1</sup>H homonuclear decoupling spectra of the acquired fraction, in combination with 2D NMR experiments (gradient <sup>1</sup>H-<sup>1</sup>H COSY, HMBC, and HSQC, as well as NOESY) enabled a complete structural assignment. The compound in question was demonstrated to possess the rare santolina irregular carbon skeleton, more specifically to be 2,5-dimethyl-4-oxo-3-vinyl-5-hexen-2-yl acetate, previously reported only once as a minor constituent of the essential oil of *Artemisia vulgaris*. The compound's restricted natural occurrence indicates its potential use as a chemotaxonomic marker.



### ACKNOWLEDGEMENT

The authors acknowledge the Ministry of Education, Science and Technological Development of Serbia for financial support (project 172061).

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## YS PP02 A synthetic approach to the identification of (iso)bornyl esters in the essential oil of feverfew (*Tanacetum parthenium* L.)

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**Keywords:** *Tanacetum parthenium*, essential oil, compound library, borneol esters, bornyl tiglate

### Abstract

*Tanacetum parthenium* L. (Asteraceae), commonly known as feverfew, is an aromatic plant native to the Balkan Peninsula. Feverfew has a long history of traditional use in the folk medicine of many nations for the treatment of fevers, migraine headaches, stomach aches, rheumatoid arthritis, etc [1].

Air-dried aerial parts of *T. parthenium* collected in the pre-anthesis phase from the slopes of Kukavica Mountain, south Serbia, produced upon hydrodistillation, a moderate yield (0.37%, w/w) of an essential oil of faintly bluish color discharged within ten minutes of exposure to air. The essential-oil composition was immediately analyzed by GC and GC-MS and these analyses resulted in successful identification of over one hundred compounds. *trans*-Chrysanthenyl acetate (30.3%) and camphor (23.8%) were the most abundant components with appreciable amounts of bornyl acetate (5.5%), bornyl angelate (5.4%), camphene (3.4%) and  $\gamma$ -terpinene (2.8%). This is in agreement with previous reports on this essential oil[2,3], especially that coming from Croatia that contained 2.2% of bornyl angelate [4].

Partial ion chromatograms of the essential oil, extracted through the use of AMDIS software, revealed that in addition to bornyl angelate, several other peaks possessed a prominent  $m/z$  83, fragmentation of borneol and also an ion corresponding to  $M^+$  (236) of bornyl angelate. We assumed that these might be esters of borneol or isoborneol and an unsaturated  $C_5$  acid, such as angelica, senecio or tiglic acids. In order to unequivocally identify these compounds, we prepared and fully characterized a library of the mentioned esters (six in total) and performed co-injection experiments. It was concluded that bornyl senecioate and bornyl tiglate were also present in the essential oil, and no esters of isoborneol were detected. Additionally, this synthetic approach allowed us to assess structure-retention index relations within this group and compile mass spectra that could allow easier identification of related compounds in the future.

### ACKNOWLEDGEMENTS

This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant No. 172061.

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## YS PP03 Antimicrobiological properties of essential oils obtained from selected *Mentha* species against *Helicobacter pylori*

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### Abstract

The aim of the study was chemical analysis of essential oils (EOs) obtained from five *Mentha* species and their cultivars and estimation of their anti-*Helicobacter pylori* potential. EOs were hydrodistilled using Deryng apparatus and analyzed by GC-MS. Compounds were identified using a computer supported spectral libraries and mass spectra of reference compounds together with the comparison of measured retention indices with reference compounds and published data. Based on the obtained chemical results, all analyzed EOs can be divided into 4 chemotypes; I - menthol, II - piperitenone oxide, III - linalool, and IV - carvone.

*H. pylori* ATCC 43504 reference strain and ten other *H. pylori* clinical strains were examined whether they were sensitive to obtained EOs and their components (menthol, menthone, linalool, eucalyptol, limonene, carvone, dihydrocarvone). The MIC and MBC values of EOs were calculated by using two fold microdilution method at concentrations ranging from 3.9 to 1000 mg/L. All tested mint EOs showed inhibitory activity against both reference *H. pylori* ATCC 43504 strain (MIC 15.6-31.3 mg/L) and clinical *H. pylori* strains (MIC<sub>50/90</sub> 62.5-250 mg/L/62.5-500 mg/L). Among reference compounds menthol (MIC<sub>50/90</sub> 7.8/31.3 mg/L) and carvone (MIC<sub>50/90</sub> 31.3/62.5 mg/L) had the highest anti-*H. pylori* activity. These results indicated that mint EOs may be a valuable therapeutic agent acting supportively against *H. pylori* infection and can reduce cost of treatment.

## YS PP04 Authentication of the naturalness of wintergreen (*Gaultheria* genus) essential oils by gas chromatography, isotope ratio mass spectrometry and radiocarbon assessment

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### Abstract

The essential oil of wintergreen can be obtained by steam distillation of leaves from different species of the genus *Gaultheria* and is mainly used in aromatherapy. Wintergreen essential oil is composed of more than 99 % methyl salicylate. This aromatic ester can be easily synthesized and used to adulterate wintergreen essential oil. In this study, we developed a methodology to authenticate the naturalness of the essential oil of wintergreen. Samples from various providers were studied and compared to commercially produced and laboratory-synthesized methyl salicylate from different chemical pathways. Different studies were carried out including (a) characterization of the samples' chemical compositions to determinate natural markers, synthetic precursors and impurities, (b) the analysis of stable isotope ratios of methyl salicylate, and (c) an assessment of the radiocarbon activity. Authentic wintergreen essential oil can be distinguished from adulterated oils by examining their compositions. The detection of methyl salicylate synthetic marker compounds (methyl-4-hydroxybenzoate, dimethyl-4-hydroxyisophthalate or dimethyl-2-hydroxyisophthalate) or the absence of several naturally occurring minor secondary metabolites (ethyl salicylate and vitispirane) contribute to the authentication. Isotopic values of bulk wintergreen essential oil have also been determined using isotope ratio mass spectrometer (IRMS). The  $\delta^{13}\text{C}$  values of authentic samples range from -36.78 to -33.36 ‰, the  $\delta^2\text{H}$  values range from -173 to -115 ‰ and the  $\delta^{18}\text{O}$  values range from -1.3 to 5.7 ‰. However, these analytical methods cannot account for the natural variability in the essential oils. To determine the boundaries of the natural isotopic values,  $^{14}\text{C}$  radioactive isotope activity assessment was undertaken, which allows for the determination of the genuineness of samples that are not assessed using multistable isotope approaches. Additional studies evaluating  $^{14}\text{C}$  activity of a noncompliant sample using IRMS identified a  $^{14}\text{C}$ -labeled synthetic methyl salicylate adulteration.

## YS PP05 Common conifer tree`s essential oils in Mongolia: Chemical profile, antioxidant and antimicrobial properties

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**Keywords:** conifer tree, essential oil, bornylacetate, *Pinus sibirica*

### Abstract

Mongolia has a quite amount of resources in the form of evergreen forest trees, especially Siberian pine and Scots pine trees. In comparison with other medicinal plant 600 species, the production of essential oil from pine trees is significantly simpler and more profitable. Especially favorable conditions are the availability of raw materials throughout the whole year and the simplicity of the procedure of essential oil production [1]. There is no industry to produce essential oils from conifers in Mongolia due to lack of investigation for biological activities on essential oils in the local conifer species.

Several researches have been done regarding antioxidant and antimicrobial activity of various species of conifers. However, there are no published reports on antioxidant properties, antibacterial and antifungal properties of essential oils obtained from Mongolian common conifers. Therefore, in the present investigation chemical profile, antibacterial, antifungal and antioxidant activity of essential oils from Mongolian common conifers (-two species: Scots pine (*Pinus sylvestris* L) and Siberian pine (*Pinus sibirica* Du Tour)) were evaluated.

The essential oils were obtained from fresh needles of the conifer species using hydro distillation and analyzed by GC-MS. The antioxidant activity was determined using different methodologies, such as ABTS radical cation (ABTS<sup>•+</sup>) scavenging activity assay and 2,20-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method; while the antibacterial activity was determined against four bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*) and two fungus (*Candida parapsilosis* and *Aspergillus niger*) using the agar diffusion method. Siberian pine`s essential oil, dominated by bornylacetate (30.1%), camphene (15.2%), limonene+ $\beta$  phellandrene (10.5%),  $\alpha$ -pinene (8.8%), 1,8-cineol (4.0%) and camphor (2.8%), was the most active antioxidant with IC<sub>50</sub> values, for DPPH and ABTS assays were 4.4 and 18.7 mg/ml respectively whereas it could not show any active against the bacteria and fungus. The main components were  $\alpha$ -pinene (29.8%), limonene+ $\beta$  phellandrene (16.1%), champhene (4.9%) d-cadinene (4.8%), bornylacetate (4.3%) in the essential oil of Scots pine. This essential oil inhibited the growth of all the tested bacteria having various degrees of inhibition zones except *P. aeruginosa*. Highest inhibitory activity was observed against both *E. coli* and *B. subtilis*, (17 mm inhibition zone), followed by *S. aureus* (15 mm) while the lowest inhibition zone was observed against the fungus with 11 mm inhibition zone. It reduced the stable free radicals DPPH with an IC<sub>50</sub> of 14.4 mg/ml and ABTS<sup>•+</sup> with an IC<sub>50</sub> of 29.3 mg/ml.

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## YS PP06 A comparative study on the essential oil composition of *Clinopodium vulgare* L. and *Micromeria dalmatica* Benth.

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**Keywords:** essential oils, *Clinopodium*, *Micromeria*

### Abstract

Most plant species of Lamiaceae family are highly valued and used as medicinal and aromatic plants. Part of their biological activity is due to the high content of essential oils. Our study presents results of comparative screening of the essential oils in two species – *Clinopodium vulgare* and *Micromeria dalmatica*. The former species is widespread, while the latter is a Balkan endemic occurring only in Bulgaria, Greece, Crete and Montenegro. *M. dalmatica* belongs to the old-world section *Pseudomelissa*, which was transferred recently to the genus *Clinopodium*. While *C. vulgare* is used only as medicinal plant, *M. dalmatica* is valued also as a spice. GC-MS analysis of the oil revealed substantial differences between the two species. The main compounds of *C. vulgare* were thymol,  $\gamma$ -terpinene, p-cymene and  $\beta$ -caryophyllene. *M. dalmatica* from Bulgaria is characterized by high quantities of essential oil with domination of monoterpene ketones: pulegone, piperitenone, menthone, piperitone oxide. Some quantitative differences in the content of the main compounds were detected in the different populations of the two species, probably depending on the local ecological conditions.

### ACKNOWLEDGMENT

The authors express their thanks for the support provided by the Bulgarian Ministry of Education and Science under the National Research Programme „Young scientists and postdocs“ approved by DO Nr.: 577/17.08.2018.

## YS PP07 Effects of immortelle essential oil on macrophage NO production

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### Abstract

The essential oil of immortelle, *Helichrysum italicum* (Asteraceae), has a long application for wound healing. Nowadays, it is one of the most popular essential oils in cosmetics as it may stimulate blood circulation and regeneration of the skin, and reduce the appearance of fine lines and wrinkles. However, several chemotypes of *H. italicum*, characterized by distinct essential-oil profiles, were described and it is unlikely that all show the same therapeutic efficacy. Recently, we found that antimicrobial activity and cytotoxicity toward macrophages of immortelle oil strongly depend on its composition.

In continuation of our research, we evaluated the effects of four commercial immortelle oils (1, 2, 3, and 4, respectively) containing differing amounts of nerol esters (23 : 43 : 12 : 21),  $\alpha$ -pinene (17 : 2 : 20 : 5),  $\gamma$ - and *ar*-curcumenes (19 : 14 : 16 : 15), and  $\beta$ -diketones (3 : 12 : 5 : 7) on LPS-stimulated NO production in macrophages. All oils suppressed NO production at the highest tested concentration (0.1 mg/mL). Oil 1, rich in mono- and sesquiterpene hydrocarbons, induced the greatest concentration-dependent decrease in NO production ( $IC_{50} = 1.5 \times 10^{-4}$  mg/mL), while oil 2, rich in nerol esters and  $\beta$ -diketones, was two-fold less active ( $IC_{50} = 1.6 \times 10^{-2}$  mg/mL). Four chromatographic fractions, enriched in mono- and sesquiterpene hydrocarbons (f1), curcumene (f2), nerol esters (f3), and  $\beta$ -diketones (f4), were also tested in order to potentially identify the carriers of the observed activity. The most potent was the curcumene fraction that reduced the production of NO to only 20% at  $1 \times 10^{-5}$  mg/mL. In comparison to the oils, fractions f1 and f2 displayed *stronger inhibitory effect* at all tested concentrations, whereas fractions f3 and f4 were less active at the highest tested concentration. Overall, these results imply that the higher content of mono- and sesquiterpene hydrocarbons in immortelle oil had the most prominent positive impact on its activity, whereas the amount of  $\beta$ -diketones had the opposite effect. A statistical (PCA) treatment of the obtained composition-activity data provided further confirmation: Strong positive correlations were observed between the activity and the amount of limonene, *trans*- $\alpha$ -bergamotene and *ar*-curcumene, while very strong negative correlations were noted for all  $\beta$ -diketones. Interestingly, in our previous study oils 1 and 3 also showed higher cytotoxicity toward macrophages than oils 2 and 4, while fractions f1 and f2 were more potent than the other two but still inferior to the oils.

### ACKNOWLEDGEMENT

This work was funded by the Ministry of Education, Science and Technological Development of Serbia (Project 172061).

## YS PP08 Gender-related differences in the xenobiotic metabolism of two *Choisya ternata* essential-oil constituents, methyl and isopropyl N-methylantranilates

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### Abstract

Two volatile protoalkaloids, methyl *N*-methylantranilate (MMA) and isopropyl *N*-methylantranilate (IMA), identified in the essential oil of Mexican orange (*Choisya ternata* Kunth (Rutaceae)) [1] exhibited polypharmacological properties [2]. Recently, we investigated their organ- [2] and urinary-metabolite profiles [3]. Herein we report on the gender-related differences in the metabolism of MMA and IMA in rats intraperitoneally treated with these two substances in a dose of 2 g kg<sup>-1</sup>. GC-MS analysis of the Et<sub>2</sub>O extracts of selected tissues homogenates (liver, kidneys, heart, brain, lungs, quadriceps femoris muscle, and spleen) of female and male rats was performed enabling identification of 12 and 16 anthranilate-related metabolites in the case of MMA and IMA, respectively. Organ-metabolite profiles of female and male rats were qualitatively similar, but there were evident gender-related differences in the quantitative distribution of particular metabolites. Generally, it seemed that male rat organs contained lower quantities of certain metabolites. This prompted us to statistically compare the compositional (quantitative) data from the GC-MS analysis by performing multivariate statistical analysis (MVA). Agglomerative hierarchical clustering (AHC) and principal component analysis (PCA) was done by utilizing the content of the analytes (mg g<sup>-1</sup>) as original variables. AHC analysis of MMA organ profiles delimited five statistically different classes of organs, where three classes were from a single organ. The metabolite profiles of the livers of female and male rats were clearly separated and formed a “pure” clade with *N*-methylantranilic acid and anthranilic acid as the most abundant anthranilates quantified. Furthermore, the organs of IMA-treated rats were grouped into three statistically different classes with one of the clades almost exclusively belonging to the organs of female rats with an exception of the kidneys of male rats, displaying unmetabolized IMA as the most abundant constituent. PCA analysis revealed strong dependencies between certain metabolites. With both MMA and IMA-treated rats, maximal correlation coefficients ( $r = 1$ ) were noted for hydroxylated metabolites, methyl and isopropyl esters of 3- and 5-hydroxyanthranilic and 3-hydroxy-*N*-methylantranilic acids. Conversely, the content of methyl- and isopropyl 5-hydroxy-*N*-methylantranilates did not strongly correlate with the aforementioned hydroxylated metabolites. It could be that the hydroxylation of *N*-methylated molecules occurs independently from the *N*-demethylated ones.

### ACKNOWLEDGEMENT

Ministry of Education, Science and Technological Development of Serbia (Grant No. 172061).

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## YS PP09 Hyssop essential oil improves quality of cooked pork sausages

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**Keywords:** hyssop, cooked pork sausage, TBARS

### Abstract

The purpose of this study was to evaluate the effect of hyssop essential oil (HEO) on the quality of cooked pork sausages during 30 days of refrigerated storage.

Cooked pork sausages were created in local industrial plant. The sausage batter consisted of meat from pork shoulder (50%), pork back fat (15%), pork skin emulsion (15%), ice water (15%), soy protein (2%), nitrite salt (2%) and spice mix (1%). HEO was added to the sausage batters at concentrations of 0.1  $\mu\text{L/g}$  (HEO1), 0.5  $\mu\text{L/g}$  (HEO2) and 1.0  $\mu\text{L/g}$  (HEO3). HEO was mixed with salt and added into the mixture prior to stuffing. The remaining batch (without HEO) was assigned as control (C). All sausages were stuffed into artificial casings ( $\text{Ø}\approx 36$  mm) and pasteurized until an internal temperature of 70°C was reached. Immediately after the heating process, sausages were cooled and stored in the refrigerator (at 4°C) until analyses.

Colour measurements were performed using MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan); D-65 lighting, 2° standard observer angle; 8-mm aperture in the measuring head. TBARS values were expressed as milligrams of malondialdehyde per kilogram of sample. The Difference-Control-Test was carried out by 10 trained assessors, who were able to discriminate samples in relation to the investigated attribute of flavour. Instrumental parameters of colour ( $L^*$ -lightness,  $a^*$ -redness,  $b^*$ -yellowness), TBARS values and sensory analysis were determined on the 1<sup>st</sup> and 30<sup>th</sup> day of storage. All three concentrations of HEO significantly ( $P<0.05$ ) increased CIE  $a^*$  values on the 1<sup>st</sup> and 30<sup>th</sup> day of storage (1<sup>st</sup> day: HEO1: 16.21, HEO2: 16.78, HEO3: 16.99, C: 15.58; 30<sup>th</sup> day: HEO1: 16.24, HEO2: 16.73, HEO3: 16.92, C: 15.54). The addition of HEO at 0.5 and 1.0  $\mu\text{L/g}$  significantly reduced ( $P<0.05$ ) TBARS values on the 30<sup>th</sup> day of storage compared to control samples (HEO2: 0.20 mg MDA/kg, HEO3: 0.17 mg MDA/kg, C: 0.36 mg MDA/kg). The flavour of sausages produced with the addition of 0.1 and 0.5  $\mu\text{L/g}$  HEO was slightly/moderately and significantly ( $P<0.05$ ) different from the control, for both storage days.

This study demonstrated that the hyssop essential oil could be used as natural antioxidant and colour enhancer in processing of cooked sausages. Further studies should be performed in order to compare its effectiveness in other food products, as well as potential replacement of synthetic food additives.

## YS PP10 Identification of new 3-phenylpropyl esters from the essential oil of *Pleurospermum austriacum* (L.) Hoffm. (Apiaceae) through the preparation of a synthetic library of isomeric hexanoates

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**Keywords:** essential oil, *Pleurospermum austriacum* (L.) Hoffm., isomeric hexanoates, GC-MS, NMR

### Abstract

*Pleurospermum* Hoffm. (Apiaceae) is a widespread, heterogeneous genus composed of 110 different taxa [1]. Although several biologically interesting natural products were isolated from *Pleurospermum* spp. (e.g. coumarins, phenylpropanoids, etc.), the genus is still poorly phytochemically investigated, especially in terms of essential-oil analysis (up to now only 4 taxa, including *P. austriacum*) [1].

One of our primary goals was to determine the exact structure of a possibly new secondary metabolite previously detected in *P. austriacum* essential oil [1]. The available data at that time allowed us to tentatively identify the constituent as an ester of the rarely naturally occurring 3-phenylpropanol and an isomer of hexanoic acid (hexanoic, 2-methylpentanoic, 3-methylpentanoic, 4-methylpentanoic, 2,2-dimethylbutanoic or 3,3-dimethylbutanoic acid). Unfortunately, the accessible spectral and GC-retention data on the mentioned esters are quite limited (only data for 3-phenylpropyl hexanoate exist in the literature). An additional problem connected to the GC-MS identification of the correct isomer is that they have similar MS-fragmentation patterns and very close RI values. For that reason, the final structural confirmation could not be done based on MS and RI data alone.

To resolve that issue, we created a small synthetic library of 42 isomeric  $\omega$ -phenylalkyl hexanoates (23 completely new compounds). GC-MS in combination with NMR, IR, and UV-Vis of the synthesized compounds provided data that led to the identification of the mentioned essential-oil constituent as 3-phenylpropyl 4-methylpentanoate (a new natural product). We hope that the spectral and chromatographic data provided in this study will make identification of these and related potentially biologically and olfactory significant compounds easier to future researchers.

### ACKNOWLEDGMENT

This work was supported by the Ministry of Education, Science and Technological Development of Serbia [Project No. 172061].

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## YS PP11 Lemon balm (*Melissa officinalis* L., Lamiaceae) essential oil prevents spontaneous and induced rat ileum contractions

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### Abstract

Lemon balm (*Melissa officinalis*) represents one of the most renowned plant species used for the treatment of different medical conditions, but it is predominantly used by patients suffering from central nervous system disorders. The gastrointestinal system function is liable to the influence of ingested substances, and this is especially true for its mechanical functions, i.e. contraction patterns. The aim of our study was to evaluate the effects of lemon balm (*M. officinalis*) essential oil on spontaneous, and acetylcholine (ACh) or potassium-induced rat ileum contractions. The essential oil was isolated from fresh plant material originating from Serbia and analyzed by GC and GC-MS. All major constituents' identities were corroborated by co-injection experiments. In order to measure the change in the contraction patterns, segments of rat terminal ileum, mounted vertically in an isolated tissue bath filled with Tyrode's solution, were connected to a force transducer. Increasing concentrations of the essential oil (1-100 µg/ml) added to the tissue bath produced a significant concentration-dependent inhibition in spontaneous contractions. In the case of ACh-induced contractions, the calculated IC<sub>50</sub> value, a concentration that prevented an increase in contractions induced by ACh (10<sup>-6</sup> mol/l), was found to be 45 ± 4 µg/ml. When the tissue segments were challenged with a high concentration of potassium (80 mmol/L) cell depolarization resulted in ileum contractions. The application of essential oil significantly prevented the potassium induced contraction occurrence, and the calculated IC<sub>50</sub> value, in this case, was 179 ± 71 µg/ml. From the present results, we can conclude that the essential oil of *M. officinalis* significantly affects spontaneous and induced rat ileum contractions, through mechanisms that are mainly mediated via acetylcholine receptors.

### ACKNOWLEDGEMENT

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant No. 172061.

## YS PP12 Odor-active compounds of yuzu (*Citrus junos* Sieb. ex Tanaka) peel oil

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### Abstract

Recently, the food and cosmetic products derived from Yuzu fruits and its essential oil have gained in popularity outside of Japan. Since several decades, the odor-active compounds of Yuzu have been investigated especially in Japan and South Korea, and yuzunone, yuzuol,<sup>[1]</sup> *trans*-4,5-epoxy-2,7-decadienal<sup>[2]</sup> and (*E*)-4-methyl-3-hexenoic acid<sup>[3]</sup> were reported as important contributors of Yuzu odor, in addition to the more classic odorants like linalool, thymol, aliphatic aldehydes, etc. However, although GC-O/AEDA analyses were reported in several articles, there is still no consensus concerning the identity of the main odorant contributors of Yuzu. In order to better understand the olfactory properties of Yuzu, we performed a detailed fractionation of the sample of cold pressed Yuzu peel oil and carried out GC-O analysis with several panelists on the oil and its fractions.

Both linalool [1-4] and limonene [5,6], the most important key odorants reported in some GC-O/AEDA studies of the literature, were detected by only a part of our panelists. On the other hand, in our study, nonanal showed the highest mean FD value and was unanimously detected by our panelists while it was often of lower importance in the published studies [1,7]. In addition to that, we synthesized some key odorants reported in the literature such as yuzunone, *trans*-4,5-epoxy-2,7-decadienal, etc., which were used for the reference compounds to confirm their existence and odor profile.

Several similar discrepancies were observed when we compared our results with the literature, and it may be explained by the differences in the chemical composition of the studied samples (due to the different producing regions, extraction methods, conditions of storage, etc.) but also in the methodological aspects of the analysis and the differences in individual olfactory sensitivity of the panelists.

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## YS PP13 Phytotoxic effect of different essential oils on *Eragrostis plana* germination

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### Abstract

Allelopathy is a chemo-ecological phenomenon involving the effect of compounds from one plant species (donor species) on another plant (target species). Among the different types of secondary metabolites with allelopathic potential, the essential oils have been studied to be explored as alternatives in the biological control of weeds. *Eragrostis plana* Ness., commonly known as annoni grass, is an exotic species that was introduced in southern Brazil in the 1950s to be used as a forage. Due to its characteristics of high prolificacy, rusticity and adaptation to poor soils, the species presented an invasive behavior, being classified as a weed, considered the most aggressive and most difficult to control in the southern fields. The phytotoxic effect of the substances is commonly verified by testing their influence on seed germination and seedling growth. Therefore, this work investigated the phytotoxic effects of *Cymbopogon citratus* (D.C.) Stapf (lemongrass), *Cymbopogon winterianus* Jowitt. (citronella) and *Rosmarinus officinalis* L. (rosemary) essential oils on annoni grass germination. For assays, 30 seeds were soaked in 3 mL of distilled water on filter paper in Petri dishes and incubated at an average temperature of 20 °C under a 12 h photoperiod. Different amounts (5 µL and 10 µL) of the essential oils were applied in a cotton ball attached to the inner face of the Petri dish lid to avoid direct contact between seeds and essential oils, allowing the oil to volatilize in the airspace inside the dish. A control group was treated with distilled water and four replicates of each treatment were performed. Germination rate and speed of germination were determined. Citronella essential oil caused greater inhibition in the germination parameters. Compared to control, citronella essential oil reduced both parameters analyzed by 90%. The application of 5 µL of lemongrass essential oil did not affect the annoni germination. However, the application of 10 µL reduced germination rate and speed of germination by 15% and 30%, respectively. The treatments with rosemary essential oil presented similar results, except for 5 µL, that reduced the germination rate of annoni grass by 14%. Scientists are interested in improving crop productivity and protecting the environment through the use of substances from environmentally friendly natural products. These results obtained will allow the elaboration of new researches aiming the development of agroecological techniques of weed management.



## YS PP14 Potent odorants contributing to the characteristic flavor of *Panax notoginseng* flower buds

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### Abstract

With the purpose of investigating the characteristic and odor active compounds contributing to the flavor of the tea infusion prepared from the flower buds of *Panax notoginseng*, an edible plant used in traditional Chinese medicine locally called SanQi flower buds (SQFBs), different extraction and analysis methods were used. The volatile compounds of SQFBs were extracted by solvent assisted flavor evaporation (SAFE), hydrodistillation (HD) and solid-phase microextraction (SPME) to investigate potent odorants. According to analyses of GC and GC-MS the main components obtained by HD and SPME were  $\beta$ -elemene, valerena-4,7(11)-diene, germacrene D,  $\beta$ -bicyclogermacrene, spathulenol while  $\alpha$ -cubebene,  $\alpha$ -humulene and spathulenol contributed to the characteristic herbal odor. The main components in the volatile concentrate prepared by SAFE are  $\gamma$ -butyrolactone, hexanoic acid, benzyl alcohol, heptanoic acid, octanoic acid and spathulenol. safranal, spathulenol and caryophyllene oxide were potent odorants responsible for the characteristic herbal odor of SQFBs.

## YS PP15 Structure elucidation of new monoterpene dimers from the essential oil of French marigold (*Tagetes patula* L.)

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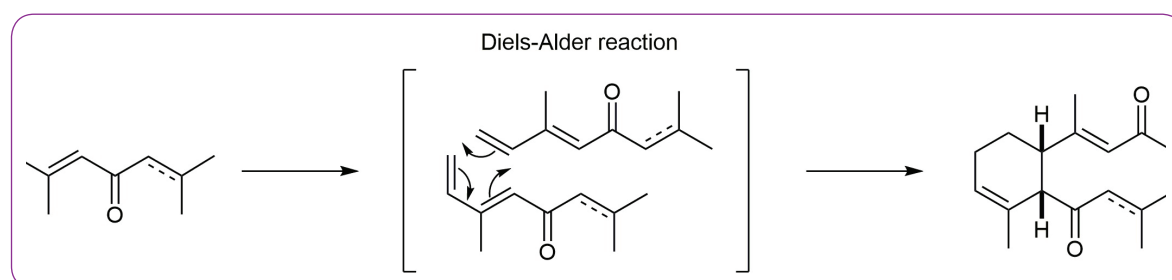
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### Abstract

*Tagetes patula* L. (Asteraceae), French marigold, is commonly used as a garden flower, but also known as a component of the popular spice in some countries (Georgia, flower petals) [1]. Medicinally speaking, many nations use infusions from dried leaves or florets of different marigold species for the treatment of various ailments [2]. Previous work also put forward a possibility that *T. patula* essential oil, analyzed on several occasions [3-5], could be used as a pesticide [6].

In this work, we performed a detailed analysis of the hydrodistilled essential oil of fresh inflorescence of *T. patula* cultivated in Serbia. The major constituents detected,  $\alpha$ -terpinolene, piperitenone, (*E*)- and (*Z*)-tagetenones (ocimenes), (*E*)- and (*Z*)-tagetones, were accompanied with a number of minor ones among which several with relatively high values of retention indices (2010 – 2260) were left unidentified in the searches of MS databases. Based on the MS fragmentation and molecular ion, one of them appeared to be a dimer of tagetenone, while the others were dihydro- or tetrahydroderivatives. The dimer of tagetenone and one dihydroderivative were previously isolated from *T. gracilis* DC. (syn. *T. multiflora* Kunth) [7] and were later on proposed to be artefacts formed during hydrodistillation [8].

In order to unambiguously identify these constituents, the essential oil was subjected to SiO<sub>2</sub> dry flash chromatography which led to fractions enriched with the peaks in question. Structure elucidation of three of these compounds, differing in presence of double bonds (shown using dashed lines in the scheme below), was performed by the analysis of various 1D and 2D NMR spectra and a complete assignment of all <sup>1</sup>H and <sup>13</sup>C NMR data was also accomplished. Based on the relative configuration of the two chiral centers and previous work [8] we believe that these dimers formed as the *endo*-products of a Diels-Alder reaction of two (*E*)-tagetenones, or (*E*)-tagetenone and (*E*)-tagetone. The regioisomer lacking the double bond in the longer chain on the cyclohexene ring represents a new compound. The tetrahydroderivative is likely to be an analogous product of two (*E*)-tagetone molecules, and if so is also a newly detected constituent of this essential oil.



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## YS PP16 Study of patchouli essential oil chemical composition as a function of part of the plant, distillation time and enzymatic treatment

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**Keywords:** patchouli, essential oil, GC-MS, enzymatic treatment.

### Abstract

Patchouli (*Pogostemon cablin*, Lamiaceae) essential oil (EO) is one of the most important raw material and has a high demand in the flavor and fragrance, food, pharmaceutical, cosmetics, and perfumery industries [1,2]. Patchouli EO quality is regulated by international norm ISO 3757 (2002). The major secondary metabolites of patchouli are  $\beta$ -patchoulene (1.80-3.50%),  $\alpha$ -guaiene (11.0-16.0%),  $\alpha$ -bulnesene (13.0- 21.0%), patchoulol (27.0-35.0%), and pogostol (1.00-2.50%). In this work, patchouli plants were cultivated in experimental plots, harvested and dried in the shade, at the CENIVAM Research Center in Bucaramanga, Colombia. EO were obtained by microwave-assisted hydrodistillation (MWHd), from different part of the plant (leaves, stems and their mixture), changing the distillation time and an enzymatic treatment. The EO chemical composition was determined by GC-MS (Agilent Technologies 6890 Plus GC, 5973 or 5975 MSD), using the linear retention indexes measured on both polar and non-polar columns and by comparison of their experimental mass spectra with those from databases (Adams, NIST and Wiley) and literature.

The EO chemical composition obtained from leaves as well as from leaves and stems were similar, patchoulol was the major compound (33.9-36.5%). However, in the EO obtained only from stems, the major compound was pogostone (31.1%). The patchouli oil yield increased with distillation time (45-150 min). The EO yield was not affected by changing of pH (7  $\rightarrow$  4) and adding enzyme (*Celluclast*). The methodology used in this work allowed obtaining a quality of patchouli oil, according to international regulations.

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## YS PP17 Trans-methyl cinnamate: Marker compound of stressed *Conocephalum conicum*

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### Abstract

Liverworts are rich sources of mono-, sesqui- and diterpenoids, as well as bi- and bis-bibenzyl acetogenin derivatives. Liverworts possess beautiful oil bodies, in which terpenoids and aromatic compounds are accumulated. The largest thalloid liverwort existing is *Conocephalum conicum* (L) Underw., belonging to Conocephalaceae family, and widely distributed in Europe, North Africa, North America, and as well in Asia. Upon squeezing those beautiful oil bodies, strong, sweet, mushroom-like scent is emitted indicating the presence of 1-octen-3-ol and 1-octen-3-yl acetate constituents [1].

Since the classification of *C. conicum* is quite complex, one of the efforts to classify the *C. conicum* is by looking at the chemical composition of this liverwort based on the different geographical origin [2]. Three classes of *C. conicum* have been identified, which are type-I, type-II and type-III [2]. Based on the marker compound interest, type-II Japanese *C. conicum* has been chosen to be studied.

Type-II Japanese *C. conicum*, which is known to have (+)-bornyl acetate as the marker compound, was placed in stressed condition to initiate biosynthesis of a phenyl propanoid; *trans*-methyl cinnamate. Analysis of the Headspace-Solid Phase Micro Extraction-Gas Chromatography-Mass Spectroscopy (HS-SPME-GC-MS) of stressed *C. conicum* showed *trans*-methyl cinnamate as the major component [3]. This phenomenon resulted in some confusion from the chemotype perspective since *trans*-methyl cinnamate is only present in type-III Japanese *C. conicum*. It shows that at the stress level, the shikimate pathway of *trans*-methyl cinnamate was activated and worked together with mevalonic acid pathway (MVA) of bornyl acetate to produce *trans*-methyl cinnamate.

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## YS PP18 Use of essential oils and natural extracts of plants that grow in Santander as anti-oxidants in a cosmetic product.

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### Abstract

Antioxidant creams are cosmetic products that seek to diminish the effects of skin aging produced by free radicals. Some aromatic plants from Chicamocha canyon in Santander (Colombia), such as *Lippia origanoides*, *Psidium sarturianum*, *Caycolpus moritzianus*, *Lantana canescens* and *Tagetes caracasana* are sources of compounds with antioxidant properties. The objective of this research work was the incorporation of essential oils and extracts of these plants as active substances, into a cosmetic product and the evaluation of their stability. The essential oils were obtained by steam distillation. The post-distilled plant material was dried and used to obtain natural extracts with solvents and supercritical CO<sub>2</sub>. The essential oils were analyzed in a gas chromatograph (GC 6890, AT, Palo Alto), equipped with mass spectrometric detector. The separation of the compounds was carried out in two columns, an apolar DB-5MS (60 m X 0.25 mm di X 0.25 μm), and a polar DB-WAX (60 m X 0.25 mm di X 0.25 μm). Compound identification was done by comparing mass spectra and linear retention indexes and those reported in literature. Extracts analysis was performed in a liquid chromatograph (1200 Series, AT) with UV-Vis diode array detector (DAD) and in a liquid chromatograph (1200 Series, AT) with a Time-Of-Flight mass analyzer (TOF 6210 G1969A, AT). Separation of the compounds was made in an apolar column (C18 Kinetex Phenomenex, Torrance), 100 mm, L x 4.6 mm, d.i. x 3.5 μm. Based on the availability of essential oils and extracts, three cosmetic products were formulated. The antioxidant activity of essential oils, extracts and creams was determined by the ORAC method. Stability tests were carried out on the formulated antioxidant creams. The essential oils incorporated were *L. origanoides*, *C. moritzianus* and *P. sarturianum* at 0.2, 0.1 and 0.2% and their major components were carvacrol, limonene and caryophyllene oxide, respectively. The pinocembrin flavonoid was common in all extracts, except for *T. caracasana*. The antioxidant activities of the formulated creams were 18, 30 and 53 μmol Trolox<sup>®</sup>/g of sample. However, 60 days later the values were 15, 19 and 17 μmol Trolox<sup>®</sup> / g sample, respectively.

## PP19 A comparative study of the chemical and aroma profile of *Rosa damascena* essential oil from Saudi Arabia and Bulgaria: An effect of the technology and geographic origin

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**Keywords:** *Rosa damascena* Mill. var. *trignipetala*, rose oil, GC-MS, aroma profile, chemometrics

### Abstract

*Rosa damascena* Miller var. *trigintipetala* Dieck is one of the most important plants from Rosaceae family, with a long historical use in the traditional medicine and as valuable oil bearing plant. It is considered as superior in terms of the essential oil quality and has been traditionally cultivated in Bulgaria and Turkey, and in lesser extent in Iran, India, Morocco and Saudi Arabia. Rose oil is one of the world's most sought-after products, with the Bulgarian rose oil being recognized as one of the world's most sought-after products for its quality and fine aroma. The botanical and geographical origin, environmental conditions, production method and storage are some of the factors affecting the chemical composition and quality of the essential oil and the sensorial and organoleptic characteristics of the product. Therefore, precise and sophisticated analytical methods for the origin assessment and quality assurance are of substantial fundamental and practical interest.

The aim of the current study is to perform a comparative study of the chemical and aroma profile of *R. damascena* essential oil from Saudi Arabia and Bulgaria to reveal the effect of the technology and geographic origin.

A comprehensive chemical profiling of seven *R. damascena* Mill. essential oil samples from Saudi Arabia (Al-Shafa, Al-Hada and Taif area) was performed by means of GC-MS and GC-FID. As a result more than 120 of them, containing C<sub>3</sub>-C<sub>27</sub> carbon atoms, mainly mono- and sesquiterpenoids, were identified by GC-MS. Fifty two components with concentration >0.01%, which represents 97.5-99.5% of the total detected compounds, were quantified by means of GC-FID. All of the analyzed rose oil samples showed similar chemical profile with identical qualitative composition and quantitative differences. The main compounds of the samples are: citronellol (19.45 - 28.13 %), geraniol (18.29 - 27.23 %), nerol (7.02 - 14.27 %), linalool (2.93 - 7.55 %), phenylethyl alcohol (1.77 - 3.87 %) and n-paraffins (13.53 - 30.85%). A sample of Bulgarian *Rosa damascena* Mill. distilled rose oil (harvest 2018) was analyzed in addition and its chemical profile was compared to those of the oils from Saudi Arabia.

The results from the chemical analysis of the rose oil samples were further analysed by chemometrical approaches (cluster analysis) and correlations between the chemical/aroma profile and the geographic origin/production technology was established.

## PP20 Amazon & Madagascar: Two tropical regions for essential oils production and opportunities for species diversification

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### Abstract

Tropical regions provide excellent settings in which to explore diversification in order to offer natural ingredients for fragrance houses. The Amazon and Madagascar tropical regions are two of these areas which preserve good conditions of luminosity and water availability. Even presenting particular weather conditions, they still offer important commercial opportunities from experimental planting to management with native communities.

Ongoing diversification programs, particularly in the Amazon and Madagascar, are focusing on achieving traceable essential oils for perfumery. Then, Symrise has started projects looking for new scents made in these tropical regions to encourage and support farmers to grow a variety of crops for essential oil production. The establishment of new supply chains creates many challenges, such as keeping production volume and quality standards.

Both regions represent important tropical places for the essential oil trade. However, they have different structures and to develop each of them it is necessary to follow local CBD/ABS legislation, local practice, respect the local biodiversity and establish an ethical trade.

## PP21 Analysis of essential oils residues in egg albumen and yolk

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**Keywords:** essential oils, eggs, GC-MS-MS, QuEChERS

### Abstract

Eggs are an affordable source of high quality proteins, minerals and essential vitamins suitable for vegetarians as well. Hence, the production has increased by 36.5% in the past decade [1]. This increase in production was supported by the use of antibiotics, but human health concerns have led to their ban in the EU since 2006. This fostered the research for alternative substances: phytogetic compounds are suggested to be promising alternatives to antibiotics [2].

Phytogetic compounds are defined as plant-derived natural bioactive compounds with positive effects on animal growth and health, and this definition applies to ground herbs and spices, or preparations like essential oils (EOs), extracts or oleoresins. These compounds are secondary plant metabolites and encompass a wide range of chemical compound classes like phenolic compounds, terpenes, alkaloids, lectins, aldehydes and ketones [3]. It is important to determine their residues in edible animal products within the scope of consumer safety assessment. We hence developed a method to determine five compounds in eggs: carvone, menthol, thymol, carvacrol and methyl salicylate. Egg albumen and egg yolk both are complex matrices.

The 27% fat content in egg yolk calls for typically laborious and time-consuming sample preparation techniques. We therefore decided to use a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) sample preparation procedure modified by an Enhanced Matrix Removal step to remove the lipids.

The QuEChERS sample preparation method coupled with the GC-MS/MS analytical technique in MRM mode improves selectivity and allows for lower detection limits by reducing the matrix background [4]. The method was validated to reach, for all the compounds in both matrices, an LOQ of <10 µg/kg and recoveries at a spike level of 20 µg/kg in the range of 85-114% with a RSD% <20%.

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## PP22 Antibacterial activity and essential oil composition of *Calendula arvensis*

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### Abstract

The genus *Calendula* L. is a member of Calenduleae tribe of Asteraceae family. It comprises of about 12–20 species native to the Macaronesian and Mediterranean regions [1]. *Calendula* L. genus is stated in Turkey by three species which are *C. arvensis*, *C. suffruticosa*, and *C. officinalis*. In European folk medicine *Calendula* L. is thought to be an immune tonic that helps avoiding sickness in winter [2]. *Calendula arvensis* L. is reported to have important biological activities such as antibacterial, antiseptic, lymphagogue, choleric, demulcent, vulnerary anti-tumor, sedative, mild anodyne anti-inflammatory, anti-parasitic, antioxidants, antiviral, analgesic properties [3]. *Calendula arvensis* L. has high medicinal properties and commercial importance and also contains natural substances which are used traditionally as herbs. Previously, the essential oil from aerial parts of *C. arvensis* was investigated.

Eighty-five components of the essential oil were identified. The major components of aerial parts were  $\delta$ -cadinene (15.1%) and  $\alpha$ -cadinol (12.4%) while the major components of the flower parts were  $\alpha$ -thujene (15.9%) and  $\delta$ -cadinene (13.1%) [4]. The objective of this study is to determine the antibacterial activity of the essential oil from aerial parts of *C. arvensis*. Essential oil of *C. arvensis* was obtained by hydrodistillation for 3 h from aerial parts. The oil composition was analyzed by means of gas chromatography-mass spectrometry (GC-MS). The analysis was carried out in triplicate. Antibacterial activity of essential oil was observed against *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 by using a broth microdilution. The experiment was started at 8 mg/mL. The yield of the oil was 0.38% (v/w). Thirty-six components were identified in the essential oil that represent  $91.8 \pm 0.1$  % ( $n = 3$ ). The major components of the essential oil were  $\delta$ -cadinene  $14.8 \pm 0.1$  %, *epi*-cubebol  $10.7 \pm 0.0$  %,  $\alpha$ -cadinol  $8.5 \pm 0.1$  %, cubenol  $7.7 \pm 0.0$  %, cubebol  $7.2 \pm 0.0$  %, 1-*epi*-cubenol  $5.4 \pm 0.0$  % and ledene  $5.1 \pm 0.0$  % ( $n = 3$ ). The essential oil showed inhibitory activity against *E. coli* and *B. cereus* at 8 mg/mL.

## PP23 Antibacterial activity of EU approved plant-bearing essential oils in vapour phase against respiratory pathogens using broth micro-dilution volatilization method.

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**Keywords:** antibacterial activity, respiratory diseases, essential oil, vapour phase

### Abstract

Essential oils (EOs) produced by aromatic herbs have been traditionally used around the world against respiratory tract infections [1]. Nowadays the Herbal Medicinal Product Committee of the European Medicine Agency approved their use in form of inhalation for the treatment of infectious cough and cold [2]. While their antimicrobial properties have been demonstrated in liquid phase, few studies have been investigated their activity in vapour phase. In this study, we aimed to determine the antibacterial activity of five essential oil-bearing aromatic herbs in vapour phase against respiratory pathogens including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *S. pyogenes* and *Haemophilus influenzae*. The EOs were extracted by hydrodistillation and antimicrobial activities were assessed using the broth microdilution volatilization method in both liquid and vapour phase [3]. The results show that thyme EO was the most effective antibacterial agent, whereas *H. influenzae* was the most susceptible bacterium with minimum inhibitory concentrations 64 and 128 for liquid and vapour phases, respectively. These outcomes indicate that EO vapours are interesting antimicrobial agents against respiratory pathogens. It also suggests that the use of EOs as an alternative to inhalation antibiotherapy is promising even though further investigation to determine their toxicity on lung cells is needed to validate their potential practical use.

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## PP24 Antibacterial, antioxidant activity and volatile composition of essential oils extracted from crude organic propolis and its residues from propolis extract production

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**Keywords:** agroindustrial residue, propolis chain, essential oil, antimicrobial growth promoter, *E. coli*

### Abstract

Propolis is well known to present interesting biological activities. However, obtaining propolis extract generates a large amount of residues in the industry. The possibility of using these residues would be interesting to aggregate value to the propolis extract chain. Essential oils (EO) because of their bioactivity are getting great attention in animal breeding to replace synthetic antimicrobials used as growth promoters. Therefore, the aim of this study was to evaluate the yield of EO extraction from crude organic propolis and its residues as well as their antibacterial and antioxidant activities and volatile composition. EOs extraction was performed by hydrodistillation for four hours. The antibacterial activity assay was carried out by microdilution method and bacterial growth was measured by absorbance reading every hour at 600 nm. Antioxidant activity was performed by ABTS and DPPH radical methods and phenolic content by Folin-Ciucateu method. Headspace GC-MS was used to investigate the volatile composition of EOs. The crude propolis EO (PEO) presented the highest yield (1.13%) while EOs from the moist residue (MREO) and dry residue (DREO) presented 0.12% and 0.16%, respectively. The antibacterial activity of EOs was tested on *Escherichia coli* ATTC 25922 and *Lactobacillus plantarum* ATTC 8014, as models of pathogenic and beneficial bacteria of occurrence in pig gut.

In overall, all EOs presented high efficiency to reduced *E. coli* growth than *L. plantarum* growth, since higher disturbances on the growth kinetics of *E. coli* than *L. plantarum* were observed. Thus, these EOs presented a selective antibacterial activity between the pathogenic and beneficial bacteria tested. However, the crude PEO compared to MREO and DREO presented the highest selective antibacterial effect. In addition, antioxidant activity by ABTS and DPPH radicals, and phenolic content were higher for the crude PEO than MREO and DREO. Volatile composition analysis showed that the crude PEO and its residues EOs presented the same major components:  $\alpha$ -pinene,  $\beta$ -pinene, and thuja-2,4(10)-diene. Nevertheless, these compounds were presented in lower proportions in the EOs from the moist and dry residues.

## PP25 Antibiofilm effect of pickering nano-emulsion of *Zataria multiflora* Boiss essential oil against *Streptococcus pneumoniae*

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**Keywords:** *Zataria multiflora*, biofilm inhibition, *Streptococcus pneumoniae*, pickering nano-emulsion

### Abstract

A biofilm comprises any group of microorganisms in which cells stick to each other and often also to a surface. Infections associated with the biofilm growth usually are challenging to eradicate. The essential oils (EOs) and their components are becoming increasingly popular in medical applications, because of their proven antibacterial effect. *Zataria multiflora* Boiss is a popular spice plant in southwestern Asia, belonging to the Lamiaceae family. Its traditional application includes anesthetic, antiseptic, and antispasmodic purposes. Previously, we determined the chemical composition of *Zataria multiflora* EO (ZEO) with GC-MS analysis, which is highly similar to thyme EO. Because in our previous work we examined the antibiofilm effect of thyme EO, we assumed, the ZEO can also inhibit the biofilm formation of *Streptococcus pneumoniae*. Our aim was to investigate the biofilm inhibition effect of ZEO. Unfortunately, due to its lipophilic character and low water solubility, the direct use of ZEO, similarly to other EOs, in a microbiological experiment is limited. Therefore, we prepared O/W type Pickering nano-emulsions stabilized with silica nanoparticles. The nanoparticles were synthesized by Stöber method [1]. Firstly, the MIC (Minimum Inhibitory Concentration) was determined with broth macrodilution test (0.4 mg/mL) against *S. pneumoniae*. The biofilm inhibition experiments were performed on the base of Peeters and co-worker's study, with the crystal violet assay [2].

Our results showed that the ZEO had anti-biofilm activity against *S. pneumoniae*, because it reduced the biomass of the bacterial biofilm. It is important to highlight that the Pickering nano-emulsions was more effective (inhibitory rate: 65.3%) than the conventional Tween 80 stabilized emulsions (inhibitory rate: 47.4%), compared to the control.

In this study, the antibiofilm effect of ZEO was investigated against *S. pneumoniae*. We can conclude that O/W type Pickering nano-emulsions of ZEO provide a new possibility for biofilm inhibition against *Streptococcus pneumoniae*.

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## PP26 Antimicrobial activity of essential oil-bearing plants against microorganisms causing spoilage of agricultural products in vapor phase

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**Keywords:** essential oil, vapor phase, antifungal property, antimicrobial property, plant disease

### Abstract

Diseases caused by plant pathogens leads to significant postharvest losses to most perishable food crops. Plant diseases are currently controlled with fungicides, however, reliance on this single control strategy leads to problems such as environmental pollution and resistance to fungicides. Therefore, there is an increasing pressure to find more natural methods of disease control. One of the prospective methods could be the use of essential oil (EO) vapors by fumigation [1]. EOs from aromatic plants have previously demonstrated antimicrobial and antifungal activity against number of plant pathogens. Despite the great antimicrobial potential EOs, growth-inhibitory effects of their vapors have poorly been investigated against microorganisms causing spoilage of agriculture products [1]. In this study we determined antimicrobial activity of EOs in liquid and vapor phase against significant plant pathogens (*Pseudomonas* spp., and *Pectobacterium* spp.) using broth microdilution volatilization method [2, 3]. EOs of five plants namely *Thymus vulgaris*, *Cinnamomum zeylanicum*, *Syzygium aromaticum*, *Allium sativum*, and *Citrus sinensis* were obtained from dried plant material using hydrodistillation. Subsequently, minimum inhibitory concentrations (MICs) were determined [3].

The result showed that the *T. vulgaris* EO produced the highest antimicrobial activity against *Pseudomonas fluorescens*, *Pectobacterium atrosepticum*, and *Pectobacterium carotovorum* with MICs ranging from 256 µg/mL to 512 µg/mL in liquid phase and 256 µg/mL to 1024 µg/mL in vapor phase. In conclusion, above mentioned EOs could be used for the development of new products for control of pathogenic microorganisms causing spoilage of agricultural products e.g. in form of controlled atmosphere or fumigation.

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## PP27 Antimicrobial activity of *Lavandula stoechas* subsp. *luisieri* essential oils against strains of fungi isolated from strawberry tree

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**Keywords:** *Lavandula stoechas*, hydrodistillation, essential oil, GC-MS, food spoilage fungi

### Abstract

*Lavandula stoechas* subsp. *luisieri* is an aromatic shrub endemic to the Iberian Peninsula and common in the semi-arid regions and mountainous areas of Southern Portugal. This plant has deserved a lot of attention due the presence of compounds derived from necrodane with high biological activity. The chemical profile and antifungal activity of *L. stoechas* subsp. *luisieri* essential oil, harvested from Beira Interior region, in Portugal, were investigated. The essential oil of each phenological stage (dormancy and flowering) of the plant was isolated by hydrodistillation and analyzed by GC-MS. The minimum inhibitory concentration (MIC) and the minimum fungicide concentration (MFC) of both essential oils were determinate to verify antifungal activity against different strains of fungi isolated from strawberry tree (*Arbutus unedo* L.), a species with economic importance in this region. The fungi tested were *Aspergillus carbonarius*, *Rhizopus stolonifer*, *Penicillium brevicompactum*, *Aureobasidium pullulans* and *Sacrothecium* sp.

Essential oils were characterized by a high percentage of oxygenated monoterpenes. 5-Methylene-2,3,4,4-tetramethylcyclopenten-2-enone (26%), fenchone (6%) and 1,8-cineole (6%) were the major compounds in the essential oil from dormancy stage, while the main compounds of the essential oil from flowering stage were trans- $\alpha$ -necrolyl acetate (27%), trans- $\alpha$ -necrodol (13%) and linalool (6%). A strong antifungal activity of the essential oils was found against all strains, although there is not much difference between both essential oils. The MIC and MFC values ranged from 0.073-0.583  $\mu$ L/mL and 1.167-9.333  $\mu$ L/mL, respectively.

## PP28 Antimicrobial activity of selected essential oils against airborne microbes

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**Keywords:** antimicrobial activity, airborne microbes, essential sandalwood oil, essential rosemary oil

### Abstract

Airborne microbes surround humans 24h a day and are a hitherto underestimated cause of risk for human health. The development of an easy and safe way to reduce the amount of airborne microbes in locations where people gather or where foods are processed is thus required.

In the presented study samples of Australian Sandalwood, East Indian Sandalwood, West Indian Sandalwood essential oils and Rosemary essential oils from Spain and Tunisia were tested against airborne microbes in small rest rooms by using an RCS Air Sampler. After determining the total microbial count with the RCS Air Sampler in the testing room by sampling air for two minutes (blank values), the essential oils were vaporized. Fifteen minutes later, the total microbial count was measured again with the same experimental setup. Ten measurements were taken for each aroma substance/essential oil and for the blank values, respectively. After incubation of the agar strips and enumeration of colonies, the total germ count in the air in CFU/m<sup>3</sup> and the average bacterial count decrease in the air were calculated.

Among numerous tested essential oils, essential Sandalwood oils as well as Rosemary essential oils were most successful in diminishing airborne microbes, with an average germ count reduction of approximately 50% [1].

As a conclusion of this study it can be stated that the convenient safe method to decrease airborne microbes by essential oils presents an additional or even alternative method to currently used air disinfectants, especially in places where people gather, such as lecture halls, waiting rooms, theatres, stations, airports, rest homes or hospitals.

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## PP29 Antimicrobial effect of edible coating incorporated with microemulsion containing essential oil of basil (*Ocimum basilicum* L.) against *Listeria monocytogenes* in ham

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**Keywords:** volatile oil, microemulsion, pathogens, *Listeria monocytogenes*, food preservation

### Abstract

Increased demand for safe and healthy food has led to demand for natural antimicrobials that can ensure their microbiological safety. Therefore, the use of essential oils has shown to be a promising alternative. These oils are volatile and need other technologies aggregated to enable their use. In the present study, edible coating of sodium alginate (1%) based on microemulsion that contain essential oil of basil (*Ocimum basilicum* L.) cultivar Maria Bonita was applied to pre-contaminated ham slices in order to verify its antimicrobial effect. The essential oil used presented in its chemical composition mainly linalool (73.39%), geraniol (15.05%) and 1,8 cineol (5.3%). Approximately 25g of slices of ham were sterilized under UV light for three minutes on each side. The slices were immersed for 5 min in 0.1% peptone solution containing approximately  $10^7$  cells of *Listeria monocytogenes* per mL. The inoculated ham slices were used to apply three treatments: control without addition of coating (Control), coated with sodium alginate without microemulsion and basil essential oil (CAW), and coating with sodium alginate incorporated with microemulsion containing basil essential oil (30 $\mu$ L/mL) (MEOB). Samples were packed in polystyrene trays coated with PVC film and stored at 6°C for two days. At the times 0, 12, 24 and 48 hours the samples were evaluated for the number of *L. monocytogenes* viable cells. In the treatments Control and CAW the number of *L. monocytogenes* viable cells, after two days, remained between  $10^5$  and  $10^6$  CFU/g, while in the MEOB treatment the viable number of *L. monocytogenes* cells was reduced to  $10^3$  CFU/g after 48h of storage. We concluded that the treatment MEOB showed the ability to reduce the microbial activity on contaminated slices of ham contaminated with against *L. monocytogenes*. This result suggests that edible coatings incorporated with microemulsion containing basil essential oil have the potential to be used in active packaging to improve food safety.



## PP30 Antioxidant and cytotoxic activities from essential oils of *Ocotea indecora* (Schott) Mez. (Lauraceae)

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**Keywords:** *Ocotea indecora*, monoterpenes, sesquiterpenes, antioxidant activity, cytotoxic activity

### Abstract

Lauraceae species are popular ingredients of spices and flavoring agents, such as cinnamon and laurel, and the essential oil of some species have been largely used in the industry. *Ocotea* Aubl. is one of the largest genera in the Neotropics, containing ca. 350 species from which 170 are found in Brazil. Among the native Brazilian *Ocotea* species, *O. indecora* (Schott) Mez., 'canela-cheirosa', whose barks are commonly used in traditional medicine as sudorific, antirheumatic and anti-syphilitic, has not yet been studied regarding its essential oil contents and biological properties. Thus, the aim of the present work was to analyse the *O. indecora* essential oil composition and to evaluate its antioxidant and cytotoxic activities.

The oil was obtained by hydrodistillation for 4 h, and the component identification was performed by GC-MS. Their antioxidant potential was determined using the diphenylpicrylhydrazyl (DPPH) free-radical scavenging method, and the cytotoxicity was evaluated against human colorectal carcinoma (HCT116) cell line. The *O. indecora* leaf oil was contained mostly of monoterpenoids, with  $\alpha$ -pinene (12.84%)  $\beta$ -pinene (12.42%) and sabinene (11.02%) as major components and spathulenol (9.06%) as the major sesquiterpenoid. On the other hand, the stem oil sesquiterpenoids were the major group, with  $\beta$ -bisabolol (12.22%) and  $\alpha$ -cuprenene (5.25%) as the main compounds. Although their chemical differences, their antioxidant potential was similar, with respective IC<sub>50</sub>'s of 151 and 178  $\mu$ g/mL. Both oils were also able to inhibit almost completely the HCT116 cell line in the concentrations of 5 and 50  $\mu$ g/mL. These results indicated that the *O. indecora* oils have a pharmacological potential to be further explored.

## PP31 Antiradical and enzyme inhibitory potential of essential oil obtained from aerial part of *Centaurea pterocaula* Trautv. and its GC-MS profile

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**Keywords:** *Centaurea pterocaula*, essential oil composition, antidiabetic activity, anti-inflammatory activity, antioxidant activity

### Abstract

The chemical composition, free radical scavenging,  $\alpha$ -amylase inhibitory, and 5-lipoxygenase inhibitory activities of essential oil obtained from aerial parts of *Centaurea pterocaula* Trautv., collected in Turkey, were studied. The essential oil obtained by hydrodistillation were tested for its chemical composition by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The in vitro biological activities of essential oil were also investigated by  $\alpha$ -amylase inhibitory, 5-lipoxygenase inhibitory and DPPH/ABTS radical scavenging methods. Hexadecanoic acid (13.9%), caryophyllene oxide (11.5%), spathulenol (11.4%), (E)- $\beta$ -damascenone (5%), hexahydrofarnesyl acetone (5%), tetradecanoic acid (4.9%) were the dominant compounds in essential oil from the aerial parts. Essential oil showed good anti-inflammatory activity, inhibiting 5-lipoxygenase with an IC<sub>50</sub> value of 86.22  $\mu$ g/mL. The oil was found to be active against DPPH and ABTS radicals with IC<sub>50</sub> values of 83.05 and 360.9  $\mu$ g/mL, respectively, as well as against  $\alpha$ -amylase with IC<sub>50</sub> value of 79.66  $\mu$ g/mL. To our knowledge, this is first report on chemical composition and biological activity of *Centaurea pterocaula* essential oil. Also, these results showed that *Centaurea pterocaula* essential oil has good  $\alpha$ -amylase inhibitory, 5-lipoxygenase inhibitory, and DPPH radical scavenging activities.

## PP32 Aroma characteristics of *Alpinia zerumbet* grown in the Ryukyu Islands using DH-TD-GC-MS

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**Keywords:** *Alpinia zerumbet*, dynamic headspace-thermal desorption-GC-MS, Ryukyu Islands, aroma characteristics

### Abstract

The aromatic perennial plant *Alpinia zerumbet* (Pers.) Burt & Smith (Zingiberaceae) is widely distributed, occurring in both tropical and sub-tropical regions. In Japan, it grows from southern Kyushu to the Ryukyu Islands. The essential oil of *A. zerumbet* has a distinct aroma and exhibits antioxidant activity. Recently, interspecific hybridization of *Alpinia* spp. has been reported in Taiwan [1], and it may also occur in Japan. This type of hybridization may produce detectable differences in essential oil characteristics. Previously, we demonstrated the contribution of *A. zerumbet* essential oil to differences in fragrance and antioxidant activity among individuals [2]. Using the dynamic headspace method combined with thermal desorption-gas chromatography-mass spectrometry (DH-TD-GC-MS), we revealed that the volatiles in the leaves clearly differed among individual plants. In addition, these observations suggested that the aroma profile of *A. zerumbet*'s valuable essential oil can be predicted [3]. Therefore, we investigated in detail the aroma characteristics of *A. zerumbet* grown in the Ryukyu Islands by using this method.

*A. zerumbet* leaves were collected from 134 individual plants from the 14 islands of middle to the south of the Ryukyu Islands, between May 2017 and April 2019. The leaves were oven-dried (40–45 °C) to a moisture content of 10% or less, and 0.5 g of the dried leaves and stems from individual plants were septum-sealed in a 27-mL gas-tight vial. After introducing air into the vial through the activated carbon trap, the volatiles were aspirated by a mini pump and adsorbed to Tenax TA (60/80 mesh, 130 mg) for 10 min at 60 °C. Chemical analysis was performed using a TD-GC-MS system. Principal component analysis (PCA) was performed using the SIMCA software to determine whether differences in chemical composition of the essential oils could be correlated with the district of their production and/or with individual plants.

The major volatiles identified in this study ( $\alpha$ -pinene, camphene, limonene,  $\beta$ -phellandrene, 1,8-cineole, *p*-cymene, camphor, linalool, and cryptone) well represented the characteristics of the essential oil of the leaf. PCA confirmed 17 distinct groups of aroma profiles of *A. zerumbet* leaves in different individuals found on the Ryukyu Islands. These aroma characteristics did not depend on the island or area, and various types were identified in each area. These results reveal the diversity of the aroma characteristics of *A. zerumbet* in the Ryukyu Islands and suggest the plausibility of selecting lineages of *A. zerumbet* to optimize future production of valuable essential oils.

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## PP33 Aroma compounds and their distribution in leaves and flowers of Macedonian *Hypericum perforatum*

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**Keywords:** *Hypericum perforatum*, aroma components, headspace, GC-MS

### Abstract

*Hypericum perforatum* has been used in traditional medicine for a long period of time [1]. It has been known as a plant characterized by a diversity of bioactive constituents that possess antioxidant, anti-inflammatory, antidepressive and cytotoxic activity [2]. Chemical composition of *H. perforatum* extracts as well as essential oil has been studied, but still very little is known about its aroma components. Therefore, this study was designed to examine aroma compounds and their distribution in leaves and flowers in the plant collected from different locations in Macedonia during the year 2014-2017. HS-GC-MS analyses of volatile aroma compounds of dried leaves and flowers of *H. perforatum* collected from Tetovo, Mavrovo and Debar resulted in the identification of 23 components representing 93.24-99.83% of total amount. In class of terpenoids, monoterpene fraction was dominated by  $\alpha$ -pinene, both in leaves (6.06-35.08%) and in flowers (14.03-31.03%). Among sesquiterpene, caryophyllene was the most abundant component (1.03-7.94% in leaves and 0.11-2.68% in flowers). Compared to this, aliphatic hydrocarbons were present in higher amount and were consisted mainly of alkanes and their derivatives. Among all identified components, isononane was predominant in all tested samples of leaves (48.82-73.10%) and flowers (57.34-75.01%). This component was followed by nonane, 3-methylnonane and 2-methyldecane which were present in higher amount in leaves (2.26-7.65%, 2.02-9.06% and 1.29-7.93% respectively) than in flowers (2.06-6.17%, 1.28-5.25% and 1.28-3.28% respectively). As high volatile aroma compounds, these identified components are probably important for the *H. perforatum* specific scent.

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## PP34 Atypical chemical profiles of Lithuanian mugwort (*Artemisia vulgaris* L.) essential oils

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**Keywords:** *Artemisia vulgaris* L., chamigrene, vulgarone B, caryophyllene oxide, germacrene D.

### Abstract

*Artemisia vulgaris* L. (Mugwort) is a well-known weed with a strong spicy odour. The plant synthesizes many bioactive compounds; among them terpenoids and flavonoids are the most important. The plant is used for culinary purposes, as an herbal remedy in a folk and modern medicine as well.

*Artemisia vulgaris* L. plants (from three to five individual plants from each population) were collected at the flowering stage (July-August, over several years) from 25 growing sites at different parts of Lithuania. The essential oils were prepared by hydro-distillation for 2.5 h of air-dried material in a Clevenger-type apparatus according to the European *Pharmacopoeia*. The yields of yellow oils with a strong characteristic odour ranged from 0.15 to 0.4% (v/w, on a dry weight basis). Chemical analyses were performed using a chromatograph Shimadzu GC-MS-QP2010, interfaced to Shimadzu GC-MS-QP2010 ULTRA mass spectrometer and a capillary column DB-5 (50 m x 0.32 mm x 0.25 µm).

Full chemical composition (twenty-eight constituents found in amounts over 3.0%) of mugwort essential oils was determined. Among three major components were conducted: arteannuic alcohol (both isomers), chamigrene, vulgarone B, caryophyllene oxide and germacrene D.

To the best of our knowledge, arteannuic alcohol, vulgarone B and 1,8-cineole+chamigrene chemotypes for *A. vulgaris* oils were described for the first time. Vulgarone B (syn. longiverbenone, a sesquiterpene ketone) being common for tansy oils, has been determined also in significant amounts in other *Artemisia* species, such as *A. annua* and *A. douglasiana* Besser [1]. The mugwort essential oils containing germacrene D and caryophyllene + its oxide as predominant constituents are quite local. It should be mentioned, that mugwort oils of caryophyllene oxide chemotype are quite rare; in the past, it was described only for the plants grown in Cuba [2]. However, germacrene D is not very common predominant compound, mugwort essential oils of this profile were described in USA [3], Egypt [4] and propagated *in vitro* of Indian origin [5].

### ACKNOWLEDGEMENTS

to Lithuanian Research Council for financial support (grant No 09.3.3-LMT-K-712-13-0164) to participate in the ISEO2019

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## PP35 Biological activities of commercial *Cistus ladanifer* essential oil from Portugal

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**Keywords:** Cistaceae, rockrose, antimicrobial, antioxidant, preservative

### Abstract

*Cistus ladanifer* is an abundant wild plant species of poor agricultural areas of the Iberian Peninsula and the number of essential oils distilleries extracting essential oil from it seems to be increasing in those areas. Therefore, finding applications for this essential oil is mandatory. A commercial *C. ladanifer* essential oil was characterized regarding the antibacterial activity against common food spoilage and pathogenic bacteria through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination and the antioxidant activity through 1,1-Diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging determination. The product presented a broad antibacterial spectrum and some antioxidant activity, showing the potential to be used as an additive or in active packaging to enhance food preservation. These possible applications may increase the valorization of the product and the plant species and thus the economic status of areas rich in the plant species.

## PP36 Brazil and essential oils: Panorama

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**Keywords:** Brazil, essential oil, panorama, research, health

### Abstract

Medicinal and aromatic plants are generally used in medicine, pharmacy, agriculture, veterinary, horticulture, cooking, perfumery, aesthetics, cosmetics, sanitary among others. At this moment, in the face of the side effects generated by synthetic drugs, it is noticed that medicine has resumed ancient customs, phytotherapy has rescued attention and the essential oils has been gaining importance in this context.

Brazil has an incomparable biodiversity and difficulties with illegal exploitation and predatory deforestation. The most accurate botanical studies in the country go back to the nineteenth century and the commercialization of brazilian essential oils permeates the international scene from the mid-twentieth century (especially with rosewood -*Aniba roseaodora*-, sassafras -*Ocotea odorifera*- and mint -*Mentha arvensis*-).

It is worth noting the work on essential oils performed by Brazilian researchers from the National Institute of Amazonian Research, Emilio Goeldi Museum, Federal Universities (from Pará, Ceará and Rural of Rio de Janeiro), University of Brasília and from São Paulo, Agronomic Institute of Campinas, Brazilian Agricultural Research Corporation and Oswaldo Cruz Foundation.

Nowadays the Brazilian citrus essential oils have more significant production. However, some native oils have been taking place in academic investigations and justifying prominence for medicinal, therapeutic and commercial interests, such as: breu (*Protium heptaphyllum*), cumaru (*Dipteryx odorata*), priprioca (*Cyperus scariosus*), pitanga (*Eugenia uniflora*), guava mountain (*Acca sellowiana*) and copaiba (*Copaifera officinalis*).

Brazil has adopted public health policies that combine integrative and complementary practices (PICS) based on traditional knowledge to prevent or control diseases. The use of essential oils in aromatherapy is one of the 29 PICS procedures foreseen in the Brazilian's Unified Health System (SUS), offered to the population in an integral and free way. Nonetheless, the application of these procedures is still under progress.

Community herbariums in health centers and public places, supply of vegetal medicines at no cost to the patients, education on medicinal and rational use of plants, access to a database aromatic plants of the Amazon, both for health professionals and for general people, are some on-going Brazilian actions that deserves publicity.

## PP37 Chemical and biological profiles of essential oil from different parts of *Myrtus communis* L. subsp. *communis* from Turkey

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**Keywords:** *Myrtus communis* subsp. *communis*, essential oil, antidiabetic activity, anti-inflammatory activity, antioxidant activity

### Abstract

The present study reports chemical composition, antidiabetic, anti-inflammatory and antioxidant potential of essential oil from leaves and twigs of *Myrtus communis* L. subsp. *communis* from Turkey. Essential oils were obtained separately from leaves and twigs of *Myrtus communis* subsp. *communis* by hydrodistillation using a Clevenger-type apparatus. Their chemical compositions were determined using Gas Chromatography-Mass Spectrometry (GC-MS). Antidiabetic, anti-inflammatory and antioxidant activities of essential oils were tested by  $\alpha$ -amylase inhibitory, 5-lipoxygenase inhibitory and DPPH/ABTS radical scavenging methods, respectively.

The major compounds of essential oil of *Myrtus communis* subsp. *communis* leaves (MCLEO) were  $\alpha$ -pinene (35.6%), 1,8-cineole (28.3%), linalool (10.5%), and limonene (8.2%), while the major constituents of essential of *Myrtus communis* subsp. *communis* twigs (MCTEO) were  $\alpha$ -pinene (30.7%), 1,8-cineole (23.5%), *p*-cymene (13.3%) and limonene (11.9%). MCLEO and MCTEO showed good and moderate radical scavenging activity with  $IC_{50}$  values of 124.40 and 390.10  $\mu$ g/mL for ABTS radical, respectively. MCLEO and MCTEO exhibited significant radical scavenging activity with  $IC_{50}$  values of 34.13 and 28.15  $\mu$ g/mL for DPPH radical, respectively. Also, MCLEO and MCTEO displayed strong and good antidiabetic activity with  $IC_{50}$  values of 29.94 and 159.80  $\mu$ g/mL against  $\alpha$ -amylase enzyme, respectively. Finally, MCLEO and MCTEO showed good anti-inflammatory activity with  $IC_{50}$  values 86.10 and 96.55  $\mu$ g/mL against 5-lipoxygenase enzyme, respectively. It can be concluded from the present study that essential oils, especially MCLEO, possess good antidiabetic, anti-inflammatory and antioxidant activities. Also, this is the first study, as far as we know, on  $\alpha$ -amylase inhibitory and 5-lipoxygenase inhibitory activities of MCLEO and MCTEO.



## PP38 Chemical characterization of essential oils of *Eplingiella fruticosa* accessions

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**Keywords:** Lamiaceae, germplasm, volatile oil, chemical constituents, variability

### Abstract

*Eplingiella fruticosa* Salzm. ex Benth. (Lamiaceae), a species popularly known as “alecrim-de-vaqueiro”, is an aromatic and medicinal plant whose antinociceptive, anti-inflammatory, vasodilatory and antioxidant effects have been attributed to its essential oil. Due to the importance and biological activity of this species, a collection of accessions of *E. fruticosa* was implemented within the Active Germplasm Bank (AGB) of Medicinal and Aromatic Plants of the Federal University of Sergipe, aiming the conservation of its variability. Aiming to initiate the studies of these accessions, the aim of this work was to analyze the chemical composition of the essential oils of the 23 *E. fruticosa* accessions conserved in the AGB. The essential oils were extracted by hydrodistillation and analyzed by GC-MS-FID. From the data of the chemical constituents of the essential oils a multivariate analysis was made. The chemical compounds present in the essential oils were responsible for the division of the accessions into three groups. The first group consisted of six accessions (EPF-001, EPF-002, EPF-005, EPF-007, EPF-008, and EPF-016) and was characterized by the presence of camphor (6.46-15.21%), spatulenol (7.29-12.68%), (E)-caryophyllene (5.31-12.05%), and bicyclogermacrene (4.08-12.16%) as the compounds of higher content. The second group was also formed by six accessions (EPF-010, EPF-011, EPF-014, EPF-017, EPF-018, and EPF-019) and was characterized by the presence of (E)-caryophyllene (2.82-18.36%), bicyclogermacrene (3.76-12.16%), 1,8-cineole (3.39-13.01%), and espatulenol (3.30-10.50%). The compounds 1,8-cineole (9.57-30.42%), espatulenol (3.62-13.38%), and (E)-caryophyllene (3.66-14.50%) were found in greater quantity in the third group and this was formed by 11 accessions (EPF-003, EPF-004, EPF-006, EPF-009, EPF-012, EPF-013, EPF-015, EPF-020, EPF-021, EPF-022, and EPF-023). The results indicate that there is variability among the *E. fruticosa* accessions of the AGB of Medicinal and Aromatic Plants of the Federal University of Sergipe regarding the chemical composition of the essential oils, making it a possible target for studies on biological properties and for the purposes of conservation of the species.

## PP39 Chemical characterization of the essential oil of dried *Hyptis suaveolens*

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**Keywords:** hydrodistillation, gas chromatography, caryophyllene oxide

### Abstract

*Hyptis suaveolens* (L.) Poit. is a plant that is considered a weed despite its high therapeutic potential and the limited amount of information about its behavior when it is subjected to agricultural production techniques, which may affect the production of biomass and metabolic products. The objective of this study was to determine the influence of the drying process on the concentration and chemical composition of the essential oil obtained from *H. suaveolens*. Drying was performed in a fixed-bed dryer connected to an automated controller system that was configured to heat to 35 and 55 °C ± 1.3 °C with regulated air velocities of 1.0 and 2.0 m s<sup>-1</sup>. To obtain the essential oil, hydrodistillation was performed in a Clevenger apparatus with dichloromethane as the solvent. To identify and quantify the components of the essential oils, a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector (FID) and a Shimadzu GCMS-QP5050A mass spectrometer were used. Helium was the sweep gas, and the total running time was 70 min. The concentrations of essential oils were influenced by the air velocity used during the drying process. The major compound caryophyllene oxide was found in its highest concentrations of 48.91 ± 7.64 and 45.60 ± 19.85% after drying at 45 °C with air velocities of 1 and 2 m s<sup>-1</sup>, respectively. The only treatment that enabled the acquisition of the compound spathulenol was drying at 35 °C with an air velocity of 1 m s<sup>-1</sup>.

## PP40 Chemical composition and antibacterial activity of *Daucus carota* (wild carrot) essential oil

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**Keywords:** *Daucus carota*, essential oil, antibacterial activity

### Abstract

*Daucus carota* L. is a member of Apiaceae. It is a vegetable used for human nutrition. It is called as “havuç tohumu”, “yere geçen”, “keşür” and “pörçüklü” in Turkish. The plant is cultivated worldwide. The essential oil of the plant is used as diuretic and stomachic. The essential oil has biological properties such as antimicrobial, hepatocellular regenerator, general tonic and stimulant, cholesterol regulator and cicatrizant [1]. The main compounds of essential oil of *Daucus carota* from Poland and Lithuanian were sabinene,  $\alpha$ -pinene and geranyl acetate. The seed oil of the plant from Turkey had carotol and daucene as main components [2]. The objective of this study is to determine the antibacterial activity of the essential oil from aerial parts of *Daucus carota*. Essential oil of *D. carota* was obtained by hydrodistillation for 3 h from aerial parts. The oil composition was analyzed by means of gas chromatography-mass spectrometry (GC-MS). The analysis was carried out in triplicate. The antibacterial activity of the oil was evaluated for Gram-negative (*Klebsiella pneumoniae* ATCC BAA-1706 and *Escherichia coli* ATCC 14169) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 19659) by using a broth microdilution. The experiment was started at 18 mg/mL. Chloramphenicol was used as a positive control for assay. All the experiments were performed in duplicate. The yield of the oil was 0.60% (v/w). Thirty compounds were identified in the oil that represent 86.7%. The main components of the essential oil from aerial parts were carotol (27.4%), elemicine (17.8%) and limonene (15.7%). The essential oil showed inhibitory activity against *K. pneumoniae* and *E. coli* and at 18 mg/mL. The oil had antibacterial activity against Gram-negative bacteria.

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## PP41 Chemical composition and antioxidant activity of *Mentha x piperita* cultivars

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### Abstract

The aim of our study is to show the chemical differences of essential oils obtained from six *Mentha x piperita* cultivars classified in menthol chemotype. The second purpose is to analyze the antioxidant activity of peppermint EO, its fractions and isolated compounds, to show what kind of EO components are responsible for antioxidant activity.

EOs were obtained from the leaves of six *M. x piperita* cultivars growing in Lublin region. All EOs were analyzed by GC-MS. The fractionation of peppermint EO was done by using classical open column (CC) and flash chromatography (FC). EO and fractions were checked for antioxidant activity.

GC-MS analysis of all six EO showed the differences in the composition and especially in the relative amount of the compounds present in these oils. Own data showed that only essential oils from two cultivars, Citaro and Chocolate, meet pharmacopoeial requirements. Fractions obtained by use of CC and FC methods were tested by TLC-bioautography, and the active fractions were evaluated by CUPRAC method. Based on the chemical composition of the active fractions, we can conclude that terpinen-4-ol, piperitone and pulegone, as well as the sesquiterpenoids are responsible for the antioxidant activity of the peppermint EO. The active components constitute only about 3% of the EO. The major components, menthol and menthone, that constitute about 70% of all components present in the peppermint EO, have no antioxidant activity.

## PP42 Chemical composition and antioxidant activity of *Cedrela odorata* L. from the north of Guatemala

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**Keywords:** *Cedrela odorata*, cubebol, Petén, sesquiterpenoids

### Abstract

*Cedrela odorata* L. (Meliaceae) is a species native of tropical America and it is distributed from the south of Mexico to Panama, the Antilles Islands and in the north of South America. In Guatemala it is known as cedar and it has been described at the provinces on the north, northwest and south of Guatemala. Trees are often 20-30 m high, the trunk often more than a meter in diameter [1]. Cedar is used in Guatemala for luxury furniture and handicrafts because it is a precious wood, so it has been extracted illegally and is included in the Appendix III or CITES. In Sao Tomé and Príncipe this plant is used for the treatment of malaria, as a febrifuge and against diabetes [2]. The purpose of the study was to determine the composition of the essential oil of the leaves of the tree that grows wild in the maya biosphere, to evaluate its potential for oil production as an alternative use for the plant.

Leaves of *C. odorata* were collected in September 2018 in a population at the north of Lake Petén Itzá in the province of Petén at 128 m above sea level and 500 km at north of Guatemala City. The essential oil was extracted by hydrodistillation yielding 0.4% (w/w). The oil analyzed by GC and GC-MS was composed mainly by sesquiterpenoids: cubebol (28.8%),  $\beta$ -cubebene (10.3%),  $\alpha$ -humulene (8.5%) and epi-cubebol (6.5%). The antioxidant activity was determined by the DPPH radical scavenging method, obtaining a higher inhibition than BHT 0.14 M but lower than BHA 0.17 M.

Previous studies on the composition of the essential oil in *C. odorata* have reported  $\alpha$ -copaene (14.4%) and  $\alpha$ -cadinol (11.2%) for the bark oil in Brazil [3] and  $\alpha$ -copaene (15.6%) and  $\delta$ -cadinene (11.7%) for the bark oil of the plant cultivated in Sao Tomé And Príncipe [2] and  $\alpha$ -santalene (9.5%) and  $\beta$ -acoradiene (7.1%) for the oil extracted from the leaves of the plant cultivated in Nigeria [4]. The results for the oil of *C. odorata* of the north of Guatemala were different as cubebol was found in the highest percentage, probably due to the different environmental conditions. Because of the promising oil yield and composition and the use of the plant as traditional medicine in Africa for the authors is worth considering the management of *C. odorata* for essential oil production and further studies on biological and pharmacological activities are recommended.

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## PP43 Chemical composition of essential oil isolated from Macedonian St. John Wort (*Hypericum perforatum* L.)

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**Keywords:** *Hypericum perforatum*, essential oil, volatile compounds, GC-MS.

### Abstract

*Hypericum perforatum* known as Saint John's Wort is a wide spread flowering plant traditionally used in medicine. Nowadays, this plant has been extensively examined for its biological activities as it has been found to be effective in treating mild to moderate depression as well as anxiety and insomnia [1]. On the other hand, the main active components responsible for the biological effects are not known well yet. Lately, great attention is being paid to the *H. perforatum* essential oil assessment thus the main goal of this study was GC-FID-MS analysis of the essential oil isolated from leaves, flowers and herbs of *H. perforatum* that grows in Western Macedonia. Essential oil was isolated from plant material collected from three locations, Tetovo, Mavrovo and Debar according to Ph. Eur. procedure.

GC-FID-MS analyses of the *H. perforatum* essential oil resulted in identification of 84 compounds representing 84.98-97.50% of the total oil. The sesquiterpene fraction was dominated in all examined oils. The most abundant components were caryophyllene (12.93-15.75% in leaves, 22.23-25.71% in flowers and 11.37-12.99% in herbs essential oil) and germacrene D (26.55-39.03% in leaves, 11.97-17.77% in flowers and 21.45-26.90% in herbs essential oil). The fraction of monoterpenes was present in smaller amount.  $\alpha$ -Pinene (0.22-0.38% in leaves, 2.86-8.75% in flowers and 3.43-4.74 in herbs essential oil) and  $\beta$ -ocimene (0.79-1.69 in leaves, 1.96-8.87% in flowers and 2.22-3.30% in herbs essential oil) were identified as predominated components. The aerial parts of *H. perforatum* collected from Western Macedonia could be considered as a source of specific essential oil with authentic chemical composition rich in sesquiterpene compound particularly caryophyllene E and germacren D.

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## PP44 Chemical composition of the leaves essential oil of three black pepper cultivars from Amazon (Brazil)

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**Keywords:** *P. nigrum* cv. *bragantina*, *P. nigrum* cv. *cingapura*, *P. nigrum* cv. *kuthiravally*, sesquiterpenes.

### Abstract

Black pepper (*Piper nigrum* L.) is the most important spices cultivated in Asian countries, Madagascar and Brazil. Pará state is the largest and third producer in Brazil and the world, respectively. The Bragantina cultivar is a hybrid obtained from South India, also known as Panniyur exhibiting large leaves, heart-shaped and long spikes with an approximate length of 14.0 cm. The Cingapura cultivar, also known as Kuching has small and narrow leaves, short spikes on average of 7.0 cm length. The kuthiravally cultivar comes from the State of Kerala (South India) and present leaves with of 10.0-12.0 and 15.75 cm to width cm and height, respectively. Long ears with larger fruits (0.49 cm in diameter). Leaf volatile concentrates from each cultivar were extracted by Likens-Nickerson apparatus using pentane as the solvent, and chemical composition was determined by GC-MS. The experiments were performed in triplicate. The chemical analysis resulted in the identification of 68, 58, and 61 compounds in the “bragantina”, “Cingapura” and “kuthiravally” cultivars, respectively. Bragantina and kuthiravally cultivars showed predominance of oxygenated sesquiterpenoids. The main compounds of bragantina cv. were  $\alpha$ -mumulol (18.40%), cubebol (11.49%) and bycyclogermacrene (6.97%). In the kuthiravally cv. the major constituents were  $\alpha$ -bisabolol (37.29%) and elemol (19.67%). Differently, the Cingapura cv. showed higher amounts of sesquiterpenes hydrocarbons such as  $\beta$ -selinene (12.48%), bycyclogermacrene (10.83%) and  $\beta$ -elemene (5.22%). These results are suggesting that, according to compound classes, the EO from these *P. nigrum* cultivars can be classified into two main groups.

## PP45 Chemical composition, antioxidative and cytotoxic activities of essential oil of *Eugenia patrisii* Vahl (Myrtaceae) from Brazilian Amazon

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**Keywords:** Ubaia, Myrtaceae, volatiles, sesquiterpenes, E-caryophyllene

### Abstract

*Eugenia patrisii* Vahl is native and non-endemic species from Brazilian Amazon, which produces fruits of beautiful appearance. However, there are few studies and a lower consume in comparison to other *Eugenia* species occurring in Brazil. The essential oil (EO) from *E. patrisii* leaves was obtained by hydrodistillation (3h), and the chemical composition analyzed by Gas Chromatography coupled in mass spectrometry (GC-MS, Shimadzu QP2010 ultra system). The DPPH radical scavenging evaluated the antioxidant activity, and cytotoxic effects against colon (HCT-116), gastric (AGP-01), and melanoma (SKMEL-19) human cancer cell lines and a normal human fibroblast cell line (MRC-5) were determined using MTT assay. The oil yield was 0.4%, which the sesquiterpenes hydrocarbons were predominant (85.7%) such as *E*-caryophyllene (32.0%), bicyclogermacrene (10.0%), 9-*epi*-*E*-caryophyllene (7.6%) and  $\beta$ -elemene (6.4%).

The essential oil showed 28.9% of DPPH radical scavenging, only seven times less than the Trolox standard (146.2 mg.TE/mL). The oil showed cytotoxic activity against all cell lines tested HCT-116 (IC<sub>50</sub> 6.7  $\mu$ g/mL), AGP-01, (IC<sub>50</sub> 3.2  $\mu$ g/mL), SKMEL-19 (IC<sub>50</sub> 5.8  $\mu$ g/mL), and MRC-5 (IC<sub>50</sub> 3.5  $\mu$ g/mL). The IC<sub>50</sub> values of doxorubicin, a positive control, were of 0.03  $\mu$ M (HCT-116), 0.2  $\mu$ M (AGP-01), 0.1  $\mu$ M (SKMEL-19) and 0.3  $\mu$ M (MRC-5). The sesquiterpene hydrocarbon *E*-caryophyllene is present in many plants worldwide and possesses significant anticancer activities, affecting growth and proliferation of many cancer cells, and a low antioxidative activity<sup>4</sup>. The goods antioxidative and cytotoxic activities of *E. patrisii* can be attributed to synergic effects and promotes the possibility of its use in the phytotherapy to cancer treatment.



## PP46 Chemical compositions, larvicidal and antimicrobial activity of essential oil from *Amomum rubidum* Lamxay & N. S. Lý (Zingiberaceae) growing in Vietnam

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**Keywords:** *Amomum rubidum*, monoterpenes, essential oil, antimicrobial activity, larvicidal activity

### Abstract

The present work aimed at the characterization of the chemical constituents, larvicidal and antimicrobial activities of essential oils from *Amomum rubidum* Lamxay & N. S. Lý. The constituents of the oils were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The larvicidal activities were determined against laboratory-reared larvae of *Culex quinquefasciatus* and *Aedes albopictus* at 24 h and 48 h. The leaf oils consist mainly of 1,8-cineole (37.7%), limonene (16.3%), d-3-carene (19.5%). while the stem oils were dominated by  $\beta$ -phellandrene (16.1%), limonene (14.4%), d-3-carene (13.9%). The main constituents of the rhizome oils were d-3-carene (21.9%), limonene (17.8%) and  $\beta$ -phellandrene (14.6%). The essential oils displayed potent antimicrobial action against Gram-positive and Gram-negative organisms, as well as larvicidal activities against larvae of *C. quinquefasciatus* and *A. albopictus*. The present study is the first of its kind on the essential oil of *A. rubidum* when compared with other studies [1-4].

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## PP47 Chemical profile of essential oils from indigenous plant species of Leyte, Philippines

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**Keywords:** Philippines, essential oil, chemical composition, GC-MS, volatile compounds

### Abstract

The Philippines are one of the biodiversity hotspot in the world [1] with several of its plant species utilized as rich sources of essential oils (EOs) having significant economic value. Some of the EO-bearing plants in the country have never been chemically analysed and some were also found to show significant biological activity [2]. The large number of plant species and potential EOs in the country would suggest myriads of chemical compounds having multitudes of potential economic importance. In this study, we aimed to determine the chemical composition of EOs isolated from five Philippine plant species, namely *Alpinia haenkei* (pericarp), *A. elegans* (pericarp and seeds), *Xanthostemon verdugonianus* (leaves), and *Cinnamomum iners* (leaves) using hydrodistillation and analysed by dual-column/dual-detector system gas chromatography-mass spectrometry (GC-MS) analysis. GC-MS analysis of the EOs revealed that trans-methyl cinnamate (70.6% w/w), 7-epi- $\alpha$ -selinene (30.8% w/w), D-limonene (18.8% w/w), cyperenone (36.8% w/w), and caryophyllene (42.4% w/w) were the major components of *A. haenkei*, *A. elegans* (pericarp), *A. elegans* (seeds), *X. verdugonianus*, and *C. iners*, respectively.

The results will deepen the knowledge on the phytochemistry of Southeast Asian plant taxa, especially on the chemical composition of essential oil-bearing plant species and their volatile compounds.

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## PP48 Chemical constituents, anti-inflammatory and anti-nociceptive activities of essential oils from Nigeria

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**Keywords:** essential oils, chemical constituents, GC-MS, anti-inflammatory activity, anti-nociceptive property activity

### Abstract

Essential oils were obtained by hydrodistillation of air dry leaves of Nigerian plants and characterized by GC and GC-MS. *Albizia lebeck* contained 2-pentyl furan (16.4%), (*E*)-geranyl acetone (15.5%), (*E*)- $\alpha$ -ionone (15.5%) and 3-octanone (11.9%) while *Mucuna pruriens* had (*E*)-2-hexenal (19.0%) and linalool (8.9%) in abundant. Hydrofarnesyl acetone (26.6%) and phytol (8.1%) occurred in *Baphia nitida* while *Casuarina equisetifolia* contained nonanal (30.4%),  $\alpha$ -zingiberene (15.5%) and (*E*)-anethole (9.4%).

The anti-inflammatory activity of *M. pruriens* increased with time increased with a peak activity observed for the 400 mg/kg ( $p^{***} < 0.001$ ). Formalin activity showed that the oil reduced edema at inflammatory stage up to 100% inhibition rate. *B. nitida* displayed a maximal antinociceptive activity ( $p^{***} < 0.001$ ) for the 100, 200 and 400 mg/kg throughout the 90<sup>th</sup> min. The formalin assay showed an inhibition range between 20-75% for all doses, and displayed high inhibition of carrageenan-induced inflammation at tested doses within the first 2 h but declined afterwards.

*Albizia Lebeck* essential oils possessed a minimal basic anti-inflammatory and anti-nociceptive actions ( $p^* < 0.5$ ). The doses 100, 200 and 400 mg/kg of the essential oils showed high inhibition at the inflammatory phase (100%), however, at the neurogenic phase, the inhibition in each treatment varies with 400 mg/kg showing the lowest inhibition (11.35%). *C. equisetifolia* displayed time dependent anti-nociceptive activity dose ( $p^{***} < 0.001$ ) with optimum activity at the 30<sup>th</sup> and 60<sup>th</sup> minutes for all doses. The formalin activity was more predominant at the inflammatory stage with inhibition maximal at 100% for the 400 mg/kg. The carrageenan-induced oedema model reveals the suppression of inflammatory mediators.

## PP49 Chemodiversity of essential oils from wild populations: Challenges and opportunities

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**Keywords:** *Thymus vulgaris*, intrapopulational variability, essential oil, screening, TLC

### Abstract

Wild populations of many species of aromatic and medicinal plants show high intrapopulational chemodiversity with respect to essential oils (EOs) profiles due to several factors [1]. It can be considered an opportunity to select genotypes whose composition can be potentially suitable for different purposes, according to the biological activity of their components [2]. Nevertheless, this intrapopulational variability might significantly affect the reliability of studies in which the sampling is based on mixing materials from a certain number of individuals from the population. Consequently, it would be convenient to carry out the sampling in such a way that the samples have a well-defined profile. This is a challenge that can be faced through an adequate selection of the gathered individuals in the samples.

In order to analyze the EOs intrapopulational variability, a method based on a preliminary screening by TLC [3] was proposed and applied to 75 individual samples collected in an area of 10 km<sup>2</sup>, in which several profiles of *Thymus vulgaris* L. were previously identified [4]. The material from the individuals showing the same profile was gathered and subjected to simultaneous extraction-distillation by a Likens-Nickerson apparatus and the extracts were analyzed by GC-MS and GC-FID.

Different well defined profiles were characterized according their major compounds: (a) 1,8-cineole (56.2%), (b) linalool (61.3%), (c) camphane skeleton: camphene (15.1%) + camphor (43.5%) + borneol (12.6%), (d) camphor (19.8 %) +  $\alpha$ -cadinol (12.9 %), and (e) camphene (11.6%) + 1,8-cineole (26.4%) + camphor (27.5%).

Two main conclusions were obtained from these results: the performed preliminary screening could be useful to (1) ensure the chemical homogeneity of the gathered individuals in sampling process in such a way that more reliable results be obtained in studies based on specific compounds quantification, (2) identify individuals with specifically suitable EO profiles whose propagation can contribute to improve the cultivation of certain species.

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## PP50 Chemometric exploration of *Rhododendron groenlandicum* reveals complex patterns

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**Keywords:** *Rhododendron groenlandicum*, chemotypes,  $\beta$ -selinene, limonene autoxidation, sabinene

### Abstract

The delicately-scented essential oil from the young leaves of *Rhododendron groenlandicum* (Oeder) Kron & Judd, wild-collected in Eastern Canada, is a growingly important commodity in perfumery and aromatherapy. A significant variability is observed amongst commercial batches of oil, making it difficult to meet compositional requirements of buyers. Although the potential occurrence of chemotypes has recently been suggested in the species based on 12 commercial oils, there has been no effort to determine whether the phenomenon indeed exists between individuals. We have therefore conducted the first exploratory study of the variability of volatile compounds of the species.

Single plants ( $n = 98$ ) were sampled within a short period in September 2018 on five sites featuring varied landscapes. Each time,  $\approx 1$  g of fresh whole leaves were macerated in  $\text{CH}_2\text{Cl}_2$  to extract the volatiles from the oil glands prior to analysis by dual-column GC-FID and GC-MS. Compounds were quantified in mg/g against methyl octanoate as an internal standard, using predicted response factors. Within-individual chemical consistency was confirmed, and comparisons between single-plant distilled oils and extracts were made. Several molecules screened (including many recurring unknowns) feature a plurimodal distribution trend across samples. Strongly correlated variables ( $r > 0.6$ ) were collapsed, underlining potential biosynthetic groups. Interestingly, the major sesquiterpene  $\beta$ -selinene, closely associated to eudesma-3,11-dien-2-one, appears to be expressed independently of all other non-oxygenated sesquiterpenes (including  $\alpha$ -selinene), which strongly correlated together. Multivariate hierarchical clustering (Bray-Curtis dissimilarity) using SIMPROF grouping was then applied to define chemotypes, defining a minimum of four samples to consider a chemotype as valid. Three „superchemotypes“ featuring either sesquiterpenes; sabinene, sabinaketon, pinocarvone and associated monoterpenoids; and limonene autoxidation products, respectively, were found. The latter type, featuring non-artifactual hydroperoxides in relatively high concentrations, is hypothesized to result from gradual within-gland chemical degradation from a limonene-rich chemotype, with biosynthesis having ceased during earlier months. Several secondary subtypes are also observed, involving variations of oxygenated sesquiterpenes such a cyclocolorenone, and/or differences in yield. Many samples (17) were statistically excluded from the seven accepted chemotypes, suggesting that more groups could be defined with more observations. Although varying in their degree of heterogeneity, no sampling site featured a single chemotype. These results suggest that determining the underlying distribution patterns of Labrador tea chemotypes will be critical to better control variability, and therefore broader sampling is warranted.

## PP51 *Clinopodium vulgare* L. essential oil – chemical composition, antioxidant and anticholinesterase potential

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### Abstract

*Clinopodium vulgare* L. (Lamiaceae) is a medicinal plant characteristic for traditional medicine of Bosnia and Herzegovina (BiH). In BiH *C. vulgare* L. is used in the treatment of digestive disorders [1], while in traditional medicine of other countries this plant is used for treatment of diabetes, cancer and viral skin diseases [2,3]. Medicinal plants are rich in bioactive compounds. Oxidative stress is related with etiology and pathogenesis of many chronic diseases and bioactive antioxidant compounds may have an important role in their prevention and treatment. Inhibitors of cholinesterase play an important role in the treatment of Alzheimer's disease and many components of essential oils and plants extracts are being tested as potential cholinesterase inhibitors.

In this work chemical composition of *C. vulgare* L. essential oil from BiH is determined using GC-MS and GC-FID technique. Inhibition of acetylcholinesterase and butyrylcholinesterase was tested by Ellman's method [4], while antioxidant potential was tested using DPPH and FRAP method.

The results of the analysis of essential oil showed that analyzed oil is rich in sesquiterpenes. The major compounds were *epi*-bicyclosesquiphelandrene (18.1%),  $\alpha$ -gurjunene (10.9%) and hexadecanoic acid (10.5%). Compared to standard compounds BHA and vitamin C, *C. vulgare* L. essential oil showed moderate antioxidative potential, as well as moderate ability to inhibit the enzymes acetylcholinesterase (40%) and butyrylcholinesterase (23%) in concentration of 1 g/L in stock solution.

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## PP52 Comparative evaluation of selected essential oils in liquid and vapor phase against *Streptococcus mutans*

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**Keywords:** essential oil, antibacterial activity, *S. mutans*, vapor phase, broth dilution

### Abstract

Among the Gram-positive streptococci, *Streptococcus mutans* is one of the major bacteria in the oral cavity which promotes the development of dental caries. The high pathogenicity and cariogenic potential of this bacterium are probably linked to its high biofilm-forming ability, acidogenicity, and acid tolerance as well. Moreover, the bacterium has developed resistance to the available antibiotics in recent years due to the inappropriate prescriptions. Therefore, especially in the case of elder and immunocompromised patients the controlling of the bacterium and the development of new inhibiting agents is highly important. Targeting several *Streptococcus* species the antibacterial activity of essential oils (EOs), as complex natural substances, have been reported earlier. Based on their volatile character it should be reasonable to investigate the potential of EO vapors as well.

Therefore, the aim of our study was the microbiological evaluation of clove, cinnamon bark, thyme, citronella, scots pine, eucalyptus, and peppermint oil against *S. mutans* (DSM 20533) by different *in vitro* techniques.

The essential oils were obtained from a Hungarian company (Aromax Inc.). Their quality was verified by gas chromatography (GC-MS, GC-FID) techniques. In the case of broth dilution method (BDM) 5% emulsion was prepared from each oil with 10% solution of Polysorbate 80 and Mueller-Hinton test medium. The MIC and MBC values were expressed in mg/mL. The vapor phase (VP) test was developed in a four-section Petri dish (PD, diameter 90 mm). After the incubation period, MIC values ( $\mu\text{L}/\text{L}$ ) considering the amount of EOs ( $\mu\text{L}$ ) were calculated referring to the free airspace (L) in our PD. All tests were carried out in triplicate.

Among our tested materials, thyme oil showed the highest inhibitory activity (MIC: 0.04 mg/mL) against *S. mutans*, which was followed by citronella, cinnamon bark, and clove (MIC: 0.17-0.41 mg/mL) in BDM. Unfortunately, scots pine oil showed activity only in relatively high concentrations. During the VP experiments, the antibacterial activity of EO's vapor was evaluated directly. As a result, we observed that *S. mutans* was less sensitive to EO volatiles (MIC: 250-500  $\mu\text{L}/\text{L}$ ). In lower concentration (MIC: 90  $\mu\text{L}/\text{L}$ ), only cinnamon bark performed potent inhibition against this pathogen. In contrast with the result of BDM, the vapor form of thyme and citronella was found moderately active. The vapor of eucalyptus and scots pine did not show any activity in our test system.

Best of our knowledge the activity of the EO's vapor used in this study was determined first against *S. mutans*. In our future study, we would also like to focus on the mode of actions and cytotoxicity of these EOs and to detect the potential of their combinations with antimicrobials as well.

## PP53 Comparison of aroma components by steam distillation for each part of *Pinus luchuensis* Mayr

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**Keywords:** Luchu pine

### Abstract

Luchu pine (*Pinus luchuensis* Mayr) is distributed in the Southwestern Islands south of Amami Oshima Island, and only Luchu pine is a pine tree species native to Okinawa [1]. It has a sweet aroma, so as with other pinaceous plants. For the purpose of elucidating the details of *P. luchuensis* aroma components, authors investigated on the aroma components of the essential oils of *P. luchuensis* obtained from leaves and branches mixed sample and from pine cones.

Aroma components were identified by GC-MS analysis (SHIMADZU GC-MS-QP 2010 Ultra). 12 kinds of peaks such as  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -phellandrene and others were detected from the essential oil by steam distillation from a sample obtained by mixing the leaves, branches and pine cones (hereinafter referred to as mixed sample). 14 kinds of peaks such as  $\alpha$ -pinene,  $\beta$ -pinene, limonene,  $\beta$ -phellandrene and others were detected from the essential oil similarly obtained by steam distillation from the pine cone sample (hereinafter referred to as pine cone sample). For comparison, commercially available essential oils obtained by steam distillation from Scots pine (*Pinus sylvestris*) cones were analyzed under the same conditions (hereinafter referred to as *P. sylvestris* sample). From the peak area ratio of each essential oil, the content ratio of the aroma component was compared. Each essential oil had the highest content of pinenes. In particular, the content of  $\alpha$ -pinene was high in the essential oils of *P. luchuensis*: 59% in mixed sample, 49% in cone sample, and 29% in essential oil of *P. sylvestris* sample. Also, the content of  $\beta$ -phellandrene was high: 9% in mixed sample, 15% in cone sample, and 3% in essential oil of *P. sylvestris* sample. The high content of  $\alpha$ -pinene and  $\beta$ -phellandrene is considered to be a feature of the aroma component of *P. luchuensis*.

In order to find out the detailed characteristics of *P. luchuensis* aroma components, it is also necessary to analyze the essential oils obtained from leaves only. Since the amount of essential oil obtained from leaves is very small, concentration analysis of aroma components will be examined in the future.

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## PP54 Composition and antifungal and antioxidant evaluation of two chemotype-oils from *Cinnamomum verum* J. Presl occurring in Maranhão, Brazil

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**Keywords:** *Cinnamomum verum*, eugenol, benzyl benzoate, (E)-cinnamaldehyde, antifungal and antioxidant activities

### Abstract

*Cinnamomum verum* J. Presl, known in Brazil as “Canela”, has been highlighted for its extensive use in the cooking of diverse cultures of the world. The composition and the antifungal and antioxidant activities of the essential oils of the wood bark and leaves of two chemical types from *C. verum* J. Presl were evaluated. GC-MS and GC-FID were used for identification and quantification of the constituents of the oils. The antioxidant activity was evaluated by the DPPH radical scavenging method, and the antifungal activity was determined by an *in vitro* test against *Colletotrichum musae*, a phytopathogenic fungus that causes the anthracnose disease in banana fruit.

In the leaf oils of Santa Inês chemotype, the main constituent was benzyl benzoate (95.3%) and in the São Luís chemotype was eugenol (93.6%). In the wood bark oils of Santa Inês chemotype was (E)-cinnamaldehyde (89.3%) and in the São Luís chemotype were benzyl benzoate (23.3%), linalool (14.0%), (E)-caryophyllene (9.1%), caryolan-8-ol (7.2%) and borneol (4.7%). Leaf essential oils showed very high antifungal activity, achieving 100% efficacy at the tested concentrations of the eugenol chemotype and above 70% efficiency in the benzyl benzoate chemotype. In the evaluation of the antioxidant activity, the eugenol chemotype oil was highlighted with an inhibitory concentration higher than 80%, presenting the best responses when compared to the equivalent antioxidant capacity of Trolox.

## PP55 Composition of *Calocedrus decurrens* essential oil and hydrolate

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**Keywords:** essential oil, hydrolate, incense cedar, GC-MS

### Abstract

*Calocedrus decurrens* known as incense cedar is a tree with a typical distribution in western Northern America and eastern Asia. It is native to California and Oregon and has travelled to China before oligocene. It is best known for its aromatic wood, industrial use in pencil manufacturing and its decorative value.

*Calocedrus decurrens* heartwood essential oil exhibited biocidal activity against various mosquitos and tick species, including ones that have developed resistance to synthetic repellents [1,2]. Thymoquinone and carvacrol are indicated as compounds causing the biocidal effect. They have been identified as major constituents of heartwood EO [3]. On the contrary, monoterpene hydrocarbons ( $\alpha$ -pinene (11.2; 56.6; 22.3%), myrcene (13.4; 8.4; 9.7%), car-3-ene (31.3; 5.2; 11.1%), limonene (6.4; 5.1; 5.5%)), were the main components of leaves, young and old branches, respectively.

In the presented research composition of essential oil (EO) and hydrolate (H) obtained by the same process of industrial hydrodistillation was investigated. Plant material (stems with needles) was collected from botanical garden in central Poland in July 2017. EO and H volatiles (isolated by solvent extraction) were analyzed by GC-FID-MS and identified according to their retention indices and mass spectra.

Main components found in *C. decurrens* essential oil were car-3-ene (38.9%), limonene (20.1%),  $\alpha$ -pinene (13.1%) and myrcene (10.5%). Recently found new pinane derivatives (such as methyl pin-2-en-8-oate, pin-2-en-8-al and pin-2-en-8-yl acetate)[4] were also identified in the EO (1.6%, 0.1% and in traces, respectively). The content of volatiles in four consecutive fractions of hydrolate was: 145, 144, 103 and 58,6 mg/L. Main compound found in all hydrolate fractions was pin-2-en-8-ol, contributing up to 30.8%. Other volatiles constituting to the composition in huge amounts were: terpinen-4-ol (up to 16.2%), myrtenol (7.8%) and methyl myrtenate (5.1%), all being oxygenated terpenes; opposite to the essential oil in which the main compounds were terpene hydrocarbons.

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## PP56 Composition of clary sage (*Salvia sclarea* L.) hydrodistillation products

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**Keywords:** clary sage, *Salvia sclarea* L., volatiles, hydrolate, essential oil

### Abstract

Clary sage (*Salvia sclarea* L.) is a perennial herb, native to Southern Europe, but it is also cultivated worldwide, especially in the Mediterranean region, central Europe, Russia, England, Morocco, and the USA. This plant was highly esteemed in Middle Ages but nowadays it fell out of use. Clary sage essential oil revealed many therapeutic properties, among others it acts antiphlogistic, antiseptic, astringent, deodorant, antidepressant, sedative, and estrogenic. Because of mild estrogenic properties it might be used as an anti-aging cosmetic ingredient. During essential oil distillation of *S. sclarea* hydrolate might be obtained. This product has not been tested so far. Due to this reason we consider it as interesting test material.

*Salvia sclarea* herb was collected in 2015 in Poland. Fresh plant material was subjected to industrial hydrodistillation and hydrolate and essential oil were produced. It revealed a pleasant, mild, herbal scent. Volatiles from every sample of hydrolate were isolated by liquid-liquid extraction with diethyl ether. The hydrolate volatiles and essential oil were analyzed by GC-FID-MS.

The total content of volatiles in representative sample of hydrolate amounted to 337 mg/L whereas in hydrolate fractions the value changed irregularly from 494 mg/L to 224.8 mg/L.

The main group of clary sage oil constituents were monoterpenes: linalyl acetate (63.9%), linalool (5.6%), and myrcene (3.6%). Germacrene D (3.9%) was also detected in large amount.

Polar compounds, especially monoterpene alcohols were identified as major group of volatile compounds in representative sample of *S. sclarea* hydrolate. Linalool (49.7%), geraniol (6.7%), and  $\alpha$ -terpineol (3.1%) were dominant. The qualitative composition of volatiles in hydrolate fractions was similar. However, some differences in the quantitative composition were observed.

According to our results *S. sclarea* herb might be raw material for valuable hydrolate production.

## PP57 Composition of the essential oil from the needles and twigs of organic Swiss stone pine (*Pinus cembra* L.) from Tyrol

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**Keywords:** swiss stone pine, Arolla pine, *Pinus cembra*, Pinaceae, essential oil composition

### Abstract

*Pinus cembra* L., also known as Swiss stone pine or Arolla pine, is a slow growing, small to medium sized evergreen conifer of the Pinaceae family, which grows in the Alps chain at altitudes from 1100 m to 2700 m above sea level [1].

The essential oil from the needles and twigs of *P. cembra* is obtained by steam distillation with yields ranging from 0.4 to 1.5% and is commercially produced in small distilleries in Tyrol, a region comprising South-West Austria and North Italy. The essential oil has a very pleasant pine-type odour with a balsamic-sweet undertone of great tenacity. It is used in medicine, aromatherapy and as a fragrance ingredient.

For this study, 56 samples of *P. cembra* essential oil have been analysed, covering a period from 2013 to 2019. The essential oil composition was determined by GC-MS and dual channel GC-FID. The enantiomeric distribution of some relevant constituents has been assessed by enantio-GC using a chiral cyclodextrin-based stationary phase.

The essential oils comprised predominantly monoterpene hydrocarbons, of which  $\alpha$ -pinene (38.4 – 46.4%),  $\beta$ -pinene (10.0 – 12.2%), limonene (11.5 – 14.2%) and  $\beta$ -phellandrene (14.5 – 21.4%) were the major constituents.

The enantiomeric distribution of the main chiral compounds was as follows:

- (1R,5R)-(+)- $\alpha$ -pinene (67.3 – 74.8%) : (1S,5S)-(-)- $\alpha$ -pinene (25.2 – 32.7%)
- (1R,4S)-(+)-camphene (12.3 – 16.2%) : (1S,4R)-(-)-camphene (83.8 – 87.7%)
- (1R,5R)-(+)- $\beta$ -pinene (3.1 – 3.9%) : (1S,5S)-(-)- $\beta$ -pinene (96.1 – 96.9%)
- (4R)-(+)-limonene (4.3 – 7.4%) : (4S)-(-)-limonene (92.6 – 95.7%)
- (4S)-(+)- $\beta$ -phellandrene (max. 0.2%) : (4R)-(-)- $\beta$ -phellandrene (min. 99.8%)

Our results for the Tyrolean *P. cembra* essential oil differ slightly from those reported in the literature [2-4]. Of special interest is the high enantiomeric purity of  $\beta$ -phellandrene. To the best of our knowledge, no enantiomeric data have yet been published for this key compound of *Pinus cembra* essential oil.

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## PP58 Determination of antibacterial activity of fennel (*Foeniculum vulgare*) aerial parts essential oil

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**Keywords:** fennel, essential oil, antibacterial activity

### Abstract

Fennel grows 1-1.8 m. It is perennial herbaceous plant. The flowers are yellow. The fruits are green or greenish-yellow [1]. It is a highly aromatic and flavorful herb with culinary and medicinal uses. The essential oils of the plant have characteristic anise odour. The main components of the oil were *trans*-anethole, fenchone, estragole (methyl chavicol), and  $\alpha$ -phellandrene [2]. The objective of this study is to determine the antibacterial activity of the essential oil from aerial parts of fennel. The oil was obtained by hydrodistillation for 3 h. Essential oil composition of *F. vulgare* oils were analyzed by means of gas chromatography-mass spectrometry (GC-MS). The antibacterial activity of the oil was evaluated for *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 19659, *Klebsiella pneumoniae* ATCC BAA-1706 and *Escherichia coli* ATCC 14169 by using a broth microdilution. The experiment was started at 18 mg/mL. Chloramphenicol was used as a positive control for assay. All the experiments were performed in duplicate. Estragole (33.64 %), limonene (24.73%) and  $\alpha$ -pinene (19.13%) were main compounds in the oil. The essential oil showed inhibitory activity against *K. pneumoniae* and *E. coli* and at 9 and 18 mg/mL, respectively. The oil had antibacterial activity against Gram-negative bacteria.

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## PP59 Compositional analysis of *Boswellia dalzielii* frankincense oleoresin essential oils from Nigeria and Burkina Faso

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**Keywords:** frankincense, olibanum, *Boswellia dalzielii*, chemical composition, cluster analysis

### Abstract

Frankincense is an aromatic oleoresin produced by members of the genus *Boswellia*. The oleoresins are used for incense and medicine, and frequently distilled into essential oils for perfumery and aromatherapy. *Boswellia dalzielii* ranges across West Africa, but has received relatively little attention as its oleoresin is little traded compared to other *Boswellia* species. We present the first analysis of the oleoresin essential oils of *B. dalzielii*, collected from individual trees in northern Nigeria and central and western Burkina Faso. All samples were hydrodistilled and analyzed by GC-MS and GC-FID by the same operators under the same conditions. Essential oil yields varied dramatically, ranging from 1.69% to 17.0% v/w. Two chemotypes were identified: one dominated by  $\alpha$ -pinene, sometimes with lesser levels of  $\alpha$ -thujene and *p*-cymene, and a second, much rarer chemotype rich in myrcene, sometimes with a significant level of limonene. The samples from Nigeria had higher levels of  $\alpha$ -pinene (42.6-72.1%) compared to those from Burkina Faso (26.0-55.1%). The Nigerian samples were also largely devoid of sesquiterpenes, while the samples from Burkina Faso did have an appreciable percentage of sesquiterpenes, most commonly  $\alpha$ -copaene (1.9-5.5%). This chemistry is similar to many species of *Boswellia*, the oleoresin essential oils of which are generally rich in  $\alpha$ -pinene and  $\alpha$ -thujene; only a few *Boswellia* species (*B. papyrifera*, *B. occulta*, *B. bullata*) show significantly different components.

## PP60 Creation of nature – identical and non allergen bergamot oil bergaptene free

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**Keywords:** nature identical, non allergen, IFRA, bergamot oil

### Abstract

Contact allergy to fragrances is a common and significant problem around the world. The studies from the SCCNFP opinion on fragrance allergy in consumers in 1999 (SCCNFP/0017/98) (SCCNFP 1999) have confirmed that 26 different fragrance raw materials identified by the SCCNFP, are fragrance allergens for consumers because of their exposure from cosmetic products [1]. The Annex III of Cosmetics Directive states that these natural extracts must be indicated in the list of ingredients, in addition to the word ‘perfume’, if their concentration exceeds 0.001% in leave-on products (e.g. a moisturizer) and 0.01% in rinse-off products (e.g. a shampoo) [2,3]. One of the natural essential oils which is widely used in fragrance industry, Bergamot Oil Bergaptene Free (CAS 68648-33-9) from the original plant *Citrus aurantium* L. subsp. *bergamia*, was analyzed by GC-MS. Several of the 26 allergens were found to be a part of Citrus Oil including d-limonene pure (CAS 5989-27-5) (5.68 %), citral (CAS 5392-40-5) (0.48 %) and linalool (CAS 78-70-6) (17.47 %). Studies were made in order to develop nature - identical of Bergamot Oil Bergaptene Free that will not contain any of these 26 contact allergens. The derived nature-identical mixture was examined and compared by GC-MS. Although the results showed differences in some constituents, only trace amounts (< 0.1%) of these contact allergens were observed. In conclusion, nature - identical perfumes resembling the odor of essential oils, which can be used without any restrictions, were created.

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## PP61 Cytotoxic effect of *Rosmarinus officinalis* essential oil on *Lactuca sativa* root meristem

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### Abstract

Essential oils are volatile mixtures obtained from plants used in the industry of food, beverages, cosmetics and hygiene products, as well for medicinal purposes. Previous studies demonstrated that *Rosmarinus officinalis* L. (rosemary) essential oil interferes negatively on root elongation of different plant species. These phytotoxic effects observed at morphological level can be explained in terms of cellular and molecular effects. Cytogenetic analysis allows the observation of the chromosomes of an organism with the main objective of identifying chromosomal alterations associated with genotoxicity and cytotoxicity. Thus, the present study aimed to evaluate the cytotoxic effect of rosemary essential oil using a plant test system.

Diaspores of *Lactuca sativa* L. (lettuce) were soaked in 3 mL of distilled water on filter paper in Petri dishes and incubated at an average temperature of 20 °C under 12 h photoperiod. After the emergence of the primary root, seedlings were exposed to 0.5 µL, 1 µL and 10 µL of the essential oil, applied on a cotton ball attached to the inner face of the Petri dish. A control group was treated with distilled water, and three replicates of each treatment were performed. After 48 h of exposure to the volatiles, root tips were cut and subsequently fixed in freshly prepared mixture of absolute ethanol and acetic acid (3: 1, v/v) for 24 h, then stored in 70% alcohol in freezer. The fixed roots were stained by the Feulgen reaction during 1 h. Slides were prepared and 6000 cells were analyzed per treatment under light microscopy. Parameters evaluated included mitotic index, metaphasic index and percentage of each mitotic phase. Compared to control, rosemary essential oil reduced the mitotic index of lettuce by 12% when 1 µL and 10 µL were applied. Regarding to metaphasic index, a reduction by 37% and 51% at 0.5 µL and 1 µL treatments, respectively, was observed. For 10 µL treatment, an increase in metaphasic index compared to 1 µL treatment was observed, indicating that this amount of essential oil applied caused an increase of the number of cells in metaphase, a key point of cell division. Compared to control, an increase in 30% of the cells in prophase and a decrease in the percentage of cells in anaphase at 0.5 µL and 1 µL treatments were observed. In contrast, it was a 2-fold increase in the percentage of cells in anaphase at 10 µL treatment. No abnormal cells were observed. Results indicated that the interference of rosemary essential oil on the cell division of lettuce cells does not occur by preventing the initiation of cell division, since there was a significant percentage of cells in prophase, but by interfering in the continuation of the process of cell division. In addition to the knowledge of the mode of action of rosemary essential oil in the lettuce root meristem, the results obtained, using a plant test system, can be used in the prediction power for the assessment of new anti-proliferative therapies.



## PP62 Creation of nature – identical and non allergen lemon oil distilled

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**Keywords:** nonallergen, nature identical, IFRA, lemon oil

### Abstract

Contact allergy to fragrances is a common and significant problem around the world. The studies from the SCCNFP opinion on fragrance allergy in consumers in 1999 (SCCNFP/0017/98) (SCCNFP 1999) have confirmed that 26 different fragrance raw materials identified by the SCCNFP, are fragrance allergens for consumers because of their exposure from cosmetic products [1]. The Annex III of Cosmetics Directive states that these natural extracts must be indicated in the list of ingredients, in addition to the word ‘perfume’, if their concentration exceeds 0.001% in leave-on products (e.g. a moisturizer) and 0.01% in rinse-off products (e.g. a shampoo) [2,3]. One of the natural essential oils which is widely used in fragrance industry is Lemon Oil Distilled (CAS 8008-56-8) from the original plant *Citrus limonum* (L). Burm. f. which was analyzed by GC-MS. Several of the 26 allergens were found to be a part of Citrus Oil including d-limonene pure (CAS 5989-27-5) (63.03 %), citral (CAS 5392-40-5) (2.03 %) and linalool (CAS 78-70-6) (%0,29 %). Studies were made in order to develop nature-identical of Citrus Oil Distilled that will not be contain any of these 26 contact allergens. The derived nature-identical solution was examined and compared by GC-MS. Although the results showed differences in some constituents, only trace amounts (< 0.1%) of these contact allergens were observed. In conclusion, nature-identical perfumes resembling essential oils in their odor, which can be used without any restrictions, were created.

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## PP63 Composition of the essential oil of accessions of *Lippia alba* in Uberlândia-MG, Brazil

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**Keywords:** Brazilian lemon balm, volatile oil, seasonality

### Abstract

*Lippia alba* (Mill), is a Brazilian aromatic plant that has essential oil (EO) with antifungal, insecticidal and repellent properties. The production of active principles in plants depends on genetic control and genotype x environment interactions, which under conditions of excess or deficiency of some environmental factor can alter the productivity, and the chemical constitution of EO.

The objective of this study was to evaluate the variation of the chemical composition of EO from seven accessions of *L. alba* during the months of August 2017 to April 2018, in Uberlândia-MG, Brazil. Leaves of the accessions were collected monthly at the experimental farm of the Federal University of Uberlândia-MG, Brazil and dried at 40°C for 5 days. EO extraction was performed in microwave heating equipment for 20 min with a power of 500W. EO samples were analyzed in CG-MS / DIC. The data were submitted to analysis of variance and the means were grouped by the Scott-Knott test ( $p \leq 0.05$ ). The EO extracted monthly from the seven accessions revealed variability in chemical composition. Citral, the main constituent of accessions LA-59 (with the highest variations in November and April, 75.34% and 74.89%, respectively), access LA-03, (with the highest variations in the months of September and April, 81.62% and 82.32%, respectively) and LA-36 access (with the highest variations in the months of September and January, 78.07% and 84.62%, respectively). The LA-24 access presented the highest percentage of linalool, ranging from 72.14% in January to 83.84% in April, and carvone, in accessions LA-13 (with major changes in September and January, 78.71% and 72.06%, respectively) and LA-57 (with the highest variations in November and December, 81.35% and 87.68%, respectively). The EO had quantitative variations of the chemical constituents, mainly the citral, linalool and carvone compounds.

## PP64 Determination of aroma characteristics of *Alpinia formosana* via DH-TD-GC-MS

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**Keywords:** *Alpinia formosana*, dynamic headspace-thermal desorption-GC-MS, Okinawa Island

### Abstract

The aromatic perennial plant *Alpinia formosana* K. Schum. (Zingiberaceae) is widely distributed in southern Japan (from southern Kyushu to the Ryukyu Islands) and Taiwan. Its rhizomes contain diterpenes and phenolic compounds, which exhibit high physiological activity [1]. This species is a hybrid between *A. zerumbet* and *A. intermedia*. However, more complicated interspecific hybridization of *Alpinia* spp. has been recently reported in Taiwan [2], and may also occur in Japan. This type of hybridization may produce detectable differences in the aroma characteristics of the leaves of *Alpinia* spp., including *A. formosana*. Previously, we examined the composition of the essential oil of *A. zerumbet*. Using the dynamic headspace method coupled with thermal desorption-gas chromatography-mass spectrometry (DH-TD-GC-MS), we revealed that the volatile compounds in the leaves varied between individual plants [3]. Therefore, this method was selected for detailed investigation of the aroma characteristics of *A. formosana* grown on the Okinawa Island.

*A. formosana* leaves were collected from 23 individual plants from the Okinawa Island between September 2017 and May 2019. The leaves were oven-dried (40–45 °C) to a moisture content of 10% or less. Thereafter, 0.5 g of the dried leaves and stems from individual plants were septum-sealed into separate 27 mL gas-tight vials. After introducing air through an activated carbon trap into the vials, the volatiles were aspirated by a minipump and adsorbed to Tenax TA (60/80 mesh, 130 mg) for 10 min at 60 °C. Subsequent chemical analysis was performed using a TD-GC-MS system. Principal component analysis (PCA) was performed using SIMCA software to determine if the differences in the chemical composition of the essential oils could be correlated to their district of production and/or with individual plants.

The major volatiles detected in the leaves included  $\alpha$ -pinene (2.0–11.6%),  $\beta$ -pinene (2.2–67.6%), camphene (0.4–7.1%), limonene (2.0–9.9%), 1,8-cineole (1.2–27.6%), p-cymene (1.8–19.3%), and camphor (0.2–11.5%). Some plants contained significant amounts of cryptone (0.5–5.3%), which is a component found in *A. zerumbet*. The major volatiles accurately represented the characteristics of the essential oil of the leaves. PCA confirmed eight distinct groups of aroma profiles for the *A. formosana* leaves collected from different individuals on the Okinawa Island. The aroma characteristics of the leaves varied between the individual plants, and also differed from those of *A. zerumbet*. These results indicated a diversity in the aroma characteristics of *A. formosana* found on the Okinawa Island as some plants exhibited aroma characteristics that closely resembled those of *A. zerumbet*, supporting the occurrence of interspecific hybridization.

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## PP65 Diverse phytotoxic effect of commercial *Origanum vulgare* essential oil against monocot and dicot model plant species

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**Keywords:** biological activity, eco-friendly pesticides, GC-MS, secondary metabolites, thymol

### Abstract

Synthetic chemical products play an important role in crop protection in agriculture [1]. The long-term use of synthetic pesticides has reflected in serious environmental problems such as the sharp decline in pollinators and, on the contrary, the resistance of pests to important pesticides. Additionally, environmental pollution and an impact on human health has increased [2]. Plant secondary metabolites are characterized by a variety of biological activity. They are practically used for their antimicrobial, antioxidant, insecticidal or phytotoxic effect. The use of natural substances in the management of undesirable plants has already been documented [3,4]. Plants offer considerable potential for selective biological activity including ease degradability of chemicals in the environment [5].

Commercial essential oil (EO) of *Origanum vulgare* was tested for its biological activity as potential eco-friendly herbicide. Composition of EO was analyzed on GC-MS. Thirty-five components were identified. Dominant components were thymol (79.9%), p-cymene (5.9 %), linalool (2.7 %) and  $\gamma$ -terpinene (2.5 %). Then, EO was diluted to twelve different concentrations (100  $\mu\text{g/ml}$  - 0.0625  $\mu\text{g/ml}$ ) and applied on two monocot species (*Triticum aestivum* and *Hordeum vulgare*) and two dicot species (*Sinapis alba* and *Lepidium sativum*) as model plants for biological assay. EO was applied on ten seeds in one Petri dish and each concentration was repeated three times and compared with control. Statistical analysis was used for evaluation influence of *O. vulgare* on different plant species seed germination and root elongation. Germination of all four species was not influenced by EO. Phytotoxic effect was statistically significant in monocot species, while in dicot species was observed completely opposite - stimulation effect, which was also statistically significant. Based on the results we can conclude that EO of *O. vulgare* presented diverse effect on monocot and dicot plant species in observation its biological activity.

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## PP66 Effect of edaphic and climatic factors on *Thymus pulegioides* $\alpha$ -terpinyl acetate chemotype

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### Abstract

Chemical polymorphism is characteristic of essential oil bearing medicinal and aromatic species *Thymus pulegioides* (Lamiaceae).  $\alpha$ -Terpinyl acetate chemotype is one of six *T. pulegioides* chemotypes found in Lithuania.  $\alpha$ -Terpinyl acetate is the main chemical compound of *T. pulegioides*  $\alpha$ -terpinyl acetate chemotype; this chemical compound has antibacterial properties. Although the chemotype of the plant is determined genetically, however, quantitative and qualitative composition of essential oils can be influenced by climatic conditions and soil chemical composition.

The aim of the study was to establish distribution individuals of  $\alpha$ -terpinyl acetate chemotype in Lithuania and to determine the influence of edaphic and climatic factors on the percentage of  $\alpha$ -terpinyl acetate in *T. pulegioides*.

One hundred and thirty-one different habitats of *T. pulegioides* were investigated in Lithuania. The study of the habitats was carried out in all climatic sub-districts of Lithuania. The individuals of  $\alpha$ -terpinyl acetate chemotype were also grown in open ground under same field conditions at the Field Experimental Station of the Nature Research Centre. Aerial parts of these individuals were collected for four years annually. Essential oils were isolated by hydrodistillation and analysed by GC-MS. Meteorological data were obtained from meteorological bulletins of closest station of meteorology of Lithuanian Hydrometeorological Service under the Ministry of Environment.

The individuals of *T. pulegioides*  $\alpha$ -terpinyl acetate chemotype are rare in Lithuania. The highest distribution of this chemotype and percentage of  $\alpha$ -terpinyl acetate were established in the Nemunas Lowland and Mūša-Nevėžis climatic sub-districts.

The amount of essential oil in the individuals of  $\alpha$ -terpinyl acetate chemotype varied from 1.18 to 1.87 % across years. The amount of  $\alpha$ -terpinyl acetate varied from 60.86 to 73.52 %. Influence of edaphic and climatic factors on the percentage of linalool was not established.

## PP67 Effect of harvest time on the drug yield and quality of Moldavian dragonhead (*Dracocephalum moldavica* L.)

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**Keywords:** moldavian dragonhead, essential oil, drug yield, geranyl acetate, citral

### Abstract

Moldavian dragonhead (*Dracocephalum moldavica* L.) is a long known annual medicinal and aromatic plant belonging to the *Lamiaceae* plant family, which is native to central Asia. The species arrived in Europe in the 16th century where it became popular rather quickly due to its lemon flavour, decorative appearance and valuable therapeutic effects. The flowering aerial parts of the plant contains essential oil and both the shoots and essential oil have appetizing, digestive, carminative, antispasmodic and sedative effects according to the traditional folk medicine. Recently its antiseptic, antibacterial and antioxidant properties are also investigated. In food industry it is used as a spice.

In our experiment the effect of harvest time on the drug yield (the leaves and flowers without stem) and its quality (essential oil content and composition) was investigated in case of eight *D. moldavica* taxa with different origin. Plant stands were established in 2017 in Soroksár in Hungary under the same environmental conditions. The sampling was carried out at different phenological stages (vegetative, floral budding, full flowering, late flowering and fruit set).

The lowest drug yield was measured before flowering in case of each taxa (5.1-7.3 g/plant), and the highest values were found in full bloom and late flowering (11.3-23.3 g/plant), but there were significant differences between the populations as well.

The essential oil content increased during flowering and reached its maximum in full bloom at majority of taxa (0.56-1.04 ml/100g) then its amount significantly decreased. The lowest accumulation levels were detected at fruiting set stage in case of every population (0.05-0.29 ml/100g). In connection with this feature we also experienced considerable differences between taxa, independently of harvest time.

In terms of essential oil composition there were no significant differences between the examined taxa with different origin. In each sample geranyl acetate, geranial (citral A) and neral (citral B) were the most important constituents. The ratio of geranyl acetate within the essential oil increased during phenological stages and reached the maximum level at fruit ripe stage (45-53%), while the accumulation of citral compounds significantly decreased at the end of the ontogenesis (citral A: from 27-39% to 19-32%; citral B: from 17-24% to 11-17%).

## PP68 Effect of planting densities, pruning techniques and shelf life on *Malus domestica* (Maçã de Alcobaça) headspace volatiles

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**Keywords:** *Malus domestica*, Maçã de Alcobaça, Royal Gala, apple, SPME

### Abstract

*Maçã de Alcobaça* is an apple trademark from Portugal, registered as protected geographical indication (PGI) under Commission Regulation (EC) No 1107/96. The fruit originates from cultivars of *Malus domestica* Borkh. (Rosaceae) of eight varieties, grown in a geographical area restricted to fourteen subdistricts of Leiria and Lisboa. The establishment, managing and growing practices used in the orchards, need to be improved in order to respect the principles of precision and organic farming. Aiming at defining scientific bases for improved growing practices, this project evaluates the effect of planting densities and pruning techniques on several parameters of the orchards.

In this study, the headspace volatiles of eight apple batches, of the Royal Gala variety, from four orchards [Campotec (C), INIAV (I), Frubaça (Fb) and Frutalvor (Fv)] were evaluated during the end of August 2018 harvesting season. Fruits were picked at the commercial harvest time, from inside (In) and outside (Ou) canopy, and analysed at two time points, T<sub>0</sub>, after harvesting, and T<sub>15</sub>, fifteen days after the first analysis, to assess shelf life at room temperature. Based on randomization lists, collective samples of seven apples per batch were inserted within a desiccator (ø 20 mm). After equilibrating the samples for 1 h at room temperature, the volatiles were collected by solid-phase microextraction (SPME) from the intact apples' headspace, under the same conditions. Volatiles desorption and analysis were performed by gas chromatography (SPME-GC) for volatiles quantification, and by gas chromatography coupled to mass spectrometry (SPME-GC-MS) for volatiles identification. Based on the desorption of the volatiles analyzed by SPME-GC, the apples' emanation rate was ranked on a scale of 1 to 16. Two replicates were performed per orchard and parameter assessed. Cluster analysis showed high correlation between samples in the same cluster, apples' volatiles being characterized by *trans*, *trans*- $\alpha$ -farnesene (37-64%), 2-methyl butyric acid hexyl ester (3-18%) and hexyl acetate (4-14%) dominance. Despite the high chemical correlation between samples collected from In and Ou canopy, T<sub>15</sub> samples showed, in some cases, higher percentages of butyl acetate or 2-methyl butyl acetate and, in general, lower emanation rate ( $\approx$  11 moderate), than T<sub>0</sub> samples ( $\approx$  16 very high).

### ACKNOWLEDGEMENTS

Thanks are due to Campotec, Frubaça, Frutalvor and INIAV for providing Maçã de Alcobaça. Thanks, are also due to PDR2020 under project OPTIMAL PDR2020-101-031442 and to FCT/MCTES for the financial support to CESAM UID/AMB/50017/2019, BioISI UID/MULTI/04046/2019, CE3C UID/BIA/00329/2019, CEAUL UID/MAT/00006/2019, and INIAV.

## PP69 Effect of *trans*-cinnamaldehyde, carvacrol and carvones on the efflux of K<sup>+</sup> ions from *Staphylococcus aureus*

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**Keywords:** monoterpenes, phenylpropanoid, antibacterial activity

### Abstract

Essential oils are substances synthesized in secondary plant metabolism. They are composed of complex combinations of terpenes and phenylpropanoids, and therefore, they present varied biological activities. Among the properties of these compounds, the antibacterial potential is emphasized. However, its mechanism of action in microorganisms has not yet been well elucidated. *trans*-Cinnamaldehyde, a phenylpropanoid, and the monoterpenes carvacrol and carvone are widely exploited in industry, mainly as flavorings, but they stand out especially for their bioactivity.

The objectives of the present study were to evaluate the influence of *trans*-cinnamaldehyde, carvacrol and carvone on the efflux of potassium ions from *Staphylococcus aureus*. *trans*-Cinnamaldehyde and carvone standards were obtained commercially from Acros Organics, and carvacrol was obtained from Sigma-Aldrich. The effect of the compounds on the release of potassium by bacterial cells was determined by flame photometry after 0, 2, 4, 6 and 24 hours of treatment. The concentrations used were 1.64 µg mL<sup>-1</sup> for *trans*-cinnamaldehyde, 1.5 µg mL<sup>-1</sup> for carvone and 0.38 µg mL<sup>-1</sup> for carvacrol. Greater potassium efflux was observed after four and 24 hours of treatment for all the compounds tested, differing statistically from the positive control at these time intervals. There was no significant variation between the evaluated times relative to those of the control. The cell wall is responsible for maintaining the integrity of the cell, being permeable to the passage of electrolytes and molecules important for the functioning of the microorganism. The extravasation of cellular contents, such as potassium ions, denotes impairment of this structure. The results obtained in the present study indicate that the patterns tested influenced the plasma membrane function. It can be inferred that these compounds are capable of increasing the permeability to potassium ions or causing rupture in the membrane and, consequently, lead to a release of these electrolytes. It was concluded that *trans*-cinnamaldehyde, carvacrol and carvone standards presented antibacterial activity against *S. aureus*, and their mechanism of action is possibly related to the efflux of ions caused by modifications in the bacterial membrane.



## PP70 Effects of arbuscular mycorrhizal fungi and phosphorus levels on the essential oil chemical composition of *Piper divaricatum* (Piperaceae)

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**Keywords:** phenylpropanoids, methyl eugenol, plant – fungi interaction

### Abstract

*Piper divaricatum* is a shrub up to 9 m in height, rich in essential oil (EO) and very employed in traditional medicine in the Amazon. The aim of this study was the evaluation of its secondary metabolites under different phosphorus levels (P) associated with arbuscular mycorrhizal fungi (AMF) inoculation. Plants were inoculated with P at concentrations of 20 and 200 mg/dm<sup>3</sup> and posteriorly with *Rhizophagus clarus* and *Claroideoglossum etunicatum* spores. After 30 days, the plant material was collected, and development parameters were analyzed such as plant height (cm), number of leaves, basal stem, number of nodes, root length and fresh weight (leaves and root). Volatile compounds were obtained from the leaves using a Likens-Nickerson apparatus (2h), and the organic fraction was analyzed by gas chromatography coupled to mass spectrometry (GC-MS). The main compound classes identified were phenylpropanoids (78.2- 88.7%) and sesquiterpene hydrocarbons (10.0%). At 30 days of growth, methyl eugenol (76. -87.5%),  $\beta$ -elemene (5.5-6.4%) and *E*- $\beta$ -ocimene (1.7-4.0%) were predominant. Methyl eugenol displayed an increase in all treatments in comparison to the control group. The amounts of methyl eugenol presented a strong Pearson correlation with the basal stem growth ( $R^2= 0.82$ ,  $p< 0.05$ ) indicating a positive relation to the treatments inoculated with only AMF and AMF associated with the highest phosphorus level (200 mg / dm<sup>3</sup>). The results are suggesting that its secondary metabolites synthesis can be optimized by inoculation of these symbionts agents and P, considering other factors as microorganism selection, nutrients available in the soil, and host plant.

## PP71 Effects of *Lavandula angustifolia* essential oil and *Mentha piperita* essential oil on body fat and autonomic nerve system

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**Keywords:** body fat, essential oil, heart rate variability, autonomic nerve, acupoint check

### Abstract

*Lavandula angustifolia* essential oil and *Mentha piperita* essential oil are famous for their calming property and activating effect, respectively. In this study, we aimed for examining the effects of these two essential oils on human autonomic nerve system, energy level and physical status including body fat.

Twenty-four female females were included, aged from 25 to 45 years. Subjects were divided in to two groups, *M. piperita* group (N=11) and *L. angustifolia* group (N=11), and were given the essential oil that they could put onto the necklace to wear. Subjects wore the necklace all day except during sleep hours. The duration was four weeks. Three parameters were checked before and after the experiment: heart rate variability was used to check the autonomic nerve system status, Acupoint Check was used to evaluate the energy level, physical status including body fat was recorded by InBody 220.

Both *M. piperita* and *L. angustifolia* showed significant enhancement of parasympathetic nerve activity and *L. angustifolia* had stronger effect. The Acupoint Check also showed balancing effect of both essential oils by decreasing overactive energy level, which is quite compatible with the findings from heart rate variability. Blood pressure and resting heart rate remained unchanged. However, the group wearing *M. piperita* essential oil showed significant decrease of body fat percentage (31.1% to 29.8%,  $P < 0.05$ ) in just four weeks.

*Mentha piperita* is considered stimulating but in this study it showed a good relaxing effect. *Lavandula angustifolia* demonstrated an even stronger effect on increasing parasympathetic nerve tone and it decreased the overactive energy. The most inspiring finding is the decrease of body fat, though the long term effect needs to be followed.

## PP72 Effects of thyme essential oils on LPS-induced inflammatory cytokines in BV-2 microglial cells

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**Keywords:** thyme essential oil, chemotypes, BV-2-microglial cells, linalool, geraniol

### Abstract

Microglia are resident immune cells of the central nervous system, which can be activated by various stimuli e.g. bacterial and viral infections, and  $\beta$ -amyloid peptide. Upon activation, microglia release various inflammatory molecules such as TNF- $\alpha$ , IL-6 or IL-1 $\beta$ . Constant presence of these inflammatory mediators leads to neuronal damage and are involved in neurodegenerative diseases. Previous studies showed that linalool inhibited inflammatory cytokine production in RAW264.7 and in BV-2 cells.

The purpose of the study was to examine the anti-inflammatory effect of different chemotypes of thyme (*Thymus vulgaris*) essential oil (EO) and their main compounds (linalool, geraniol, thujanol and thymol) on BV-2 neuronal immune cells.

The composition of EO chemotypes were analysed with GC-MS. Stock emulsions were made from the four chemotypes and their standards by adding dimethyl sulfoxide to the EOs. The stock solutions were diluted in phosphate buffer saline 10, 100, 200, 500, 1000 and 2000 times. Cell viability measurements were carried out using a CCK-8 kit according to the manufacturer's protocol. To investigate the anti-inflammatory effect of the EOs on BV-2 cells, we established different treatment conditions (e.g. pretreatment of the cells with EO, simultaneous treatment of the cells with LPS and EO, etc.). The mRNA expressions of IL-6 and TNF $\alpha$  were determined by real time PCR. Cytokine secretions of the BV-2 cells were measured using IL-6 and TNF $\alpha$  specific ELISA kits according to the manufacturer's protocols. The protein levels of NF $\kappa$ B and P-C/EBP $\beta$  transcription factors were examined using Western blot.

The chemotypes of the EO and the main compounds decreased the IL-6 and TNF $\alpha$  mRNA expression levels compared to the controls. After the co-treatment of microglia with LPS and EOs it was revealed, that all of the chemotypes decreased both the IL-6 and TNF $\alpha$  mRNA expressions, while only the geraniol standard was able to decrease them compared to the LPS treated cells. After the pretreatment with LPS, only the geraniol, thymol and thujanol chemotypes, and geraniol standard were able to decrease cytokine expressions. After the pretreatment with EOs only the EOs were successful in decreasing the LPS induced cytokine expressions, not the main compounds. The ELISA measurements revealed that there was a delay between transcription and protein syntheses and secretion of the cytokines. Therefore, the most powerful EOs inhibiting inflammatory cytokine production were linalool and geraniol chemotypes and their main compounds. The Western blot analyses showed that the aforementioned EOs could influence both NF $\kappa$ B and phospho-C/EBP $\beta$  transcription factors which are the major regulators of IL-6 and TNF $\alpha$  transcriptions.

In summary, the chemotypes of thyme EO might be suitable as a complimentary therapy against neuroinflammation.

## PP73 Essential oil composition of *Ballota nigra*

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### Abstract

The genus *Ballota* is a member of the Lamiaceae family. *Ballota* genus is represented in Turkey by 16 species. *Ballota* genus has been traditionally used against nausea, vomiting, nervous dyspepsia, as well as sedative, mild astringent, and as an antimicrobial agent. Generally, the aerial parts of the plant are used medicinally. Previously, the essential oil composition of *B. nigra* subsp. *anatolica* was reported. The main compounds were 1-hexacosanol (26.7%), germacrene D and caryophyllene oxide (9.3%). The essential oil from flowering aerial parts of *B. nigra* L. ssp. *foetida* was investigated. The main compounds were  $\beta$ -caryophyllene (20.0%), germacrene D (18.0%) and caryophyllene oxide (15.0%). Essential oil of *B. nigra* was obtained by hydrodistillation for 3 h from aerial parts. The oil composition was analyzed by means of gas chromatography-mass spectrometry (GC-MS). The analysis was carried out in triplicate. The essential oil of the plant afforded very low oil yield (<0.01% (v/w) yield). Forty-four compounds were identified in the oil that represent 91.6%. The main component of the essential oil from aerial parts was phytol (32.0%). The essential oil composition observed in the current study showed a different composition in comparison to the literature. The essential oil composition identified from *B. nigra* originating from Istanbul clearly points out a new chemotype of this species.

## PP74 Essential oil composition obtained from *Cannabis sativa* growing wild in the urban area of Vienna, Austria

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**Keywords:** hemp, Cannabaceae, essential oil, cannabidiol,  $\alpha$ -pinene,  $\beta$ -caryophyllene

### Abstract

*Cannabis sativa* L., hemp (Cannabaceae), occurs spontaneous in the eastern part of Austria in the Pannonian climate region. Such plants growing preferentially on waysides are classified as var. *spontanea*. Altogether hemp is a cultivated plant since ancient times for multiple purposes. It is well known as source of narcotic drugs but has also numerous traditional and renewed uses as technical fibre plant, as seed fatty oil supply and for additives in the food and feed industry. For the present work plants were collected on a ruderal site in the northern part of Vienna before flowering, dried and separated in stems and leaves.

Essential oils were obtained by hydrodistillation and microdistillation. The essential oil composition was characterized by GC-MS and GC-FID. With less than 0.1% in the stems and about 0.15% in the leaves, the plants were low in essential oils. The leaf oil contained mainly  $\beta$ -caryophyllene (26.2%),  $\alpha$ -humulene (13.1%), cannabidiol (11.4%),  $\beta$ -selinene (5.0%), caryophyllen oxide (4.5%) and  $\alpha$ -selinene (4.4%). Stem essential oil contained  $\alpha$ -pinene (20.2%), cannabidiol (18.0%),  $\beta$ -caryophyllene (8.3%),  $\beta$ -pinene (7.0%) and myrcene (6.1%). Further compounds accounting for less than 2.5% each were E- $\beta$ -ocimene, trans-bergamotene, E,E- $\alpha$ -farnesene, selina-4,11-diene, humulene epoxide II and  $\alpha$ -bisabolol. So, the essential oils presented widely occurring monoterpenes and sesquiterpenes. The hemp-typical compound in both plant parts was cannabidiol, a non-psychoactive terpene phenol with spasmolytic, anxiolytic and antiinflammatory properties. In sum the present oil composition was close to leaf essential oils from spontaneously occurring hemp in Hungary [1].

### ACKNOWLEDGEMENT

Funding was received from the Hochschuljubiläumsstiftung der Stadt Wien (H-02328/2007)

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## PP75 Essential oil composition of *Galium verum*

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### Abstract

The genus *Galium* L. is member of Rubiaceae family. *Galium* has 101 species in 10 sections in Turkey. *Galium verum* L. is used as a diuretic, choloretic, antidiarrheal and sedative in folk medicine. There are very few reports on the phytochemistry of the *Galium* L. species in the literature. Previously, essential oil from *G. verum* flower was investigated. The main components of the essential oil from flower were *cis*-3-hexen-1-ol (29.77%), squalene (20.82%), diethyleneglycol monomethyl ether (10.17%) and benzyl alcohol (7.85%). Additionally, antioxidant properties of methanol extracts of *G. verum* L. in Serbia was reported. Methanol extracts showed very strong scavenger activity. According to a study from Turkey, essential oil composition of *G. aparine* and *G. odoratum* aerial parts were reported. The main component of *G. aparine* was hexadecanoic acid (22.3%) and the main components of *G. odoratum* were thymol (30.6%) and isothymol (22.8) [5]. Essential oil of *G. verum* was obtained by hydrodistillation for 3 h from aerial parts. The oil composition was analyzed by means of gas chromatography-mass spectrometry (GC-MS). The yield of the oil was 0.03% (v/w). Fifty compounds were identified in the oil that represent 93.0%. The main component of the oil was hexadecanoic acid (51.1%). The essential oils of *G. verum* were dominated by fatty acid derivatives.

## PP76 Essential oil composition of *Hypericum adenotrichum* Spach., an endemic Turkish species

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### Abstract

The *Hypericum* genus of Hypericaceae is represented in Turkey by about 89 species of which 43 are endemic [1]. The species of this genus have been used as traditional medicinal plants due to their various medicinal properties for hundreds of years [2]. Phytochemical investigations on *Hypericum* species have shown that they contain flavonols (catechins), xanthenes, coumarins, glycosides, anthraquinones, phloroglucinols, flavonoids, flavonolglycosides, lactones, pyrones, lipids, triterpenes, tannins, and essential oils [3]. *Hypericum adenotrichum* Spach, a herbaceous perennial growing wild in dry grassland and stony places in Turkey, is one such endemic species and commonly known as “Kızılçikotu” [4].

This study was conducted to evaluate the essential oil composition of *H. adenotrichum*. The essential oils obtained by hydrodistillation from the root and herba of Turkey native *H. adenotrichum* were analyzed by GC-MS-FID.

The compounds were identified in the essential oils of root of *H. adenotrichum* with undecane (64.4 %), hexadecanoic acid (5.3 %) and  $\alpha$ -copaene (3.9 %) as main constituents. Hexadecanoic acid (11.3 %),  $\delta$ -cadinene (6 %), and  $\alpha$ -copaene (5.9 %) were identified as major compounds of the herb. Chemical profiling using volatiles may be useful in taxonomical classifications.

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## PP77 Essential oil composition of *Juniperus oxycedrus* in Bulgaria

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**Keywords:**  $\alpha$ -pinene, limonene, d-3-carene, monoterpenes, oxygenated hydrocarbons

### Abstract

*Juniperus oxycedrus* (also known as red juniper) from Cupressaceae family is found in countries with Mediterranean climate, including Bulgaria. It is easily distinguishable from the other junipers by its red galbuli (cones, berries). All plant parts (leaves, galbuli, stems) contain essential oil (EO). *Juniperus oxycedrus* exhibits significant morphological and EO chemical variation, which is the reason for its debatable taxonomic status. The objective of this study was to assess the EO yield and chemical profile of *J. oxycedrus* populations from different floristic regions of Bulgaria.

Over 40 leaf and 20 galbuli samples were collected from around 15 *J. oxycedrus* natural populations across Bulgaria. The EO was extracted via hydrodistillation and analyzed on GC-MS. Sixty-eight constituents were identified in the leaf EO samples and 39 constituents were identified in the galbuli EO. Overall, monoterpenes were the major chemical group in the leaf EO, with  $\alpha$ -pinene (bicyclic monoterpene) and limonene (monocyclic monoterpene) being the major constituents. Other major constituents in the leaf EO included  $\beta$ -caryophyllene (bicyclic sesquiterpene), germacrene D (monocyclic sesquiterpene), caryophyllene oxide (bicyclic monoterpene), and manoyl oxide (tricyclic sesquiterpene). The major constituents of the galbuli EO were  $\alpha$ -pinene and  $\beta$ -myrcene (acyclic monoterpene). Other major constituents of the galbuli EO included limonene,  $\beta$ -caryophyllene,  $\alpha$ -caryophyllene (monocyclic sesquiterpene), germacrene D, and  $\delta$ -cadinene (bicyclic sesquiterpene).

The results demonstrated significant variation in the EO yield and the concentrations of the major EO constituents, suggesting the presence of several chemotypes within *J. oxycedrus*. These chemotypes with distinct EO composition may have differential bioactivity and consequently diverse applications. The results will benefit the industry utilizing the *J. oxycedrus* EO. The group will continue with the assessment of the biological activities of these different EO of *J. oxycedrus*.



## PP78 Essential oil composition of *Prasium majus* L. from Northern Cyprus

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### Abstract

Hydrodistilled essential oil of the dried aerial parts of *Prasium majus* L. (Lamiaceae) was analyzed by GC-FID and GC-MS. 30 compounds were characterized comprising 99.1% of the oil. Germacrene D (27.5%) and kessane (23.0%) were found as main constituents.

## PP79 Essential oil contents and components of *Artemisia annua* L.

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**Keywords:** *Artemisia annua*, essential oil, GC-MS

### Abstract

*Artemisia annua* is a perennial plant from Asteraceae family. The species originated from Chinese flora. However, many African countries cultivate it for commercial purposes. The plant grows up to 2.4 m, leaves are dark green to brownish green. *Artemisia annua*'s odor is characteristic and aromatic besides the taste of the leaves are bitter. The species are best known for its artemisinin content. Artemisinin is effective for multidrug-resistant malaria and cerebral malaria. *Artemisia annua* essential oil is rich in mono and sesquiterpenes.

In the study cultivated *A. annua* plants from Hatay province were analyzed to determine its essential oil contents and components. Dry leaves and dry flowers of plants were used as plant material. Harvesting of materials was done in October. Samples were hydro distilled for two hours with neo-Clevenger apparatus. Essential oil rates found in leaves as 1% and flowers 2.3%. The essential oils were analyzed with gas chromatography-mass spectrometry (GC-MS) device with HP-Innowax column (60 m). 1 µl essential oil was injected directly for the analyses. Artemisia ketone was found as a major component in both leaf and flower essential oil followed by camphor, eucalyptol, and camphene. The rate of artemisia ketone was 55.21% in leaf essential oil and 64.21% in flower essential oil. Camphor was found higher in leaf than flower as 12.30% and 7.10%, respectively. Eucalyptol and camphene were found as 4.38% and 1.58% in dry leaf essential oil. In flower, essential oil eucalyptol found as 2.70% and camphene found as 1.36%.

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## PP80 Essential oil of *Citrus sinensis* (L.) Osbeck: Chemical characterization and antifungal activity

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**Keywords:** natural products, *Aspergillus carbonarius*, *Aspergillus flavus*

### Abstract

Essential oils are volatile substances of complex, lipophilic mixtures, usually odoriferous, colorless and derived from the secondary metabolism of plants. Moro sanguine orange is a fruit of the species *Citrus sinensis* (L.) Osbeck and stands out because of its expressive antioxidant activity. In this study, the objective was to analyze the antifungal activity of the essential oil of Moro sanguine orange peels against the filamentous fungi *Aspergillus carbonarius* and *A. flavus*.

Oranges were purchased from the Active Germplasm Bank of the APTA Citros Sylvio Moreira/IAC Center in Mogi-Mirim, SP (Brazil). The essential oil was extracted by the hydrodistillation process using a modified Clevenger apparatus. Two hundred grams of chopped fresh peels were used. The hydrolate was collected in a test tube, and the oil was separated by centrifugation, transferred to an amber glass vial and stored protected from light at low temperature. Parallel to the extraction of the oil, the determination of the moisture of the sample was achieved. The characterization and chemical quantification of essential oil constituents was performed by CG-MS and CG-FID, respectively. The analysis of the inhibitory effect was performed using the disk diffusion test method. The major constituents identified and quantified were limonene (91.50%), linalool (7.24%) and myrcene (0.43%). The minimal inhibitory concentration of the essential oil from Moro orange was 125  $\mu\text{L}\cdot\text{mL}^{-1}$  for both fungi. Antifungal activity was observed for the essential oil at a high concentration, which can be attributed to the high concentration of limonene present in the sample.

## PP81 Essential oil of *Lavandula pedunculata* from Portugal: Chemical profile and biological activities

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**Keywords:** Lamiaceae, Spanish lavender, essential oil, antibacterial, antioxidant

### Abstract

*Lavandula pedunculata* (Miller) Cav. is an autochthonous and abundant plant from poor agricultural soils of Iberian Peninsula. The chemical composition and *in vitro* biological activities of *L. pedunculata* essential oil, harvested in Center of Portugal, were evaluated. The essential oil was obtained by steam distillation and the chemical compounds were analyzed by GC-MS. The antibacterial activity of essential oil was performed against some pathogenic bacteria and common food spoilage microorganisms through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination. The antioxidant activity was evaluated by 1,1-Diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging determination. *L. pedunculata* essential oil revealed fenchone (38.7%) and camphor (34.9%) as the predominant compounds. Some antioxidant potential ( $IC_{50}$  44 mg.mL<sup>-1</sup>) and a broad antibacterial spectrum of the essential oil were presented, showing the possible application of this plant product as additive in food preservation. These results showed the potential of *L. pedunculata* essential oil which may have several applications and the valorization of this plant species and the poor areas where they grow.

## PP82 Essential oil volatile profile and antibacterial activity of *Salvia x accidentalis*, a hybrid described in the region of Murcia (Spain)

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**Keywords:** *Salvia x accidentalis*, *Salvia lavandulifolia* subsp. *oxyodon*, *Salvia officinalis* L., essential oil, antimicrobial activity

### Abstract

The conjunction of *Salvia officinalis* L., naturalized in the Iberian Peninsula, and the variability of *Salvia lavandulifolia* Vahl. (Spanish salvia), for which three subspecies have been defined in the Murcia region (*lavandulifolia*, *vellerea* and *oxyodon*), justify the existence of spontaneous hybrids, including *Salvia x accidentalis* Sanchez Gómez & R. Morales (*S. lavandulifolia* subsp. *oxyodon* x *S. officinalis*) (Sanchez Gómez, P. *et al.*, 2015). The hybrid vigor of *Salvia x accidentalis* and the expression of morphological characters clearly intermediate between the parents make it an interesting species for study. In addition, since *S. lavandulifolia* is by far the most exploited sage in Spain at the commercial level, it is interesting to study the potential medicinal use of this hybrid as a new alternative or complement to the traditional commercial crops. On the basis of this, *the main goal of the present work* was to evaluate the chemical composition of *Salvia x accidentalis* and its bacterial growth inhibition curves against *Bacillus cereus* strain CECT 131, *Listeria monocytogenes* strain CECT 911, *Salmonella enterica* subsp. *enterica* strain CECT 443, *Shigella sonnei* strain CECT 413, and *Staphylococcus aureus* subsp. *aureus* CECT 59. The essential oils were extracted by hydrodistillation and the qualitative and quantitative composition were analyzed by a gas chromatograph coupled to a mass spectrometer (GC-MS). Ten plants were collected from the collection of wild plants owned by the University of Murcia.

The volatile profile of the essential oils from *Salvia x accidentalis* underlines the hybrid character of these plants: namely, the identification of  $\beta$ -thujone and  $\alpha$ -thujone along with the high relative concentrations at which eucaliptol and camphor were quantified, the values being intermediate with respect to those observed in the parents. The growth inhibition was monitored for 48 h, with the essential oil concentrations ranging from 2,500 ppm to 20,000 ppm. The essential oils of *Salvia x accidentalis* had their highest effect against *S. enterica* and *S. aureus*. At a concentration of 5,000 ppm the growth was inhibited for 48 and 39 h, respectively, and with 2,500 ppm for 19 and 35 h, respectively. The effect on these two bacteria was greater than with *S. lavandulifolia* subsp. *oxyodon*, which, at 5,000 ppm, inhibited growth for 29 h (*S. enterica*) or 15 h (*S. aureus*). However, similar essential oil concentrations were needed from both sages to act against *S. sonnei* (10,000 ppm), *L. monocytogenes* (5,000 ppm), and *B. cereus* (20,000 ppm). These strains seemed to be more sensitive to high concentrations of eucaliptol and camphor; components which are present at similar levels in both the hybrid and the parent.

From the results it can be concluded that essential oils from *Salvia x accidentalis* show higher activity against *S. enterica* and *S. aureus* mainly due to the differential presence of thujones that come from the other parent, *S. officinalis*.

## PP83 Essential oil yield and composition of *Satureja montana* subsp. *kitaibelii* and *S. coerulea* (Lamiaceae) from Bulgaria

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**Keywords** Balkan endemic, *Satureja montana* subsp. *kitaibelii*, *Satureja coerulea* essential oils

*Satureja montana* subsp. *kitaibelii* (Wierzb. ex Heuff.) P.W.Ball and *S. coerulea* Janka in Velen. (Lamiaceae) are species found in the Bulgarian flora. *Satureja coerulea* is a Balkan endemic plant species. The two species are known for their pleasantly aromatic and pharmacologically active essential oil (EO). Both species are found on rocky outcrops on limestone base in Stara Planina (the Balkan Mountains), the Rhodope Mountains, and the Thracian Lowland. The objective of this study was to assess the variability of EO content and composition of *S. montana* subsp. *kitaibelii* and *S. coerulea* populations in the Bulgarian flora.

The EO was isolated through hydrodistillation and analyzed for chemical profile by gas chromatography (GC). Overall, the EO content in the dried aboveground biomass of *S. montana* subsp. *kitaibelii* was 0.11- 0.48% and that of *S. coerulea* was 0.1-0.16%, respectively. Thirty-three EO constituents were identified in the *S. montana* subsp. *kitaibelii* EO and 27 constituents in *S. coerulea*. The identified EO constituents belonged to the groups of monoterpenes and sesquiterpenes. Overall, there was significant variation in the EO content and composition between different populations of *S. montana* subsp. *kitaibelii*. The results showed the presence of two chemotypes in this species; (1) p-cymen and terpinen-4-ol type, and (2) thymoquinone and geranyl acetate type. Generally, the EO composition of *S. coerulea* was also highly variable, with major constituents p-cymen and terpinen-4-ol. The results from this study contribute to our understanding of the chemical variability within the two *Satureja* species, and could also be used by breeders for the development of new cultivars of *S. montana* subsp. *kitaibelii* and *S. coerulea*.

### Acknowledgement

This study was supported by the Agricultural University Plovdiv, Bulgaria. Project Nr. 11-18.

## PP84 Essential oil, aqueous and ethanol extracts of *Mangifera indica* (L.) bark: Potentiality as phytomedicine

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**Keywords:** *Mangifera indica* bark, chemical composition, antibacterial activity, antioxidant activity, toxicity

### Abstract

Ethnobotanical survey has revealed the use of *Mangifera indica* Linn. (*Anacardiaceae*) bark for the treatment of infectious diarrhea. In order to assess this traditional use, *M. indica* bark was collected at the University of Yaoundé I (Cameroon) for chemical and biological investigations purpose. Fresh bark was used to get essential oil by hydrodistillation (0.04% yield) while aqueous and ethanol extracts were obtained by maceration of dried bark with 7.4% and 1.8% yields respectively. Our first experiments revealed that the essential oil was characterized by the association of two major sesquiterpenes: (E)- $\beta$ -caryophyllene (60.3%) and  $\alpha$ -humulene (36.7%). The antimicrobial activity was measured for all samples against Gram-negative and Gram-positive bacteria and the best inhibitory effects was observed with the essential oil. On the other hand, the aqueous and ethanol extracts of *M. indica* bark were characterized by high contents of total phenol compounds and high radical scavenging activity compared to the essential oil [1]. Additional chemical and biological tests were performed on these samples. The aqueous and ethanol extracts were submitted to LC-MS and HPTLC analyses, showing the presence of xanthonoids, mainly mangiferin and derivatives. The cytotoxicity of the samples was estimated on a normal skin fibroblast cell line (CCD-45 SK) as well as on liver (HepG2) and breast (MCF-7) cancer cell lines using the tetrazolium salt MTT colorimetric test. The aqueous and ethanol extracts were non cytotoxic on CCD-45 SK and MCF-7 cell lines at 0.2 mg/mL while toxicity was observed with the essential oil (IC<sub>50</sub> 0.063 and 0.052 mg/mL, respectively). On the other hand, the three samples were cytotoxic on liver cancer cells (IC<sub>50</sub> 0.065-0.1 mg/mL). Finally, acute toxicity was studied on rat model (OECD Guidelines for the testing of chemicals: 423, 2001). No noticeable sign of toxicity was observed at 1000 mg/kg of essential oil and 3000 mg/kg of the aqueous and ethanol extracts. In conclusion, our results can be considered as a scientific support for traditional use.

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## PP85 Essential oils and biological activities of Malaysian piper species (Piperaceae)

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### Abstract

Malaysia is blessed with one of the most diverse and oldest flora in the world. Therefore, its flora is a rich source of medicinal plants and bioactive molecules possessing interesting pharmacological activities. Piper is the genus of the family Piperaceae. The Piper species were mostly woody perennial climbers and rarely shrub. Members of the Piper genus are of commercial, economical and medicinal importance. They have been used for a number of practical applications like remedies in many traditional medicinal systems such as traditional Chinese medicine, the Indian Ayurvedic system and folklore medicines of Latin America and West Indies. The chemistry of Piper species has been widely investigated and the phytochemical investigations have led to the isolation of a number of physiologically active compounds. They have been extensively investigated as a source of new natural products with potential antitumoral, antimicrobial, antifungal and insecticidal activities. The research focus and interest of our group is the studies on essential oils, bioactive and structurally complex molecules from Piper species. This presentation will discuss briefly about the findings on Piper species from the Malayan flora.



## PP86 Essential oils and hypoglycemic activity: Bio-guided fractionation approach looking for plant bioactive secondary metabolites with alpha-amylase inhibition capacity

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**Keywords:** essential oils, Diabetes Mellitus, in vitro alpha-amylase inhibition assay, bio-guided fractionation

### Abstract

Essential oils are complex mixtures of volatile and semi-volatile secondary metabolites obtained by hydro-, steam- or dry-distillation or by a suitable mechanical process without heating (for citrus fruits) of a plant or of some parts of it [1]. Plant secondary metabolites are rich sources of bioactive compounds, but despite of this evidence, few studies are reported about the hypoglycemic activity of essential oil components. Diabetes mellitus is a chronic metabolic disorder characterized by a difficulty in the maintenance of blood glycaemia. A possible therapeutic approach is the inhibition of  $\alpha$ -amylase (i.e. a carbohydrate hydrolyzing enzyme), thus stopping carbohydrate digestion, and as a consequence, reducing glycaemia. Examples of such inhibitors used in the clinical practice for treating diabetes are acarbose, miglitol and voglibose [2].

The aim of this study is the screening of eighty-four essential oils obtained by distillation of different plant species and botanical families looking for new  $\alpha$ -amylase inhibitors deriving from plant secondary metabolism. A bio-guided fractionation approach, based on an *in vitro*  $\alpha$ -amylase inhibition assay, was adopted to isolate and identify the active fractions/compounds in different essential oils. Three essential oils (i.e. *Eucalyptus radiata* A. Cunn. ex DC., *Laurus nobilis* L. and *Myristica fragrans* Houtt.) resulted to be particularly active with an inhibitory capacity slightly higher than acarbose, chosen as positive control. An interesting number of both hydrocarbon and oxygenated compounds were characterized by a good  $\alpha$ -amylase inhibition, around 30% (i.e. 4-terpineol, linalool,  $\alpha$ -terpineol and  $\alpha$ -pinene). These preliminary results demonstrate that essential oils may represent a promising source of potential  $\alpha$ -amylase inhibitors.

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## PP87 Essential-oil composition of *Cnidium silaifolium* (Jacq.) Simonk. from Serbia

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**Keywords:** *Cnidium silaifolium*, essential oil, 2,3,6-trimethylbenzaldehyde, ferulol

### Abstract

The genus *Cnidium* (Apiaceae) comprises six species spread across Europe and Asia (Polat *et al.*, 2013). Species of this genus are traditionally used to treat various diseases; antitumor, cytotoxic and antioxidant activities of these taxa are reported in the literature (Polat *et al.*, 2013 and refs. cited within; Li *et al.*, 2015). *Cnidium silaifolium* is distributed in Southern Europe, from southern France to Greece (Lemmich, 1996). Volatile constituents of this species were the subject of two previous papers. The essential oil obtained by Kapetanos and coworkers from material collected in the Central Balkans was predominantly composed of  $\alpha$ -pinene and sabinene (Kapetanos *et al.*, 2008). The major constituent of *C. silaifolium* essential oil from Turkey was the sesquiterpene kessane, in addition to being characterized by a high abundance of other sesquiterpenoids (almost 80%).

The essential oil of the aboveground parts of *C. silaifolium* in the pre-anthesis phase from Serbia was obtained by hydrodistillation and analyzed by GC and GC-MS. 2,3,6-Trimethylbenzaldehyde was an overwhelming major (53.2%) constituent identified, followed by the isomeric 2,3,4-trimethylbenzaldehyde (4.4%) and several monoterpenes (terpinen-4-ol, 3.5%;  $\alpha$ -pinene 2.7%; and sabinene 2.6%). The presence of the two mentioned aromatic aldehydes in our oil (and of the corresponding alcohols and acids) was, however, not surprising - it was shown that they are artefactually produced from ferulol and isoferulol derivatives (previously found in Apiaceae taxa) during hydrodistillation (Kubeczka and Ullmann, 1981). Although our results differed significantly from the previous reports, the occurrence of ferulol derived volatiles is in agreement with the distribution of this metabolite among Apiaceae genera (Kubeczka and Ullmann, 1981).

### ACKNOWLEDGEMENT

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project 172061).

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## PP88 Evaluation of antimicrobial activity of oximes derived from natural volatile compounds

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**Keywords:** volatile carbonyl compounds, oximes, antimicrobial activity

### Abstract

New active agents against microorganisms are currently being sought to overcome the challenge of antimicrobial resistance. We wanted to address this societal challenge by evaluating the antimicrobial activity of low-molecular oximes, which haven't been extensively studied with regard to this issue. Fifty-three oximes and their substrates were screened *in vitro* for their growth inhibitory activity against seven strains of microorganisms: *E. coli* (ATCC 10536), *S. aureus* (ATCC 6538), *E. hirae* (ATCC 10541), *P. aeruginosa* (ATCC 15442), *L. pneumophila* (ATCC 33152), *A. brasiliensis* (ATCC 16404), and *C. albicans* (ATCC 10231). The MIC parameters were determined using the micro-plate Alamar Blue® assay.

Against moulds and yeasts best results were obtained by *trans*-cinnamaldehyde both carbonyl and oxime compounds - 112.5 µg/ml and 37.5 µg/ml for *A. brasiliensis* and 37.5 µg/ml and 75 µg/ml for *C. albicans*. Against Gram-negative bacteria best results were obtained by (-)-campher (*E. coli*), α-ionone both carbonyl and oxime, dihydro-α-ionone oxime, dihydro-β-ionone oxime and β-ionone which obtained MIC of 37.5 µg/ml. The most significant, among Gram-positive bacteria, MIC parameter (18.75 µg/ml) was that of α-isomethylionone oxime against *E. hirae*. Other promising results were gained by pseudoionone both carbonyl compound and oxime (*B. cereus* and *S. aureus*). Against *S. aureus* encouraging results had α-hexylcinnamaldehyde oxime, phenylacetaldehyde and β-ionone oxime (37.5 µg/ml).

## PP89 Evaluation of essential oils from piper species - potential use in the control of phytopathogen *botrytis cinerea* of strawberry

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**Keywords:** piper, phytopathogen, essential oil, *Botrytis cinerea*

### Abstract

The strawberry (*Fragaria x Ananassa* Duch) is very perishable and susceptible to fungal infection, resulting in economic loss. One of the main diseases affecting strawberry is gray mold caused by fungus *Botrytis cinerea* Pers. A good alternative to control this fungus in strawberry fruit are essential oils because it has been recognized as potential compounds in preventing fungal infections [1-3].

The objective of this study was the use of essential oils from five species of *Piper* to control *B. cinerea* of strawberries. The leaves (50 g each) from *Piper aduncum*, *P. amalago*, *P. caldense*, *P. mikanianum* and *P. mollicomum* were subjected to hydrodistillation separately and in triplicate in a modified Clevenger-type apparatus for four hours, yielding 1.13; 0.81; 1.28; 1.89 and 0.05%, respectively. The analyzes were performed by GC-MS and GC-FID, with a Shimadzu gas chromatograph, model GC-2010 Plus and an Agilent gas chromatograph model 7890A. The identification of the main constituents was based on the arithmetic index, calculated considering a series of *n*-alkanes, comparing the mass spectra with the literature [4] and NIST-14 Data Base. The main constituents of essential oils are: the arylpropanoids (53.58 and 74.51%) for *P. aduncum* and *P. caldense*, respectively, the sesquiterpenes (77.58%) for *P. amalago*, the oxygenated sesquiterpenes (60.45%) for *P. mikanianum* and an alkenylphenol (80.12%) for *P. mollicomum*. The evaluation of fungicidal activity of these essential oils against *B. cinerea* was made by Minimum Inhibitory Concentration (MIC). The MIC was determined by the microdilution technique in 96 well plates, using potato dextrose broth and dimethyl sulfoxide as solvent [5]. The essential oils were evaluated at concentrations ranging from 1.00 to 1,200.00 µg mL<sup>-1</sup>, while procymidone (1 - 20 µg mL<sup>-1</sup>) and mancozebe (6 - 50 µg mL<sup>-1</sup>) were used as the positive control. The tests were performed at 24 and 96 hours in quintuplicate, with a Bio tek Spectrophotometer model ELX800 GIDX. The essential oils of *P. aduncum*, *P. amalago* and *P. mollicomum* showed weak activity (above 250 µg mL<sup>-1</sup>), while the essential oils of *P. caldense* and *P. mikanianum* showed a strong activity against *B. cinerea*, with MIC of 20 µg mL<sup>-1</sup>. These results were excellent since their MIC were smaller than those of the positive controls besides these compounds can offer low toxicity and the environmental preservation. This has demonstrated the potential use of essential oils from *Piper* for control agricultural pests such as *B. cinerea*.

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## PP90 Evaluation of hemolytic activity of methyl and isopropyl N-methylantranilates

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**Keywords:** methyl and isopropyl N-methylantranilates, medicinal plants, bioactive compounds, hemolytic

### Abstract

Two volatile alkaloids, methyl (MA) and isopropyl N-methylantranilates (IA), identified in the essential oil of *Choisya ternata* Kunth (Rutaceae), have been proven to possess polypharmacological properties (antinociceptive, anti-inflammatory, gastro-, hepato-, nephroprotective activities, anxiolytic and antidepressant properties, and likewise an effect on diazepam-induced sleep). The membrane of erythrocytes can be affected by consumption of bioactive compounds from herbs and medicinal plants. The present study was carried out to evaluate the hemolytic activity of the MA and IA on human and rat erythrocytes. The toxicity of MA and IA on human and rat erythrocytes was measured by *in vitro* hemolytic assay. The absorbance of hemoglobin release was read by spectrophotometer at 540 nm. The concentrations ranged from 0.127 to 1.27  $\mu\text{M}$  for MA and 0.026 to 0.26  $\mu\text{M}$  for IA.

Our results show that hemolytic activity of MA and IA on rat and human erythrocytes is comparable to isotonic solution. Only the highest dose (0.26  $\mu\text{M}$ ) of IA showed statistically significant higher hemolysis compared to saline control on rat red blood cells. This was also the highest observed hemolysis but still relatively low at 4.5%. The present study shows that MA and IA manifest low hemolytic activity < 5%. These results explain that parameters of red blood membrane cells are not altered if therapeutic formulations from these substances are used at studied concentration, but a great attention will be taken with higher concentrations.

## PP91 Evaluation of lavender (*L. angustifolia* Mill.) and lavandin (*L. x intermedia* Emeric.) cultivars in Hungary

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**Keywords:** Lavandula cultivars, volatile oil, GC-MS, Hungary, flowering phase

### Abstract

In Hungary, an increasing tendency has been observed concerning cultivation of *Lavandula* species, however, growers have limited knowledge on the performance of cultivars available. The aim of our studies was to compare the volatile oil properties of Hungarian and foreign varieties belonging to *L. angustifolia* (LA: 'Budakalászi'-HU, 'Hidcote', 'Munstead', 'Maillette') and *L. x intermedia* (LI: 'Judit' -HU, 'Grosso', 'Grappenhall'), respectively. Our investigations were carried out in Budapest, in the summer of 2018, in 2-year-old experimental plots of *Lavandula* cultivars. Variety samples were collected in different flowering phases (bud phase, full flowering, overblown), then dried in natural way. Volatile oils were isolated by hydro-distillation and analysed by GC-MS.

Concerning mean values of true lavender (LA) cultivars, 'Maillette' was proven to be the highest essential oil (EO) yielding one (4.416 ml/100 g DW), while 'Hidcote' showed the lowest values (2.216 ml/100 g DW). In the case of hybrid lavender (LI) varieties, the highest average EO amount was found in the samples of 'Judit' (7.094 ml/100 g DW), while that of the lowest at 'Grappenhall' (4.948 ml/100 g DW). As a general rule, peak EO values of LA varieties were found in overblown phase (3.802 ml/100 g DW), while those of LI cultivars, in full flowering (5.710 ml/100 g DW). The overall variety averages and standard deviations of EO amount of LI cultivars (5.710±2.365 ml/100 g DW) were much higher than that of LA varieties (3.101±0.835 ml/100 g DW). Four subsets of varieties could have been distinguished according to the statistical analysis of volatile oil levels.

Each cultivar involved had specific essential oil composition, which was subjected to moderate changes during flowering. Beside linalool and linalyl acetate, certain varieties had additional major compounds, such as 1,8-cineol (13.26-17.52 %: Judit, Grappenhall), camphor (7.40-19.32: Judit, Grappenhall) or lavandulyl acetate (9.50-18.34 %: Budakalászi, Hidcote). Linalool percentage ranged between 18.88-62.45 % at LA while from 30.04% to 45.01% at LI cultivars, respectively. Some compounds showed increasing tendency in the course of generative phases (e.g. linalool, 1,8-cineol, camphor, borneol, etc.) while others had lower percentage in overblown phase (linalyl acetate, lavandulyl acetate, beta-ocymene, beta-caryophyllene, etc.). In general, better volatile oil quality could be detected in full flowering of all cultivars involved than in overblown phase. Several additional monoterpenes have been identified in bud phase, however, they decrease or disappear during flowering. According to our preliminary results on essential oil properties, Maillette and Budakalászi belonging to *L. angustifolia* as well as Grosso or Judit of *L. x intermedia* cultivars can be suggested for growing in Hungary.

## PP92 Exploiting ionic liquids as GC stationary phases in the essential oil field, the uncommon selectivity of trihexyl(tetradecyl)phosphonium chloride

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**Keywords:** essential oil analysis, stationary phases, routine quality control

### Abstract

Essential oils are complex mixtures of volatiles whose composition is fundamental to define their quality and economic value as well as to quantify their bioactive or regulated markers. Gas chromatography is the technique of election for their analysis and must guarantee efficiency and selectivity suitable to provide a baseline separation of all markers in a single analytical run. Search for new stationary phases (SPs) with uncommon selectivity and, at the same time, with good chromatographic properties, is therefore a constant challenge. Room-temperature ionic liquids (ILs) were successfully introduced as GC SPs in 2010 in several fields because of their unique and tunable selectivity, low vapor pressure and volatility, high thermal stability (over 300°C), and good chromatographic properties. Phosphonium based ILs are a class of ionic liquids that were first systematically studied by Breitbach and Armstrong in 2008 [1] and whose features make them suitable as gas chromatographic stationary phases.

This contribution reports the results of an in-depth study on trihexyl(tetradecyl)phosphonium chloride ([P66614<sup>+</sup>][Cl<sup>-</sup>]) as stationary phase for the essential oil analysis. This IL is characterized by a strong retention and a peculiar selectivity based on the analyte functional groups that can be successfully exploited in the flavour, fragrance and essential oil fields [2]. In view of a possible routine application, columns coated with [P66614<sup>+</sup>][Cl<sup>-</sup>] were also characterized in terms of maximum operative temperatures and long-term stability. Furthermore, the column geometry (length, inner diameter and film thickness) was carefully tuned in order to exploit the main features of the stationary phase to make it applicable for routine analyses in the flavor, fragrance and essential oils fields including the use in fast GC.

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## PP93 Extraction of phytochemicals from feed mixed with essential oil based feed additives

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### Abstract

Phytochemical feed additives are getting more and more interesting for the livestock industry, especially since authorities and consumers concern related to their use as AGPs (and the correlation to high antimicrobial resistance) have increased all over the world. Phytochemicals are functional plant ingredients with both sensory and biological properties that either support feed intake or play an important role to gut health and overall health of the animals, which results in better animal performance. Phytochemical feed additives can contain whole plants, part of plants, plant extracts, and essential oils or their compounds. BIOMIM, a supplier for phytochemical feed additives, invested in the development of an analytical method to quantify the major active substances out of essential oils used in the products. The possibility to rely on a sound and validated analytical method is critical to ensure the quality of the products brought to the market but also to recover and analyze the active substances in feeds to which the products have been added.

The quantification of the substances of interest is straightforward for the product itself but posed a challenge for the feed matrix. Different extraction solvents and parameters such as extraction temperature, extraction time and use of ultrasonic bath have been tested. However, it was not possible to reach satisfying recoveries and even more unsatisfying, the recoveries varied between different feed matrices.

To solve this issue, an automated extraction device (Speed Extractor E-916, Büchi) was used. The specific device can apply high pressure and temperature to the feed sample. The duration of the extraction was about 25 minutes. Ten ml extraction cells were used into which 2.5 g of feed sample were weighted and the rest of the cell was filled up with quartz sand. The extraction took place at 100 bar pressure and 100°C. The samples were extracted applying two extraction cycles with ethyl acetate as solvent. Using these device recoveries of the analytes of interest (Linalool, Carvone, Thymol, Carvacrol) of 80 - 120% for different feed matrices (feed for pigs, broilers, fish) in a concentration range from 0.25 - 100 mg/kg were achieved.

The extraction was followed by GC-MS for quantification. For the separation a WAX column was used and substance-specific ions were recorded in SIM mode. The method was successfully in-house validated and is now routinely applied to feed samples in our lab.



## PP94 Histochemical evaluation of *Campomanesia rufa* leaves, chemical characterization of their essential oil

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**Keywords:** oxygenated sesquiterpenes, volatile oils, Myrtaceae, *Campomanesia*

### Abstract

*Campomanesia* is a genus with about 42 species catalogued, composed by woody fruit plants, with shrub and arboreal habit. Some plants from this genus are known for their medicinal properties and bioactive components with biological potential on its composition. The aims of this work were to evaluate the yield and chemical composition of the essential oil of *C. rufa* leaves, besides describing the storage sites of the terpenes in the leaf tissue. Leaves were collected at the Campus of the Federal University of Lavras on a mild autumn morning with no precipitation. They were cleaned and chopped. The essential oil was obtained by hydrodistillation for two hours using the modified Clevenger apparatus. The hydrolate was collected in a test tube and the oil was separated by centrifugation, then transferred to an amber glass and stored under light protection at low temperature. In parallel, the extraction yield was determined by the humidity test, based on the methodology of Pimentel. The chemical composition of the essential oil was characterized in a GC-MS and quantified in a GC-FID by area normalization. Mature and completely expanded leaves were collected from the third node in order to perform the histochemical tests. Hand-free cross sections of the leaves were made with the aid of a steel blade. For analysis of the terpenes in the plant tissue, the cross sections were immersed in NADI reagent (0.1%  $\alpha$ -naphthol + 1% dimethyl- $p$ -phenylene diamine hydrochloride + 0.05M sodium phosphate buffer) and held for one hour in the dark. Thereafter, the sections were immediately washed in a buffer solution (0.1 M sodium phosphate). The yield of the essential oil of *C. rufa* leaves was 0.3% w/w MFB, and the moisture content of the leaves was 42.43%. The major constituents found in the essential oil of *C. rufa* leaves were (2E, 6E)-farnesal (41.64%), (2E, 6Z)-farnesal (27.74%), (2E, 6Z)-farnesol (3.54%),  $\beta$ -pinene (3.38%) and limonene (3.25%). The histochemistry of the leaf tissue showed the presence of terpenes in the mesophyll and in the midrib. In the mesophyll, the terpenes were located in epidermal cells of the adaxial leaf surface, palisade parenchyma, lumen and epithelial cells of the secretory cavities and midrib, cells of the abaxial epidermis and secretory cavities.

## PP95 *Hypericum* species in Bulgaria as sources of valuable essential oils

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**Keywords** *Hypericum hirsutum*, *Hypericum maculatum*, *Hypericum perforatum*, essential oil

### Abstract

The genus *Hypericum* is one of nine genera forming the family Hypericaceae (Guttiferae) and comprises around 469 species distributed throughout the world. Twenty-two *Hypericum* species are found in the Bulgarian flora. Many of the *Hypericum* species have been used in traditional medicine to alleviate a number of diseases and have been reported to possess antispasmodic, diuretic, antimigraine, antiepileptic, and other bioactivities. The objective of this study was to assess the variability of EO content and composition of *Hypericum hirsutum* L., *H. maculatum* Crantz, and *H. perforatum* L. populations in the Bulgarian flora.

The EO was isolated through hydrodistillation and analyzed for chemical profile by gas chromatography (GC). Overall, the EO content in the dried aboveground biomass of *H. hirsutum*, *H. maculatum*, and *H. perforatum* was 0.12%, 0.10% and 0.18%, respectively. The identified EO constituents belonged to the groups of monoterpenes and sesquiterpenes. Overall, there was a significant variation in the EO content and composition between different species of *H. hirsutum*, *H. maculatum* and *H. perforatum*. Generally, the EO composition of the basic chemical composition of the three species was similar to that reported in the literature, with *cis*-caryophyllene,  $\beta$ -caryophyllene, caryophyllene oxide, and  $\alpha$ -cinene as the major oil constituents.

### Acknowledgement

This work was supported by the Bulgarian Ministry of Education and Science under the National Research Program "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018"

## PP96 Identification of volatile compounds in seeds, flowers and leaves collected from *Elaeagnus angustifolia* tree

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**Keywords** Igde seed, flower, leaf, SPME, volatile compounds

### Abstract

*Elaeagnus angustifolia*, a member of the Elaeagnaceae family, is mainly grown in subtropical regions of Asia, Europe, some parts of North America and in wild region of Turkey. *Elaeagnus angustifolia* fruit is named as 'Igde' in Turkey and also 'Russian-olive', 'Russian silverberry' or 'Oleaster' in Europe and Asia. Leaves and flowers of *Elaeagnus angustifolia* have been used to treat various diseases such as nausea, vomiting, flatulence, gastric disorders, asthma and jaundice. In this study, we objected to investigate VCs in the seeds, flowers and leaves collected from *Elaeagnus angustifolia* trees. Volatile compounds were extracted using solid phase microextraction (SPME) and analyzed with gas chromatography-mass spectrometry (GC-MS). A total of 117 volatile compounds, including 32 esters (15.5- 93.6%), 18 aldehydes (0.7- 20%), 17 alcohols (1.2-31.6%), 14 terpenes (0.6-2.5%), 8 alkanes and alkenes (0.1-5.6 %), 8 ketones (0.7-2.4%), 8 phenyls and phenols (2.1-14.1%), 3 furanes (0-0.9 %), 2 acids (0-0.8 %) and 7 other compounds (0.8-6.8%), were identified in flowers, seeds and leaves of *Elaeagnus angustifolia*. Ester chemical group was identified in seed and flower as major VC, which accounted for approximately 72% and 94% of total compounds, respectively. Alcohols were the most abundant chemical group identified in leaves, in terms of their percentage proportions. Acid chemical group was detected in the leaves only. 2-Propenoic acid 3-phenyl ethyl ester was the principal volatile compound identified in the seed and flower, accounted for approximately 64% and 83% of total volatile compounds, respectively. It is probably that 2-propenoic acid 3-phenyl ethyl ester is responsible for characteristic odour of *Elaeagnus angustifolia* tree. Interestingly, 3,6,9,12,15-pentaoxanonadecan-1-ol constituted the majority of VCs identified in the leaves, accounting for approximately 17% of total compounds. As a result, VCs identified in the seeds, flowers and leaves collected from *Elaeagnus angustifolia* were different, in terms of their number and their percentage proportions.

## PP97 Implementation of an ultrasound-assisted extraction/dispersive liquid–liquid micro-extraction method to study diurnal changes in volatile composition of *Mentha longifolia* (L.) L.

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**Keywords:** *Mentha longifolia*, inflorescences, volatile, liquid-liquid extraction, SPME

### Abstract

The volatile composition of the inflorescences of aromatic plants is a decisive factor to understand the plant-insect interactions. Likewise, their own interest as compounds with potential uses in food, pharmacy or perfumery industries could be considered. Therefore, the availability of analytical methods to monitor their diurnal or seasonal variations may be of increasing interest [1]. In addition, given the high intrapopulation chemodiversity, common in many aromatic plants, monitoring of individuals shows an additional interest. For this reason, the implementation of analytical methods that make feasible a large number of determinations to be carried out simultaneously, susceptible of adequate statistical treatment, can be very useful.

In order to fulfil these requirements, a method based on ultrasound-assisted extraction/dispersive liquid–liquid microextraction method [2] coupled with GC-MS and GC-FID analysis has been tested in this work. Three samples of inflorescences from each of the four tested individuals of *M. longifolia* were collected three times per day at 8 AM, 2 PM and 8 PM and analyzed at once. Average values for each sampling time were calculated and subjected to one-way variance analysis.

The major compound was found the same one that in leaves: piperitenone oxide, which did not show significant changes (69.1; 75.5; 61.3 %). Despite of the high variability among individuals, some minor but not negligible and ecological relevant components showed a significant decrease over the morning: piperitone oxide (3.3; 0.1; 0.0 %), menthalactone (2.1; 0.3; 0.1 %) and nepetalactone <math>\alpha</math>- (2.1; 0.3; 0.5 %). However, limonene (2.0; 2.5; 6.0 %) and germacrene-D (1.4; 2.1; 3.6 %) showed the opposite behavior. In general, when considered individual changes, they were similar for the four plants.

In order to check the usefulness of this methodology, the chromatographic profiles were compared with those obtained "in vivo" by static solid phase microextraction.

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## PP98 Improved characterisation of fragranced products by GCxGC-TOF MS with Tandem Ionisation

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**Keywords:** GCxGC, TOF, Soft ionisation, flow modulation

### Abstract

Compliance with the EU Cosmetics Directive requires that allergenic compounds are identified and quantified accurately - a considerable challenge due to the complex fragrance compositions, diverse matrices, wide concentration ranges involved and the ever-expanding list of target compounds. To tackle this issue, we demonstrate the use of comprehensive two-dimensional GC coupled with time-of flight mass spectrometry (GCxGC-TOF MS). The enhanced separation capacity copes with the most complex of matrices, while the commercialisation of simple, consumable-free flow modulation devices has made routine use more feasible.

This study focuses on the use of GCxGC-TOF MS incorporating a novel ion source design to provide both hard (70 eV) and soft (10-20 eV) electron ionisation simultaneously - in a technique we have termed 'Tandem Ionisation'. The ability of soft EI to provide enhanced molecular ions whilst retaining structurally-significant fragment ions also allows for greater orthogonality between the mass spectra of isomeric compounds, thus simplifying compound identification and reducing reliance on retention indices. Furthermore, the reduction in "common" fragments at low eV provides reduced demand on dynamic range and can also simplify the use of data mining algorithms, such as deconvolution.

Here, we show how flow-modulated GCxGC-TOF MS with Tandem Ionisation can provide robust quality control through the use of repeatable retention times in two dimensions ( $^1t_R$  and  $^2t_R$ ), retention indices and confirmatory ion ratios from both the 70 eV and soft EI datasets.

## PP99 Influence of the essential oil from *Lippia origanoides* on ergosterol synthesis

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### Abstract

Synthetic products are marketed to reduce the damage caused by micro-organisms, but research has demonstrated the increase of resistant strains. Considering the consequences of fungal infections and the difficulties encountered in their treatment, the use of essential oils constitutes a possible alternative to traditional treatment because they have important biological activities. The objectives of this work were to characterize the essential oil constituents of leaves of *Lippia origanoides* and to evaluate their influence on the ergosterol synthesis of the fungus *Aspergillus flavus*. The extraction of the essential oil was performed by the hydrodistillation method, and the components were subsequently identified and quantified by CG-MS and CG-FID, respectively. The inhibitory effect of the essential oil on ergosterol synthesis was analyzed by HPLC. The data were submitted to analysis of variance, and the means were compared by the Tukey test at the 5% probability level. The chemical constituents found in the essential oil of *L. origanoides* were carvacrol (41.51%), *p*-cymene (18.36%) and  $\gamma$ -terpinene (12.73%). The essential oil inhibited 100% of the ergosterol biosynthesis by *A. flavus* at a concentration of 0.98  $\mu\text{L mL}^{-1}$  and reduced the sterol concentration by 90.19, 84.64 and 73.34% at concentrations of 15.62; 7.81 and 3.91  $\mu\text{L mL}^{-1}$ , respectively. It was verified by t analysis of variance that there was a significant difference between the percentage of inhibition of ergosterol at all of the concentrations of the essential oil tested. The essential oil was shown to be a promising natural agent in the control of fungi and in the development of new drugs acting on ergosterol biosynthesis.

## PP100 Injection of essential oils: New tool for the management of sap-sucking pest in apple and pear orchards

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### Abstract

Apple aphid (*Disaphys plantaginea*) and pear psylla (*Cacopsylla pyri*) are responsible for significant yield declines in apple and pear orchards through sap-sucking and disease spreading. To manage them, farmers have so far used a wide range of phytosanitary products, sometimes harmful to both environment and human health. Therefore this research aims to develop alternative product based on essential oils injected into the tree vascular system (xylem).

The objective of this work is to first select the essential oil (or mixture) with the strongest insecticidal and/or harmful properties (anti-feedant, repellent, anti-oviposition). Secondly, the phytotoxicity will be assessed through eco-physiological measures such as electrolyte loss, chlorophyll fluorescence and photosynthetic activity (infra-red gas analyser). Third, the translocation/kinetics of volatile organic compounds (VOCs) and their impact on the plant will be ensured by measuring the VOCs profile contain as well as emitted by leaves using dynamic headspace and gas chromatography coupled to mass spectrometry (DHS-GC-MS).

Research is still in its very beginning stages of development. Nevertheless a first test of injection using Baxter bag and 23Ga needles was performed with injection of 1 % and 0.1% of *Cinnamomum cassia* essential oil emulsified with Tween 20 (1:4) and 20mM EDTA in a climatic chamber. The emulsion was treated with an Ultra turax and a high pressure homogeniseur to ensure sufficient stability and lead to a mean of particle size distribution of  $139.33 \pm 10.53$  nm.

Chlorophyll fluorescence as well as photosynthetic activity was monitored everyday, during the five days that the experiment last, to assess the abiotic stress occasioned to the tree (jonagold variety of one year) at three different stages of the canopy. Beside two trees were entangled with Nalophan bag connected to pump (50ml/min; 16h) and 2 sorbent tubes filled with TENAX TA to monitor the emitted VOCs (one injected the other not).

The VOCs emitted presented great differences though a small contamination of the non injected. The trans-cinnamaldehydes emitted by the leaves reach value of  $19.63\text{mg/m}^3$  after 96h of injection.

Nevertheless the results gathered concerning the vitality of the plant suggest with a photosynthetic activity close to  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $F_v/F_m < 0.3$  after 72h the death of the tissue. These results implying that this essential oil is not suitable for this kind of application and/or that the formulations present strong phytotoxic properties.

## PP101 Integral use of *Lippia origanoides* grown in Colombia

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**Keywords:** *Lippia origanoides*, essential oil, integral use of plant material, steam distillation

### Abstract

*Lippia origanoides* is an aromatic shrub 1 to 3.5 m tall, endemic to Central and South America. It is popularly known as mountain oregano. It is a promising species for the development of functionalized natural ingredients, because both its essential oil and extracts possess phenylpropanoids such as thymol, carvacrol, and flavonoids such as naringenin, pinocembrin and apigenin, with proven biological activity. The EO of this species is used as a growth promoter and antibacterial agent in broilers production, replacing synthetic antibiotics. Its high antioxidant capacity is used in cosmetic and food industries. The hydrosol, a distillation residue, is used as an agricultural biopesticide for fungi and pests control, in coffee, strawberry and corn crops.

This work studies the result of applying different distillation and extractive methods of leaves and stems of LO, with the aim to find a configuration of unit operations for integral use of plant material (PM). Fresh leaves, dried, milled and enzyme pre-treated PM were extracted. Distillation techniques such as Steam Distillation (SD), Hydrodistillation (HD) and reduced pressure fractional distillation were utilized. Extractive techniques with agro-solvents (ASE) and sonication using ethanol-water mixtures at different ratios and supercritical CO<sub>2</sub> extraction (SC-CO<sub>2</sub>) with and without ethanol as co-solvent were also employed. GC-MS, GC-FID, HPLC-DAD and HPLC-ESI-MS were used, depending on the sample. Drying the PM increased the concentration of thymol and carvacrol in the essential oil (EO) due to the absence of monoterpenes that are present in the EO of fresh PM. Fractionated EO obtained from fresh PM had a thymol and carvacrol content similar to that of the EO obtained from dry PM. By crystallization at -15 °C from the heavy fraction of the essential oil, thymol of 92% purity was obtained. The enzyme pre-treatment increased the extraction yield up to 24%. ASE was the technique with the highest extraction yield and showed better results than SC-CO<sub>2</sub>. However, when ethanol was used as co-solvent, the extract composition was similar. The integral use of PM requires consecutive unit operations of SD, rectification, drying, crystallization, milling, and ASE with ethanol or SC-CO<sub>2</sub>. Commercial value products that can be obtained from LO are: EO enriched in *p*-cymene and  $\gamma$ -terpinene ( $W_i=0.58$ ), with an annual production of 300 kg/ha; EO enriched with either thymol ( $W_i=0.81$ ) and carvacrol ( $W_i=0.62$ ), thymol crystals ( $W_i=0.92$ ), ASE extract (302 mg flavonoids/g extract) and SC-CO<sub>2</sub> extract (242 mg flavonoids/g extract). The hydrosol and the post-distillation plant material can be used as biopesticide and fertilizer or biofuel, respectively. The integral use of *L. origanoides* could generate an alternative of rural development in Colombia, to maintain a stable and lasting peace.



## PP102 Interpopulation diversity of essential oils from arnica (*Lychnophora pinaster* Mart.)

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**Keywords:** essential oil, phenylpropanoids, sesquiterpenes, savanna

### Abstract

In Brazil, the Cerrado phytogeographical domain is home to a rich biodiversity of aromatic and medicinal species, which are subjected to severe conditions of the abiotic environment, such as *Lychnophora pinaster* (Asteraceae). The species is native and endemic to the State of Minas Gerais, occurring in regions with different soil and climatic conditions and altitude (800 – 2,000). It is known as *arnica* and the alcohol extract of the aerial parts is used in contusions, bruises and insect bites [1]. Essential oil from leaves collected in the southern region of the state showed antimicrobial activity against *Streptococcus mutans* [2]. This study aimed to investigate the chemical composition of leaf essential oils from populations of *L. pinaster* occurring in three geographic regions with different environmental conditions. Leaf samples were collected from 77 genotypes of *L. pinaster*, distributed in a population in the southern region (Pop 1: 1043 m altitude), one in the northern region (Pop 2: 753 m) and another in the metropolitan region of Belo Horizonte (Pop 3: 1498 m). The essential oils were extracted by hydrodistillation (2h) and analyzed by GC-FID, GC-MS, NMR and multivariate statistical analysis. The essential oils of P1 population were characterized by a high level of phenylpropanoids, with (*E*)-methyl cinnamate as the major constituent (mean % Pop 1: 79.0%). In the northern and metropolitan regions of Belo Horizonte populations, the sesquiterpenes class was the most abundant and among the major substances, 14-hydroxy-(*Z*)-caryophyllene (mean % Pop 2: 20.8%) and 14-acetoxy- $\alpha$ -humulene (Pop 3: 60.90%), and (*E*)-methyl cinnamate was not observed. In the multivariate analysis, the populations were distributed in three groups, Group I was formed by the population of the southern region (Pop 1) and Groups II and III by the populations of the northern and metropolitan regions of Belo Horizonte, Pop 2 and Pop 3, respectively.

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## PP103 Intrasubspecific chemical variability of *Salvia lavandulifolia* Vahl. (Spanish sage) - The need for a revision of Norma UNE 84310.

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**Keywords:** Spanish sage, essential oil, intrasubspecific chemical variability

### Abstract

In the Western Mediterranean area, in the Iberian Peninsula, *Salvia lavandulifolia* Vahl. (Spanish sage) is cultivated and exploited for its essential oil production. This species, according to the several bibliographic references published, exhibits a high chemical intraspecific variability, associated with the lack of a selection of individual plants previous to their corresponding breeding program. But, apart from this, a real concept that should be taken into account before the selection of these plants, is the existence of five different subspecies of *S. lavandulifolia*: *lavandulifolia*; *vellerea* (Cuatrec.) Rivas Goday & Rivas Mart.; *oxyodon* (Webb & Heldr.) Rivas Goday & Rivas Mart.; *mariolensis* (Figuerola) Alcaraz & de la Torre; and *blancoana* (Webb & Heldr.) Rosúa & Blanca. Attending to this, in the present work the chemical composition of these five Spanish sage subspecies are described and compared to the Spanish Reference UNE 84310, which defines, among other facets, the chemical volatile profile that this species officially exhibits. For this, a prospection of Spanish sage natural populations, considering the habitats for each subspecies, was performed around South-Eastern Spain. Five individual plants per population, from three different locations, were prospected, making a total of 15 plants per subspecies to be analysed. The essential oil (of every individual plant) was extracted by hydrodistillation and the qualitative and quantitative composition was analysed by gas chromatography coupled to mass spectrometry. By analysing the volatile profiles of the plants prospected, just the *Blancoana* subspecies was found to meet the requirements of the reference profile, although sabinyl acetate (1.3% relative concentration) was only detected in two plants out of the 15 analysed. A similar composition was quantified in the *Mariolensis* subspecies, but in this case none of the plants analysed had sabinyl acetate in the profile. By contrast, subspecies *lavandulifolia* and *vellerea* exhibited a more distant volatile profile: in *lavandulifolia*, eucalyptol (44.3%), linalool (0.1 %), linalyl acetate (not detected, n.d.), sabinyl acetate (n.d.) and terpenyl acetate (n.d.) were outside the percentiles suggested by the Norma, and in *vellerea*,  $\alpha$ -pinene (1.6%) and  $\alpha$ -terpenyl acetate (11.6%) were outside the range established along with sabinyl acetate again, since none of the plants showed this component at detectable levels in their volatile profiles. In South Eastern Spain, at the commercial level, *oxyodon* is the subspecies that can be found more frequently in the cultivated lands; in relation to its volatile profile,  $\alpha$ -pinene (2.7%) was outside the range and, once again, sabinyl acetate was only detected in one plant out of 15. These results point out the need for a revision of the normative regarding the Spanish sage volatile profile since none of the 75 individual plants analysed met the quality profile defined in this Norma UNE 84310.

## PP104 Is *Santalum yasi* a promising source of sandalwood essential oil?

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**Keywords:** sandalwood, essential oil, santalum yasi

### Abstract

*Santalum yasi* is a species that grows in the South Pacific region, on Fiji, Niue and Tonga islands. For several reasons, the heartwood of *S. yasi* has not been used yet as a source for the industrial production of essential oil, despite its relatively high content of a- and b-santalol. GC-MS analysis of the essential oil of *S. yasi* confirms that it could be a valuable candidate as a substitute for the commercial oils produced from *S. album*, *S. austrocaledonicum*, *S. spicatum* and *S. paniculatum*, on both quantitative (yield) and qualitative standpoints. A comparison with the corresponding cold solvent extract, reveals similar features and differences observed previously with other sandalwood oils. Using an ionic liquid stationary phase, a GC analysis of *S. yasi* heartwood essential oil shows a clean separation of (*E,E*)-farnesol, the main suspected allergen present in sandalwood essential oil.

## PP105 Modelling of fractional batch distillation of *Lippia origanoides* essential oils

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**Keywords:** fractional distillation, data reconciliation, carvacrol, thymol, *Lippia origanoides*

### Abstract

Essential oils that contain carvacrol and thymol as their main constituents have diverse applications in final consumer products that benefit from their antioxidant and microbicidal properties. Reduced-pressure fractional distillation is a common approach to reduce composition variability in these ingredients. Using *Lippia origanoides* essential oil (with carvacrol and thymol among its major constituents) as a model essential oil, a methodology was developed to predict fraction compositions in a multipurpose batch distillation column for a variety of essential oils. Nine *L. origanoides* essential oils from different crops, were distilled in a fractional micro distillation apparatus, and the GC-FID determined fraction compositions were adjusted by data reconciliation to satisfy material balances. Seven main oil constituents (carvacrol, thymol, *trans*- $\beta$ -caryophyllene, *p*-cymene, humulene,  $\gamma$ -terpinene,  $\beta$ -myrcene) were monitored directly and amounts of the remaining oil components were combined into a single figure, which had a relative amount below 10%. The reconciled data were compared with simulated compositions determined by solving a rigorous batch distillation model, obtaining a considerably good fit,  $R^2=0.84$  and  $MSE=0.006$ . Out of 154 concentration values, 11 cases corresponding to highly volatile components and to anomalously large experimental error were poorly predicted by this model. Their removal improved model performance to  $R^2=0.97$  and  $MSE=0.001$ . This methodology can be extended to other essential oils and serves as a starting point to develop dynamic optimization strategies and to determine column design parameters.

## PP106 New 3-methoxycuminyl esters from the essential oil of *Pulicaria dysenterica* (L.) Bernh.

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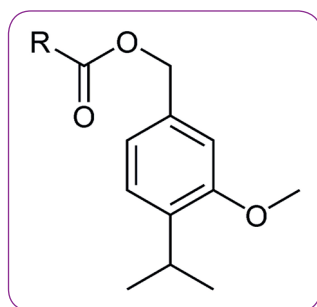
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### Abstract

The genus *Pulicaria* (Compositae) consists of ca. 100 species with a distribution from Europe to North Africa and Asia [1]. Although some of the flavonoids, sesquiterpenoids, and diterpenoids from *Pulicaria* species possess significant bioactivities, the genus is still poorly phytochemically and pharmacologically studied [1].

Analyses by GC and GC-MS of the two essential oil samples of aerial parts of *Pulicaria dysenterica* (L.) Bernh. showed the presence of 3-methoxycuminyl isobutyrate as the main constituent (25.5 – 31.1%). This composition turned out to be slightly different from the one presented in previously published reports [2-3] and that motivated us to perform a more detailed analyses which led to the identification of two other 3-methoxycuminyl esters: 2-methylbutanoate (a new natural product) and 3-methylbutanoate (a rare natural product that was identified only as a constituent of *Inula viscosa* essential oil [4]). In order to confirm the tentative identification, a small library of 5 esters (3-methoxycuminyl 2-methylpropanoate, butanoate, 2-methylbutanoate, 3-methylbutanoate, and pentanoate) was created. The synthesized esters were fully characterized by spectral (MS, IR, UV-Vis, 1D, and 2D NMR) and chromatographic (GC and GC-MS) techniques. To assess the safety and potential beneficial pharmacological uses, acute toxicity of *P. dysenterica* essential oil, selected constituents of the essential oil (3-methoxycuminyl isobutyrate, 2-methylbutanoate, and 3-methylbutanoate), as well as, the additional newly synthesized 3-methoxycuminyl esters (butanoate and pentanoate) were tested in the model of *Artemia salina* (brine shrimps). *P. dysenterica* essential oil and the tested esters showed low to moderate toxicity. Antimicrobial testing of the essential oil and synthesized compounds (in total, against 18 Gram-positive and Gram-negative bacteria, yeast, and mold strains) showed prominent activity against all tested microorganisms, where active concentrations ranged from 0.01 – 4.00 mg/mL.



### ACKNOWLEDGEMENT

This work was supported by the Ministry of Education, Science and Technological Development of Serbia [Project No. 172061].

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## PP107 New natural products from *Asphodelus albus* Mill. essential oil

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**Keywords:** *Asphodelus albus*, essential oil, new natural products

### Abstract

*Asphodelus albus* Mill. (white asphodel, or čapljan in Serbian) is an ornamental perennial plant belonging to the genus *Asphodelus* (Asphodelaceae), a circum-Mediterranean genus represented by 16 species [1]. Since ancient times, and even today in some regions, white asphodel has been used as a traditional ritual plant in many cultures in the Mediterranean – in the tradition of the ancient Greeks, native Corsicans, as well as in Spain, southern France, Turkey and cultures in this region [2]. Beyond this cultural-historical context, white asphodel has also been used medicinally in the folk medicine of the Mediterranean area due to many healing properties ascribed to this plant species. The culinary use of this taxon is also known – in Spain, leaves, tender shoots and tubers have been consumed stewed [3]; in Italy, the young shoots are consumed boiled, while the leaves are used to wrap Burrata cheese [4]; in Bosnia and Herzegovina, the underground parts rich in starch are used to prepare mush and bread [5]; in France, the root is regarded as a famine food called the “bread of the poor” and it was of particular importance before the introduction of potato [2,6]. Its fruits and leaves are also used in livestock feed [7]. Previous phytochemical investigations of *A. albus* mainly focused on the isolation and identification of anthraquinones [8-10], that are considered to be important chemotaxonomic markers at intergeneric level [11]. A comprehensive chemical analysis of the essential oil isolated from the aerial parts of this taxon performed in this work, in combination with detailed spectral analyses and chemical synthesis of selected compounds, has led to the identification of a series of new natural products (mainly esters of 3-furylmethanol and short-chained fatty acids) with possibly important olfactory properties.

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## PP108 OILPROTECT – Use of essential oil as insecticides for grain storage

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**Keywords:** essential oil, insecticid, grain storage, *Sitophilus granarius*, *Oryzaephilus surinamensis*

### Abstract

Cereals are consumed in our alimentation through different forms. Between the harvesting in fields and the human/livestock consumption, cereals can be stocked for long months in silos. During this period, these cereals are potential food resources for many insects (*S. granarius*, *O. surinamensis*, *R. dominica*, ...) that can cause 10-30% losses in silos. On a global scale, it concerns millions of tons of cereal per year. Consequently, many insecticides have been developed on the market to overcome this issue. Even if current molecules are less toxic than before, the residues that are present are a major challenge for public and animal health, as well as for environment.

The OILPROTECT project takes place in this context by trying to find a less toxic alternative to current insecticides. Plants have developed natural defence mechanisms and specific compounds against pests in the course of their evolution. These compounds have the particularity of being non-toxic for vertebrate considering the recommended use dose and are often present in our food condiments.

The main goal of this project is to formulate an efficient and natural insecticide with different essential oils and to obtain a residue-free product on grain. In that context, essential oils were chemically characterized and their toxicities were determined. Then, an in-depth study of cereal silos eco-systems was carried out in the lab to determine the potential protection of this kind of treatment with plant extracts.

## PP109 New volatile free fatty acids from Balkan immortelle essential oil

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### Abstract

The growing use of *Helichrysum italicum* (Asteraceae) or immortelle essential oil (EO) in glamorous perfumes and luxurious skin care products led both to an increase in price and production of this EO. The most consumed immortelle EOs are the Corsican neryl acetate/ $\beta$ -diketones chemotype and the Balkan  $\alpha$ -pinene/ $\gamma$ -curcumene chemotype. Both of these chemotypes have a rather complex aroma and previous GC-O studies revealed the identity of several key odorants [1,2].

Volatile free fatty acids (VFFAs) are generally present in trace amounts in EOs. However, because of their very low detection threshold VFFAs could have an important impact on the overall EO olfactory properties. The minor (+)-*cis*- and (+)-*trans*-olibanic acids provide the characteristic old churchlike endnote of frankincense and are good examples of this [3].

Hence, we decided to study the VFFAs composition of commercial immortelle oil from Serbia (Balkan chemotype). An EO sample was submitted to basic liquid-liquid extraction and the isolated VFFAs were converted to the corresponding methyl esters (MEs) with  $\text{CH}_2\text{N}_2$ . Concurrent GC-MS analysis enabled the identification of over 30 MEs. Particularly intriguing for us were MEs of 2-methyl fatty acids (2-Me-FAs; readily distinguishable by a base peak at  $m/z$  88 (McLafferty ion) in their MS). Alongside those of 2-Me-FAs (1-4; Fig. 1) four MEs containing additional branching were noted. The difference in RI values of these unknown and MEs of 2-Me-FAs implied that they should be MEs of 2,3-dimethylbutanoic (5), 2,4- (6) and 2,3-dimethylpentanoic acids (7 and 8; 2 diastereoisomers). This tentative identification was corroborated by synthesis (via  $\alpha$ -methylation of Li-enolates of the corresponding mono-Me-branched MEs) and co-injection experiments. A detailed literature survey revealed that 6-8 represent new natural products. Previously, only 1 and 2 were found in Corsican oils [1]. It is known that 2-Me-FAs ( $\text{C}_4$ - $\text{C}_7$ ) have very low thresholds and that their aroma could significantly vary (e.g. from sweet/apple-like to rotten fruit/cheese-like) dependent on the structure and concentration [4]. Thus, it would be interesting to investigate the impact of additional branching in these newly found VFFAs on their olfactory properties.

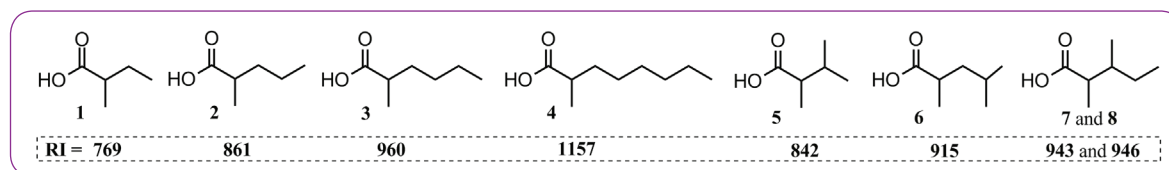


Figure 1: 2-Methyl fatty acids from immortelle oil and the RI values of the corresponding methyl esters

### ACKNOWLEDGEMENT

This work was funded by the Ministry of Education, Science and Technological Development of Serbia (Project 172061).

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## PP110 Ovicidal activity of essential oils and their components on phytoparasitic nematodes

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**Keywords:** essential oils, phytoparasitic nematodes, eggs

### Abstract

Propagation and survival of the most economically relevant phytonematode species mainly rely on eggs, due to a prolonged survival and presence of protective structures such as gelatinous matrices or cysts. Therefore, eggs represent a main target of any innovative nematode control tools, such as potential formulations based on essential oils (EOs) or their active components. In this study, a large number of EOs and, at a less extent, of their single constituents have been tested at different concentrations and exposure times on the egg masses of the root-knot nematode *Meloidogyne incognita* and the encysted eggs of cyst nematodes *Globodera rostochiensis* and *Heterodera carotae*. The ovicidal activity of tested EOs largely varied according to their composition and source plant. The EOs from *Ruta graveolens* and *Cinnamomum zeylanicum* were highly active on *M. incognita* eggs, as causing 76 and 88% mortality rates, respectively, since after a 24-hour egg exposure to 500 µg mL<sup>-1</sup> EOs' solutions. Adversely, a lower egg hatch inhibition was observed for the EOs from *Schinus molle* and *Pelargonium graveolens*. Egg hatch inhibition also varied among the EOs from the same plant genus, as in a comparative study on two different *Monarda* species the ovicidal activity was always significantly higher for *M. didyma* than for *M. fistulosa* EO. A study on single EOs' components revealed a strong activity on *M. incognita* eggs for carvacrol, as determining an almost complete egg mortality after a 48-hour exposure of egg masses to a 500 µg mL<sup>-1</sup> carvacrol solution, as well as for thymol. Adversely, a low activity on root-knot nematode eggs was recorded for g-terpinene and o-cymene. The activity on cyst nematode eggs varied either among the EOs and the nematode species. The EO from *S. molle* was much more suppressive on *H. carotae* than on *G. rostochiensis* eggs, whereas the *E. caryophyllata* EO exerted a similar activity on both nematode species. Data demonstrated that effect of EOs is extended to all the infective and propagative stages of phytonematode species, as confirming the potential of these plant compounds for the formulation of new nematicides.

## PP111 Phytochemical analysis of the *Anthriscus caucalis* M. Bieb volatiles

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**Keywords:** *Anthriscus caucalis*, essential oil, headspace volatiles, GC-MS

### Abstract

*Anthriscus caucalis* M. Bieb, also known as burr chervil, bur-chervil or bur beak chervil is a species from the carrot family, native to Western, Southern and Central Europe, Western Asia and Northern Africa. Through the centuries, it has been introduced into the North America, New Zealand, and South America. For this reason, *A. caucalis* has many synonyms that place him in other genera like *Torilis*, *Scandix*, *Chaerophyllum* etc. It is similar in appearance to chervil, the common cooking herb from the same genus. It sends up thin, hollow stems and bears umbels of white flowers. The light green leaves are triangular and made up of many leaflets.

Above ground parts of the *A. caucalis* were collected in May 2014, at the vicinity of Niš, village Hum, Serbia. From the fresh specimen, essential oil was isolated by Clevenger type hydrodistillation. The chemical composition of the oil was analyzed by GC and GC-MS, and the content of the volatile components in the native sample by static headspace GC-MS. In the essential oil of the aerial parts 34 components were found, what makes 97.1% of the total oil. Headspace analysis showed the presence of 28 components with a share of 99.3% of the total. The major class of compounds, in both investigated specimens, was found to be monoterpenoids with the share of 88.3% in oil and 92.8% in headspace sample, but in different proportions with respect to the ratio of hydrocarbon to oxygenated compounds (13.9% to 74.4% and 58.2% to 34.6%, respectively). In particular, just like in previous report on the *A. caucalis* essential oil composition from Vienna [1], the most dominant compounds were *cis*-chrysanthenyl acetate and *cis*-chrysanthenol which accounts nearly 3/4 parts of the total. In addition, compounds present in a significant percentage in essential oil were: (*Z*)- $\beta$ -ocimene (5.1%), myrcene (3.9%) and (*E*)- $\beta$ -farnesene (2.2%). On the other hand, chemical composition of the *A. caucalis* essential oil from China [2] was completely different with the sesquiterpene hydrocarbons as the most dominant class of compounds (89.8%). In headspace specimen, the most abundant components were: *cis*-chrysanthenol (28.4%), myrcene (17.7%),  $\alpha$ -pinene (15.1%), (*Z*)- $\beta$ -ocimene (11.2%) and limonene (6.1%).

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## PP112 Phytochemical profiles and antioxidant properties of hydrosols, obtained from Bulgarian *Rosa alba* L. and *Rosa damascena* Mill.

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**Keywords:** antioxidant capacity, chemicals, *Rosa alba* L.; *Rosa damascena* Mill., hydrosol

### Abstract

This study assessed phytochemical profile and antioxidant capacity of hydrosols, obtained by water-steam distillation of *Rosa alba* L. and *Rosa damascena* Mill. plants, grown in experimental field of the Institute of Rose and Essential Oil Plants, Bulgaria. The identification of hydrosols' compounds was performed using GC-MS, the total phenolic content (TPC) was determined quantitatively using the Folin-Ciocalteu reagent, with gallic acid as the standard. The antioxidant capacity of both hydrosols in concentration from 0.3% up to 15.0% were tested in liposomal suspension, and in systems generating either hydroxyl and superoxide anion radicals. The results showed that hydrosols were rich in geraniol (42.55 % for *Rosa alba* L., and 27.25 % for *Rosa damascena* Mill.) and citronellol (28.70 % in both hydrosols). The hydrosolic TPC were found to be 72 and 32.52 µg/ml of the gallic acid equivalent, respectively. Both hydrosols demonstrated good capacity to inhibit Fe<sup>2+</sup>/ascorbic acid - induced lipid peroxidation in egg liposomal model system as the effect of white rose hydrosol in all tested concentrations was higher than those of pink rose. However *Rosa damascena* Mill demonstrated better ·OH scavenger effect than that of *Rosa alba* L. These results suggest that the hydrosols from both oil-bearing roses have well-expressed antioxidant properties that could be used as antioxidant supplements in conditions with oxidative stress ethology.

## PP113 Variability in the composition of essential oils from fresh aerial parts of *Anthemis arvensis* L. collected in the beginning and the end of the flowering phase

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**Keywords:** *Anthemis arvensis* L., essential oil analysis, essential oil comparison

### Abstract

Corn chamomile, *Anthemis arvensis* L. (Asteraceae), is an herbaceous annual plant species, that as an invasive weed that can be found throughout most of Europe and parts of Asia and North Africa, growing both on sand and in cultivated fields. Aboveground parts have been used in traditional medicine as a vermifuge and a topical analgesic [1]. This taxon has been the subject of several phytochemical studies, but up to this point, only a few studies dealt with the composition of its essential oil [2,3].

Herein, we present a detailed analysis (by GC and GC-MS) of the essential oil of aerial parts of *A. arvensis* L. collected in the beginning and at the end of the flowering phase (beginning and end of May 2016), from a wild-growing population in Serbia (Bovan lake, near Niš). Hydrodistillation of fresh plant material yielded, in both cases, a small amount of practically odorless light green essential oil (0.03% and 0.04%, w/w, respectively). The analyses enabled the identification of more than 180 compounds in each oil sample. The essential oil obtained from the plant at the beginning of the flowering phase had germacrene D (14.2%),  $\delta$ -elemene (4.0%) and tricosane (3.0%) as the major constituents, whereas the most abundant compounds in essential oil from the end of the flowering phase were found to be *cis*-chrysantenyl acetate (11.3%),  $\tau$ -muroolol (8.0%) and linalool (7.0%) A more detailed comparison of these two essential oils obtained from the same locality showed other noticeable differences in qualitative and quantitative chemical composition.

### ACKNOWLEDGEMENT

The authors acknowledge the Ministry of Education, Science and Technological Development of Serbia for financial support (project 172061).

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## PP114 Potential anti-quorum sensing activity of selected essential oils against *Chromobacterium violaceum*

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**Keywords:** anti-quorum sensing, *Chromobacterium violaceum*, zone of turbidity, essential oil

### Abstract

The use of essential oils as traditional remedy for the treatment of various infections dates back to the middle ages. There is a corpus of scientific literature documenting the anti-infective properties of essential oils with a focus on bactericidal or bacteriostatic activity. Other antimicrobial drug targets with the potential to reduce antimicrobial resistance are somewhat neglected. The current study investigated the potential anti-quorum sensing (AQS) activity of selected commercial essential oils where quorum sensing is a drug target with the potential to reduce antimicrobial resistance. The AQS activity of 40 essential oils (EOs) was determined using the agar well diffusion method, micro-dilution assay and spectrophotometric quantification of violacein production in *Chromobacterium violaceum*. Noteworthy activity of essential oils was reported as Zones of Turbidity (ZOT)  $\geq$  eugenol (positive control), percentage inhibition of violacein production  $\geq$  90% and low minimum quorum sensing inhibitory concentration (MQSIC) comparable to eugenol. The results demonstrate that 17 out of the 40 EOs possess moderate AQS activity (ZOT: 3.00–5.50 mm) compared to eugenol (ZOT = 12.00 mm). Low MQSIC values of 0.06 mg/ml were also reported for 10 out of the 40 EOs thus demonstrating the potency and ability of the oils to inhibit QS at low concentrations. The essential oils that exhibited noteworthy activity at the lowest concentration of 0.1 mg/ml were; *Citrus limon*, (70.7%), *Cymbopogon flexuosus* (88.4%), *Cymbopogon martini* (70.0%), *Eugenia caryophyllus* (69.6%), *Gaultheria procumbens* (91.4%), *Origanum compactum* (78.6%) and *Thymus vulgaris* (79.7%). The results demonstrate the ability of essential oils to interfere with bacterial QS thus providing a possible alternative drug target to combat infections.

## PP115 Relationships between amount of essential oil and anatomical characters of leaves in *Thymus pulegioides*

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**Keywords:** *Thymus pulegioides*, essential oil amount, leaf anatomy, glandular trichomes, anatomical markers

### Abstract

Analysis of essential oil amount in individual plants is long and expensive process. Therefore, search of quick and cheap preliminary essential oils evaluation method is important, because it could facilitate initial selection of plants from natural habitats. Individual plants of widely spread *Thymus pulegioides* (Lamiaceae) growing in same environmental conditions can accumulate different amount of essential oil; it is determined genetically. The aim of this study was the search for *T. pulegioides* anatomical markers on leaves, which could correspond to amount of essential oil.

The impress method and inverted microscope was used for investigation of the following anatomical characteristics: density and diameter of glandular trichomes, stomata density and the number of epidermal cells around the glandular trichome in lower and upper epidermises of *T. pulegioides* leaves. The essential oils were isolated by hydro-distillation, using a Clevenger-type apparatus.

Results showed that variation of density and diameter of glandular trichomes between *T. pulegioides* individuals was 8.3–21.7 glandular trichomes in 1 mm<sup>2</sup> (CV=20%), 46.3–72.4 μm (CV=8%) in lower epidermis and 6.8–19.0 glandular trichomes in 1 mm<sup>2</sup> (CV=17%), 50.4–79.9 μm (CV=8%) in upper epidermis, respectively. In average 18 epidermal cells surrounded each glandular trichome. Investigated *T. pulegioides* individuals accumulated very different percentage of essential oils – from 0.16% to 2.09%. Statistically significant (p<0.05) positive correlation was established between amount of essential oil and diameter of glandular trichomes in upper epidermis. Therefore diameter of glandular trichomes in upper epidermis of leaves could be used as anatomical marker for selection of *T. pulegioides* plants with higher percentage of essential oil.

## PP116 Rose and lavender essential oils chiral components as important markers for quality and authenticity assessment

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**Keywords:** *R.damascena* Mill., *L. angustifolia*, essential oil authenticity control, enantioselective gas chromatography, chiral compounds

### Abstract

Essential oils (EOs) have been widely used all over the world and their use is constantly increasing because of the strong demand for pure natural ingredients in many fields (in fine perfumery, as natural cosmetics and cosmeceuticals, as food supplements and in clinical aromatherapy). EOs are of high-added value and, therefore, they are often subject of adulteration by adding non-volatile ingredients, synthetic compounds, or cheaper essential oils. Bearing in mind the extremely complex chemical composition of the EO, sophisticated analytical techniques are required ensuring quality control and consumer safety.

The development of stable chiral phases for GC has allowed detailed study of enantiomeric composition of the volatile compounds. ES-GC has become an important technique for detection of adulterants, essential oil profiling and quality control, based on the fact that plants produce metabolites mainly as chiral molecules. Thus, diastereomer and enantiomer composition, which is characteristic for the geographic and botanical origin, allows detection of accidental or deliberate addition of a synthetic products or even the discrimination of different geographic or botanical origin.

Therefore, the aim of the current study is to explore composition of some chiral components, which are important quality and authenticity markers in Bulgarian lavender and rose oil samples by means of ES-GC/MSD/FID. In this respect linalool, rose oxides and citronellol in rose oil and linalool, camphor and linalyl acetate in lavender oil samples have been studied and an analytical protocol for detection of synthetic citronellol, linalool and linalyl acetate was developed.

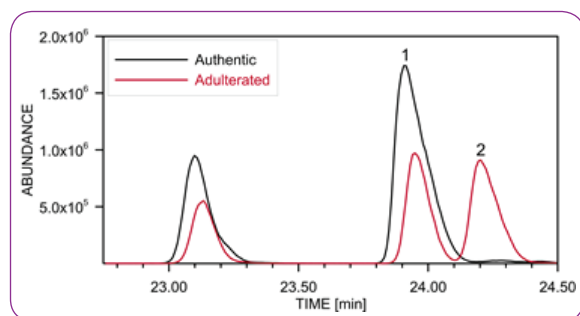


Figure 1: Citronellol enantiomers distribution in genuine and adulterated rose oil: 1. (3S)-(-) Citronellol, 2. (3R)-(+)- Citronellol.

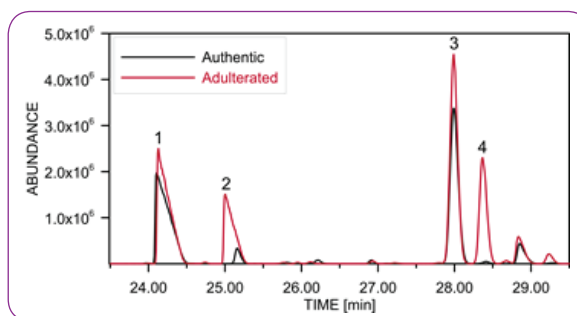


Figure 2: Linalool and linalyl acetate enantiomers distribution in authentic and adulterated lavender oil: 1. (R)-(-) Linalool, 2. (S)-(+)- Linalool; 3. (R)-(-) Linalyl acetate, 4. (S)-(+)- Linalyl acetate.

## PP117 Scent from *Cattleya wallisii*, an orchid from the Amazon

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**Keywords:** *Cattleya wallisii*, Orchidaceae, floral scent, methyl salicylate

### Abstract

The genus *Cattleya* (Orchidaceae) comprises around 60 species with neotropical distribution. Many are considered to be endangered species, mostly due to anthropic pressure. Several species have been studied regarding their volatile composition [1]. *Cattleya wallisii* (Linden) Linden ex Rehb.f. (Orchidaceae) is an epiphyte herb native to the Amazon [2]. To the best of our knowledge, this is the first investigation on the volatiles of *C. wallisii*.

The plant was originally collected in the Amazon area. A voucher was deposited at the herbarium of the University of Brasilia (UBPires, JM8271). A greenhouse cultivated specimen was covered with a roaster (cooking food) plastic bag, and the volatiles were collected by dynamic headspace and trapped into a homemade micro tube (2 cm x 1 mm i.d.) filled with Porapak Q. After the collection period (1 h), 50 µL of hexane were used to wash the volatiles to an ampoule. The same flower was sampled three times, in consecutive days, at the same day period (14 to 15 h). Octadecane was added as internal standard. GC-FID and GC-MS analyses were performed in Agilent 7890B and 5975C, respectively. Theoretical response factors were used to correct FID areas. Identification was based on mass spectra and linear retention indices.

The main compounds found in the volatile fraction were methyl salicylate (27.7%), nerol (14.0%), (*E,E*)- $\alpha$ -farnesene (6.2%),  $\beta$ -bisabolene (6.0%), and linalool (4.9%). Many acetyl and benzyl esters were also identified. Methyl salicylate was reported to be the major compound in the volatiles from *Cattleya velutina*, and it is also present in the scents from *C. schilleriana*, *C. jenmanii* and *C. mossiae* (1).

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## PP118 Seasonal variation of essential oil composition in *Thymus caespititius*, carvacrol chemotype

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**Keywords:** *Thymus caespititius*, seasonal variation, chemotypes, essential oils

### Abstract

*Thymus caespititius* is an aromatic species, endemic from the NW Iberian Peninsula, and Madeira and Azores archipelagos. Several studies have shown chemical variability in essential oils from this species, with the existence of well-defined monoterpene-rich chemotypes: the most common  $\alpha$ -terpineol type, identified in plants grown in several locations, and the sabinene, thymol and carvacrol types, that occur only in plants grown in the Azores. Studies on the genetic inheritance of chemotype identity as well as on the seasonal variation of the essential composition were not performed in this species until now. To achieve this, controlled crosses among chemotypes were established, to help understand the genetics of chemotype identity. In this case study, plants of the carvacrol chemotype, collected on June 2008, at Flores Island, Azores, Portugal, were grown in pots for three years after what crosses were performed. Both parent plants and offsprings were established on September 2011 in a garden at Faculdade de Ciências da Universidade de Lisboa (FCUL) and grown for five years under open-air conditions, without irrigation or fertilization. To verify the stability of the chemotypes over time, the essential oils from two plants (F1A, F1B) obtained from controlled crosses and from one wild plant (CW) grown in the FCUL garden, were evaluated monthly for two years (September 2016 to August 2018). GC and GC-MS analyses showed that the two plants resulting from controlled crosses (F1A, F1B) of carvacrol chemotype parents produced an essential oil rich in carvacrol (F1A, 20-47%; F1B, 20-44%), like the cultivated wild plant (CW, 16-33%). Relative amounts of  $\tau$ -cadinol were also similar (F1A, 6-18%, F1B, 4-13%, CW, 4-11%). However, some differences in essential oils composition were noteworthy, namely in the relative amounts of sabinene (F1A, traces-0.2%, F1B, traces-0.1%, CW, 0.3-2.0%),  $\alpha$ -terpineol (F1A, 0.1-2%, F1B, traces-1%, CW, 2-11%), and carvacryl acetate (F1A, 7-51%, F1B, 15-58%, CW, 1-8%). Furthermore, fluctuations in the relative amount of the main monoterpene components (sabinene, *p*-cymene,  $\alpha$ -terpineol, carvacrol, carvacryl acetate) throughout the two years did not show a defined pattern. These results stress the importance of specifying the several parameters, namely the harvest time, plant stage (vegetative or floral) when assessing chemotype determination.

### ACKNOWLEDGEMENTS

Thanks are due for the financial support to CESAM (UID/AMB/50017/2019), to FCT/MCTES through national funds.

## PP119 Soil influence on the chemical composition of the *Geranium macrorrhizum* L. rhizome essential oil

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**Keywords:** *Geranium macrorrhizum*, essential oil, rhizome, chemical composition, soil

### Abstract

*Geranium macrorrhizum* L. occurs in the Southern Alps, Apennine and Carpathian Mountains, extending to southern Greece in the Balkan Peninsula. In Europe it is increasingly cultivated on account of its ornamental flowers. Being winter-hardy it naturalizes easily in parts of central Europe. *G. macrorrhizum* is highly valued in ethnopharmacology of the Balkan region, for its aromatic essential oil, and it is known in Serbia as wild geranium or "zdravac", with reference to its Serbian name zdravats, meaning "health". It possesses a wide range of beneficial properties such as antimicrobial, hypotensive, spasmolytic, astringent, cardiotoxic, antioxidant, and sedative activities. As the specific epithet implies, *G. macrorrhizum* has a stout horizontal rhizome sheathed in old stipules; the branched rhizome, mainly subterranean, is also partly above ground.

The rhizome samples of the *G. macrorrhizum* were collected in January 2019 in plant hibernation phase, on three different localities in Serbia. Each specimen originated from the different soil type: sample ST from the Mt. Stol - conglomerate; DE hill near the town of Despotovac - limestone and CE from the Mt. Čemernik - shale. Essential oils from the fresh rhizomes were isolated by Clevenger type hydrodistillation. Chemical composition of the oils was analyzed by GC and GC-MS. In the essential oils of the investigated samples, 89 components were identified in total, what makes a little bit over 90% of present compounds. The most dominant class of compounds in all investigated specimens was found to be sesquiterpenoids with the share of 87.0% in ST, 91.0% in DE and 90.7% in CE sample, but in different proportions with respect to the ratio of hydrocarbon to oxygenated compounds, primarily in relation to the sample ST (66.3% to 20.7%, 45.6% to 45.4% and 44.9% to 45.8%, respectively). Significant differences were also observed in the abundance of the main components of the essential oils. The percentage of the most valuable compound, germacrone, was 8.2%, 21.3% and 12.2%, while the abundance of the main component,  $\alpha$ -bulnesene, was 49.1%, 32.4 and 23.8% in the ST, DE and CE samples, respectively. On the other hand, just like in previous report on the *G. macrorrhizum* rhizome essential oil composition from the same locality [1], the major components were present almost in same percentage despite the fact that they were collected at a notable time interval. In addition, compounds found in a significant percentage (over the 5%) in the *G. macrorrhizum* rhizome essential oils were:  $\alpha$ -guaiene, eremophilone and selin-11-en-4 $\alpha$ -ol.

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## PP120 *Spicaria divaricata* as a novel source of sesquiterpenes

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**Keywords:** *Spicaria divaricata*, *Paecilomyces divaricatus*, sesquiterpenes, HS-SPME, volatile profile

### Abstract

One of the commonly occurring fungi causing food spoil is *Spicaria divaricata* (syn. *Paecilomyces variotii*, *Paecilomyces divaricatus*) [1]. It is a cosmopolitan species, found in soil, plants, animal and indoor environments. Characteristic is that this fungus is heat-resistant, which means that products subjected to pasteurization can also be infected. Isolates of *S. divaricata* were used in tannase production and whole cells are used for the biotransformation of various types of compounds, e.g. coumaric acid and DHEA derivatives [2,3].

During the biotransformation by *Spicaria divaricata* AM 423, it was noticed that the strain used in the experiments has a characteristic smell. Therefore a first study was carried out to determine the aromatic profile of the fungus. Headspace solid-phase microextraction (HS-SPME) coupled with GC-MS technique was used to identify volatile compounds present in fungus headspace. As a result, more than thirty compounds were identified, mainly belonging to sesquiterpenes group. Literature overview has shown that numerous of identified compounds possess various properties like anti-inflammatory, antimicrobial and antioxidant ones [4,5].

The obtained results may indicate the potential antimicrobial and antioxidant activities of the strain *S. divaricata* AM423.

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## PP121 Structure-odor relationships of pinene derivatives

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**Keywords:** pinene, odor, structure-odor relationships

### Abstract

The aromas of natural odor materials consist of many odor compounds. In some cases (sandalwood and frankincense), we have found that the odors of the materials arise from a group of constituents with similar structures [1]. Recent studies on the olfactory mechanism show each odorant interacts with many different receptors and each receptor interacts with many different odorants. There are also antagonistic interactions between odorants with similar structures and the receptors. The relationship between the odorant and the receptors is complex [2]. The interactions of several constituents with similar structures are important for the aroma profile. We focus on a change in the response pattern of olfactory receptors indicating a change in odor when the structure of an odorant molecule was changed. We studied the structure-odor relationships on the basis of the following concepts. (1) Similar odors among odorants means olfactory receptors recognize a similar structure. (2) Different odors among odorants means olfactory receptors recognize a different structure. We conducted experiments with pinene, a cyclic terpene, to obtain systematic knowledge about the terpene. We synthesized several odor compounds from alpha-pinene and beta-pinene. Detailed examination of odors for the obtained compounds revealed that the crosslinking structure is affecting the characteristic aroma of pinene. The obtained results indicated that the receptors distinguish between endocyclic and exocyclic double bond. Endocyclic double bond was hardly recognized by the olfactory receptor. And it was confirmed that the position of the functional group (OH and OMe) affects the aroma in addition to the type of the functional group even in the case of pinene derivatives

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## PP122 The effect of aroma compounds on airborne microbes for the enhancement of the comfort of vehicle interior

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### Abstract

In Japan, car companies such as Toyota Motor Corporation and Fuji Heavy Industries Ltd. have focused on the reduction of airborne microbes and fungi in the vehicle compartment. They are also interested in supplying fragrance to enhance comfort in the vehicle interior. We have reported the use of aroma compounds to be pleasant and less harmful than using disinfectants to reduce airborne microbes. In our country car users prefer weaker scents; therefore, we tried to improve our established method to provide for their needs. Then we discovered that even much less amount of aroma compounds is effective to reduce airborne microbes [1,2]. When 100 ml of terpineol (mixture of isomers,  $\alpha$ -terpineol 65%;  $\beta$ -terpineol 10%;  $\gamma$ -terpineol 20%) was spread by using an air pump for one minute every five minutes for three times, approximately 90 % of airborne microbes were reduced ( $p=0.002$ ). The intensity of the smell was quite low, although the test was performed while the engine was turned off. Therefore, this new method would satisfy car users' needs. Some other aroma compounds were also tested because drivers' preference should be considered.

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## PP123 The effect of thyme (*Thymus vulgaris* L.) essential oil in endotoxin-induced acute airway inflammation model of knock-out mice

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**Keywords:** thyme oil, airway inflammation, LPS, TRPV1/TRPA1, myeloperoxidase activity

### Abstract

Respiratory tract diseases associated with bacterial infection and inflammation affect a large number of people from every age group worldwide. Because of volatility, essential oils (EOs) can easily reach the upper and lower parts of the respiratory tract via inhalation. However, the anti-inflammatory effect of EOs is poorly studied *in vivo*. Transient Receptor Potential Vanilloid1 (TRPV1) and Ankyrin1 (TRPA1) ion channels are expressed on the sensory neurons and epithelial cells of the airways and play a role in sensory-immune interactions.

Therefore, we aimed to examine the chemical composition and effects of thyme oil (TO) inhalation in the model of lipopolysaccharide (LPS)-induced airway inflammation and the potential role of TRPA1/V1 ion channels in mediating TO effect. This oil was selected on the basis of its potent antibacterial activity and preliminary research with this model in wild type (WT) mice.

The chemical composition of TO was determined by GC-MS. Lung inflammation was evoked by the intratracheal administration of 60 µL LPS (*E. coli* 083: LPS) in female TRPA1/V1<sup>-/-</sup> (KO) mice. TO or the control oil was inhaled three times for 30 min during the 24-h period of the experiments. Airway function was measured in awake, spontaneously breathing animals by unrestrained whole body plethysmography. Lung myeloperoxidase (MPO) activity was determined by spectrophotometry. The histopathological alterations were evaluated from hematoxylin-eosin stained lung sections by semiquantitative scoring.

Thymol (46.3%) and p-cymene (22.1%) were the two main components of TO. TO inhalation significantly aggravated airway hyperreactivity in KO mice. Minute ventilation was increased by TO inhalation in the intact group but not in the LPS-treated mice. Other respiratory parameters were not significantly affected by TO. Histopathological parameters were not affected significantly by TO inhalation. The increased MPO activity was not reduced by TO inhalation in KO mice.

The preliminary comparison of the WT and KO results refer that TO can be considered as a potential treatment in airway inflammation, and its protective effect is potentially mediated by TRPA1/V1 ion channels.

## PP124 The effect of aroma compounds on the discomfort while icing

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### Abstract

Icing is one of the effective methods to reduce fatigue and to repair athletic injury. However, some athletes avoid icing because they dislike discomfort such as pain during cooling. We focused on the fact that we feel pain only few minutes after soaking our hand or leg in cold water. That means athletes only need to endure for first few minutes with the help of any method of relief.

In previous studies we reported that 1,8-cineole and (R)-(-)-linalool were effective to reduce physical stress which was induced by cold pressor test. In this research the effect of aroma compounds on the athletes' discomfort during icing was examined.

Ten athletes participated in this experiment. They soaked their hand in the cold water of approximately 0~3°C for 50 seconds. An electrocardiogram was employed and R-R Interval (RRI) was used as a stress index. 1,8-Cineole which stimulates human body, (R)-(-)-linalool which has a sedative effect, and essential oils which was favored by each subjects were tested as fragrance compounds. The experiment was conducted under four conditions, such as control and supplying three fragrances for each person.

Because of space of limitations the results of one male subject are described here. Under the control condition his RRI value during icing decreased approximately 8% compare to that of before icing ( $p < 0.001$ ). When 100 $\mu$ l of 1,8-cineole and (R)-(-)-linalool were supplied, RRI values increased about 12% and 8 %, respectively ( $p < 0.001$ ,  $p < 0.001$ ). On the other hand, RRI value decreased approximately 4% when grapefruits oil was supplied ( $p = 0.006$ ). This indicates that some aroma compounds might be effective to reduce discomfort during icing.

## PP125 The essential oil composition of *Croton gratissimus* Burch

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**Keywords:** GC-MS, chemometrics, principal component analysis

### Abstract

*Croton gratissimus* is widely used in traditional medicine to treat a range of conditions including malaria, hypertension, diabetes, arthritis, urinary tract infections, gonorrhoea and impotence. The young branches of *C. gratissimus* are pleasantly aromatic and it is recorded that Bushman girls used them to make perfume. The chemistry of the non-volatile constituents has been widely investigated. However, scientific data on the volatile constituents are lacking. Essential oils were isolated using hydrodistillation from the aerial parts of *C. gratissimus* ( $n = 62$ ) collected at different locations in South Africa and analysed with one-dimensional gas chromatography coupled simultaneously to a mass spectrometer (MS) and a flame ionisation detector (FID) in order to determine chemical variation. The essential oil (EO) profile showed the presence of mono- and sesquiterpenes as the major compound classes. The most abundant compounds were  $\alpha$ -pinene (4.1–38.2%), camphene (4.2–32.1%), sabinene (4.3–41.8%), myrcene (4.5–28.9%),  $\alpha$ -phellandrene (4.7–48.1%), oxidohimachalene (n.d–54.6%) and *E*- $\beta$ -ocimene (7.5–39.7%). Untargeted analysis of GC-MS data using SIMCA-P+ 14.0 revealed three major chemotypes. Principal component analysis (PCA) analysis loadings and contribution plots identified oxidohimachalene as the main chemical marker for Chemotype 1. Chemotype 2 was dominated by  $\alpha$ -phellandrene and  $\alpha$ -pinene, while Chemotype 3 was characterised by low concentrations of  $\alpha$ -phellandrene and oxidohimachalene. The study revealed mostly quantitative rather than qualitative variation of the volatile constituents of *C. gratissimus*.



## PP126 The impact of plant volatiles on bacterial quorum sensing

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**Keywords:** *Chromobacterium violaceum*, quorum sensing, essential oil, stereochemistry

### Abstract

Studies describing the use of essential oil constituents as antimicrobial agents have steadily increased; however, some phyto-constituents are often overlooked due to unfavourable minimum inhibitory concentration (MIC) values. Virulence depends on transcriptional factors which are regulated by cell-to-cell communication called quorum sensing (QS). This study was undertaken to evaluate the antimicrobial and anti-QS properties of 29 compounds commonly found in essential oils using two bioreporter strains. QS-inhibitory activity was assessed qualitatively by agar diffusion and quantitatively by spectrophotometric assays. MICs of all the tested compounds ranged from 0.032 to >5 mg/ml.

Twenty-two compounds displayed varying levels of QS inhibitory activity with zones of violacein inhibition ranging from 9 to 16 mm. Majority of tested molecules inhibited violacein and pyocyanin production in *Chromobacterium violaceum* and *Pseudomonas aeruginosa*, while seven compounds increased violacein and pyocyanin production. Interestingly, it was observed that the (+)-enantiomers of carvone, limonene and borneol increased violacein and pyocyanin production, while their levorotary analogues inhibited this production.  $\alpha$ -Terpineol and cis-3-nonen-1-ol exhibited >90% violacein inhibition, suggesting their potential as QS inhibitors. This preliminary study indicates that plant volatiles have the potential to impede or promote bacterial communication and further studies need to be undertaken to explore the contribution of structural analogues and stereochemistry of molecules in this process.

## PP127 The impact of soil herbicides on the yield and chemical composition of rose oil

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**Keywords:** *Rosa damascena* Mill., herbicides, essential oil, quality

### Abstract

During the period of 2014-2015, the herbicidal effect and selectivity of isoxaflutole (Merlin 750 WG), oxadiargyl (Raft 400 SK), imazamox (Pulsar 40) were studied on rose plantation. The treatment was performed on the *Rosa damascena* Mill. experimental field, in the Institute for Roses and Aromatic Plants, Bulgaria. The population variety plants were used. The herbicides were introduced before the beginning of vegetation and the doses were chosen as optimal, based on data of other agricultural crops. The results were compared to those of the untreated control.

The two-year study showed that the application of oxidiazinyl at a dose of 64 g/decare (daa) resulted in a 49% and 61% increase in oil yield. Imazamox at a dose of 6 g/decare (daa) and isoxaflutole at a dose of 4.5 g/decare (daa) increased the yield to a lower degree, respectively to 51% and 26%.

The main components of the odor ranged for citronellol and nerol from 24.9-32.1%, for geraniol from 18.9-26.5%, and for linalool from 0.8-2.5%. The paraffins varied from 21.3% to 28.3%. The quality was discussed, based on relation of terpene alcohols/paraffins and year conditions.

## PP128 The influence of various dehydrating techniques on quality of yarrow (*Achillea millefolium* L.) essential oils obtained from flowers

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**Keywords:** *Achillea millefolium*, drying, essential oils, quality

### Abstract

Yarrow (*Achillea millefolium* L.) is a worldwide known medicinal plant from *Asteraceae* family, which is native for Europe, western Asia and northern America. Yarrow is especially valuable due to its richness of essential oils (EOs) (approximately 2.0%) which main constituents are artemisia ketone, linalyl acetate, camphor and 1,8-cineole [1,2]. Those and other substances with lower content makes yarrow EOs and its' extracts useful as a preservative agent against foodborne pathogens, insects attractant and insects repellent, as well as an effective antioxidant and anti-inflammatory agent [2,3]. Nevertheless, the activities of medicinal plants are highly dependent on their quality, dictated by plant chemotype, cultivation and harvest conditions and post-harvest treatments among which the biggest impact has the preservation method [2]. The most common procedure used nowadays is convective drying, which unfortunately does not guarantee the quality of the final product. Due to that the study focused on the impact of various dehydrating techniques was performed with yarrow cultivated in Poland. Three techniques of dehydrating were applied: (i) convective drying (CD) at 50, 60 and 70°C, (ii) microwave-vacuum drying with powers 240, 360 and 480W and (iii) combined convective pre-drying at 60°C followed by microwave-vacuum finish drying with power 360 W. The impact on plant material was examined by headspace solid-phase microextraction (HS-SPME) technique and analysis of EOs obtained with Deryng apparatus, both coupled with GC-MS technique. Around one hundred compounds were identified in yarrow EOs, with artemisia ketone, sabinene, chamazulene,  $\beta$ -pinene and terpinen-4-ol as a major ones. Based on the EOs retention, which vary between 48% to 60% regarding to applied technique, and their main constituents' quantification the dehydrating techniques and various parameters were evaluated and the optimal methods were defined.

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## PP129 The volatile components emitted from *Marchantia paleacea* subsp. *diptera*

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**Keywords:** (S)-(-)-perillaldehyde, *Marchantia paleacea* subsp. *diptera*, Headspace GC-MS analysis

### Abstract

The volatile components from the thalloid liverwort, *Marchantia paleacea* subsp. *diptera* were investigated by HS-SPME-GC-MS. The monoterpene aldehyde, perillaldehyde was identified for the first time as the major component and its content was around 50% of the volatiles. In addition,  $\alpha$ -pinene (2.57%), limonene (15.3%),  $\beta$ -caryophyllene (6.3%),  $\alpha$ - and  $\beta$ -selinene (3.4 %) were identified. Until now the major components emitted from the *MPD* have been reported as limonene, 1-octen-3-yl acetate, and 1-octen-3-ol by Y. Asakawa, one of the authors, but there have been no reports about the existence of perillaldehyde in *MPD*, liverwort.

Using the multidimensional GC-MS analysis equipped with a chiral column on the second column, the chiralities of both perillaldehyde and limonene considering as the precursor of perillaldehyde, were analyzed. We found that on both compounds, the content of (S)-(-)-enantiomer was determined over 99.0 % and its (R)-enantiomer was detected below 0.5 %. This is the first report of the existence of the monoterpene aldehyde perillaldehyde emitted from liverwort like *MPD* and its stereochemistry was determined (S)-(-)- form to be predominant.

*MPD* in this study was cultured on either Nippi soil or Gamborg's B5 medium. During cultivation of *MPD*, a strong "Perilla frutescens leaf note" was emitted when the thallus was either crushed by fingers or softly scratched by a wood-made brush.

## PP130 Three new sesquiterpene alcohols from the essential oil of the liverwort *Conocephalum conicum* (L.) Dum.

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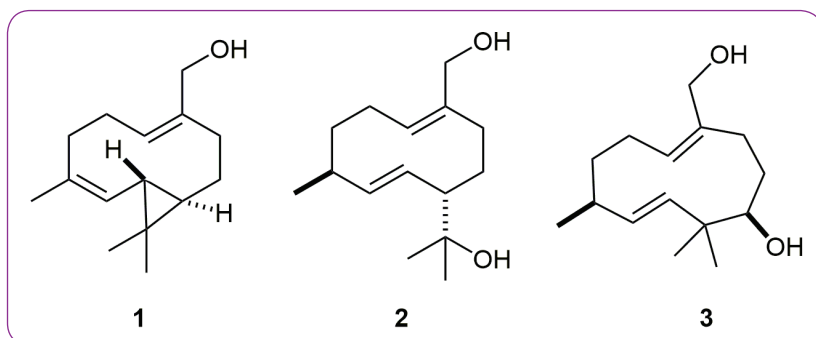
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**Keywords:** *Conocephalum conicum* (L.) Dum. (Conocephalaceae), lepidozene, structural elucidation

### Abstract

*Conocephalum conicum* (L.) Dum. (Conocephalaceae, Marchantiales), snake liverwort, is the largest of the thalloid liverworts known as a prolific source of biologically active metabolites, many of which belong to rare carbon skeletons almost exclusively found in liverworts [1]. Previous phytochemical investigations of *C. conicum* revealed a great variation of its secondary metabolism, both due to the complex genetic makeup, reflected in the fact that this liverwort comprises a complex of five cryptic species (A, C, F, J, L), and the high dependence of the secondary metabolite profile on the plant material geographical origin [2]. In this work, we wish to report on the identification of three new volatile natural products: macrocyclic sesquiterpenes germacrane, humulane, and lepidozane skeletons. These compounds were isolated and identified in the essential oil of *C. conicum* from a single population collected on the slopes of Jastrebac Mountain, Serbia. Structure elucidation was accomplished by spectral means (various 1D and 2D NMR experiments in CDCl<sub>3</sub> and C<sub>6</sub>D<sub>6</sub>, IR, UV, and MS) and the relative configuration and the complete assignment of all <sup>1</sup>H and <sup>13</sup>C NMR signals of the compounds were additionally verified by spectral simulation.



### ACKNOWLEDGEMENT

The authors acknowledge the Ministry of Education, Science and Technological Development of the Republic of Serbia for the financial support (Project 172061).

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## PP131 The volatile components of *Lindera umbellata*, “Kuromoji” oil, and its application for the touch care treatment

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**Keywords:** volatile components, kuromoji, *Lindera umbellata*, linalool, touch care treatment

### Abstract

The volatile components of kuromoji oil (*Lindera umbellata* Thunb.) produced in Shizuoka Pref. were analyzed by GC-MS. As the major components, linalool (~30%), 1,8-cineole, 4-terpinenol,  $\alpha$ -terpineol, piperitone, geranyl acetate, geraniol, dihydrocarvone, and *trans*-nerolidol were identified. The enantiomeric ratios of linalool and limonene in this oil were determined for the first time. In linalool, the ratio of (*R*)-(-)-enantiomer vs (*S*)-(+)-enantiomer was 68:32, while, in limonene, the ratio of (*R*)-(+)- vs (*S*)-(-)- was 22:78.

The touch care was performed for the cancer patients in our hospital while presenting this oil, then it became clear that the relaxation effect continues to appear longer even after the treatment compared with touch care with no aroma. The effect of aroma on touch care was determined objectively by measuring the change of heart rate during the treatment, the change of stress hormone such as  $\alpha$ -amylase activity (Amy) and chromogranin A (CgA) in saliva before and after treatment.

The HR decreased during the treatment and returned to the original level after the treatment in the absence of aroma, but it maintained a constant value after treatment whereas it decreased when the aroma was present. The Amy in saliva after treatment was decreased compared with the value before treatment, in both conditions (with aroma and no aroma). However, the amount of CgA in saliva after treatment with aroma (kuromoji oil) was decreased surprisingly compared with it of before treatment, only in touch care treatment.

## PP132 Thymol, a monoterpenoid phenol, moderately ameliorates high-dose L-arginine-induced rat gastric damage

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**Keywords:** thymol, L-arginine, gastric damage, inflammation

### Abstract

Acute pancreatic damage, such as the one following the application of a high dose of L-arginine, is frequently associated with gastric mucosa damage. Application of natural-based agents to prevent different illnesses, including gastric tissue damage, has found its way into the medicinal practice worldwide. Thymol is a monoterpenoid phenol with numerous known beneficial properties, present in a number of different essential oils. The aim of the present study was to investigate the effects of thymol in rats, applied in a single dose of 10 mg/kg, on acute gastric damage induced by L-arginine (3.5g/kg). The administration of L-arginine caused significant damage to the rat gastric mucosa characterized by numerous small dotted lesions of the mucosa mainly localized in the antral portion of the stomach. Lesions in this group of animals were graded with a score of 5/10 and the depth of mucosa affected by the lesions was  $68.8 \pm 14.1\%$ . When the animals were pre-treated with thymol, the number of lesions decreased and their localization was predominantly in the corpus of the stomach. Additionally, the lesion score for this group was 3.5/10 and the affected depth of the mucosa was  $72.3 \pm 3.6\%$ . When compared to the L-arginine-treated rats, the stomachs of those treated with thymol and L-arginine displayed a reduction only in the extent of tissue necrotization, while the present tissue edema and inflammatory-cell infiltrate remained unaffected. The results of the present study indicate that thymol has a limited effect in preventing gastric damage induced by L-arginine even though some previous studies claimed otherwise.

## PP133 Three seemingly compositionally identical pine essential oils (*Pinus nigra*, *P. mugo* and *P. sylvestris*) possess differing cytotoxic and antimicrobial potentials

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### Abstract

Although for centuries essential oils are readily used to improve oral health, their efficacy and safety are not completely investigated. In the present study, we aimed to evaluate *in vitro* toxicity of *Pinus* spp. essential oils towards human gingival epithelial cells and gingival exudate anaerobic bacterial isolates. Three pine essential oils (*Pinus nigra*, *P. mugo* and *P. sylvestris*) were analyzed using GC and GC-MS and their constituents were identified based on MS and RI data, and wherever possible by co-injection experiments. The main oil constituents of all three oils included  $\alpha$ -pinene,  $\beta$ -pinene, myrcene and  $\Delta^3$ -carene in very similar ratios. Cytotoxicity of the essential oils, tested at 0.375-1.5 mg/ml, on human gingival epithelial cells, sampled using a cotton swab, was determined by viable cell counting; viable and dead cells were stained with fluorescein diacetate and ethidium bromide, respectively, following the treatment. Antimicrobial potential (minimal inhibitory concentration, MIC) of the essential oils was assessed in the case of four gingival exudate anaerobic bacterial isolates (*Veillonella* sp., *Bacteroides* sp., *Peptostreptococcus* sp., and *Streptococcus intermedius*) using a standard microdilution method. Cell viability was most significantly affected by *P. sylvestris* essential oil (1.5 mg/ml caused death of 80% of cells), while *P. nigra* essential oil was found to be the least toxic which in the highest tested concentration produced a decrease of only 17% of cell viability. The same trend of activity was observed in the antimicrobial assay, where *P. sylvestris* essential oil exerted the lowest MIC values towards all four tested bacteria, while *P. mugo* was shown to be the weakest antimicrobial agent. We could suggest that based on our findings *P. nigra* essential oil might be considered moderately safe for use in mouthwashes since it exerted limited cytotoxicity in the highest tested concentration and achieved good antimicrobial activity when compared to the other two tested essential oils. In addition, it appears that some minor constituents are the cause of such differing biological profiles of essential oils with very similar relative abundances of the major constituents, and this deserves further study.

### ACKNOWLEDGEMENT:

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant No. 172061



## PP134 Thymol, carvacrol, and cuminol are ligand-specific antagonists of human aryl hydrocarbon receptor (AhR)

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**Keywords:** aryl hydrocarbon receptor, AhR, antagonism, phenolic compounds

### Abstract

Aryl hydrocarbon receptor (AhR) is a ligand-activated transcriptional factor that plays a pivotal role in many physiological and pathophysiological processes. AhR is activated by a number of exogenous and endogenous compounds, including those found in a diet.

In the current work, we identified thymol, carvacrol, and cuminol, monoterpenoid-like phenolic compounds found in essential oils of thyme, oregano, and cumin, as novel ligand-specific modulators of the transcriptional activity of human AhR. Our experiments were performed in concentrations that mimic the amount of tested compounds occurring in our diet, thus providing the realistic outlook on the effects of these compounds on the human AhR. We used 2,3,7,8-tetrachloro-dibenzo-dioxin (TCDD), benzo[*a*]pyrene (B(a)P) and 6-formylindolo[3,2-*b*]carbazole (FICZ) as model AhR agonists, to determine the ligand-specific antagonist effects of tested compounds. Half maximal inhibitory concentrations (IC<sub>50</sub>) were calculated. Cuminol inhibited B(a)P-induced AhR transcriptional activity (IC<sub>50</sub> 104.5 ± 14.0 μM), but potentiated TCDD- and FICZ-induced activity. On the other hand, carvacrol and thymol displayed antagonist activities against TCDD (IC<sub>50</sub> 1.9 ± 0.5 μM and 4.1 ± 0.6 μM, respectively) and B(a)P (IC<sub>50</sub> 78.2 ± 7.4 μM and 72.0 ± 12.1 μM, respectively), but strongly potentiated the FICZ-induced AhR activity. These results suggest a differential mechanism of antagonism. Antagonist activities were confirmed by the means of RT-PCR by analysing the ligand-inducible expression of AhR target gene CYP1A1 in human cancer cell lines.

In conclusion, we show that thymol, carvacrol, and cuminol are novel ligand-specific antagonists of human AhR, with potentially diverse mechanism of action.

## PP135 Use of essential oils from invasive species as vector control agents in Vietnam

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**Keywords:** Dengue fever, mosquito, larvicidal

### Abstract

Mosquitoes have been and continue to be the deadliest creatures on earth. *Culex quinquefasciatus* Say (Diptera: Culicidae), the southern house mosquito, is a vector of lymphatic filariasis as well as several arboviruses such as West Nile virus and St. Louis encephalitis virus and possibly Zika virus. The *Aedes* group of mosquitoes includes the Asian tiger mosquito, *Aedes albopictus* (Skuse), and the yellow fever mosquito, *Ae. aegypti* (L.). Both *Ae. albopictus* and *Ae. aegypti* are known vectors of yellow fever virus, chikungunya virus, dengue virus, and Zika virus. Dengue fever epidemics are frequent and widespread in Vietnam and chikungunya and Zika infections have been reported.

Insecticide resistance in mosquitoes has been increasing worldwide and could lead to a re-emergence of mosquito-borne diseases. In addition to insecticide resistance, environmental impacts of synthetic insecticides have been a chronic problem for several decades. There is a clear need for complementary vector control methods and essential oils may provide renewable and environmentally-safe alternatives to synthetic insecticides.

Non-native invasive plant species are generally detrimental to the local environments where they have been introduced. They can outcompete native plant species and reduce biodiversity. They can alter ecosystem functions, and can have substantial economic impacts. Control methods for invasive plants have generally included application of herbicides, physical cutting, or burning. However, harvesting invasive species for beneficial uses as a method for control of invasive species may provide economic incentives to offset eradication costs.

In this work, we present the essential oil compositions and larvicidal activities of invasive weedy species collected in Vietnam.

## PP136 *Pinus cembra* essential oils from three sites in the Austrian Alps

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**Keywords:** Swiss stone pine,  $\alpha$ -pinene,  $\beta$ -phellandrene, germacrene D.

### Abstract

*Pinus cembra* L., Swiss stone pine (Pinaceae), a large coniferous tree, is native in the higher regions of the Alps, Carpathians and Transylvanian Mountains. The tree is rich in resins and volatile compounds that encounter many applications in medicine, aromatherapy, cosmetics and local liquor production. Twigs with needles are the most used plant parts to extract essential oils. Several literature reference mention  $\alpha$ -pinene, limonene and  $\beta$ -phellandrene amongst the main essential oil compounds [1] but essential oils from plants originating from the Austrian Alps are not yet well documented. For the present study three locations (Styria, Carinthia and East Tyrol) were chosen to collect branches with needles as individual samples from 10 trees. The essential oils were distilled in a wood-fired copper distillery with a 80 litre boiler and a 40 litre sample container column. The essential oil composition was characterized by GC-MS and GC-FID. For all three sites, the main compounds in the essential oils from individual trees were  $\alpha$ -pinene (27.5-44.8%),  $\beta$ -phellandrene (19.4-37.8%),  $\beta$ -pinene (3.5-11.9%) and germacrene D (1.0-10.4%). For  $\alpha$ -pinene,  $\beta$ -pinene and  $\beta$ -phellandrene the East Tyrol population showed lower variability than the two other populations. Significant differences between the three populations were found for germacrene D, myrcene,  $\delta$ -3-carene,  $\delta$ -cadinene and  $\gamma$ -cadinene. Although the essential oils appeared similar regarding the main compounds, a discriminant analysis could correctly assign the samples to the collecting sites.

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## PP137 Variability of thujone content and accumulation of essential oil due to plant development and organs of sage (*Salvia officinalis* L.)

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**Keywords:** sage, ontogenesis, flowering, chemotype, chemical variability

### Abstract

The monoterpene ketones,  $\alpha$ - and  $\beta$ - thujones, are natural substances found in plants, commonly used for flavoring of foods and beverages. According to the recent regulation of the European Parliament and Council (2008), thujone in chemically pure form is not allowed to be added to foods, but it may be introduced indirectly into foods by using plants containing thujone. The EMA (European Medicines Agency) suggests a daily limit of 5.0 mg thujone/day/person for thujone in sage (*S. officinalis*) for a maximum duration of use of two weeks. While thujone is receiving more and more attention from researchers and producers, reliable data on its variability are both scarce and contradictory. Therefore, the aim of our study was to detect the influencing factors on the variation of thujone-containing essential oil (EO) depending on plant organ and harvesting time (plant development) of sage (*Salvia officinalis* L.).

Sage was harvested in 2018 at the vegetative, floral budding, flowering and after flowering phases; flowers and leaves were sampled separately. The EO content is always higher (by 30% - 182%) in the flowers, than in the leaves. The highest yield of EO was obtained from flowers in floral budding phenophase (2.82 ml/100 g) but the lowest one was also detected in flowers at after flowering stage (0.73 ml/100 g). Decreasing EO yields were indicated in both organs during the developmental phases.

The total area of the identified components in sage EO varied between 91.5% and 94.1%. During evaluation of the components, ten constituents which were higher than 1% of the GC area were identified. Leaf oils and flower oils showed different profiles.  $\alpha$ -Thujone (16.7-24.7%) was the major component of leaves while it was found only in lower concentrations in the flower oils where viridiflorol was detected as the main component (20.9-28.6%).  $\beta$ -Thujone was present both in leaves and flowers and varied from 2.9% (in flowers) to 8.6% (in leaves). Both isomers of thujone always showed higher ratios in leaves than in flowers at the same time. During the sampled phenophases, in the leaf samples the highest level of  $\alpha$ -thujone accumulation was registered at the first two developmental phases (23.15-24.70%), and was decreased significantly during the flowering period. In flowers, the same dynamics of  $\alpha$ -thujone ratio was detected with a peak at the floral budding stage (14.2%), although the differences were not significant.

The study confirmed that to reach lower thujone levels in *Salvia folium*, harvesting sage in a later phenological stage seems to be advantageous.

### ACKNOWLEDGEMENT

This research was supported by the Higher Education Institutional Excellence Program (20430-3/2018/FEKUT-STRAT) awarded by the Ministry of Human Capacities within the framework of plant breeding and plant protection researches of Szent István University.

## PP138 Variation of essential oil composition within the *Pinus cembra* tree and between trees from a population in Salzburg

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**Keywords:** Swiss stone pine,  $\alpha$ -pinene, germacrene D, cembrene, intraindividual variation

### Abstract

*Pinus cembra* L., Swiss stone pine (Pinaceae), is an Alpine coniferous tree rich in essential oils and resins. The multiple uses include wood for construction and furniture, while aromatic fractions are valuable in medicine, aromatherapy, cosmetics and local liquor production. However the essential oil diversity in the tree is not yet well documented [1]. Seven 120-170 years old trees that grew on 2000 m above sea level in the Salzburg Alps were sampled. The distance between the trees was 5 to 700 m. The analyzed plant parts include shoots with needles and twigs without needles from treetop, shoots with needles and twigs without from the lower third of the tree, shoot with needles from cone bearing twigs, one year-old cones from treetop, bark, heartwood and sapwood from the lower part of the trunk. Essential oils were obtained by hydrodistillation and volatile fraction by SPME and were characterised by GC-MS-FID. Highest essential oil contents were present in cones and needles while woody parts were low in essential oils. Needle essential oils from all parts of the crown were rich in  $\alpha$ -pinene,  $\beta$ -phellandrene,  $\beta$ -pinene, limonene and germacrene D. In cone essential oil,  $\alpha$ -pinene,  $\beta$ -pinene and limonene prevailed while twig essential oils presented usually more  $\beta$ -phellandrene than  $\alpha$ -pinene. Bark contained more essential oil than sapwood and heartwood. These oils contained 25-50% diterpenes as cembrene, cembratrienol and methyl daniellate. In sapwood oils also up to 30% alkane derivatives (mainly nonanoic acid, nonylaldehyde) were found. A multivariate statistical analysis showed that there were also differences in essential oil composition between individual trees. SPME analysis extracted preferentially the more volatile monoterpenes and only traces of diterpenes. This technique appeared suitable to characterize needle and cone oils.

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## PP139 Volatile compounds and phenolic compounds and phenylalanine ammonium liase activity in *Peperomia pellucida* (Piperaceae) after rhizobacteria inoculation

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**Keywords:** rhizobacteria, essential oil, phenolic compounds, phenylalanine ammonia-lyase

### Abstract

*Peperomia pellucida* (L.) Kunth is widely distributed throughout the Neotropics, Africa, Southeast Asia, and Oceania. Several studies have described its antimicrobial, cytotoxic, antidiabetic, and other bioactivities. This study aimed at the evaluation of secondary metabolism of *P. pellucida* during association with rhizobacteria. Plants from Amazon were cultivated for 30 days in a greenhouse and inoculated with *Pantoea* sp. Leaves were collected at seven, 21 and 30 days post inoculation (dpi) and its essential oil (EO) was extracted using the Likens-Nickerson apparatus, and chemical composition was performed by GC-MS. At 30 dpi, phenylpropanoids and derivatives increased from 47.3% to 52.1% and oxygenated sesquiterpenes decreased from 28.5% to 23.8% in comparison to control plants. The main constituents were 1,2-dimethoxy-4-(2-methoxyethenyl)benzene (1), ishwarane (2) and dillapiole (3). The compound 1 had an increase of 20% at 30 dpi (26.1%-31.4%). However, the amounts of compound 2 were constant in inoculated plants (27.5%). Total phenolic content (TPC) was measured by the Folin-Ciocalteu method, and phenylalanine ammonia-lyase (PAL) enzyme activity was evaluated by quantification of *trans*-cinnamic acid. The TPC increased 11.2% (26.4-29.4 mg EAG.g<sup>-1</sup> of extract) and 30.3% (26.9-35.1 mg EAG.g<sup>-1</sup> of extract) in inoculated plants at 21 and 30 dpi, respectively. Furthermore, PAL enzyme activity displayed an increase of 30% and 38% at 21 (23.0-30.8 μU.mL<sup>-1</sup>) and 30 dpi (29.0-40.0 μU.mL<sup>-1</sup>). These changes were attributed to the role of secondary metabolism in the induction of plant defense responses to bacterial colonization.

## PP140 Volatile compositions and antifungal activities of native American medicinal plants; focus on the Asteraceae

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**Keywords:** American Indian, traditional medicine, antifungal activity

### Abstract

Native Americans have had a rich ethnobotanical heritage for treating diseases, ailments, and injuries. Unfortunately, much of the traditional medicine knowledge of Native North American peoples has been lost due to population decimation and displacement from their native lands by European conquerors. Nevertheless, there are still some remaining sources of information about Native American ethnobotany. In this work, we have consulted the ethnobotanical literature for members of the Asteraceae used in Cherokee and other Native American traditional medicine that are native to the southeastern United States. The essential oils have been obtained by hydrodistillation and analyzed by GC and GC-MS. In addition, we have carried out antifungal screening of the essential oils.

## PP141 Volatile compounds of wild-growing *Rosa canina* fruit

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**Keywords:** *Rosa canina* L., rosehip, pulp, seeds, volatiles

### Abstract

*Rosa canina* L. (Rosaceae), a dog rose, is one of the most widespread wild species of the genus *Rosa* which grow almost everywhere in Croatia, especially in its Mediterranean region. The plant is appreciated for its shiny red fruits, called rosehips, whose consumption is popular in Croatia mainly in form of marmalade, jam and flavour teas. It is well known that rose hips of *R. canina* are rich source of various bioactive compounds such as vitamins (especially vitamin C), phenolic compounds (especially flavonoids), carotenoids and fatty acids, with potential positive effect on human health. Most studies of *R. canina* rosehips have been focused primarily on those compounds and their antioxidant and anti-inflammatory properties. However, literature data regarding volatile compounds of *R. canina* hips are rather limited. Hence, the aim of this study was to characterize the volatile compounds of *R. canina* hips originating from Croatia.

The volatile compounds, commonly designated as aroma compounds, are naturally present in fruits. Those compounds, together with non-volatile compounds responsible for taste, are responsible for the flavour of fruits. A great variety of aroma compounds is present in fruits. Regarding various rose hip species, volatile compounds comprise mostly alcohols, aldehydes, ketones, monoterpenes and sesquiterpenes. According to literature, the sampling of the rosehips' volatile compounds was achieved by various techniques, such as hydrodistillation, extraction in Soxhlet apparatus, headspace solid phase microextraction and immersion solid phase microextraction, always followed by gas chromatography-mass spectrometry (GC-MS) analysis.

Hydrodistillation, the most usual method for the essential oils isolation, *i.e.* volatile compounds, was chosen for the isolation of volatile compounds from *R. canina* fruits. As far as we know, only one study reported the volatile compounds of *R. canina* obtained by hydrodistillation of the entire rosehips. In this study, the entire rosehips were used for the isolation of volatiles, as well as seeds and fleshy pulp separately. The chemical composition of the essential oils was analyzed by GC-MS.

The dominating compound in *R. canina* essential oil, obtained from entire rosehips, was norisoprenoid vitispirane followed by ketone 6-methylhept-5-en-2-one. Vitispirane was identified in *R. canina* fruit pulp, too, but in much smaller amount, while the content of 6-methylhept-5-en-2-one was almost the same. Other quantitatively important components in this sample were C<sub>9</sub> - C<sub>22</sub> hydrocarbons, and aldehydes hex-2-enal, furfural and hept-2-enal. The essential oil isolated from *R. canina* seeds was characterized by a high content of phenylacetaldehyde, moderate content of benzyl isothiocyanate and absence of vitispirane and 6-methylhept-5-en-2-one. Regarding terpenes, the typical constituents of essential oils, monoterpene limonene was dominated component in all essential oils.



## PP142 Volatile compounds profile and trichome micromorphology of the endemic *Veronica saturejoides* Vis. ssp. *satpurejoides*

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### Abstract

Genus *Veronica* is the largest genus of the Plantaginaceae family and is divided into 13 subgenera according to morphological and molecular evidence. *Veronica saturejoides* Vis. ssp. *satpurejoides* is endemic to Croatia and it belongs to Central European hemicyptophytes. Aerial parts of the flowering *Veronica saturejoides* Vis. ssp. *satpurejoides* were collected from Dinara mountain in July 2017 and air dried for three weeks in a single layer in a well ventilated room, and protected from direct sunlight. The profile of volatile compounds and micromorphology of the trichomes of this species endemic to Croatia was investigated. Clevenger-type apparatus was used for the isolation of the essential oil (EO). Water distilled volatile compounds (VC) from the essential oil and hydrolate have been analyzed by GC and GC-MS using a VF-5ms capillary column. The total yield of VC in the EO was 0.04%, based on dry weight of the samples. Thirteen compounds were determined representing 84.5% of the total VC. The most abundant compounds identified were hexadecanoic acid, hexahydrofarnesyl acetone and docosane. In the hydrolate using headspace injection needle, eight compounds were identified representing 84.3% of the total VC. The most abundant compounds were trans-1(7),8-p-mentadien-2-ol, allo-aromadendrene, and Z-methyl isoeugenol. Scanning electron microscopy was conducted and non-glandular (unbranched, bi-cellular to multicellular) and glandular (composed of one stalk cell and two elliptically formed head cells) trichomes could be observed on stems, leaves, and the calyces. In our review we found just few *Veronica* species that were investigated in the field of volatile compounds profile. GC-MS studies have been performed on *Veronica thymoides* subsp. *pseudocinerea* where the most abundant constituent was hexatriacontene (21%) [1]. In another research, Feng [2] identified essential oil components of *Veronica linariifolia* Pall. ex Link, and found that the main constituents were cyclohexene (25.83%),  $\beta$ -pinene (11.61%), 1S- $\alpha$ -pinene (10.65%),  $\beta$ -phellandrene (10.49%),  $\beta$ -myrcene (10.42%), and germacrene D (4.99%). In our previous research in the oil of *V. spicata* the most abundant compound was phytol [3]. Given the diversity of the presented results we can say that the present study gives additional knowledge about volatile compounds and trichome micromorphology of the genus *Veronica*.

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## PP143 Volatile organic compounds in hydrosols obtained from some wild growing plants in Lithuania

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**Keywords:** *Betula pendula*, *Sambucus nigra*, *Fillipendula ulmaria*, *Pinus mugo*, *Tilia cordata*

### Abstract

Hydrosols (known as aromatic or plant waters) are obtained after distillation procedure during essential oil production. Usually, hydrosols are enriched not only with small amounts of terpenoids, but also with valuable non-volatile but water-soluble compounds, such as: polyphenols, flavonoids, tannins, sugars, organic acids or vitamin C. Only a few plants growing wild in Lithuania synthesize enough quantities of essential oils that it would be cost-effective to extract them. In contrast, most low amount essential oil bearing plants are ideal for hydrosols production. This product is much safer to use for children, pregnant women or allergic people.

The work represents data of several year of research on volatile chemistry of five hydrosols obtained from some plants (silver birch, mountain pine, small-leaved linden, common elder and meadowsweet) growing wild in Lithuania.

Volatile organic compounds (VOCs) of the hydrosols were extracted using liquid-liquid extraction [1] and the qualitative GC-MS analyses were performed on a chromatograph Shimadzu GC-2010 PLIUS interfaced to Shimadzu GC-MS-QP2010 ULTRA mass spectrometer and fitted with a Rxi-5MS (30mx0.25mmx0.25mm) capillary column. Major VOCs determined in analysed samples are listed below:

Table 1. Major VOCs in analysed hydrosols

Plant botanical name	Major compounds (%)
<i>Betula pendula</i>	Eugenol (9.4-14.5), $\alpha$ -cadinol (3.6-10.5), linalool (6.0-11.1)
<i>Pinus mugo</i>	$\alpha$ -Terpineol (17.3-19.5), terpinen-4-ol (11.9-15.2), bornyl acetate (5.6-17.8)
<i>Tilia cordata</i>	Phenylethyl alcohol (7.5-13.3), hotrienol (6.0-8.6), 4-methoxyphenylethanol (4.8-9.0)
<i>Sambucus nigro</i>	Hotrienol (26.3-38.0), <i>cis</i> -linalool oxide (pyranoid) (11.7-27.7), linalool (5.2-8.5)
<i>Fillipendula ulmaria</i>	Salicyl aldehyde (12.6-66.7), methyl salicylate (6.6-50.6), linalool (0.5-1.9)

Despite that all investigated samples showed good radical scavenging abilities evaluated by DPPH<sup>•</sup> assay, a significant amount of total phenolic compounds was found only in *Fillipendula ulmaria* hydrosol.

### ACKNOWLEDGEMENTS

Authors are grateful to UAB Kvapu namai for samples, technical and financial support.

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## PP144 Volatiles of *Lunaria annua* L. obtained by conventional and microwave-assisted isolation and their cytotoxic activity

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**Keywords:** *Lunaria annua*, isothiocyanates, glucosinolates, microwave-assisted isolation, cytotoxic activity

### Abstract

*Lunaria annua* L. (annual honesty) belongs to Brassicaceae family, which is highly characterized by glucosinolates (GSL) [1]. It is native to the Balkans and southwest Asia. Unmodified *L. annua* oil has been used on a small scale as an industrial lubricant and as a source of nervonic acid used in pharmaceutical and nutraceutical applications. The goal of the present study was the isolation of the volatiles by conventional methods which include hydrodistillation using a Clevenger type apparatus, CH<sub>2</sub>Cl<sub>2</sub> extraction after autolysis as well as by modern technique using microwave (MW) distillation and extraction. Isothiocyanates (ITCs), the main volatiles identified by GC-MS, originated from GSL degradation, i.e. isopropyl ITC from glucoputranjivin (1), 5-(methylsulfinyl)pentyl ITC from glucoalyssin (2) and 6-(methylsulfinyl)hexyl ITC from glucohesperin (3). Two other ITCs, namely pent-4-enyl ITC and hex-5-enyl ITC were also detected indicating the presence of two more corresponding GSLs. However, LC-PDA-MS/MS analysis of desulfoGSLs confirmed the presence of only 1, 2, and 3. Thus, it can be suggested that pent-4-enyl ITC and hex-5-enyl ITC are formed due to the thermal instability of 5-(methylsulfinyl)pentyl ITC and 6-(methylsulfinyl)hexyl ITC, respectively during GC-MS analysis.

Hydrodistillate, CH<sub>2</sub>Cl<sub>2</sub> extract after autolysis as well as MW extract were evaluated for their cytotoxic activity against two tumor cell lines. The activities were observed after four, 24, 48, and 72 h and expressed as IC<sub>50</sub>. Hydrodistillate, CH<sub>2</sub>Cl<sub>2</sub> extract and MW extract showed the best cytotoxic activity on human lung cancer cell line A549 during incubation time of 72 h (IC<sub>50</sub> 152.8, 18.79, and 33.46 µg/mL, respectively), while the best effect on breast cancer cell line MDA-MB-231 was after 48 h (IC<sub>50</sub> 67.44, 6.0 and 11.8 µg/mL, respectively). Following the Al-Gendy, et al. [2] criteria, who categorized the activities against the tested cell lines based on IC<sub>50</sub> values, CH<sub>2</sub>Cl<sub>2</sub> extract and MW extract may be considered as highly active, while hydrodistillate as moderately active.

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## PP145 Volatiles of *Piper crocatum* Ruiz & Pav (Piperaceae): Chemical differences between maroon- and green-underside leaves

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**Keywords:** red betel leaves, *Piper crocatum* Ruiz & Pav, essential oil, *trans*-sesquisabinene hydrate, linalool

### Abstract

The genus *Piper* is a large member of Piperaceae, a family of plants that has undergone a long history of evolution and diversity in response to various environmental factors. Our investigation into the chemistry of the genus led to a serendipitous discovery of a local chemovar of *Piper crocatum* Ruiz & Pav, a species which is endemic to Peru in South America and also commonly found in Indonesia. To the best of our knowledge, this is the first report of its occurrence in Malaysia. The climbing vine is a rare species, and highly prized for its colourful leaf as an exotic indoor plant. A distinct morphological trait of the vine is that it has leaf upper surface that is olive green, heavily spotted with pinkish silver markings, while the leaves' undersides are deep maroon in colour. Initially, we found out that some branches on the vine bore leaves with green undersides, a phenomenon believed to be due to different maturity levels of the leaves. However, upon further examination, it was observed that the occurrence of green-underside leaves was not homogenous across the plant. It rather seemed to occur at random. In addition, the odour of the leaves, when crushed, was also distinctly different, suggesting possible differences in their volatile constituents. Hence, we investigated the volatile constituents that could contribute to the odours. GC-MS analysis of the essential oil profiles revealed that sesquiterpenoids made up 82.0% of the constituents in the maroon-underside leaves, while the green-underside leaves were made up of 52.5% sesquiterpenoids and 47.2% monoterpenoids. Among the sesquiterpenoids, 7-*epi-trans*-sesquisabinene hydrate and *trans*-sesquisabinene hydrate were common to both types of leaves, but were 5.6% and 13.5% higher, respectively, in the maroon-underside leaves. Among the monoterpenoids, linalool, terpinen-4-ol and  $\alpha$ -terpineol were common to both, but were 13.4%, 3.6% and 10.0% higher, respectively, in the green-underside leaves. Phytol was the only diterpenoid, detected in both types, at a higher abundance (1.1%) in the maroon-underside leaves compared to the green-underside leaves (0.3%). From a total of 68 constituents identified in both types of leaves, we did not identify any constituents derived via the phenylpropanoid biosynthetic pathway. The phenylpropanoids, notably eugenol and isoeugenol, are common in other *Piper* species such as *P. betle* L., *P. lanatum* Roxb., *P. pedicellosum* Wall., *P. penangense* C. DC. and *P. sarmentosum* Roxb.. The absence of such constituents in *P. crocatum* Ruiz & Pav may be of chemotaxonomic significance.

## PP146 Forest medicine in aromatherapy

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**Keywords:** forest medicine, shinrin yoku, Siberian fir needle oil isobornylacetate, mood, olfactory adaption

### Abstract

Forest medicine is a new interdisciplinary science, which finds its origin in Japan. Especially forest bathing (“shinrin-yoku” – walking in forest environments) is experiencing an increasing importance in complementary medicine even in Europe. Studies have shown that forest environments like shinrin-yoku have several beneficial effects on human health. It has been reported that walking in forest environments not only decreases the risk of psychosocial stress-related diseases, but also enhances human immune function due to the increase of human natural killer (NK) activity, number of NK cells, and the intracellular levels of anti-cancer proteins, suggesting a preventive effect on cancers. Furthermore, shinrin yoku reduces blood pressure, heart rate and stress hormones and increases the activity of parasympathetic nerves and reduces the activity of sympathetic nerves. These findings raised the question of whether aromatherapy could achieve similar psychophysiological effects by inhaling essential oils due to the hedonic and semantic impact of their odor.

In the present pilot-study, Siberian fir needle oil (*Abies sibirica*) and isobornyl acetate were tested. Due to the well-known phenomenon of “olfactory adaptation” it was also investigated whether a permanent exposure to an odor, as used in aromatherapy, changes its psychophysiological effect on humans compared to an intermitted odor confrontation.

Forty-eight healthy nonsmokers, 24 men and 24 women, volunteered for this study. They were randomly divided into two groups (“fir oil”, “isobornyl acetate”) and compared with a control group without odor. Each group consisted of 12 males and 12 females, respectively. To assess the adaptation-effect, an intensity rating was performed every five minutes within each session of 30 minutes. Blood pressure, heart rate and mood were determined in time (prior to and after odor exposure). Hedonic valence, familiarity and expected effect of presented odors (fir oil, isobornyl acetate, control) were rated at the end of the second session. Data will be presented, indicating a difference between odors on the effect on humans.

### ACKNOWLEDGEMENT

Authors are thankful to all participants who volunteered for this study.

## PP147 Comparison of the human skin sensitization potency to the CLP regulatory classification using fragrance substances

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### Abstract

In the past decades, a wealth of research was done to identify non-animal integrated testing strategies equivalent to results obtained by using in vivo methods. It was recognized that sensitization potency represents a particularly challenging endpoint for which further sub-categorization in non-sensitizer, weak and strong sensitizer is not always evident. Known for their sensitizing properties, fragrance substances are suitable testing materials for the development and validation of alternative methods to animal testing.

In this work, 50 fragrance substances were used to compare the regulatory classification for skin sensitization hazard under the EU CLP Regulation versus the relative human skin sensitization potency categorization resulting from the HPIRT or HMT data. This comparison shows that the EU CLP classification underperformed in differentiation between irritating and sensitizing effect including differentiation of the sensitization potency for „moderate“, „weak“, „very weak“ or „non-sensitizers“. Cases of overclassification result mainly from notified classifications under the EU CLP Regulation or from evaluations under REACH where the outcome points at other hazardous endpoints such as eye irritation, respiratory sensitization or acute toxicity, in contrary to the positive data from HRIPT or HMT. Substances with “moderate” (Category 3), “weak” (Category 4) and “very weak” (Category 5) sensitizing potency based on the HRIPT or HMT data, but not classified for skin sensitization under CLP Regulation, were scrutinized in more detail. With one outlier, these substances are predicted as weak sensitizers. The present research is addressing the inherent limits of the EU CLP classification on the endpoint skin sensitization. While objectively assessing skin sensitization potency is an important toxicological target, it is premature to seek for an adequate regulatory alignment and complementation by using regulatory classification on this endpoint up to the frontiers of toxicological progress. Unless new regulatory provisions would contribute to such an alignment, risk management measures should not depend on the potency quantification of the hazard on skin sensitization where the potency is between „moderate“ to „very weak“.

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## PP148 Evaluation of antibacterial activities and chemical compositions of *Citrus medica* L. var. *sarcodactylis* Swingle essential oils

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**Keywords:** *Citrus medica* L. var. *sarcodactylis* Swingle, Rutaceae, essential oil, antibacterial activity

### Abstract

*Citrus medica* L. var. *sarcodactylis* Swingle (Rutaceae) known as “Buddha’s hand” or “fingered citron” used in folk medicine as tonic, antispasmodic, antiemetic, expectorant and inhaler.

In present study, *C. medica* leaves and fruits were hydrodistilled for 3 h by Clevenger-type apparatus. The components of essential oils were analysed by Gas chromatography (GC) and Gas chromatography-Mass spectrometry (GC-MS), simultaneously. Limonene (47.2, 49.6, 47.6 %) and geranial (13.5, 2.2, 4.3 %) were found as a major components for essential oils of leaves, ripe fruits and unripe fruits, respectively. Additionally, major component for essential oil of *C. medica* leaves was found neral (9.7%).  $\gamma$ -Terpinene was identified as a major component for ripe fruits (29.8%) and unripe fruits (27.9%). The potential in vitro antibacterial activity of the essential oils were evaluated using the broth microdilution assay. A panel of oral human pathogenic strains *Escherichia coli* NRRL B-3008, *Salmonella typhimurium* ATCC 13311, *Bacillus cereus* NRRL B-3711, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538 were used. Minimal Inhibitory Concentrations (MIC) of the samples were determined, where chloramphenicol and ciprofloxacin were used as a positive controls in the experiments. Essential oils were found relatively less effective against all pathogenic strains (20 mg/mL). Essential oils of leaves and unripe fruits were found more effective against *P. aeruginosa* (10 mg/mL) than other samples (>20 mg/mL).

## PP149 Workability and quality of extended Safety Data Sheets

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### Abstract

The workability and quality of the safety data sheet (SDS) implies a smooth information exchange on the safe handling of hazardous substances between registrants under REACH and the downstream users in the supply chain. In this respect, the SDS can be considered as the most important source of information for the users on the safety data generated under REACH verified against reality. The addition of exposure scenarios to an existing SDS resulted in extended SDS. Besides of controlling exposure, this addition aimed at increasing the transparency and facilitation of the communication in the supply chain. In the same time, the resulting eSDS is characterized with a higher level of complexity which requires specialized technical expertise and represents a burden for SMEs especially when safety information for mixtures needs to be provided. Several initiatives are ongoing on improving the workability and quality of extended Safety Data Sheets. On one side, REF-5 enforcement project is dealing with the compilation, communication and implementation of safe use information in the supply chain. Target substances were those registered and for which exposure scenarios were required. A dedicated project REACH2SDS developed a criterion based scheme for assessing the availability of information concerning workplace exposure, risk and risk management measures (RMMs) in the Chemical Safety Report (CSR) and the consistency to extended SDS. On the other side, the Exchange Network of Exposure Scenarios is working with several sector associations to ensure that a right communication approach is being implemented up and down the supply chain.

During the second REACH review a concrete action was formulated, the REACH Review Action 3, on which it was then further elaborated in a Workshop on Improving the workability and quality of extended Safety Data Sheets (March, 2019) to (i) focus on user-targeted information, (ii) support the introduction of minimum requirements for the exposure scenarios for substances and mixtures in SDS and (iii) develop a methodology for SDS of mixtures. The current status and results of this work are presented and discussed herein. A new tool known as a “dynamic SDS” will become available to facilitate the extraction of information from SDS based on the needs and uses by the actors along the supply chain. It needs to be acknowledged that minimum standards provide for a horizontal applicability, e. g. applicable to every situation requiring safety handling. Sector specific needs will require dedicated input from interested actors (SMEs and downstream users) and consistency of exposure scenarios. At present, the most practical solution is associated with the development of sector specific user maps and harmonization of linguistic challenges having in mind the link to the Occupation, Safety and Health.



## PP150 Chemical characterization of *Cnidium silaifolium* ssp. *orientale* (Boiss.) Tutin essential oils and *in vitro* biological evaluation

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**Keywords** Apiaceae, *Cnidium silaifolium* ssp. *orientale*, antimicrobial, antioxidant

### Abstract

*Cnidium silaifolium* ssp. *orientale* (Boiss.) Tutin of the Apiaceae is the only representative of the species and known as “galyabişotu” in Turkey. The chemical composition of the essential oils obtained by hydrodistillation from the aerial parts, fruits and roots of *Cnidium silaifolium* ssp. *orientale* were analyzed using GC-FID and GC-MS, simultaneously. In addition, *in vitro* antimicrobial and antioxidant activities of the essential oils were evaluated.

A total of 109 volatile compounds were identified in the essential oils of *C. silaifolium*, where the main component was characterized as  $\alpha$ -pinene (50.3 %) in root; germacrene D (20.3%) in fruit; and  $\beta$ -caryophyllene (18.7%) in aerial parts, respectively. The *in vitro* broth microdilution method was used against *Salmonella typhi* ATCC 6539, *Acinetobacter baumannii* ATCC 19606, *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19115, *Helicobacter pylori* ATCC 43504 and *Mycobacterium avium* ssp. *avium* ATCC 25291. Mild minimum inhibitory concentrations were observed against some of the tested human pathogens. While the best inhibitory activity of the root and aerial part essential oils was against *S. aureus* (0.039 and 0.156 mg/mL), fruit essential oil was more effective against the pathogen *B. cereus* (0.078 mg/mL) among the tested microorganisms. The DPPH and ABTS radical scavenging activity tests showed no antioxidant activity compared to the standards. Further biological evaluations are ongoing.

## PP151 Comparison of volatile constituents of *Lippia javanica* obtained by microwave-assisted and conventional hydrodistillation methods

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### Abstract

*Lippia javanica* is a well-known aromatic shrub indigenous to South Africa. The plant is commonly referred to as lemon bush or 'fever tea' and is used traditionally for treatment of respiratory complaints and skin infections. The essential oil isolated from *L. javanica* exhibits healing properties including antiseptic action, treatment of insect bites, head lice and scalp infections. Microwave-assisted hydrodistillation (MAHD) and the conventional Clevenger-type hydrodistillation (CHD) methods were used to obtain the volatile constituents of *L. javanica* aerial parts. The two methods were compared in terms of extraction time and chemical composition of the essential oil. *Lippia javanica* samples (n= 5) were collected from five different locations in the Gauteng province of South Africa and the essential oils obtained using the two hydrodistillation methods. Gas chromatography-mass spectrometry-flame ionisation detection (GC-MS-FID) analysis was performed to obtain the chemical profiles of the oils. Microwave- assisted took shorter duration of time (15-30 min) compared to CHD where oils were distilled for 180 min. Furthermore, the oil yield with MAHD was lower compared to the conventional method. The composition of the oils obtained by the two methods showed qualitative and quantitative differences. Although the major constituents in both cases included; myrcene, ipsenone, E-tagetone, Z-tagetone, myrcenone and ocimenone. MAHD showed profiles consistent with high amounts of ipsenone (47.4%), Z-tagetone (18.9%) and myrcenone (13.4%) as major constituents, while the CHD essentialoil was dominated by myrcene (28.3%) and myrcenone (42.7%). These results clearly indicate that the essential oil composition is highly variable depending on the isolation procedure involved.

## PP152 Distillation time effects on essential oil compositions of *Satureja cuneifolia* Ten.

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### Abstract

*Satureja cuneifolia* Ten., belonging to Lamiaceae family, is generally used in food industry as spices, flavoring agents, herbal teas besides essential oil production [1,2]. *S. cuneifolia* was collected in August 2018 from Sütçüler/Isparta (Turkey). Aerial parts of the plant were subjected to hydrodistillation using a Clevenger apparatus. The oils were obtained at the given four different distillation times (5, 30, 60 and 180 minutes) and analyzed by GC-MS/FID, separately.

Total distilled essential oil contains 42 components identified and yield of the total oil is found as 2.7%. The essential oil taken in the first five minutes contains 42 compounds and yield of the first fraction is found as 0.72%, while last fraction (180 minutes) contains 39 compounds and yield of the last fraction is determined as 0.06%. Major compound is carvacrol 48.1% in total oil. Main component is carvacrol and ranged from 38.9 to 61.8% depending on distillation times. The other major components were p-cymene 2.3-19.2%,  $\gamma$ -terpinene 6.4-11.0% and geraniol 2.3-10.1%.

The results showed that amount of essential oil and compositions of them were affected by distillation times. The results showed that 30 minutes distillation time was the most appropriate time for higher carvacrol content, while 60 minutes distillation time was the most appropriate time for yield of the essential oil.

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## PP153 Ginger essential oil improves microbiological quality of cooked pork sausages

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### Abstract

The aim of the present study was to apply ginger essential oil (GEO) as additive to improve microbiological quality of cooked pork sausages during 30 days of refrigerated storage.

Cooked pork sausages were created in local industrial plant. The sausage batter consisted of meat from pork shoulder, pork back fat, pork skin emulsion, ice water, soy protein, nitrite salt and spice mix. GEO which was of commercial origin, was added to the sausage batters at concentrations of 0.1  $\mu\text{L/g}$  (GEO1), 0.5  $\mu\text{L/g}$  (GEO2) and 1.0  $\mu\text{L/g}$  (GEO3). GEO was mixed with salt and added into the mixture prior to stuffing. The batch without added GEO was assigned as control (C). After stuffing in artificial casings, all sausages were pasteurized until an internal temperature of 72°C was reached. Thereafter, the sausages were cooled and stored at 4°C until an analyses.

Microbiological analyses were performed on three samples from each sample group. Twenty grams of sample were homogenized for 10 min at 200 rpm in 180 mL 1 g/L buffered peptone water and then serial of decimal dilution were prepared. One milliliter of each dilution was placed in a sterile Petri dish and poured with convenient media depending on the type of investigated microorganism. The following microbial analyses were performed: total aerobic mesophilic bacteria count, *Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes*. Results were expressed as the number of colony forming units per gram (cfu/g).

The highest concentration of GEO significantly ( $P<0.05$ ) reduced the total number of aerobic mesophilic bacteria (16.7 cfu/g) comparing to control samples (33.3 cfu/g) at the first day of storage. However, there was no significant ( $P<0.05$ ) difference between GEO1, GEO2 and C samples. *Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes* were not detected in any investigated samples at the first day of storage.

As expected, for all treatments the total aerobic mesophilic bacteria count significantly ( $P<0.05$ ) increased during 30 days of storage. All three concentrations of GEO resulted in significantly ( $P<0.05$ ) reduced values of total aerobic mesophilic bacteria count (GEO1: 133.3, GEO2: 113.3 and GEO3:96.7 cfu/g) compared to control samples (273.3 cfu/g) at 30th day of storage. GEO2 samples were not significantly ( $P<0.05$ ) different from GEO1 nor GEO3 samples, however there was significant ( $P<0.05$ ) difference between GEO1 and GEO3 samples. The analysed foodborne pathogenic bacteria (*Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes*) were not detected in any of the examined samples of cooked pork sausages at 30th day of storage.

This research demonstrated that the application of ginger essential oil used at different concentrations reduced the microbial growth of cooked pork sausages. GEO at concentration of 1.0  $\mu\text{L/g}$  was the most effective in retarding the microbial growth.”

## PP154 Volatile components of two *Onosma* from Turkey

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### Abstract

The genus *Onosma* L. (Boraginaceae) is represented in Turkey by 105 species (110 taxa), 53 of them and 1 variety are endemic for Turkey [1-3]. Some species of *Onosma* are used as herbs, traditional medicine such as against burns, wounds and ailments [4,5].

Hydrodistilled essential oils of the aerial parts of *Onosma isaurica* Boiss. & Heldr. And *Onosma bulbotrichum* DC. were analyzed by GC and GC-MS systems, simultaneously.

The oil of *O. isaurica* contained hexahydrofarnesyl acetone (20.3%), phytol (19.0%), farnesyl acetone (8.1%) and neophytadiene isomer I (7.0%) as main constituents. The oil of *O. bulbotrichum* was characterized by the occurrence of hexahydrofarnesyl acetone (11.6%), farnesyl acetone (9.9%), hexadecanal (8.8%) (E)-geranyl acetone (7.4%) and neophytadiene isomer I (7.3%) as major components.

In a previous study, main component of the essential oil of *O. sieheana* roots was found p-cymene, while *O. microcarpum* was reported tymol, carvacrol and n-heptane as major components [6,7]. *Onosma echioides* (L.) L. var. *columnae* Lacaita was determined hexadecanoic acid and phytol were major components in flower oils, while phytol and hexahydrofarnesyl acetone were the main components in the leaf oils [8].

To the best of our knowledge, this is the first report on the GC and GC/MS determination of the essential oil composition of the *O. isaurica* Boiss. & Heldr. and *O. bulbotrichum* DC. species studied.

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