

NATURAL VOLATILES & ESSENTIAL OILS

A Quarterly Open Access Scientific Journal



46th INTERNATIONAL
SYMPOSIUM
ON **ESSENTIAL OILS**
13-16 September 2015

ABSTRACTS

NV&EO

Publisher: BADEBIO Ltd.



NVEO**NATURAL VOLATILES
& ESSENTIAL OILS***A Quarterly Open Access Scientific Journal***Editor in Chief**

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NVEO is a major forum for the publication of new findings and research into natural volatiles and essential oils. It is created by the Permanent Scientific Committee of ISEO (International Symposium on Essential Oils). The journal is principally aimed at publishing proceedings of the ISEOs, but is also a peer reviewed journal for publishing original research articles and reviews in the field of natural volatiles and essential oils including wide ranging related issues on the analysis, chemistry, biological and pharmacological activities, applications and regulatory affairs, etc.

Published four times per year, NVEO provides articles on the aromatic principles of biological materials such as plants, animals, insects, microorganisms, etc. and is directed towards furthering readers' knowledge on advances in this field.

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WELCOME

We are pleased to welcome you to the 46th International Symposium on Essential Oils here in the historic city of Lublin. Each year, the Symposium gathers expert professionals and scientists in the field of essential oils and natural volatiles. ISEO symposia have been organized annually in Europe since 1969. The main purpose of the first meeting, which was held in Leiden (The Netherlands), was a discussion on the analytical and associated issues in essential oil research. In 1971, representatives of the essential oil industry were invited to join the scheduled congresses. The scope of these meetings has significantly broadened over the years. Although the main topics of the ISEO symposia have concerned with the methods and techniques of essential oil analysis and the structure elucidation of the oil constituents, further topics, such as the biogenesis of volatile compounds, the quality control and standardization of essential oils, the medicinal properties of essential oils and their components, the safety of essential oils and volatile compounds, and the asymmetric synthesis of the constituents have been discussed.

The symposium attracts the attention of participants from European countries and the broader scientific world engaged in essential oils, as evidenced by the presence of scientists and industry representatives from Japan, Iran, Israel, Egypt, South Africa, Turkey, Brazil, Chile, and the United States, among others.

The venue of the 46th ISEO is Lublin, the biggest town in Eastern Poland, with a population of 347,000 inhabitants. It is an old and historic city, with numerous architectural monuments, a unique atmosphere, and a very picturesque location. Lublin, with its 700-year-old tradition, is a city where history intertwines with the modern times, and where the rich cultural heritage influences our everyday lives. The magical atmosphere of Lublin is especially felt in the Old Town, where numerous restaurants and quaint pubs are steeped in the history of the Lublin historic tenements.

The Symposium will include plenary lectures, oral presentations, young scientists' lectures, poster sessions, and social activities. Our main goal is to bring together scientists from universities, research centers and industries from all over the world, and offer to all a meaningful and attractive symposium, blending different cultures and knowledge together, and promoting new connections and collaborations to advance the diverse field of essential oil research for the benefit of society.

Abstracts of the symposium are published in the quarterly open Access Journal of ISEO, Natural Volatiles and Essential Oils (NVEO). The journal will be pleased to accept review and research papers in related fields.

We wish all of the ISEO 2015 participants a very successful and enjoyable symposium, and many wonderful memories of a very pleasant stay in Lublin. Thank you for joining this meeting.

Dr. Agnieszka Ludwiczuk

President of the Organizing Committee of ISEO 2015

Prof. Dr. K. Hüsnü Can Baser

Editor-in-Chief, NVEO

ISEO 2015 COMMITTEES

ISEO 2015 Organizing Committee

President: Agnieszka Ludwiczuk

Secretary: Krystyna Skalicka-Woźniak

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Aleksandra Józefczyk
Małgorzata Kozyra
Wirginia Kukuła-Koch
Marek Kwietniewski
Tomasz Mroczek
Elwira Sieniawska
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Krzysztof Kamil Wojtanowski
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International Symposium on Essential Oils (ISEO) Permanent Scientific Committee

Yoshinori Asakawa (Tokushima, Japan)
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Éva Németh-Zámboriné (Budapest, Hungary)

ISEO 2015 ORGANIZERS



Medical University of Lublin



Chair and Department of Pharmacognosy with Medicinal Plant Unit
of Medical University of Lublin



Polish Academy of Sciences

SUPPORTING ORGANIZATIONS



International Federation of Essential Oils and Aroma Trades (IFEAT)



Polish Society of Aromatherapy



Section of Herbal Drugs of the Polish Pharmaceutical Society

SPONSORS and EXHIBITORS

Main Sponsor



“SHIM-POL A.M. Borzymowski”
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Sponsor



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YOUNG SCIENTISTS FELLOWSHIPS

As a result of support of the *International Federation of Essential Oils and Aroma Trades* (IFEAT) and the *Organizing Committee of ISEO 2015*, for which we are grateful, twenty-one *Registration Fellowships* were granted to assist young researchers to attend the 46th International Symposium on Essential Oils.

As a result of this generosity, a group of young scientists are now able to experience and participate in the ISEO symposium, and to contribute to the development of the essential oils and natural volatiles research field. It is also an excellent opportunity to bring together the next generation of scientists and experts from around the world who are interested in responding appropriately to new challenges in the field, to engage in professional dialogue, and to have new experiences, and be exposed to new ideas.

After an intense evaluation and selection procedure, the Young Scientists ISEO 2015 *Registration Fellowship* Selection Committee has accepted twenty-one contributions. Among these, six were chosen for oral presentation and the remaining fifteen were established for poster presentation.

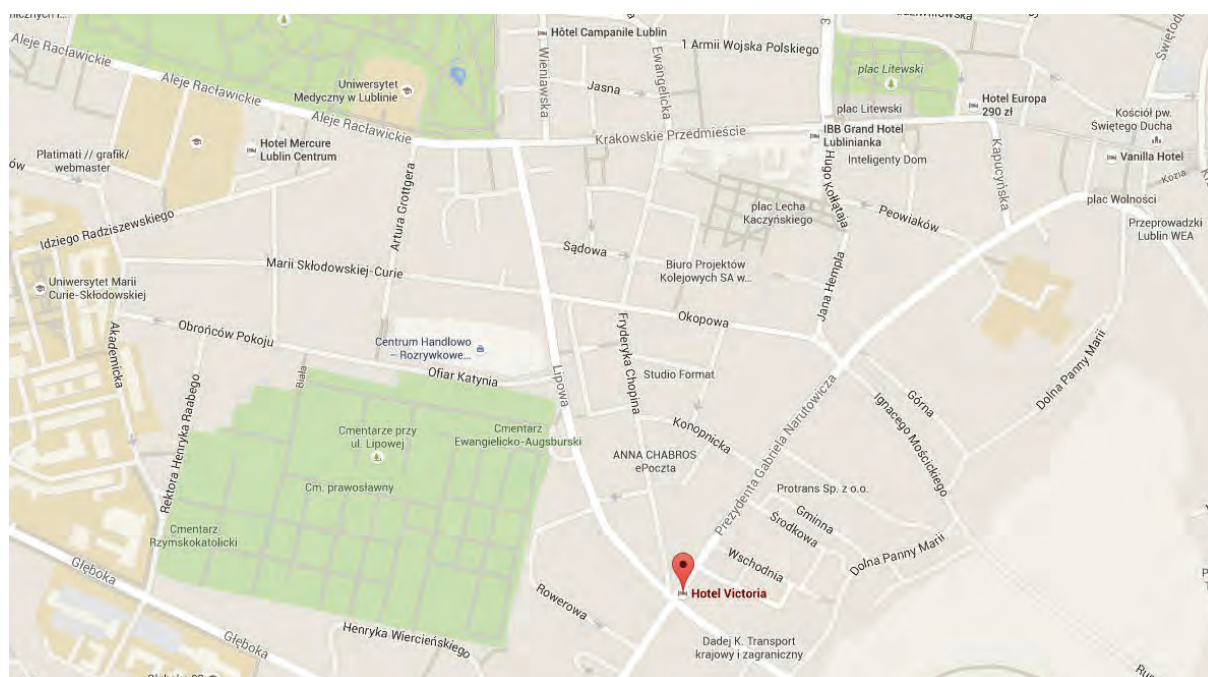
The *International Federation of Essential Oils and Aroma Trades* (IFEAT) and the *Organizing Committee of ISEO 2015* supported the registration fees of the following young scientists:

Opeyemi N. Avoseh	University of Fort Hare, South Africa
Alicja Gawarecka	West Pomeranian University of Technology, Poland
Mónika Homa	University of Szeged, Hungary
Adriana Jara	University of Talca, Chile
Srna Jovic	University of Belgrade, Serbia
Erika Beáta Kerekes	University of Szeged, Hungary
Sára Kindlovits	Corvinus University of Budapest, Hungary
Johan Linde	Tshwane University of Technology, South Africa
Ali Medbouhi	University of Tlemcen, Algeria
Małgorzata Niemiec	Medical University of Lublin, Poland
Vladimíra Ocelová	Institute of Animal Physiology, SAS, Slovak Republic
Idris Oyemitan	Walter Sisulu University, South Africa
Carmen M. Sinche Ambrosio	University of Sao Paulo, Brazil
Nikola M. Stojanović	Faculty of Medicine, University of Niš, Serbia
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Łukasz Świątek	Medical University of Lublin, Poland
Urszula Świerczek	Wrocław University of Technology, Poland
Waed Tarraf	University of Bari, Italy
Vaida Vaičiulytė	Nature Research Centre, Lithuania
Anita Vidács	University of Szeged, Hungary
Magdalena Walasek	Medical University of Lublin, Poland

GENERAL INFORMATION

Symposium Venue

Hotel Victoria Lublin
 58/60 Narutowicza Street
 20-016 Lublin, Poland
 tel.: +48 81 532 70 11-13
www.hotel.victoria.lublin.pl



By <https://www.google.com/maps/>

Registration

The registration desk is located in the entrance to the Conference Centre of Hotel Victoria

Registration hours

Sunday, September 13	16.00 – 19.00
Monday, September 14	08.00 – 18.00
Tuesday, September 15	08.00 – 18.30
Wednesday, September 16	08.00 – 12.30

Symposium Language

The official language of the symposium is English. There will be no simultaneous translation.

Badges

Participants are requested to wear their badges at all times during the symposium, lunch and all social events.

Plenary Lectures, Oral Presentations, and Young Scientists' Lectures

The Plenary Lectures are limited to 35 min, while Oral Presentations and Young Scientists Lectures to 20 min, including discussion.

Posters

Posters will be exhibited according to their designated poster numbers. Odd (1, 3, 5, ...) and even (2, 4, 6, ...) numbers will be displayed Monday and Tuesday, respectively. The authors are requested to stand by their posters during the poster sessions.

Currency

Polish currency is Polish Zloty (PLN). You can exchange currency upon your arrival at the airport, or at banks in Lublin. Most banks are open during business hours, from 8 a.m. until 6 p.m., Monday through Friday. Most major credit cards are accepted at businesses, restaurants, and hotels, including Visa, MasterCard, American Express and Diners Club.

Electricity

The electrical power supply in Poland is 230 volts at 50 Hz. Japanese, UK, USA and other visitors: please note that in Poland Type E and C electrical plugs are used.

Liability and Insurance

The Organizers are in no respect responsible for any accommodation problem, not for any accident, injury, theft, loss and property damage to any person during the Symposium.

SCIENTIFIC PROGRAMME

13th September 2015, Sunday		
16.00 – 19.00	Registration	Victoria Hotel
19.00 – 21.00	Welcome reception	Victoria Hotel
14th September 2015, Monday		
08.00 – 9.30	Registration	Victoria Hotel
09.30 – 10.10	Opening Ceremony	Victoria Hotel
10.10 – 10.40	Coffee Break	
10.40 – 12.15	Session I	Chair persons: Patrizia RUBIOLLO (Turin, Italy) Jan KARLSEN (Oslo, Norway)
10.40 – 11.15	PL-01	Gerhard BUCHBAUER (Vienna, Austria) <i>“Essential oil constituents – metabolism and interactions”</i>
11.15 – 11.35	OP-01	Adam FEYAERTS (Leuven, Belgium) <i>“A novel top-down approach to assess the antimicrobial activity of 121 essential oils against 8 diverse pathogenic bacteria and fungi”</i>
11.35 – 11.55	OP-02	Györgyi HORVÁTH (Pécs, HUNGARY) <i>“Essential oils: are they effective antibacterial and anti-inflammatory agents in the treatment of respiratory tract infections?”</i>
11.55 – 12.15	OP-03	Cécile LABADIE (Vallauris, France) <i>“Hydrosols of Citrus aurantium and Rosa sp. support the growth of a diverse spoilage microflora impacting volatile compounds”</i>
12.15 – 13.45	Lunch	
13.45 – 15.20	Session II	Chair persons: Gerhard BUCHBAUER (Vienna, Austria) K. Hüsnü Can BASER (Eskişehir, Turkey)
13.45 – 14.20	PL-02	Niko S. RADULOVIĆ (Niš, Serbia) <i>“The interplay between chemical synthesis and analysis and biological activity: What can a research group from Serbia offer to the essential-oil community?”</i>
14.20 – 14.40	OP-04	Toshio HASEGAWA (Saitama, Japan) <i>“The aroma profile of galangal containing cinnamic acid derivatives as main components”</i>
14.40 – 15.00	OP-05	Temel ÖZEK (Eskişehir, Turkey) <i>“Chemical composition and in-vitro activities of essential oils of Oliveria decumbens Vent. from Turkey”</i>
15.00 – 15.20	OP-06	Agnieszka LUDWICZUK (Lublin, Poland) <i>“Essential oil composition of selected Mentha species and cultivars and their bacteriostatic activity against Staphylococcus epidermidis”</i>
15.20 – 15.50	Coffee Break	
15.50 – 17.50	Poster session I	(Odd numbers like 1, 3, 5...)
18.00 – 19.30	Meeting time	

15th September 2015, Tuesday		
09.00 – 10.35	Session III	Chair persons: Éva NEMETH-ZAMBORINE (Budapest, Hungary) Tomasz LIGOR (Toruń, Poland)
09.00 – 09.35	PL-03	Alvaro V. VILJOEN (Pretoria, South Africa) <i>"Scents of Africa – exploring the aromatic flora in a botanical utopia"</i>
09.35 – 09.55	YSL-01	Johan LINDE (Pretoria, South Africa) <i>"Chemical constituents and biological properties of Marchantiophyta from South Africa"</i>
09.55 – 10.15	YSL-02	Waed TARRAF (Bari, Italy) <i>"Mycorrhizal symbiosis as a potential biotechnology for medicinal and aromatic plants improvement and sustainable production of essential oils in Lamiaceae family"</i>
10.15 – 10.35	YSL-03	Vladimíra OCEĽOVÁ (Košice, Slovak Republic) <i>"Thymus vulgaris essential oil as feed additive in broiler chickens"</i>
10.35 – 11.05	Coffee Break	
11.05 – 12.40	Session IV	Chair persons: Paola DUGO (Messina, Italy) Stanisław LOCHYŃSKI (Wrocław, Poland)
11.05 – 11.40	PL-04	Fanis BULJUBASIC (Heidelberg, Germany) <i>"The scent of human diseases: specific volatile organic compounds as disease biomarkers"</i>
11.40 – 12.00	YSL-04	Erika KEREKES (Szeged, Hungary) <i>"Cinnamon and thyme essential oils and their major components as anti-quorum sensing and anti-biofilm forming agents"</i>
12.00 – 12.20	YSL-05	Łukasz ŚWIĄTEK (Lublin, Poland) <i>"Cytotoxicity, antiviral activity and GC/MS analysis of commercially available eucalyptus, pine and Indian melissa essential oils"</i>
12.20 – 12.40	YSL-06	Daniel STRUB (Wrocław, Poland) <i>"Main constituents of Carum carvi seeds and Mentha spicata essential oils as substrates for preparation of novel fragrant compounds with preserved terpene backbone"</i>
12.40 – 14.00	Lunch	
14.00 – 15.55	Session V	Chair persons: Alvaro V. VILJOEN (Pretoria, South Africa) Nicolas BALDOVINI (Nice, France)
14.00 – 14.35	PL-05	Tomasz LIGOR (Toruń, Poland) <i>"Breath sampling and identification of VOCs by GC-TOF-MS"</i>
14.35 – 14.55	OP-07	Paweł STALICA (SHIM-POL, Izabelin, Poland) <i>"On-line LC-GC×GC-MS/MS: A powerful unified separation-science tool"</i>
14.55 – 15.15	OP-08	Danilo SCIARRONE (Messina, Italy) <i>"Simultaneous multi collection in each chromatographic dimension of highly pure volatiles from complex samples exploiting a versatile LC-GC-GC-GC-PREP"</i>
15.15 – 15.35	OP-09	Barbara SGORBINI (Torino, Italy) <i>"Determination of free and glucosidically-bound volatiles in plants"</i>

15.35 – 15.55	OP-10	Paola DUGO (Messina, Italy) <i>"Characterization and isolation of limonoids in by-products of Citrus essential oils industry by means of liquid chromatography"</i>
15.55 – 16.30	Coffee Break	
16.30 – 18.30	Poster session II	(Even numbers like 2, 4, 6...)
20.00 – 24.00	Symposium Dinner	
16th September 2015, Wednesday		
09.00 – 10.15	Session VI	Chair persons: Yoshinori ASAKAWA (Tokushima, Japan) Fatih DEMIRCI (Eskişehir, Turkey)
09.00 – 09.35	PL-06	Władysław S. BRUD (Warsaw, Poland) <i>"So called 'potential allergens' in essential oils"</i>
09.35 – 09.55	OP-11	Krisztina SZABÓ (Budapest, Hungary) <i>"Survey of the essential oil profile of Origanum vulgare subsp. vulgare populations and their stability among different environmental conditions"</i>
09.55 – 10.15	OP-12	Jaime USANO-ALEMANY (Bonn, Germany) <i>"Study of essential oil yield and quality of a heterogeneous Salvia lavandulifolia crop monitored at different phenological stages throughout four years"</i>
10.15 – 10.45	Coffee Break	
10.45 – 11.45	Session VII	Chair persons: Kazimierz GŁOWNIAK (Lublin, Poland) Władysław S. BRUD (Warsaw, Poland)
10.45 – 11.05	OP-13	Diego CARNAROGLIO (Bergamo, Italy) <i>"Lab and pilot scale microwave-assisted recovery of volatile fraction from citrus peels waste"</i>
11.05 – 11.25	OP-14	Yoshinori ASAKAWA (Tokushima, Japan) <i>"Volatile components of two Japanese scale insects, Ceroplastes japonicus and Ceroplastes ribens"</i>
11.25 – 11.45	OP-15	Michael KNOTT (Windhoek, Namibia) <i>"Isolation, characterization and in vitro cytotoxic bioactivity of volatile polyhalogenated monoterpenes from Southern African marine algal Plocamium species"</i>
11.45 – 12.30	Closing Ceremony	
12.30 – 14.00	Lunch	
14.30	Excursion	Information and registration on desk

POSTER PRESENTATIONS

Poster sessions: *session I:* Odd numbers like 1, 3, 5... (Monday, Sept. 14th)

session II: Even numbers like 2, 4, 6... (Tuesday, Sept. 15th)

* Registration Fellowships founded by IFEAT and ISEO 2015 Organizing Committee

- | | | |
|----------------|---|--|
| PP-001 | Sherifat ABOABA
(Nigeria) | <i>Volatile constituents and larvicidal activity of the essential oils from three Nigerian medicinal plants</i> |
| PP-004 | Kamilla ÁCS
(Hungary) | <i>Vapour-phase method: a possible solution for in vitro antimicrobial evaluation of volatile substances</i> |
| PP-007 | Oyuttsetsen AMARSANAA
(Mongolia) | <i>Essential oil composition of four Artemisia species from Mongolian Trans-Altai Gobi</i> |
| PP-008* | Opeyemi N. AVOSEH
(South Africa) | <i>Volatile profiles and anti-inflammatory property of Cinnamomum camphora (L.) Nees & Eberm from Eastern Cape, South Africa</i> |
| PP-009 | Karima BABA AISSA
(Algeria) | <i>Effect of thyme and oregano essential oil products on different aphids</i> |
| PP-010 | Tomasz BAJ
(Poland) | <i>Chemical composition, cytotoxicity, antioxidant activity and the influence on FaDu cell cycle of clove essential oil and its main constituent</i> |
| PP-011 | Tomasz BAJ
(Poland) | <i>Antimicrobial and antioxidant activity of carrot (Daucus carotta L.) seed essential oil</i> |
| PP-012 | Magdalena BARTNIK
(Poland) | <i>GC-MS analysis of essential oil and headspace from aerial parts of Peucedanum tauricum with PCA and HCA statistical interpretation</i> |
| PP-013 | Magdalena BARTNIK
(Poland) | <i>GC-MS analysis of essential oil and headspace of Heracleum dissectum Ledeb. herb</i> |
| PP-015 | Humberto R. BIZZO
(Brazil) | <i>Chemical composition and fungicide activity of the essential oil from the leaves of Psidium laruotteanum Cambess. (Myrtaceae)</i> |
| PP-016 | Humberto R. BIZZO
(Brazil) | <i>Scents from Brazilian Cerrado: The essential oil from leaves of Bidens segetum Mart. ex Colla (Asteraceae)</i> |
| PP-018 | Vicente CASTELL
(Spain) | <i>Variability of essential oil composition of wormwood (Artemisia absinthium L.) affected by harvest date</i> |
| PP-020 | Fatih DEMIRCI
(Turkey) | <i>Synergistic essential oil combinations against Staphylococcus aureus and Propionibacterium acnes</i> |
| PP-021 | Ewa DREWNOWSKA
(Poland) | <i>Extracting of limonene from the peels of lemons and limes and the determination of the purity of the obtained compound</i> |
| PP-022 | Paola DUGO
(Italy) | <i>Characterization of essential oils and plant extracts secondary metabolites by using Gas Chromatography hyphenated to Carbon Isotope Ratio Mass Spectrometry (GC-C-IRMS)</i> |
| PP-023 | Paola DUGO
(Italy) | <i>Traceability of vanilla flavoured food by means Head Space-Solid Phase Micro Extraction (HS-SPME) coupled to Gas Chromatography-Isotope Ratio Mass Spectrometry (GC-IRMS)</i> |
| PP-024 | Amina DZAFIC
(Germany) | <i>Quality monitoring of selected essential oils upon storage under real-time condition</i> |
| PP-026 | A. Cristina FIGUEIREDO
(Portugal) | <i>Plant biotechnology in phytonematicidals screening (PlantBioNem)</i> |
| PP-027 | A. Cristina FIGUEIREDO
(Portugal) | <i>α-Pinene synthase gene expression following nematode invasion in Pinus pinaster and P. pinea with Bursaphelenchus xylophilus in vitro co-cultures</i> |
| PP-028 | Kazimierz GŁOWNIAK
(Poland) | <i>Chemical composition of the essential oil and some biological effect of Artemisia ordosica Krasch. from Mongolian Gobi</i> |

- PP-029*** Alicja GAWARECKA
(Poland) *Grapefruit peels as a source of limonene - research on isolation and purity of limonene*
- PP-032** Toshio HASEGAWA
(Japan) *Aroma profiles of common Japanese trees: Japanese cypress, camphor tree, Japanese cedar, and hiba arborvitae*
- PP-033*** Mónika HOMA
(Hungary) *Antifungal effect of essential oils against clinically relevant fusaria*
- PP-035*** Adriana JARA
(Chile) *Essential oil composition of Azorella spinosa of Maule Region, Chile*
- PP-036*** Srna JOVIC
(Serbia) *Antimicrobial activity of the essential oil of cultivated Micromeria pulegium (Roche) Benth. (Lamiaceae)*
- PP-038** Agnieszka KICEL
(Poland) *Chemical composition of essential oil of Helichrysum arenarium (L.) Moench*
- PP-039*** Sára KINDLOVITS
(Hungary) *Essential oil and proazulene content of 11 different Achillea collina Becker accession*
- PP-040** Sára KINDLOVITS
(Hungary) *Effect of sowing density and harvesting time on the root production and essential oil content of Pimpinella peregrina L.*
- PP-041** Małgorzata KOZYRA
(Poland) *Chemical components and variability of the essential oils isolated from inflorescences of Carduus species*
- PP-042** Wirginia KUKUŁA-KOCH
(Poland) *Supercritical carbon dioxide extraction coupled with pressurized liquid extraction in the recovery of terpenes and phenolics from the aerial parts of Cretan barberry*
- PP-043** Wirginia KUKUŁA-KOCH
(Poland) *Influence of digestion and thermal processing on the volatile composition of Zingiber officinale and its products*
- PP-044** Eisuke KURAYA
(Japan) *A new technique for the measurement of antioxidant activity in essential oils using bicontinuous microemulsions*
- PP-045** Eisuke KURAYA
(Japan) *Chemical and aroma profiles of Northern Limit Yuzu (Citrus junos) peel oils from different producing districts*
- PP-046** Juan A. LLORENS-MOLINA
(Spain) *Essential oil composition of leaves and berries of Juniperus oxycedrus L. growing in siliceous and calcicolous soils from Valencia (Spain)*
- PP-047** Juan A. LLORENS-MOLINA
(Spain) *Essential oil composition of leaves and berries of Pistacia lentiscus L. growing in siliceous and calcicolous soils from Valencia (Spain)*
- PP-048** Agnieszka MACIĄG
(Poland) *Chemical composition of SFE CO₂ extracts produced from marigold (Calendula officinalis L.), sage (Salvia officinalis L.) and chamomile (Matricaria chamomilla L.)*
- PP-049** Agnieszka MACIĄG
(Poland) *Composition of stinging nettle (Urtica dioica L.) hydrolate*
- PP-050** Edyta MAKUCH
(Poland) *Methods of the isolation of the clove oil (Oleum Caryophylli) and identification of its main component (eugenol) by the gas chromatography and infrared spectroscopy methods*
- PP-051** Anna MALM
(Poland) *Thymol as anti-adhesive and anti-biofilm agent preventing formation of biofilm in vitro by Candida albicans strains isolated from oral cavity*
- PP-052** Svitlana MARCHYSHYN
(Ukraine) *Investigation of essential oils of decorative herbs*
- PP-054*** Ali MEDBOUHI
(Algeria) *Chemical composition and biological activities of hydrosol extract of Micromeria inodora (Desf.) Benth. from Algeria*
- PP-058** Olena MYKHALIUK
(Ukraine) *Investigation of essential oils of Schizandra chinensis Baill.*
- PP-059** Agnieszka NAJDA
(Poland) *Composition and biological activity of essential oils from the leaves and stems of stevia (Stevia rebaudiana Bertoni) from eastern Poland*
- PP-061** Éva NÉMETH-ZÁMBORINÉ
(Hungary) *Changes in yield and essential oil compounds of four Lamiaceae species due to different water supply*

- PP-062** Éva NÉMETH-ZÁMBORINÉ (Hungary) *Evaluation of different Perilla frutescens (L.) Britt. origins in Hungary*
- PP-063*** Małgorzata NIEMIEC (Poland) *Fingerprint analysis of essential oils from Achillea sp. by high-performance liquid chromatography*
- PP-064*** Idris OYEMITAN (South Africa) *Central nervous system activities and chemical composition of essential oil of fresh fruits of Piper guineense (Piperaceae) in mice*
- PP-065** Kaan POLATOĞLU (Turkey) *Essential oil composition and insecticidal activity of Inula viscosa (L.) Aiton. from Cyprus*
- PP-066** Kaan POLATOĞLU (Turkey) *Acetylcholinesterase inhibitory and PRAP activities of the essential oils on selected Tanacetum species*
- PP-067** Ain RAAL (Estonia) *Content and composition of essential oil in Betula spp. leaves growing naturally in Estonia*
- PP-068** Agnieszka K. RAJCA-FERREIRA (Brazil) *Antioxidant activity and chemical composition of essential oils from two Guatteria elliptica R. E. Fries (Annonaceae) specimens*
- PP-069** Marek RENČO (Slovak Republic) *Nematicidal activity of essential oils against the potato cyst nematode Globodera rostochiensis*
- PP-070** Patrizia RUBIOLO (Italy) *In vitro anti-herpes activity of Salvia desoleana Atzei & V. Picci essential oil*
- PP-071** M. Pilar SANTAMARINA (Spain) *In vitro and in vivo control of Fusarium oxysporum and F. proliferatum by commercial Laurus nobilis L. and Syzygium aromaticum L. Merr. & Perry essential oils*
- PP-072** M. Pilar SANTAMARINA (Spain) *Antifungal activity and potential use of commercial essential oils against Curvularia hawaiiensis isolated from rice*
- PP-073** Nicola SASANELLI (Italy) *Effect of essential oils on the hatch of eggs of cyst-forming phytoparasitic nematodes*
- PP-075** Olga SHELEPOVA (Russia) *Chemical composition of the essential oils of the chemotypes Mentha spicata L.*
- PP-076** Ken SHIMOJIMA (Japan) *Improving functional ingredients of seed oil by underwater shock wave*
- PP-077** Elwira SIENIAWSKA (Poland) *The evaluation of the cytotoxicity of carrot seed essential oil and HPLC separation of its main constituents*
- PP-078*** Carmen M. SINCHE AMBROSIO (Brazil) *In vitro antibacterial activity of essential oils on pathogenic and probiotic microbiota of poultry and swine*
- PP-079** Natalia SKUBIJ (Poland) *Essential oil content in thyme (Thymus vulgaris L.) depending on potassium and calcium carbonate fertilization*
- PP-080** Brett SMITH (USA) *Chemical differentiation of Boswellia sacra and Boswellia carterii essential oils by gas chromatography and chiral gas chromatography-mass spectroscopy*
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- PP-082*** Nikola M. STOJANOVIĆ (Serbia) *Influence of Hypericum perforatum essential oil on the mice central nervous system*
- PP-083*** Urszula ŚWIERCZEK (Poland) *Biotechnological potential of ionic liquids containing components of essential oils*
- PP-084** Attila SZKUKALEK (Slovakia) *New chemotype of spearmint, Mentha spicata L. from Slovakia*
- PP-085** Ayumi TAKEMOTO (Japan) *Comparison of high efficiency of essential oil extraction using instantaneous high pressure process in hydrodistillation method and microwave method*
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- PP-093 Jürgen WANNER**
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(Germany)
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- PP-095 Rafał WAWRZYŃIAK**
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Differences in composition of volatile compounds in cryptic species B of the Aneura pinguis complex (Marchantiophyta, Metzgeriidae)
- PP-096 Jarosław WIDELSKI**
(Poland)
Analysis of essential oils from three species from the genus Ferula (Apiaceae)
- PP-097 Jarosław WIDELSKI**
(Poland)
Volatiles from herb and fruits of Peucedanum luxurians and their antimicrobial activity
- PP-098 Katarzyna WIELGUSZ**
(Poland)
Fungistatic properties of selected essential oils for controlling Fusarium wilt in flax
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(Ukraine)
Investigation of the volatile oil in modified roots, flowers and leaves of Hemerocallis species
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Histopathological effects of eucalyptol on the midgut of larvae Alphitobius diaperinus Panzer (Coleoptera: Tenebrionidae)
- PP-101 Klaudyna HEBDA**
(Poland)
Historical and ethnobotanical review of labdanum resin usage

PLENARY LECTURES

PL-01. Essential oil constituents – metabolism and interactions

Walter Jäger and Gerhard Buchbauer*

Department of Pharmaceutical Chemistry, Faculty of Life Sciences, University of Vienna, Center of Pharmacy,
A-1090 Vienna, Althanstrasse 14, Vienna, Austria

*Corresponding author. Email: gerhard.buchbauer@univie.ac.at

All over the world people use a variety of medicinal plants in order to treat diseases. Together with the increased appearance of common and self initiative usage of herbal preparations there is also an increased need of knowing and understanding possible interactions with concomitantly taken drugs. There is a general belief that herbal products, thus essential oils too, are safe because they are natural. As it turned out this is a wrong simplification. Since essential oils are mixtures of multiple active ingredients, while synthetic drugs usually contain single chemical entities, it is theoretically more likely for essential oils to interact with drugs than it is for one drug to interact with another. The following discourse will reveal some volatile oils or their ingredients and the way they interact with drugs. Most of them are pharmacokinetic ones, while pharmacodynamic interactions seem to be less studied. While some of these interactions may have a negligible impact on to the effect of drugs, particularly when used aromatherapeutically, others may be a serious threat to health. It could also be possible to use the effects of these interactions to the benefits of people, especially when antibiotics are used concomitantly with essential oils against bacteria, e.g. against MRSA.

This lecture provides an overview of available evidence about the influence of different essential oils on conventional medicinal administration. The aim was also to demonstrate the impact of volatile oils on the effect of drugs if applied together (Golos, 2014).

Keywords: ADME, Antibiotics, CYP450, CYP inducers/inhibitors, Essential oils, Multi drug resistance, Pharmacokinetics.

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PL-02. The interplay between chemical synthesis and analysis and biological activity: What can a research group from Serbia offer to the essential-oil community?

Niko S. Radulović*

Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

*Corresponding author. Email: nikoradulovic@yahoo.com

Organic synthesis and analysis (OSA) group is a research group situated at the University of Niš (Serbia), consisted of both chemists and biology-oriented scientists, that has gained experience in the field of essential oils for almost 15 years. In that time we have investigated several hundreds of plant taxa from many different plant families and this resulted in more than 1000 successfully identified volatile constituents. During these studies essential oils have been proven to be as either a prolific source of new and interesting chemical compounds with promising biological/pharmacological activities or a fingerprint of the plant genetic makeup. Both approaches to volatile plant metabolites (medicinal chemistry or chemotaxonomy) require the application and development of cutting edge analytical methodologies, whereas the first includes both organic synthesis and a full array of *in vivo* and *in vitro* pharmacological and toxicological assays. In this lecture I would like to provide a glimpse into the most relevant results of the OSA group: the application of small synthetic libraries of compounds for the identification and biological evaluation of essential-oil constituents and the utilization of the average mass scan of the total ion chromatograms of essential oils in chemotaxonomy.

If only a fragment of the structure of an interesting essential-oil constituent could be inferred from the available data (MS and retention index (RI)), or if the tentative identification could be narrowed down to a number of isomers (e.g. by biosynthetic considerations), the only comprehensive approach in this situation (alongside chromatographic isolation which is not always possible) would be to create a small synthetic library of all possible compounds. Such libraries provide the possibility to investigate structure–activity/property relationships within the group of synthesized compounds and provide important (spectral) data for future investigations. Even if a synthesized library compound does not turn out to be the natural product of the currently studied plant species (organism) it might not be a long wait for it to be identified from another source because there would then be data available in the literature (MS libraries, etc.) that allow an easier identification and dereplication.

Plant volatiles have been repeatedly shown to provide valuable insight into the evolutionary relationships among plant taxa on various taxonomical levels. The number of variables available from GC–MS analyses of these plant metabolites usually represents a large data set. The comparison of such data sets requires the use of multivariate statistical analyses (MSA) but with several serious shortcomings. We developed a more reliable and faster approach to multivariate statistical comparison of essential oils by using relative abundances of m/z values of the average mass scan (AMS) of the total GC chromatograms instead of the traditionally used variables-percentages (peak areas) of individual oil constituents. MSA of complex volatile mixtures, using the corresponding directly obtainable AMS, could be considered as a promising time saving tool for easy and reliable comparison purposes. The AMS approach gives comparable or even better results than the traditional method – it reflected the natural relationships between observations within the studied groups of oils.

Keywords: Small synthetic libraries of compounds, average mass scan of the total ion chromatogram, biological/pharmacological activity, chemotaxonomy, structure-activity/property relationship.

Acknowledgments

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PL-03. Scents of Africa - exploring the aromatic flora in a botanical utopia

Alvaro M. Viljoen^{1,*}, Guy P.P. Kamatou¹ and Sandy F. van Vuuren²

¹ Department of Pharmaceutical Sciences, Private Bag X680, Tshwane University of Technology, Pretoria, South Africa

² Department of Pharmacy and Pharmacology, 7 York Road, Parktown 2193, University of the Witwatersrand, Johannesburg, South Africa

*Corresponding author. Email: ViljoenAM@tut.ac.za

With over 24 000 species of flowering plants South Africa is considered to be the third most biodiverse area in the world and 10% of the world's total flora is contained within its boundaries. Many of these species are aromatic and are extensively used in local traditional healing practices. The rich indigenous knowledge systems which have developed on local plant use acted as a catalyst to explore the medicinal aromatic flora of South Africa. Using a multidisciplinary approach we initiated a project in 1999 to explore the exceptional flora of South Africa with an acute focus on plant volatiles. This research culminated in the very first special issue of Journal of Essential Oil Research, which was devoted to discoveries on South Africa's aromatic plants. Our research is not only aimed at profiling the chemical composition of essential oils but also unraveling the biological properties of these oils and their constituents with a special interest in synergy research and gaining a better understanding of structure activity relationships. The complexity of essential oils make them ideally suited for multivariate data analysis and remains a valuable independent test of taxonomy which is often based on morphology alone. Using classical GC-MS data in tandem with vibrational spectroscopy several examples will be presented to illustrate how essential oil data has assisted to unravel taxonomic problems. The application of this data in the quality assurance of essential oils will be highlighted. Our research approach has always been to integrate the various research themes in a multidisciplinary approach to explore and catalogue the composition and biological properties of South Africa's unique aromatic flora. This paper is a reflection on the research achievements over the past 15 years and illustrates the abundant opportunities, which this discipline may offer to scientists seeking a stimulating and rewarding research career.

Keywords: South Africa, essential oils, chemometrics, quality control.

PL-04. The scent of human diseases: specific volatile organic compounds as disease biomarkers

Fanis Buljubasic*

General Hospital Mannheim, Department of Internal Medicine, University of Heidelberg, Heidelberg, Germany

*Corresponding author. Email: fidelio_bih@yahoo.com

The use of VOCs (volatile organic compounds) as specific biomarkers of human diseases has been the focus of scientific research in recent years. The VOCs are collected from the exhaled breath of patients and mainly detected and identified by GC-MS and electronic noses.

The aim of the studies on VOCs is to develop a useful and first of all a non-invasive method for diagnosing human diseases, many of which are cancers, but also include some metabolic and infectious diseases.

The results show that both methods, used in detecting and identifying the VOCs, could create a unique chemical signature or "smellprint", that can be detected in many diseases. The VOCs analyzed by GC-MS could deliver a VOC-pattern, characteristic of bacterial infections (*M. tuberculosis* and *C. difficile*), as well as bronchial asthma, COPD and schizophrenia. With the introduction of NA-NOSES, which are more sensitive than classic electronic noses, VOCs characteristic of lung cancer, ovarian carcinoma, mesothelioma and COPD, have been detected and quantified. For example, the VOC-pattern characteristic of lung cancer has been identified and surprisingly, detected in its first stage, which is always curable and which cannot be always diagnosed by standard diagnostic tools (computertomography, bronchoscopy).

However, many problems in using VOCs as diagnostic biomarkers still exist. The analytical equipment is expensive and most techniques are time-consuming. The largest problem is a lack of information about the biological pathways, that produce relevant VOCs in patients with the above-mentioned diseases. In diseases such as lung cancer or breast cancer VOCs are released due to lipid peroxidation or oxidative stress, but in many diseases the biological pathways are still unknown, thus leaving no information as to the origins, physiological and exhalation kinetics of the VOCs.

Therefore, further studies on VOCs might be useful not only in diagnosing and mentoring human diseases as biomarkers, but also in providing pathological mechanism of diseases and also in developing novel therapies.

Keywords: biomarkers, VOCs, disease diagnosis, e-nose, healthcare applications.

PL-05. Breath sampling and identification of VOCs by GC TOF MS

Tomasz Ligor*, Bogusław Buszewski

Chair of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University,
Gagarina 7, 87 100 Toruń, Poland

*Corresponding author. Email: msd2501@chem.umk.pl

Exhaled breath contains a large number of different volatile substances. This includes inorganic gases (N_2 , O_2 , CO_2 , CO, NO) and volatile organic compounds: hydrocarbons - ethane, pentane, hexane, methyl hexanes, isoprene, toluene, alcohols, ketones, aldehydes, additionally nitrogen and sulfur containing substances. VOCs might be useful biomarkers for the screening of respiratory diseases. Additionally, breath analysis is a non-invasive and painlessness procedure, and its sampling does not require skilled medical staff. Moreover, exhaled gas test might be complementary method to blood and urine analysis. A number of studies have been carried out in order to searching of volatiles related to different diseases by means of chromatographic techniques. Breath analysis presents many problems such as: exhaled compounds which are present in very low concentrations (typically ppb, ppt level) and are found in a matrix where interference from numerous exogenous compounds is expected.

An analytical procedure generally involves few steps, such as: sampling, preconcentration, separation, detection and identification. Nevertheless, such approach is complicated, time consuming, needs skilled staff and some compounds can be missed before chromatographic analysis. Additionally, GC/MS cannot be used at bed side. Therefore, various *on-line* techniques, such as sensors, e-noses, laser spectroscopy are developed to overcome these drawbacks.

In the current study, the solid phase microextraction (SPME) and sorption tubes (TD) were used for enrichment of volatiles from breath. Gas chromatography and mass spectrometry were applied for identification. Especially, TOF-MS is efficient technology for automatic peak deconvolution and peak finding, which which is important for chromatographically not resolved compounds.

Keywords: VOCs, human breath, sample preparation.

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PL-06. So called “potential allergens” in essential oils

Władysław S. Brud^{1,*} and Iwona Konopacka-Brud²

¹Academy of Cosmetology and Health Care, 00-252 Podwale 13, Warsaw, Poland,

²Pollena-Aroma Ltd, Przemysłowa 4, 05-100, Nowy Dwór Mazowiecki, Poland

* Corresponding author. Email: wsbrud@post.pl

Term “potential allergens” for fragrance substances and essential oils containing these substances was introduced in official documents by EU directive in 2003 and appears in numerous documents, opinions and literature. Its controversial effect especially in the area of use of essential oils in fragrances and flavours as well as many other areas is based on disputable sources of information as well as materials tested in various research works, which resulted in conclusion of allergenic properties of the oils. Main problem in existing and prepared EU directives is well known diversity of so called Natural Complex Substances and difficulties with proper identification of real source of adverse properties of the material under scientific examination.

Keywords: Potential allergens, essential oils, natural complex substances, regulations.



ORAL PRESENTATIONS

OP-01. A novel top-down approach to assess the antimicrobial activity of 121 essential oils against 8 diverse pathogenic bacteria and fungi

Adam Feyaerts^{1,2,*}, Helene Tournu^{1,2}, Yves Briers³, Daniele Pepe⁴, Rob Lavigne³, Walter Luyten⁵ and Patrick Van Dijck^{1,2}

¹VIB Department of Molecular Microbiology, KU Leuven, Belgium

²Laboratory of Molecular Cell Biology, KU Leuven, Kasteelpark Arenberg 31, 3001, Leuven, Belgium

³Department of Biosystems, KU Leuven, 3001, Leuven, Belgium

⁴Department of Chemistry, B6c, University of Liège, 4000 Liège, Belgium

⁵Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, 3000, Leuven, Belgium

*Corresponding author. Email: adam.feyaerts@mmbio.vib-kuleuven.be

In this exploratory study the antimicrobial activity (AMA) of 121 essential oils (EOs) and 2 EO components (EOCs) were assessed against eight diverse human (multi-resistant) pathogenic bacteria and fungi. In general the bioactivity of EO(C)s is difficult to assess because they are intrinsically highly volatile and poorly water-soluble. Therefore classical standardised assays such as the disk diffusion assay and the minimal inhibitory concentration assay were first adapted and optimised before screening. In addition, as several microorganisms are capable of growing as a biofilm, where they are often less sensitive to drugs, the AMA against fungi biofilm was also evaluated (Hood et al, 2003; Hyldgaard et al, 2012).

Differences in the methods used to assess AMA of EOs and the choice of test organism(s) as well as the lack of detailed information about the composition of the EO(s) complicate the comparison between data from different publications. In a novel top-down approach, we assembled a library of 121 EOs whose compositions are fully known, to investigate their AMA in a comparable way. *In silico* data mining techniques, such as clustering and principal component analysis, were applied to chemical composition of the EOs and their corresponding biological activity. The outcome was consistent with previous findings, hence validating not only this new approach but also confirming these earlier findings with the stronger statistical power of this study.

As a direct result we could assign specific AMA to similar EOs and identify corresponding classes of EOCs. Moreover, we could identify at least one known EOC with an unknown interesting and specific AMA. However, we expect that the analysis of more complex patterns will uncover additional interesting bioactivities of EOCs.

The applications of this method are diverse: (1) for an industrial application, three dissimilar EOs were selected from various natural products after an *in vitro* evaluation to replace some harmful synthetic compounds, (2) combining the chemical and biological data with data mining makes it possible to predict the AMA of an EO with a known composition.

Finally, the method is not limited to AMA data but can be applied to other bioactivity data.

Keywords: essential oil, antimicrobial activity, clustering analysis, principal component analysis, biofilm.

Acknowledgments

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OP-02. Essential oils: are they effective antibacterial and anti-inflammatory agents in the treatment of respiratory tract infections?

Györgyi Horváth^{1,*}, Kamilla Ács¹, Eszter Csikós¹, Amir Reza Ashraf¹, Andrea Böszörményi², Béla Kocsis³, Kata Csekő⁴, Ágnes Kemény⁴ and Zsuzsanna Helyes⁴

¹ Department of Pharmacognosy, Medical School, University of Pécs, 7624, Pécs, Hungary

² Institute of Pharmacognosy, Faculty of Pharmacy, Semmelweis University, 1085, Budapest, Hungary

³ Institute of Medical Microbiology and Immunology, Medical School, University of Pécs, 7624, Pécs, Hungary

⁴ Institute of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, 7624, Pécs, Hungary

*Corresponding author. Email: gyorgyi.horvath@aok.pte.hu

Respiratory tract infections (RTIs) affect a large number of people in every age group worldwide. Essential oils (EOs) can easily reach the upper and lower parts of the respiratory tract via inhalation due to their volatility. Unfortunately, there are only a few number of articles in which the antimicrobial activity of EOs against respiratory tract pathogens were tested. Furthermore, the anti-inflammatory effect of EOs are weakly studied as well. Therefore, the purpose of our study was to examine the chemical composition and *in vitro* antibacterial activity of cinnamon bark, clove, thyme, scots pine, and citronella EOs. Moreover, our study aimed at the investigation of anti-inflammatory effect of thyme EO in endotoxin-induced acute airway inflammation *in vivo* (mouse) model.

The chemical composition of EOs was determined by GC-MS. The antibacterial activity of EOs was tested with vapour-phase technique using *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pneumoniae* (DSM 20566), and *Moraxella catarrhalis* (DSM 9143). The values of MIC were expressed in $\mu\text{L/L}$ referred to the air space above the microorganism in the Petri dish. *In vivo* experiments were performed on female C57BL/6 mice ($n=7-10/\text{group}$). Lung inflammation was evoked by intratracheal endotoxin (*E. coli* 083 LPS). Mice inhaled thyme EO vaporized 3 times for 30 min. Airway responsiveness in breathing animals was measured by whole body plethysmography.

trans-Cinnamaldehyde (74%), eugenol (88.6%), thymol (46.3%), α -pinene (39.4%), and citronellal (36.2%) were the main compounds in cinnamon bark, clove, thyme, scots pine, and citronella EOs, respectively. Cinnamon bark and thyme EOs showed the highest activity against *P. aeruginosa* (31.25 $\mu\text{L/L}$). Clove and citronella oils could inhibit the growth of *S. pneumoniae* and *M. catarrhalis* in different concentrations (50-150 $\mu\text{L/L}$). Scots pine was inactive in our test. In *in vivo* experiments, thyme oil could reduce the airway responsiveness in the treated groups compared to animals in the control groups, but it had no effect on other investigated parameters (e.g. inspiration and expiration time, relaxation time, etc.).

Based on our results, cinnamon bark, thyme, clove, and citronella EOs may be regarded as effective agents in the treatment of RTIs.

Keywords: essential oils, GC-MS, vapour-phase, antibacterial, airway inflammation.

Acknowledgments

This work was supported by the OTKA PD 104660 (Hungarian Scientific Research Fund) grant.

OP-03. Hydrosols of *Citrus aurantium* and *Rosa sp.* support the growth of a diverse spoilage microflora impacting volatile compounds

Cécile Labadie^{1,2*}, Christian Ginies², Marie-Hélène Guinebretiere², Catherine Renard², Céline Cerutti¹, Frédéric Carlin^{2*}

¹ Albert Vieille SAS, 06227 Vallauris, France

² INRA, Avignon Université, UMR408 Sécurité et Qualité des Produits d'Origine Végétale, F-84000 Avignon, France

*Corresponding authors. E-mail: ce.labadie@hotmail.com; frederic.carlin@avignon.inra.fr

Citrus aurantium, *Rosa centifolia* and *Rosa damascena* hydrosols are hydrodistillation products mainly used as food flavoring agents or ingredient in cosmetics. We analyzed 22 hydrosol samples collected from manufacturers around the Mediterranean basin and in Eastern Europe, at storage time between 0 (sampling at processing day) and 13 months. These hydrosols contained essential oil (EO) at median concentrations of 116 mg/L in the *Rosa centifolia* hydrosols, and of 677 mg/L in the *Citrus aurantium* hydrosols. The EO concentrations in hydrosols were similar to previously reported minimal inhibitory concentrations against diverse pathogenic bacteria, i.e. from 20 to 200 mg/L for neroli oil. However bacteria detected in the tested hydrosols were able to grow when inoculated in filter sterilized hydrosols, showing that EO are not in concentrations sufficient to prevent their multiplication. Maximal bacterial counts were in the range 10^6 - 10^7 CFU/mL and did not comply with professional standards which recommend bacterial counts lower than 10^2 CFU/mL. Only hydrosols stored in a sterile packaging showed the lowest count during a three months period (<5 CFU/mL). This suggests that contaminations likely occur during handling of hydrosols in the open air, i.e. in non-sterile conditions. The 58 hydrosol bacterial isolates were divided into 4 major branches: a *Pseudomonas sp.* branch, a *Burkholderia cepacia* complex branch, and two undefined species branches belonging to *Acetobacteraceae* and *Rhodospirillaceae*. These bacteria require low nutrients for growth and are usually described as environmental contaminants. A few of them, inoculated in filter-sterilized hydrosols, were able to metabolize EO compounds such as alpha-terpineol and geraniol, with a concomitant production of 6-methyl-5-hepten-2-one, and 2-phenylethyl acetate with a concomitant production of 2-phenylethanol. Challenge tests with pathogenic or reference micro-organisms (*Bacillus cereus*; *Escherichia coli*; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Listeria monocytogenes*; *Salmonella* Typhimurium, *Candida albicans* and *Aspergillus brasiliensis*) showed that only *P. aeruginosa*, and *A. brasiliensis* were able to multiply at 30°C in at least one of the tested hydrosol. None of these microorganisms has been found among hydrosol isolates. In conclusion packaging in aseptic conditions could contribute to the microbiological stability of hydrosols during long-term storage. If not, a chemical stabilization with preservatives should be considered.

Keywords: Hydrolat, orange blossom water, rose water, essential oil, challenge-tests, pathogenic bacteria, spoilage bacteria.

OP-04. The aroma profile of galangal containing cinnamic acid derivatives as main components

Momohiro Hashimoto¹, Toshio Hasegawa^{1,*}, Takashi Fujihara¹, and Hideo Yamada²

¹ Department of Chemistry, Graduate School of Science and Engineering, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama 338-8570, Japan

² Yamada-matsu Co., Ltd., Kamigyo-ku, Kyoto 602-8014, Japan

*Corresponding author. Email: toshih@mail.saitama-u.ac.jp

Cinnamic acid derivatives are important odorants due to their characteristic scent. Some materials, such as cinnamon bark, matsutake mushrooms, and *Kaempferia galanga* L. rhizome (galangal), contain several cinnamic acid derivatives as important odor constituents. The main constituent of galangal is (*E*)-ethyl 4-methoxycinnamate (**1**). Previously, we studied the aroma profile of star anise, which consists of (*E*)-anethole and structurally similar compounds, by our laboratory's method considering the intermolecular interactions of the compounds (Hasegawa et al., 2014). Here we report the aroma profile of galangal, which was investigated by the same method. Odorants in galangal were extracted by hexane extraction, steam distillation, and headspace sampling. A difference in odor was observed between the hexane extract and the steam distillate. Next, we searched for the key compounds in the aroma profile of galangal by separating the constituents of the hexane extract, which had an odor similar to original material's. Bulb-to-bulb distillation of the hexane extract produced a fraction with galangal-like odor. The main component of this fraction was not **1** but ethyl cinnamate (**2**). Moreover, GC-MS analysis revealed that this fraction contained benzenoids and hydrocarbons in addition to **2**. These results indicate that **2**, which lacks a methoxy group, is more important than **1**, which contains a methoxy group, in the aroma profile of galangal. We synthesized cinnamic acid derivatives to examine the importance of the odor expression of cinnamic acid derivatives in the odor characteristics of galangal. Cinnamic acid derivatives lacking a *p*-methoxy group had a strong fruity odor. At the *para* position, replacement of the hydrogen atom with a methoxy group resulted in a different, weaker odor. These results were notably different from our results for star anise, whose key odor compounds were benzenoid compounds with a *p*-methoxy group. We found that the presence of a *p*-methoxy group in cinnamic acid derivatives played an important role in the aroma profile of galangal.

Keywords: *Kaempferia galanga* L., cinnamic acid derivatives, aroma profile, structure-odor relationship.

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OP-05. Chemical composition and *in-vitro* activities of essential oils of *Oliveria decumbens* Vent. from Turkey

Temel Özek^{1,*}, Gülmira Özek¹, Fatih Göger¹, Ahmet Duran², and K. Hüsnü Can Baser^{1,3}

¹ Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskişehir, Turkey

² Department of Biology, Faculty of Science, Selcuk University, 42075, Konya, Turkey

³ Botany and Microbiology Department, College of Science, King Saud University, 11451, Riyadh, Saudi Arabia

*Corresponding author. Email: tozek@anadolu.edu.tr

Oliveria decumbens Vent. (Apiaceae) was collected from Urfa-Ceylanpinar in 2014. Essential oils from leaf-flower (L&F) and stem (S) were obtained by hydrodistillation in a Clevenger type apparatus. The yields of the oils were 4.1% and 0.1% (moisture free basis) from L&F and S respectively. The essential oils were analyzed by GC-FID and GC-MS techniques. Monoterpene (C₁₀H₁₆) hydrocarbons and their oxygenated forms predominated in the oils with γ -terpinene, isothymol, thymol and *p*-cymene as major constituents in both oils. Enantiomeric ratios of chiral compounds in the oils were studied by using a chiral column. The essential oils were also subjected to preliminary test for free radical scavenging activity, Cuprac reducing antioxidant capacity, anticholinesterase activity and α -Amylase inhibitory activity.

The present work is the first detailed contribution into the chemistry and *in-vitro* activity studies of the essential oils of *Oliveria decumbens* from Turkey.

Keywords: Apiaceae, *Oliveria decumbens*, essential oil, GC/MS, antioxidant, CUPRAC, anticholinesterase, α -Amylase inhibitory.

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OP-06. Essential oil composition of selected *Mentha* species and cultivars and their bacteriostatic activity against *Staphylococcus epidermidis*

Agnieszka Ludwiczuk^{1,*}, Anna Kiełtyka-Dadasiewicz², Rafał Sawicki³, Joanna Golus³ and Grażyna Ginalska³

¹ Chair and Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodźki Str., 20-093 Lublin, Poland

² Garden of Cosmetic Plants and Raw Materials, Research and Science Innovation Center, Tarasowa 4/96 Str., 20-819 Lublin, Poland

³ Department of Biochemistry and Biotechnology, Medical University of Lublin, 1 Chodźki Str., 20-093 Lublin, Poland

*Corresponding author. Email: aludwiczuk@pharmacognosy.org

Mint are herbaceous, perennial aromatic herbs that are cultivated for their essential oils (EO) used for medicinal, cosmetic and aromatic purposes (McKay & Blumberg, 2006). The mint species are not clearly distinct and estimates of the number of species varies from 13 to 18. Hybridization between some of the species occurs naturally. Many hybrids, as well as numerous cultivars, are known in cultivation (Gobert et al., 2002; Saric-Kundalic et al., 2009).

The aim of our work was chemical analysis of 15 samples belonging to four mint species and its cultivars. These were *M. x piperita* L. (9 cultivars), *M. spicata* L. (3), *M. x gracilis* Sole (1), and *M. rotundifolia* L. (2). GC/MS analysis of EO hydrodistilled from all mint samples showed considerable variability in chemical composition. Analyzed mints were classified based on the dominant monoterpene compound into four groups. Group I was characterized by the presence of menthol and menthone as typical compounds, carvone is the major constituent of group II, linalool of group III, while piperitenone oxide characterize group IV.

Staphylococcus epidermidis is the most frequent cause of infections on indwelling medical devices. The reason is ability of the bacteria to form biofilm extremely resistant to antibiotics. Growing antimicrobial agents resistance of *Staphylococci* and other pathogenic species force to seek new active substances also among essential oils. Reference strain of biofilm forming *Staphylococcus epidermidis* ATCC 35984 was tested against fifteen EO hydrodistilled from different mint samples. Two of them, *M. rotundifolia* L. 'Variegata' and *M. x piperita* L. 'Almira' exhibit significant bacteriostatic activity. MIC (Minimal Inhibitory Concentration) was 0.25% and 0.5% respectively in compare to average MIC 4% of other tested mint EO. Both active EO are characterized by the presence of piperitenone oxide as the major component.

Keywords: *Mentha* cultivars, piperitenone oxide, *Staphylococcus epidermidis*.

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OP-07. On-line LC-GC×GC-MS/MS: A powerful unified separation-science tool

Paweł Stalica*

“SHIM-POL A.M. Borzymowski” E. Borzymowska-Reszka, A. Reszka Registered Partnership, 5 Lubomirskiego Str. 05-080 Izabelin, Poland

*Corresponding author. Email: pawels@shim-pol.pl

The latest results demonstrates the potential of the on-line combination of high performance liquid chromatography (HPLC), cryogenically modulated comprehensive two-dimensional gas chromatography (GC×GC), and triple quadrupole mass spectrometry (MS/MS). The selectivity of the HPLC dimension enabled the separation of chemical classes; each fraction, transferred to the GC×GC instrument *via* a syringe-based interface, was subjected to a specific programmed temperature vaporizer (PTV) GC×GC-MS/MS untargeted or targeted analysis.



Fig. 1 LC-GC×GC-MS/MS Shimadzu instrumentation

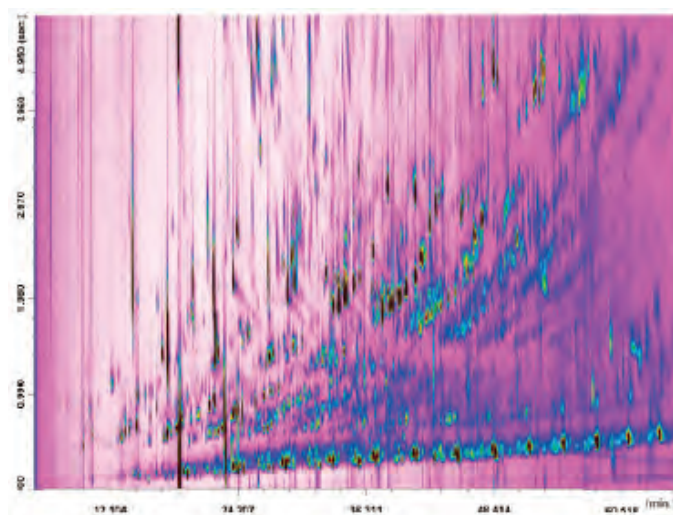


Fig. 2 Example of TIC-GC×GC-MS Shimadzu chromatogram

Keywords: two-dimensional gas chromatography, triple quadrupole mass spectrometry, syringe-based interface.

OP-08. Simultaneous multi collection in each chromatographic dimension of highly pure volatiles from complex samples exploiting a versatile LC-GC-GC-GC-PREP

Danilo Sciarrone^{1*}, Sebastiano Pantò¹, Paola Dugo^{1,2,3}, Luigi Mondello^{1,2,3}

¹ Scienze del Farmaco e Prodotti per la Salute” Department, University of Messina, viale Annunziata, 98168 – Messina, Italy

² University Campus Bio-Medico of Rome, Via Álvaro del Portillo, 21 - 00128 Rome, Italy

³ Chromaleont s.r.l., c/o “Scienze del Farmaco e Prodotti per la Salute” Department, University of Messina, viale Annunziata, 98168 – Messina, Italy

*Corresponding author. Email: dsciarrone@unime.it

The correct structural elucidation of a single component from natural samples is often a very hard task due to the fact that they are often characterized by the presence of a high number of compounds belonging to different chemical classes. In this case, the isolation of pure compounds becomes necessary in order to allow the use of elucidation techniques as NMR or MS. However, both the reliable identification and isolation of pure solutes can be hindered by a non-sufficient chromatographic separation, which can be considered as a critical point.

A new versatile multidimensional MDGC preparative system, coupled to an LC pre-separation step operated in normal phase (whenever required by the complexity of the sample), demonstrated to be capable of collecting sample components in a wide range of concentrations, in a short time period, while maintaining a high purity degree for the collected components. When necessary, the LC dimension allowed the injection of higher sample amounts and the transfer of simplified sub-samples to the MDGC-prep system.

Based on the sample complexity and on the chromatographic issues related to the purification of each component to be collected, the system was exploited for the simultaneous collection of different compounds in each of the three chromatographic dimensions, either exploiting or not the LC pre-separation, based on the need. In order to demonstrate the versatility and capability of the system, different cases of study are discussed relative to several components of interest isolated from natural complex samples and collected in the first, second or third GC dimension.

Keywords: MDGC, preparative GC, multidimensional GC, collection.

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OP-09. Determination of free and glucosidically-bound volatiles in plants

Barbara Sgorbini^{*}, Cecilia Cagliero, Lorenzo Boggia, Carlo Bicchi, Patrizia Rubiolo

Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via Pietro Giuria 9, I-10125 Torino, Italy

^{*}Corresponding author. Email: barbara.sgorbini@unito.it

Plant volatiles are in general isolated by submitting fresh or dried plant matrices to hydrodistillation to obtain the essential oil (EO). However, EO is not always representative of what the plants produce, because a part of the plant components are dissolved in the residual distillation water. Moreover, volatiles can be present in a plant in a glucosidically-bound form that are not recovered by hydrodistillation (Bruneton 2009).

This study aims i) to investigate exhaustively the volatile composition of two model plants peppermint aerial parts (*Mentha x piperita* L., Lamiaceae) and dried cloves (*Syzygium aromaticum* (L.) Merr. & L.M.Perry, Myrtaceae) ii) to evaluate the distribution of eugenol and L-menthol in aglycone form in the essential oils and in the residual distillation water and to compare their distributions to the total amounts in the original matrices, and iii) to analyze the two selected markers in their glucosidically-bound forms. L-Menthol and eugenol were selected because a) they are highly abundant markers in the plants investigated, b) they may be taken as representative of monoterpene alcohols and phenolic compounds, i.e. two of the groups of secondary metabolites often present in glycoside form in several species, c) they may be taken as representative of compounds with different water solubility, being their octanol/water partition coefficient (K_o/w) respectively 3.38 for L-menthol and 2.73 for eugenol (Episuite 2012), and d) the two plant matrices are known to provide widely differing essential oil yields. High-concentration-capacity sample preparation techniques (SBSE, and HS-SPME and in-solution SPME) to run quali-quantitative analysis without sample manipulation, in combination with GC-MS, and direct LC-MS glucoside analysis, have been employed to achieve this goal and to cross-validate the results.

The results showed that hydrodistillation is unable to isolate in full the aglycone in the essential oil and that about 23% of L-menthol and 8% of eugenol remained in the residual distillation water in the form of glucoside. The results are of great interest to achieve a better knowledge of the composition of aromatic plants and indicate the possibility to exploit residual hydrodistillation water as by-product in cosmetic, pharmaceutical and food fields.

Keywords: Essential oils; Residual distillation waters; glucosides; L-Menthol; Eugenol.

Acknowledgments

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OP-10. Characterization and isolation of limonoids in by-products of *Citrus* essential oils industry by means of liquid chromatography

Paola Dugo^{1,2,3*}, Marina Russo¹, Adriana Arigò¹, Maria Luisa Calabrò¹ and Luigi Mondello^{1,2,3}

¹"Scienze del Farmaco e Prodotti per la Salute" Department, University of Messina, viale Annunziata, 98168 – Messina, Italy

²University Campus Bio-Medico of Rome, Via Álvaro del Portillo, 21 - 00128 Rome, Italy

³Chromaleont s.r.l., c/o "Scienze del Farmaco e Prodotti per la Salute" Department, University of Messina, viale Annunziata, 98168 – Messina, Italy

*Corresponding author. Email: pdugo@unime.it

Citrus essential oils and their derived products are widely used in food, cosmetics, flavor and fragrance industry, for preparation of soft drinks, alcoholic beverages, ice creams, cookies, candies, air freshness, pharmaceutical preparations, cleaning products as well as colognes, fine perfumes and other cosmetic products. The by-products obtained from the extraction of *Citrus* essential oils are represented by peels, pulps, seeds and juices. These are characterized by the presence of bioactive molecules such as flavonoids, limonoids, phenolic acids, coumarins, furocoumarins, polymethoxyflavones, and carotenoids. Limonoids are human health promoters, and have many pharmacological properties, such as: anticancer, antioxidant, antibacterial and antifungal. Limonoids occur in *Citrus* both in glucosidic and in aglyconic form. Limonoid aglycones are water-insoluble and responsible for a bitter taste of the *Citrus* fruits, whereas limonoid glucosides are water-soluble and tasteless. These molecules are present in considerable amounts in *Citrus* fruits, and although the highest concentration was found in seeds, other components are also present in peels, pulps and juices. This work reports the qualitative and quantitative composition of limonoids (both aglycones and glucosides) in juice, peels, pulps and seeds of some *Citrus* fruits (bergamot, lemon, sweet orange and blood orange) by means of RP-HPLC/PDA/MS. Moreover a multidimensional HPLC system coupled with a photodiode array and a mass spectrometer detectors was used to isolate two limonoid aglycones: limonin and nomilin from lemon seeds, and three limonoid glucosides: nomilin glucoside, limonin glucoside and nomilinic acid glucoside from a concentrated bergamot juice. Isolation of limonoids from bergamot juice and lemon seeds represent a way to re-evaluate these by-products generated by the bergamot and lemon essential oils production. Also, unlike other *Citrus* juices, bergamot juice has no an appreciable taste and consequently is difficult to put it on market. Bergamot juice can be easily concentrated and used to isolate limonoid glucosides, which can be used as antioxidant in different functional food preparations or as purified nutraceuticals..

Keywords: Citrus, essential oil, limonoids, HPLC/PDA/MS, preparative HPLC.

Acknowledgments

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OP-11. Survey of the essential oil profile of *Origanum vulgare* subsp. *vulgare* populations and their stability among different environmental conditions

Beatrix Cserhádi, Szilvia Tavaszi-Sárosi, Péter Rajhárt and Krisztina Szabó*

Department of Medicinal and Aromatic Plants, Corvinus University of Budapest, H-1118 Budapest, Villányi str. 29-35. Hungary

*Corresponding author. Email: szabo.krisztina@uni-corvinus.hu

Essential oil content and composition of *Origanum vulgare* subsp. *vulgare* populations were examined for two years in their wild habitat (2010-11) and for two years in cultivation (2011-12). The aim of our work was to screen the essential oil characteristics of the species in natural habitats with different elevations, soils types, precipitation and average temperature; then to follow up the same characteristics by establishing and examining the progeny populations among the same environmental circumstances at our Research Field.

The 9 wild populations were situated in the northern part of Hungary following a zone of 300 km distance. The Research Field is situated approximately in the middle of this line representing a uniform environment for the progenies. The sampling was done in all cases in the full flowering period harvesting 20 individuals/population. The shoots were dried in shadow in room temperature and handled as mass samples after shredding. 20 grams of samples were distilled following the Hungarian Pharmacopoeia (Ph.Hg. VII.) using a Clevenger type apparatus. The composition of the essential oil was evaluated by GC-MS 6890 N Gaschromatograph equipped with 5975 inert mass selective detector, Agilent Technologies, injector temperature was 230 °C; split ratio: 30:1; transfer line: 240 °C; colonna – HP-5MS (5 % phenyl methyl siloxane) length: 30 m, id: 250 µm, film thickness: 0.25 µm; carrier gas: helium (constant speed of 1 ml/min); temperature programme: 60-240 °C by 3 °C/min; ionization energy was 70 eV. Detection of the compounds was done by comparing their mass spectra to librarian references (NIST) and by calculating their linear retention indexes (LRI).

The essential oil content of our populations (both in wild habitat and cultivation) ranged between 0.005 and 0.402 ml/100g. Decreasing tendency was proved, almost all the populations showed much lower essential oil content in cultivation circumstances. Differences among the essential oil compositions were remarkable only in case of the second year for the progenies. The precipitation was revealed as the most influencing environmental factor for the shift towards the oxidized sesquiterpenes in the typical sesquiterpene chemotypes.

Keywords: sesquiterpene, interaction, environment, natural habitat, genotype.

Acknowledgments

TÁMOP 4.2.1./B-09/01/KMR/2010-0005 and TÁMOP 4.2.2./B-10.1-2010-0023.

OP-12. Study of essential oil yield and quality of a heterogeneous *Salvia lavandulifolia* crop monitored at different phenological stages throughout four years

Jaime Usano-Aleman^{1,*}, Jesús Palá-Paúl² and David Herráiz-Peñalver³

¹ Institute of Crop Science and Resources Conservation, INRES-Horticultural Sciences, University of Bonn, 53121, Bonn, Germany

² Department of Plant Biology I (Botany and Plant Physiology), Faculty of Biology, Complutense University, 28040, Madrid, Spain

³ Agricultural Centre of Albaladejito, Junta de Comunidades de Castilla-La Mancha, 16194, Cuenca, Spain.

*Corresponding author. Email: jusanoal@uni-bonn.de

The question of finding references regarding the essential oil chemical composition of aromatic and medicinal plants species does not involve particularly great difficulties. Nevertheless, very few studies have been conducted with the objective of monitoring essential oil quality and quantity throughout different cropping seasons considering, in addition, the intra-specific phytochemical diversity within the species (Rahimmalek et al, 2009; Herráiz-Peñalver et al, 2010). Our main objective was to obtain a clear picture about the year-by-year evolution of the essential oils of Spanish sage (*Salvia lavandulifolia*) as an established crop in Spain. To this end, we grown replicas in a single cultivation plot of different mother plants coming from a broad prospection of wild populations from the centre of Spain. Essential oils from Spanish sage have shown over four years of cultivation the following main compounds: 1,8-cineole > β -pinene + myrcene > camphor > α -pinene > limonene. The average content of 1,8-cineole is further enhanced until reaching 50% of relative content with some genotypes reaching as much as 69.5%. On the other hand, the high content of compounds such as 1,8-cineole, limonene and camphor can be cleared allocated to concrete genotypes. As an example of this, individuals placed above the average line on Figure 1 may be considered as 1,8-cineole chemotypes. In this respect, we proved firstly that the content of the previous major compounds is highly related to the genotype and secondly that the climatic conditions have mostly influence on the essential oil yield. Lack of rainfall when new leaves are beginning to grow increased remarkably the essential oil yield in full bloom stage. On the other hand, the phenological stage showed minor influence on the essential oil chemical composition. By this way, samples collected over time showed more similar essential oil chemical composition regarding the year on which they were collected rather than the phenological stage (Usano-Aleman et al, 2014).

Keywords: Spanish sage, 1,8-cineol, essential oil yield, cultivation.

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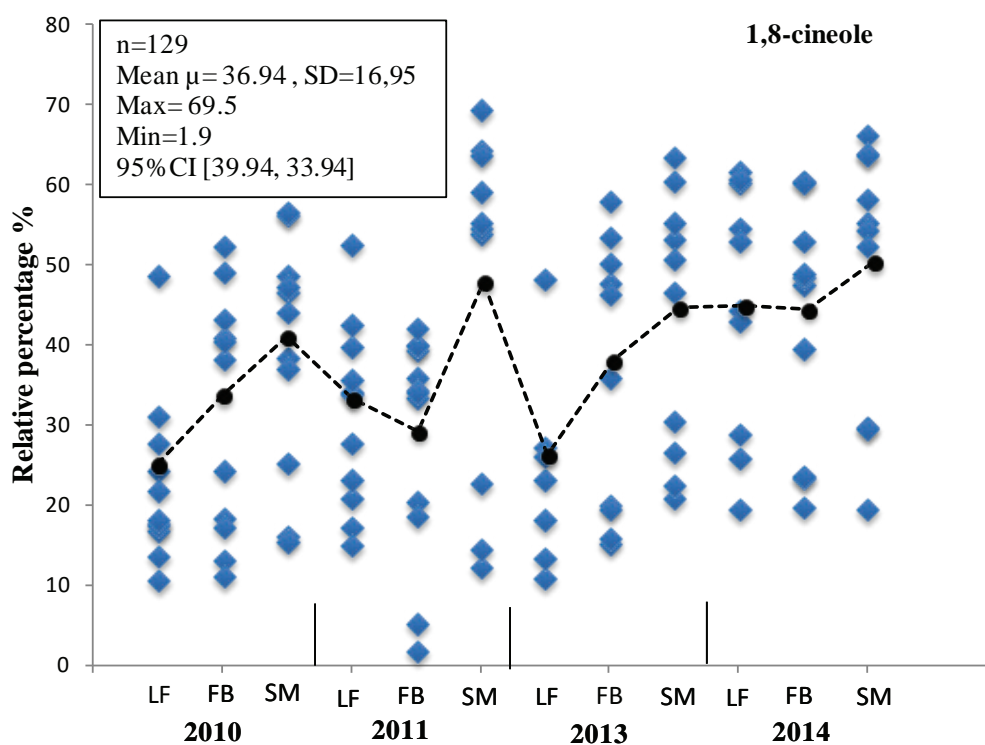


Fig. 1. Relative percentage content of 1,8-cineole in the *Salvia lavandulifolia* essential oil monitored during four years. The central curve represents average data at each collection time while diamonds represents individual samples. Different letters indicate the phenological stage as follows: LF for new leaves formation stage, FB for the full bloom stage and SM for the seed maturation stage.

OP-13. Lab and pilot scale microwave-assisted recovery of volatile fraction from citrus peels waste

Diego Carnaroglio^{1,2}, Giorgio Grillo², Emanuela Calcio Gaudino², Farid Chemat³, Mario Pagliaro⁴, Giancarlo Cravotto^{2*}

¹ Milestone srl, Via Fatebenefratelli 1/5, I-26010 Sorisole, Bergamo, Italy

² Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin, Via P. Giuria 9, 10125 Turin (Italy)

³ GREEN Extraction Team, INRA, UMR 408, University of Avignon, F-84000 Avignon, France

⁴ Istituto per lo Studio dei Materiali Nanostrutturati, CNR via U. La Malfa 153, 90146 Palermo, Italy

*Corresponding author. Email: synthesis@milestonesrl.com

Citrus peel is one of the most abundant underutilized agri-food waste with 15.6 million metric tons from 31.2 million metric tons of processed citrus fruit annually. A profitable exploitation of this biomass is a challenging task for researchers and industry. Orange oil (D-limonene 94–96%) obtained by skin cold-pressing or hydrodistillation is widely employed as flavoring agent. In this study microwave hydrodistillation (MWHD), solvent-free microwave-assisted extraction (SFME) and microwave hydrodiffusion and gravity (MHG) were investigated to speed up the process. Dedicated MW reactors are commercially available both for lab (NEOS, NEOS-GR) and pilot scale (MAC75) applications. Aim of this study was to evaluate the advantages of MW-assisted methods in the production of citrus oil from *Citrus sinensis* (L.) peels waste in term of oil quality, time and energy consumption. The production scale was increased from hundreds grams to several kilograms, developing a fast and efficient MW-assisted integrated processes with or without a steam flow. Besides orange oil, the bio-refinery approach enabled a quantitative pectin recovery and the residual cellulose was converted to bioplastics in further MW-assisted steps.

Keywords: Green extraction, essential oil, microwave, citrus peels waste, d-limonene, Scale-up.

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OP-14. Volatile components of two Japanese scale insects, *Ceroplastes japonicus* and *Ceroplastes rubens*

Yoshinori Asakawa^{1*}, Masao Toyota¹, Kazutoshi Sakurai², Kenichi Tomiyama² and Yoshihiro Yaguchi²

¹ Faculty of Pharmaceutical Sciences, Tokushima Bunri University, 180 Yamashiro-cho, Tokushima 770-8514, Japan

² Corporate Research and Development Division, Takasago International Corporation, Hiratsuka, Kangawa 254-0073, Japan

*Corresponding author. Email: asakawa@ph.bunri-u-ac.jp

Scale insects are generally covered by thick wax and epiphytically live on deciduous and ever green plants. Most of them damage many fruit trees. When *Ceroplastes japonicus* (Coccidae) is crushed, it emits cherry, hop, woody and green scent. *C. rubens* lives epiphytically on the twigs of *Ilex integra* and *Laurus nobilis* etc. It also emits sour, sweet and woody odor. We investigated volatile components of the female *C. japonicus* and *C. rubens* from the view point of identification of their characteristic odor. *C. japonicus* and *C. rubens* (0.80g) were smashed and immersed into pentane for 1 hr, respectively and each extract was dried over sodium sulfate, followed by evaporation (Kuderna Danish) at 42°C to give each crude extract. Each insect was also extracted with ether and the former and the ether extracts were analyzed by TLC, GC/MS and NMR. The major component of *C. japonicus* is α -humulene and δ -cadinene. The ether extract of the former species contained α -fenchene, α -selinene, α -copaene, β -bourbonene, β -elemene (12%), β -caryophyllene, β -copaene, α -humulene (57%), germacrene D, δ -cadinene, 1,2-humulene oxide and pimaradiene. The *n*-pentane extract of *C. rubens* produced β -selinene and octadecanoic acid as the predominant components, together with the same components as those found in *C. japonicus*. The ether extract of *C. rubens* also showed the presence of β -bourbonene, β -elemene, selina-5,11-diene, *ar*-curcumene, β -selinene, α -selinene, pimaradiene, labda-7(17),12,14-diene and octadecanoic acid. Odor-contributing components on GC/O analysis *Ceroplastes japonicus* and *C. rubens*: The odor of *C. japonicus* is stronger than that of *C. rubens* when both insects were crushed by fingers. This fact is corresponding to the difference of volatile components of both insects. The odor of the former insect emits more sweaty and cheesy odor which might be due to the presence of butyric acid and 4-methyl-(3*E*)-hexanoic acid. On GC/O analysis, decan-1,4-olide (γ -decalactone) with milky coconut-like note and sotolon with brown sugar lump-like note were also detected as the characteristic cherry-like sweet and sour note of these two insects.

Keywords: Scale insects; *Ceroplastes japonicus*; *C. rubens*; sesquiterpenes; γ -decalactone; sotolon.

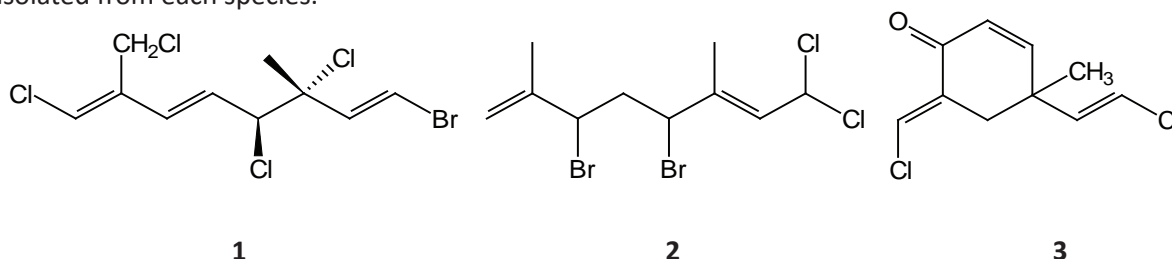
OP-15. Isolation, characterisation and *in vitro* cytotoxic bioactivity of volatile polyhalogenated monoterpenes from Southern African marine algal *Plocamium* species

Michael Knott*

School of Pharmacy, University of Namibia, Private Bag 13301, 340 Mandume Ndemufayo Avenue, Pionierspark, Windhoek, Namibia, 9000

*Corresponding author. Email: mknott@unam.na (M.G. Knott)

In our continuing efforts to discover new lead compounds for the treatment of cancer, various Southern African *Plocamium* species have been investigated. Bioactive volatile secondary metabolites were isolated using column chromatography and normal phase HPLC and characterised by means of one and two dimensional NMR spectroscopic data and MS analysis. Compounds **1-2** were isolated from *P. surhii* and *P. corallorhiza* respectively, and demonstrated good *in vitro* activity against WHCO1 oesophageal cancer cell lines ($IC_{50} = 6.6 \mu\text{M}$ and $IC_{50} = 9.3 \mu\text{M}$) compared to cisplatin which had an $IC_{50} = 13 \mu\text{M}$ for the same test (Antunes et al., 2011; Knott et al., 2005). Compound **3** was isolated from *P. maxillosum* and had an $IC_{50} = 12 \mu\text{M}$ against MDA-MB-231 breast cancer cell lines (Knott et al., 2011) compared to tamoxifen which had an $IC_{50} = 0.1 \mu\text{M}$. Interestingly, in terms of chemotaxonomy, the major metabolites isolated from selected Southern African *Plocamium* species are unique to each species, however, some degree of overlap appears to occur with regards to some of the minor secondary metabolites isolated from each species.



These results highlight the potential of these compounds as important leads in the development of new anticancer drugs as well as the need for investigating their SAR's and mode of action.

Keywords: *Plocamium*; halogenated monoterpenes; cytotoxic.

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YOUNG SCIENTIST LECTURES

YSL-01. Chemical constituents and biological properties of Marchantiophyta from South Africa

Johan Linde^{1,*}, Sandra Combrinck², Agnieszka Ludwiczuk³, Sandy van Vuuren⁴, Jacques van Rooy⁵ and Ntebogeng Mokgalaka¹

¹Department of Chemistry, Tshwane University of Technology, Private Bag X680, Pretoria, South Africa

²Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria, South Africa

³ Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodzki Str., 20-093 Lublin, Poland

⁴ Department of Pharmacy and Pharmacology, University of the Witwatersrand, 7 York Rd, Parktown, South Africa

⁵ South African Biodiversity Institute, Private Bag X101, Pretoria, South Africa

*Corresponding author. Email: LindeJH@tut.ac.za

Liverworts (Marchantiophyta) are small leafless, flowerless, spore-producing plants. They are considered to be the oldest terrestrial plants, with 6000 species occurring globally and 1200 species in southern Africa. Liverworts produce secondary metabolites in abundance. At present more than 1500 terpenoids and 350 aromatic compounds (excluding flavonoids) have been isolated from or detected in the Marchantiophyta (Asakawa, 1982, 1995, 2008; Asakawa et al., 2013). Several of these constituents are unique to liverworts (Buck and Goffinet, 2000; Asakawa et al., 2013) and exhibit interesting biological activities, such as antibacterial, antifungal, cytotoxic, insect repellent, insecticidal, muscle relaxing, as well as some enzyme inhibitory and apoptosis-inducing activities (Zinsmeister et al., 1991, Asakawa, 1995; Mues, 2000; Ludwiczuk and Asakawa, 2008).

Liverwort specimens were collected from several localities in South Africa. Volatile compounds of nine species, extracted using solvent-based extraction, were identified by gas chromatography-mass spectrometry (GC-MS). Solvent extracts (MeOH: CHCl₃) were evaluated for their antimicrobial activities against *Candida albicans* (ATCC 10240), *Cryptococcus neoformans* (ATCC 1416), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 8740), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923). The most active extract was that from *Plagiochasma rupestre* against *P. aeruginosa* and *E. faecalis*, with minimum inhibitory concentrations (MICs) of 0.5 mg/mL and 1.8 mg/mL respectively. Reversed phase preparative high performance liquid chromatography (HPLC) purification of the extract from *Dumortiera hirsuta* yielded a pure compound, which was tentatively identified as a dumortane-type derivative, using GC-MS. Structure elucidation is currently underway using one and two dimensional spectra obtained from nuclear magnetic resonance spectroscopy and infrared analysis. This is the first comprehensive study of the chemical profiles and biological properties of southern African liverworts.

Keywords: Liverwort, volatiles, antimicrobial activity.

Acknowledgments

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YSL-02. Mycorrhizal symbiosis as a potential biotechnology for medicinal and aromatic plants improvement and sustainable production of essential oils in Lamiaceae family

Waed Tarraf*, Claudia Ruta, Anna Tagarelli, Francesca De Cillis, Giuseppe De Mastro

Department of agricultural and environmental science, University of Bari "Aldo Moro", Via Amendola, 165/A- 70126, Bari, Italy

*Corresponding author. Email: waadtaraff@yahoo.co.uk

Medicinal and aromatic plants have gained momentum over the world due to their phytochemicals, especially essential oils; they are used widely for pharmaceutical, food, cosmetic and fragrance purposes. To meet the increased demand for high productivity and standard quality of essential oils, the research in sustainable agriculture has focused on less chemical inputs along with development of new technology i.e. arbuscular mycorrhiza fungi (AMF), as a powerful candidate to improve nutrients uptake by host plant.

To evaluate the efficiency of different AM fungi on quantity and quality of essential oils (EOs) from *Salvia officinalis* L., *Thymus vulgaris* L. and *Origanum vulgare* L. a greenhouse experiment was conducted, including two mycorrhizae treatments, *Funneliformis mosseae* and commercial Symbivit applied alone or combined with two phosphorus levels (P_1 and P_2), and untreated control.

The highest EOs content was induced by Symbivit (2.70, 1.44, and 1.23 % v/w) for thyme, oregano and sage respectively, while a slight decrease was recorded in *T. vulgaris* (2.27%) and *O. vulgare* (1.06%) by *F. mosseae*. However, the P addition reduced the EOs content regardless of the host plant except for sage; where it was increased in the treatments of *F. mosseae* combined with (P_1 or P_2). Sage EO composition was altered by inoculation; manool was the major constitute which increased in all the treatments over the non-inoculated one. Its content varied between the two fungi, *F. mosseae* plants obtained 39.33% whereas Symbivit had only 16.44%. For oregano, carvacrol content of mycorrhizal plants was comparable to control. Symbivit induced higher carvacrol (77.46%) compared to *F. mosseae* (69.41%) and also *F. mosseae × P_1 increased carvacrol (79.70%). For thyme, the symbiosis increased relatively thymol (66.66 and 67.38%) by Symbivit and *F. mosseae*, respectively. All the combined treatments had no considerable changes to non-colonized plants (63.47%).*

Generally, the results exhibit the roles of mycorrhizae as biofertilizers on both quantity and quality of EOs, thus enhancing the sustainability of production even in low soil fertility. Also, they suggest using exclusive inoculums as great potential for specific host, to achieve high economic benefits since the response to AMF was varied with fungal species.

Keywords: Lamiaceae, essential oil, *mycorrhizae*, phosphorus, biofertilizers.

YSL-03. *Thymus vulgaris* essential oil as feed additive in broiler chickens

Vladimíra Ocelřová^{1,*}, Remigius Chizzola², Jana Pissarřřikřovřř¹, Oksana Ivaniřřinřřvřř¹, Miroslav Ryzner¹, Ľubomíra Greřřřkovřř¹, řřtefan Faix¹, Iveta Plachřř¹

¹ Institute of Animal Physiology, SAS, řřoltřřsovřř 4, 040 01 Kořřice, Slovak Republic

² Institute of Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine Vienna, Veterinřřrplatz 1, 1210 Vienna, Austria

*Corresponding author. Email: ocelova@saske.sk

Bioactive components of *Thymus vulgaris* essential oil (EO) can improve health parameters in animals. Positive effect of thyme EO is needed especially in poultry production during the fattening period and intensive growth. Numerous studies have already confirmed thyme EO components beneficial qualities but not much is known about the pharmacokinetics of the main compound thymol. Absorption and metabolism of phytogetic feed additives in poultry is related to metabolism and deposition of their main compounds in tissues intended for food production for humans.

Forty-eight 1-day old non sexed broiler chickens (Ross 308) were divided randomly into 6 groups with different levels of thyme EO in feedstuff. Qualitative and quantitative analysis of the thymol in studied oil was performed by high performance liquid chromatography (HPLC). Thymol was presented at high concentration (47.85 %) in examined volatile oil. Basal diet for broiler chickens was supplemented with 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.1% *T. vulgaris* EO respectively and consumed by broiler chickens during four weeks. Considering that compounds of EO are volatile at room temperature, evaporation of thymol was studied by using HPLC after mixing the thyme EO to feedstuff. Amount of thymol was relatively stable for three days according to our results.

Levels of thymol after enzymatic cleavage were investigated in plasma and kidney from broiler chickens by using SPME followed by gas chromatography coupled to mass spectrometry. Concentrations of thymol in kidney were higher in comparison to concentrations in plasma. Our results seem to indicate intensive metabolism of thymol in kidney tissue what corresponding with suggestion that kidney can play important role in metabolism of phenols (Kohlert et al., 2002).

Phenols derived from phytogetic feed additives are easily absorbed in gastrointestinal tract and conjugated with sulfate and glucuronic acid in tissues. Overdose of feed additive can overcome capacity of conjugation and alternative metabolic pathways can produce highly reactive metabolites (EFSA, 2012). The concentration of feed additives which is beneficial for animals and safe for consumers is needed to be found out.

Keywords: *Thymus vulgaris*, essential oil, thymol, plasma, kidney.

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YSL-04. Cinnamon and thyme essential oils and their major components as anti-quorum sensing and anti-biofilm forming agents

Erika Beáta Kerekes¹, Maria Iacob², Judit Papp², Viktória Kósa³, Csaba Vágvölgyi¹, Judit Krisch³

¹ Department of Microbiology, University of Szeged, Faculty of Science and Informatics, Szeged, Hungary

² Department of Biology and Geology, University of Babeş-Bolyai, Hungarian Institute of Biology and Ecology, Cluj-Napoca, Romania

³ Institute of Food Engineering, University of Szeged, Faculty of Engineering, Szeged, Hungary

*Corresponding author. Email: kerekserika88@gmail.com

Biofilm development and quorum sensing (QS) which regulates biofilm formation represent a serious problem by lowering the shelf life of foods and causing severe health problems (Liu et al., 2006, Bakkiyaraj et al., 2012). Application of essential oils (EOs) in the food industry as natural antimicrobials for extension of shelf life has become widespread (Burt, 2004). In our study, the anti-QS effect of cinnamon (*Cinnamomum zeylanicum*) and thyme (*Thymus vulgaris*) EOs and their main components were evaluated using *Chromobacterium violaceum* and *Serratia marcescens* biomarkers. Anti-biofilm forming effect was also tested against biofilms of *Escherichia coli* and *Pseudomonas putida*. Anti-biofilm tests were conducted with MIC/2 values of EOs in microtiter plates and anti-QS effect was measured by paper disc diffusion assay. Our results showed that after using cinnamon EO the rate of biofilm reduction was around 55% in case of *Ps. putida* and 40% for *E. coli*. Thyme EO reduced these biofilms with 66% and 27% respectively. *S. marcescens* QS inhibition zones were the highest after using 1µl of cinnamon (5 mm) and 5µl of thyme EO (14 mm). The inhibition of *C. violaceum* QS was concentration dependent (inhibition zones from 0.5 mm to 5 mm). In conclusion, these EOs are promising candidates to be used as natural food preservatives in the future.

Keywords: essential oils, biofilms, quorum sensing.

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YSL-05. Cytotoxicity, antiviral activity and GC/MS analysis of commercially available eucalyptus, pine and Indian melissa essential oils

Łukasz Świątek^{1,*}, Barbara Rajtar¹, Tomasz Baj², and Małgorzata Polz-Dacewicz¹

¹ Department of Virology, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

² Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

*Corresponding author. Email: lukasz.swiatek@umlub.pl

Essential oils (EO) have been previously reported to possess antiviral properties. (Venturi et al., 2015) In this study commercially available essential oils from eucalyptus (*Eucalyptus globulus*), pine (*Pinus sylvestris*) and Indian melissa (*Cymbopogon citratus*) were tested for cytotoxic properties and antiviral activity against HHV-1 (Human Herpesvirus type 1). Furthermore, the influence of EO on cell cycle was evaluated.

Cytotoxicity was assessed on VERO (green monkey kidney) and FaDu (human pharynx squamous cell carcinoma) cell lines using the MTT method. EO in non-toxic concentrations were then incubated with HHV-1 infected Vero cell line until the cytopathic effect (CPE) was observed in the positive control. Subsequently, the virus titre was measured using end-point dilution assay. The influence of EO on cell cycle of FaDu cell line was tested using two-step cell cycle protocol and NucleoCounter NC-3000 image cytometer. The composition of EO was tested with the use of GC/MS (Shimadzu GC-2010 Plus, Shimadzu QP2010 Ultra MS). The main components of eucalyptus oil were: 1,8-cineole (59.6%), *p*-cymene (13.7%), limonene (11.9%); pine oil: alpha-pinene (37.9%), beta-pinene (19.5%), 3-carene (15.4%); Indian melissa oil: citronellal (34.9%), geranial (18.6%), citronellol (12.7%).

Indian melissa oil showed low toxicity on both VERO and FaDu (IC₅₀ 196.9 and 183.7 µg/ml, respectively). Eucalyptus oil was also non-toxic to VERO (IC₅₀ 277.6 µg/ml) but showed selective toxicity towards cancer cell line FaDu (IC₅₀ 49.2 µg/ml). The highest toxicity on FaDu was observed for pine oil (IC₅₀ 6.2 µg/ml). Among tested EO, only Indian melissa oil in the concentration of 75 and 125 µg/ml inhibited CPE formation in HHV-1 infected VERO cell line, resulting in the decrease of the virus titre by 0.99 and 5.02 log, respectively.

Cell cycle of FaDu was altered by pine EO, showing increase in the S phase after 7, 10 and 12 hours of incubation, and decrease in the G2/M phase after 7 and 10h. After 24h an increase in the G2/M phase was observed. However, after 28h all phases were similar to control cells.

Indian melissa and eucalyptus EO possess low toxicity on VERO cell line. Pine EO showed significant toxicity on cancer cell line and should be further studied to assess its anticancer potential. Indian melissa EO possesses antiviral activity against HHV-1.

Keywords: essential oil, cytotoxic activity, antiviral activity, HHV-1.

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YSL-06. Main constituents of *Carum carvi* seeds and *Mentha spicata* essential oils as substrates for preparation of novel fragrant compounds with preserved terpene backbone

Daniel Jan Strub^{1,*}, Józef Kula², Stanisław Lochyński^{1,3}

¹ Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wyb. Wyspiańskiego 27, 50-370 Wrocław, Poland

² Institute of General Food Chemistry, Faculty of Biotechnology and Food Sciences, Technical University of Łódź, Żeromskiego 116, 90-924 Łódź, Poland

³ Institute of Cosmetology, Wrocław University College of Physiotherapy, Tadeusza Kościuszki 4, 50-038 Wrocław, Poland

*Corresponding author. Email: daniel.strub@pwr.edu.pl

Terpenes and their oxygenated derivatives are commonly used as substrates for the synthesis of new semisynthetic compounds with olfactory properties. Oximes are rarely applied as constituents of fragrance compositions due to their lower volatility and only seven of them are used commercially (Zviely & Li, 2013). Studies regarding Structure-Odour Relationship (SOR) of terpenoid oxime ethers are even scarcer than parental oximes. Synthesis, olfactory evaluation and application of simple oxime ethers are described only in three patents (Kaiser et al., 2002; Narula et al. 2005; 2006).

Our current research is focused on the synthesis of new terpenoid oxime ethers with preserved terpene backbone. We have obtained over 40 compounds with fenchane and *p*-menthane moieties. Substrates for syntheses of final compounds were (+)- and (–)-fenchone (Strub et al., 2014) and (+)- and (–)-carvone. Synthetic details and olfactory evaluation of all novel oxime ethers will be presented. Significant fragrance diversity is observed between homologous series of oxime ethers. More noticeable are (–)-*O*-glycidyl fenchane derivative with floral profile (gerbera and aster), *O*-methyl derivatives with preserved *p*-menthane system (pickled cucumbers scent), and compounds with *O*-amyl and isoamyl side chain with fruity profiles (black currant and pear).

Keywords: Oxime ethers, olfactory evaluation, terpenoids.

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POSTER PRESENTATIONS

PP-001. Volatile constituents and larvicidal Activity of the essential oils from three Nigerian medicinal plants

Sherifat Aboaba^{1,*}, Kolade Ibrahim² and Guido Flamini³

¹ Department of Chemistry, University of Ibadan, Ibadan, Nigeria

² Department of Zoology, University of Ibadan, Ibadan, Nigeria

³ Dipartimento Di Farmacia, Pisa., Italy

*Corresponding author. Email: saboaba@gmail.com

Larvicides are products that kill mosquito larvae. Essential oils and plant extracts are emerging natural sources of larvicides, insecticides and insect antifeedant (Duke, 1990). The essential oils from the leaves and stem barks of three Nigerian medicinal plants; *Anthocleista vogelii*, *Morus mesozygia* and *Sterculia tragacantha* were obtained by hydrodistillation and characterized using GC and GC-MS analyses; thereafter the constituents were identified by comparing their mass spectra with NIST 1998 library data of the GC-MS system as well as by comparison of their retention indices (RI) with relevant literature data (Adams, 2007). The oils were tested for their ability to kill mosquito larva using 3rd instar stage of female *Culex* mosquito and the LC₅₀ values were calculated using Probit analysis (Finey, 1971).

The yields of the essential oils varied from 0.46–0.73% (w/w). The characterised constituents ranged from 90.3–96.9% representing the total identified compounds in the entire oils. *A. vogelii* leaves was dominated by 1,8-cineole (24.4%) and viridiflorol (24.2%) while the stem bark had in abundance 2-pentylfuran (13.4%) and 1-octen-3-ol (9.3%); the leaves of *M. mesozygia* leaves was dominated by (E)- β -ionone (12.4%) and β -elemene (11.7%) and the stem-bark had in abundance 2-dodecanone (77.0%) and hexahydrofarnesylacetone (13.0%), on other hand, the major constituents in the leaf essential oil *S. tragacantha* was β -bisabolol (13.1%) and (E)-geranylacetone (11.3%); hexahydrofarnesylacetone (32.5%) and 1,8-cineole (14.7%) were the most abundant in the stem-bark of *S. tragacantha* oil. The general profile of the six essential oils indicates they are rich in apocarotenoids.

The oils also displayed different levels of toxicity to the larva of *Culex* mosquito, the LC₅₀ values varied in the range 83.52 – 1131.97 μ g/mL indicating high toxicity to low toxicity.

The studied essential oils are dominated by compounds that have displayed biological activities and the mosquito larvicidal assay provides evidence that the oils could be good mosquito larvicidal agents useful to combat the spread of filariasis.

Keywords: Medicinal plants, hydro distillation, GC-MS, Apocarotenoids, *Culex* mosquito.

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PP-004. Vapour-phase method: a possible solution for *in vitro* antimicrobial evaluation of volatile substances

Kamilla Ács^{1*}, Klaudia Kulcsár¹, Béla Kocsis², Andrea Böszörményi³, Györgyi Horváth¹

¹ Department of Pharmacognosy, Medical School, University of Pécs, H- 7624, Pécs, Rókus str. 2., Hungary

² Department of Medical Microbiology and Immunology, Medical School, University of Pécs, H- 7624, Pécs, Szigeti str. 12., Hungary

³ Semmelweis University, Faculty of Pharmacy, Institute of Pharmacognosy, H-1085, Budapest, Üllői str. 26., Hungary

*Corresponding author. Email: kamilla.acs@aok.pte.hu

The recurrent respiratory infections (RIs) and narrowing possibilities of antibiotic therapy are producing serious problems in medicinal health care nowadays. Therefore, the discovery of new alternative agents, which can directly pass into the respiratory tract (e.g. volatile substances), would be highly important. The antimicrobial potentials of essential oils (EOs) were studied by several *in vitro* techniques before, but these methods usually focused on the antimicrobial activity of the EOs in liquid media instead of volatile phase. Thus, the investigation of the antibacterial activity of EOs in vapour-phase (VP) would be very reasonable for the supplementary treatment of RIs.

The aim of our study was the microbiological evaluation of cinnamon bark, clove, thyme, scots pine, eucalyptus, peppermint, and citronella EOs by *in vitro* VP technique.

The EOs were obtained from a Hungarian drugstore (Herbaria) and analysed with GC-MS. The antibacterial activity was tested against respiratory tract pathogens: *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pyogenes* (1116), *Streptococcus pneumoniae* (DSM 20566), and *Moraxella catarrhalis* (DSM 9143). We used four-section Petri dish (PD, VWR Int. Ltd.) poured with Mueller-Hinton- (MHA, Oxoid Ltd.) or blood agar. The EOs were evaporated through a filter paper in the PD. The minimum inhibitory concentrations (MIC) were determined after 48 h incubation in 37°C. The values of MIC were expressed in µL/L referred to the free space in the PD. All tests were carried out in triplicate.

Cinnamon oil was the most effective against all of the investigated bacteria in 25-75 µL/L concentrations. Against *P. aeruginosa*, cinnamon and thyme showed better activity (31.25 µL/L) in comparison with eucalyptus oil (125 µL/L). In case of *Streptococcus* species and *M. catarrhalis*, thyme, clove, peppermint, and citronella were also effective in higher concentrations (50-250 µL/L). We found eucalyptus oil moderately active against both *M. catarrhalis* and *S. pneumoniae*. Scots pine EO was inactive against the investigated bacteria.

VP technique can be optimized for determination of antibacterial effect of different EOs against respiratory tract bacteria. In our further studies, we would like to focus on the mode of action and anti-inflammatory effect of EOs in *in vivo* (mouse) models, determine the active components of EOs, and include other respiratory tract pathogens.

Keywords: Essential oil, vapour-phase, antibacterial activity, respiratory tract.

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PP-007. Essential oil composition of four *Artemisia* species from Mongolian Trans-Altai Gobi

Altantsetseg Shatar¹, Oyuttsetsen Amarsanaa^{2*}, Javzmaa Namshir¹, Shatar Sandui¹

¹ Institute of Chemistry and Chemical Technology Mongolian Academy of Sciences, 13330 Peace avenue, MAS 4th building, Bayanzurkh district, Ulaanbaatar, Mongolia

² School of chemistry and chemical engineering, National university of Mongolia. Ulaanbaatar. 14261, Mongolia

*Corresponding author. Email: oyuta11@gmail.com

The genus *Artemisia* (Asteraceae) which contains many useful aromatic and medicinal plants comprises of about 400 species found in the northern hemisphere. In Mongolia the genus commonly called "Wormwood" with about 103 species is mostly occurring in the Mongolian Desert-Gobi (Gubanov, 1996; Shatar & Altantsetseg, 2011). These herbs are used world wide in tonic, stomachic and stimulant beverages and as antiphlogistics in antiseptic oils or tinctures applied for the relief of rheumatic pains (Shatar & Altantsetseg, 2011).

Aerial parts of four *Artemisia* species were collected from Mongolian Trans-Altai Gobi at the flowering and fruiting stage. The oils were isolated by hydrodistillation in Clevenger-type apparatus for 3 hours. The yields obtained of the oil were: *A. scoparia* Waldst. et Kit (0.6%), *A. macrocephala* Jacquem. ex Besser (0.4%), *A. anethifolia* Web. ex Stechm (0.9%) and *A. dracunculus* L (1.0%). The oils were analyzed by GC and GC-MS. Many constituents were identified by comparing their retention indices with those of authentic compounds or with data published in the literature (Adams, 1995). Twenty four compounds were identified in the essential oil (EO) of *A. scoparia* representing 93.6% of all components. The major compound was p-cymene (11.3%), together with limonene (10.3%), myrcene (9.6%), α -pinene (8.6%), and γ -terpinene (7.8%). The most characteristic compounds present in EO obtained from *A. macrocephala* were γ -terpinene (15.7%), α -terpineol (14.7%), borneol (10.8%), and 1,8-cineole (10.7%). Totally, thirty six compounds were identified. Forty nine components were identified in the oil of *A. anethifolia* representing 94.6% of the oil. The main components of this oil were camphor (26.1%), α -thujone (10.1%), borneol (5.1%), and β -thujone (4.4%). Methyl eugenol (31.0%) was the most characteristic components of EO hydrodistilled from *A. dracunculus*. Besides this compounds the presence of myrcene (27.8%), elemicin (25.3%), and (E)-nerolidol (12.3%) were confirmed. Sixty seven compounds were identified in this essential oil.

The differences observed in the chemical composition of these four *Artemisia* species essential oils from Mongolian Trans-Altai Gobi compared to those from different origins could be attributed to their genetic potential, ecological conditions and climatic variations (Shatar & Altantsetseg, 2011).

Keywords: *Artemisia* species, essential oil composition, p-cymene, γ -terpinene, camphor, methyl eugenol.

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PP-008. Volatile profiles and anti-inflammatory property of *Cinnamomum camphora* (L.) Nees & Eberm from Eastern Cape, South Africa

Opeyemi N. Avoseh¹, Adebola O. Oyedeji², and Ope-oluwa O. Oyedeji^{1*}

¹ Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, Alice, South Africa

² Department of Chemistry, School of Applied and Environmental Sciences, Walter Sisulu University, South Africa.

*Corresponding author. Email: ooyedeji@ufh.ac.za

Cinnamomum camphora (L.) Nees & Eberm commonly called camphor tree is known worldwide as a source of camphor. The traditional doctors in South Africa use the infusion of dried leaves as ritual concoction (Van Wyk et al, 1997) and anti-inflammation. Volatiles constituents of the leaves and stem were isolated using hydro-distillation method and were characterised using Gas Chromatography and Gas-Chromatography/Mass Spectrometry. The stem bark essential oils majorly consist of Camphor 54.98% and cis- β -terpineol (20.74%) while the leaf is characterised with caryophyllene oxide (7.65%) and camphor (25.63%). The constituents of *C. camphora* essential oils from Eastern Cape, South Africa had been observed from those of the same species from Cuba, Australia, and Madagascar which are camphor based chemotypes (Chalchat & Valade, 2000; Pino & Fuentes, 1998; Stubbs, et al, 2004). Oral administration of essential oils at the dose of 2% showed significant ($p < 0.001$) anti-inflammatory properties in the albumin induced test model in rats. Essential oils from the fresh leaves and dry stems inhibited inflammation beyond 4 h post treatment. The potent anti-inflammatory activity of essential oils of *C. camphora* hereby confirmed its traditional use in treating various inflammatory diseases.

Keywords: *Cinnamomum camphora*, camphor, anti-inflammation, Eastern Cape, cis- β -terpineol.

Acknowledgments

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PP-009. Effect of thyme and oregano essential oil products on different aphids

Karima Baba Aissa*, Sarah Belkhoumali, Messaouda Belhani, Kamel Moussaoui and Eddine Djazouli Zahr

Department of Biotechnology, Saad Dahleb University of Blida, Route de Soumaa, BLIDA 09000, Algeria

*Corresponding author. E-mail: krimouss67@aol.fr

The use of the chemicals for the control of insects raised more worries connected to the environment, human health, non-target species and development of the resistant populations. (Regnault-Roger et al., 2005). Now the aromatic plants have a considerable asset thanks to the progressive discovery of the application of their essential oils in the biological fight against the pests of the cultures. (Auger et al., 1999).

The present study related to the evaluation of the effectiveness of the essential oils formulated (in solution with the addition of an organic builder, water and stabilizers) containing thyme and oregano in comparison to synthetic product (Methomyl). All samples were tested against two citrus fruits aphids (*Aphis citricola* and *Aphis gossypii*) and the plant lice of the black poplar (*Chaitophorus leucomelas*).

The results of this study showed that all the essential oils tested had a repressive effect on the pests targeted whith a supremacy of effectiveness of essential oil containing thymol compared to the others biopesticides applied. On the other hand the thymol is less effective than synthetic products, with the lowest rate abundance of aphid populations.

The same results allowed, to highlight a shock effect and temporal toxicity of all the samples tested. The estimation of the effectiveness of doses applied, revealed that all doses had a crackdown on the abundance of the target aphid populations, but the full doses (D=1/1000ml) reveal significantly more effective than half-doses (DD=0.5/1000ml).

Key words: toxicity, essential oils formulated, thyme, oregano, *Aphids*.

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PP-010. Chemical composition, cytotoxicity, antioxidant activity and the influence on FaDu cell cycle of clove essential oil and its main constituent

Tomasz Baj^{1,*}, Łukasz Świątek², Barbara Rajtar² and Małgorzata Polz-Dacewicz²

¹ Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

² Department of Virology, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

*Corresponding author. Email: tbaj@pharmacognosy.org

Essential oils (EO) have been previously reported to possess antioxidant and cytotoxic properties. (Kasrati et al., 2015; Zorzetto et al., 2015) In this study commercially available essential oil from clove (CEO) and its main constituent – eugenol (EUG) were tested for cytotoxic properties and antioxidant activity. Furthermore, the influence of CEO and EUG on the cell cycle of FaDu (human pharynx squamous cell carcinoma) cell line was evaluated.

The composition of EO was tested with the use a Shimadzu GC-2010 Plus GC instrument coupled to a Shimadzu QP2010 Ultra mass spectrometer. Compounds were separated on a fused-silica capillary column ZB-5 MS (30 m, 0.25 mm) with a film thickness of 0.25 mm (Phenomenex). The retention indices were determined in relation to a homologous series of *n*-alkanes (C8–C24). Compounds were identified using a computer-supported spectral library, mass spectra of reference compounds, as well as MS data from the literature. The main compounds of CEO were: eugenol (83.23%), β -caryophyllene (11.56%), α -caryophyllene (2.39%).

Cytotoxicity was assessed on VERO (green monkey kidney) and FaDu cell lines using the MTT method. The influence of EUG and CEO on cell cycle of FaDu cell line was tested using two-step cell cycle protocol and NucleoCounter® NC-3000™ image cytometer.

The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method.

The results of cytotoxicity testing are shown in the table. IC_{50} , IC_{10} , IC_1 are the concentrations inhibiting the cell proliferation by 50%, 10% and 1%, respectively.

EO	VERO		FaDu		
	IC_{50} ($\mu\text{g/ml}$)	IC_{10} ($\mu\text{g/ml}$)	IC_{50} ($\mu\text{g/ml}$)	IC_{10} ($\mu\text{g/ml}$)	IC_1 ($\mu\text{g/ml}$)
EUG	108.2	50.9	92.1	33.2	23.1
CEO	75.2	48.6	59.9	28.2	14.3

Cell cycle of FaDu was measured after incubation of cells with IC_{50} , IC_{10} and IC_1 concentrations for 5h. When exposed to IC_{50} concentrations of both EUG and CEO, a noticeable decrease of G1 phase, by approx. 20pp (percentage points), and increase of S phase, by approx. 12pp could be observed. The G2 phase was slightly increased by 4pp.

EUG and CEO were moderately toxic to both tested cell lines without showing any significant selectivity towards cancer cell line. Interestingly, CEO was more toxic than its major constituent. Neither EUG nor CEO selectively inhibited a single stage of cell cycle.

Antioxidant activity EC_{50} values of EUG and CEO were 198 $\mu\text{g/ml}$ and 156 $\mu\text{g/ml}$ respectively. Both preparations showed high antioxidant activity.

Keywords: essential oil, cytotoxic activity, antioxidant activity, cell cycle analysis.

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PP-011. Antimicrobial and antioxidant activity of carrot (*Daucus carotta* L.) seed essential oil

Tomasz Baj^{1,*}, Izabela Korona-Glowniak², Elwira Sieniawska¹ and Anna Malm²

¹ Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

² Department of Microbiology, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

*Corresponding author. Email: tbaj@pharmacognosy.org

Essential oil from the seeds of carrots becomes more popular and more common in herbal stores. It possesses wide spectrum of properties including toning, nourishing, regenerating, aphrodisiac, stimulant and antiseptic activities.

The aim of the present study was to evaluate the antimicrobial activity and the antioxidant properties of commercially available wild carrot seed essential oil. Declared by the manufacturer, the country of origin of the essential oil was Morocco.

The antimicrobial assay of carrot oil was carried out towards reference strains using a serial dilution method to obtain the MIC (Minimal Inhibitory Concentration) values (Wiegand et al., 2008). Carrot oil showed moderate activity against Gram-positive bacteria (MIC 125-500 µg/mL): staphylococci (*Staphylococcus aureus* ATCC25923, *Staphylococcus aureus* ATCC6538, *Staphylococcus epidermidis* ATCC12228); micrococci (*Micrococcus luteus* ATCC10240), bacilli (*Bacillus subtilis* ATCC6633, *Bacillus cereus* ATCC10876) and streptococci (*Streptococcus pyogenes* ATCC19615, *Streptococcus pneumoniae* ATCC49619, *Streptococcus mutans* ATCC25175) as well as against the tested yeasts (125-1000 µg/mL) - *Candida albicans* ATCC10231, *Candida parapsilosis* ATCC22019. No bioactivity (MIC >1000 µg/mL) of carrot oil against Gram-negative rods (*Escherichia coli* ATCC25922, *Salmonella* Typhimurium ATCC14028, *Klebsiella pneumoniae* ATCC13883, *Proteus mirabilis* ATCC12453, *Pseudomonas aeruginosa* ATCC9027) was observed. The low values of MBC/MIC ratio (1-4) for carrot oil suggested its bactericidal power against most of the tested reference Gram-positive bacteria.

The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method. The essential oils showed low antioxidant activity EC₅₀ 8.6 mg/ml. These data confirm the poor antioxidant activity of the essential oil from carrot (Ksuori et al., 2015) and its moderate bioactivity to Gram-positive bacteria and yeasts (Rokbeni et al., 2013).

Keywords: essential oil, wild carrot, *Daucus carota* L., antioxidant activity, antimicrobial activity.

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PP-012. GC-MS analysis of essential oil and headspace from aerial parts of *Peucedanum tauricum* with PCA and HCA statistical interpretation

Magdalena Bartnik^{1,*} and Łukasz Komsta²

¹ Chair and Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

² Chair and Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland

*Corresponding author. Email: mbartnik@pharmacognosy.org

Presented study is a part of investigations of *Peucedanum tauricum* M.B. (Apiaceae) related to the variability of secondary metabolites in phenological period, which is interesting from both phytochemical and chemotaxonomic points of view. This study enabled us to indicate optimal harvesting time for an isolation of secondary metabolites, especially two compounds, guaia-1(10),11-diene and guaia-9,11-diene, which were newly isolated from fruits of *P. tauricum* (Bartnik 2004, Tesso et al., 2005). These compounds can be isolated for further biological studies and could be considered as chemotaxonomic markers of this species.

HS-SPME/GC-MS were used for analysis of volatiles (HS) from *P. tauricum* and compared with GC-MS analysis of the hydrodistilled essential oil (HD). Fresh flowers (FL), immature and mature fruits (IF, MF) and leaves (L) in the time of harvesting of generative organs, were parallelly investigated. Statistical analysis (PCA and HCA) showed the differences between HS and HD, and also between different plant parts. Reproducible organs (FL, IF, MF) show different chemical profiles (both in HS and HD) in comparison to leaves collected in the same time of the phenological period.

As shown in Fig. 1A, PC1 represents the trend differentiating reproducible organs (positive PC1 values) and leaves (negative PC1 values). PC3 represents mainly differences between HS and HD and positive value of this component is observed for HS, whereas negative for HD extraction (Fig. 1B).

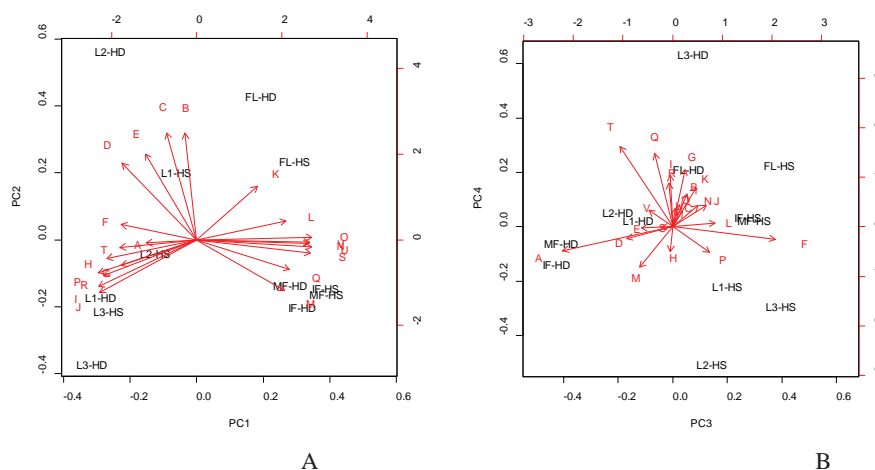


Fig. 1. PCA of the restricted matrix from GC-MS analysis of *P. tauricum*.

As the conclusion we can found, that generative organs, mostly immature fruits contain highest amount of two targeted major sesquiterpene compounds (constituted c.a. 66% of the total hydrodistilled essential oil obtained from IF). It is also worth to underline, that our study is the very first report about the analysis of the plant from *Peucedanum* genus, where analysis of the headspace and of the essential oil was performed during plant phenological period, and fresh aerial parts of the plant; leaves, flowers and fruits were the subject of investigations.

Keywords: *Peucedanum tauricum*, HS-SPME, essential oil, GC-MS, Principal Component Analysis.

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PP-013. GC-MS analysis of essential oil and headspace of *Heracleum dissectum* Ledeb. herb

Magdalena Bartnik^{1,*} and Katarzyna Targowska-Duda²

¹ Chair and Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

² Chair of Chemistry, Department of Biopharmacy, Medical University of Lublin, Chodźki 4A, 20-093 Lublin, Poland

*Corresponding author. Email: mbartnik@pharmacognosy.org

Heracleum dissectum Ledeb. (Apiaceae) Fl. Altaic. 1: 301. 1829. (Cowparsnip) is widely distributed from Western Siberia, Middle Asia, Mongolia to Far Russia, Japan and northern China. Collected from the wild and cultivated as a fodder crop and food plant for its leaves and young shoots (pickled or cooked in soups) (Pemberton & Lee, 1996). In Mongolia *H. dissectum* leaves and flowers are used in bacterial and infection fever and in stomach and intestinal disorders (Boldsai Khan & Sanchir, 2004). Composition of the volatiles from young shoots of this plant were not investigated before, so the aim of our study was to evaluate the chemical composition of the headspace (HS) and the essential oil (HD) obtained by hydrodistillation from this plant part. Hydrodistillation of the essential oil was carried out by use of Deryng apparatus (3 h), with addition of m-xylene. For HS-SPME nonpolar polydimethylsiloxane (PDMS) 30 µm fiber (Supelco) was used. Gas chromatography of HD and HS were done by use of GC-MS (Carlo Erba GC 6000, Italy) ITS-40 (Finnigam MAT, USA), MS (EI-70 eV). Analyzed compounds were analyzed on a DB-5 fused silica capillary column (30 m length, 0.25 mm I.D., 0.25 µm film thickness, J&W Scientific, USA).

The amount of the hydrodistilled oil was c.a. 0.11% of the dry wt. and 54 components (mainly sesquiterpenes) were detected, from which 46 were identified. The major constituents in the obtained essential oil were limonene and β -phellandrene, that constitute 42.55% of the total oil. α -Pinene (7.5%), β -myrcene (3,7%) and kessane (9,5%) also were present.

In the HS fraction 34 compounds were found and identified. The main compound was octyl acetate (26.8%). Sabinene (14.4%), 1-octanol (15.3%), isobutyric acid octyl ester (5.3%), limonene and β -felandrene (5.4%), and octyl valerate (5.5%) also were identified.

Keywords: *Heracleum dissectum*, HS-SPME, essential oil, GC-MS.

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PP-015. Chemical composition and fungicide activity of the essential oil from the leaves of *Psidium laruotteanum* Cambess. (Myrtaceae)

Fernando C. M. Medeiros¹, Cláudio H. S. Del Menezzi¹, Roberto F. Vieira²,
Marcelly C. S. Santos³, Humberto R. Bizzo^{3,*}

¹ University of Brasilia, Brasilia, DF, Brazil

² Embrapa Genetic Resources and Biotechnology - Brasilia, DF, Brazil

³ Embrapa Food Technology - Avenida das Américas, 29501 Rio de Janeiro 23020-470 RJ, Brazil

*Corresponding author. Email: humberto.bizzo@embrapa.br

Cerrado (savannah-like vegetation) is the second largest Brazilian biome, covering almost 2 million km² (the same size as Western Europe). It concentrates a huge amount of endemic species, many of which have great potential for producing essential oils. In this work, the composition of the essential oil from the leaves of *Psidium laruotteanum* Cambess was analyzed and the oil was tested against to xylophagus fungi, *Gloeophyllum trabeum* and *Trametes versicolor*. Leaves from five individuals of the same population were collected at the Fazenda Água Limpa, Brasilia, Brazil. A voucher specimen was deposited in the herbarium of Embrapa Genetic Resources and Biotechnology (registry: CEN 84485). Fresh leaves were subjected to hydrodistillation in a Clevenger-type apparatus for 2 hours. The oil was analyzed by GC/FID and GC/MS in an Agilent 5973N system, with a HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 μm). Hydrogen and helium were used respectively as carrier gas (1.0 mL/min). Oven temperature was raised from 60 to 240°C at 3°C/min. Mass detector was operated in electronic ionization mode at 70 eV. The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature (Adams, 2007). Biological assays were performed by a modified well-diffusion method (Saad et al., 2013). A hole was made in the center of the culture medium in which 20 μL of a solution of the essential oil in ethanol (12.5% to pure oil) were added. The mycelia of the fungi were placed 6 cm from the edges of the Petri dish. After a period of incubation at 27°C and relative humidity of 70%, the inhibition index was measured through photo and image processing.

The essential oil was obtained in 0.3% yield. Most of the oil components were monoterpenes, and major compounds identified were *p*-cymene (34.8%), 1,8-cineole (11.9%) and α-pinene (11.4%). Limonene (7.9%), γ-terpinene (7.0%), terpinen-4-ol (6.1%) and α-terpineol (5.3%) were also detected. To the best of our knowledge, this is the first report on the essential oil of *P. laruotteanum*. No inhibition on fungi growing was observed even when the pure essential oil was applied.

Keywords: *Psidium laruotteanum*, Myrtaceae, essential oil, Brazilian Cerrado.

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PP-016. Scents from Brazilian Cerrado: The essential oil from leaves of *Bidens segetum* Mart. ex Colla (Asteraceae)

Rafael F. Silva¹, Rafaela G. Cavalcanti², Claudia M. Rezende³, Dijalma B. Silva⁴, Roberto F. Vieira⁴, Marcellly C. S. Santos⁵, Humberto R. Bizzo^{5,*}

¹ Institute of Research on Natural Products, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

² Faculty of Pharmacy, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

³ Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

⁴ Embrapa Genetic Resources and Biotechnology - Brasilia, DF, Brazil

⁵ Embrapa Food Technology - Avenida das Américas, 29501 Rio de Janeiro 23020-470 RJ, Brazil

*Corresponding author. Email: humberto.bizzo@embrapa.br

The Brazilian savannah vegetation, occurring in Central region of the country, is called Cerrado. This biome is very varied in form - many physiognomic forms have been described. The Cerrado is considered one of the 25 most important biodiversity hotspots in the world and has numerous herbs, including several aromatic plant families, many of which have never been subjected to chemical study (Myers et al., 2000). In this work, the analysis of the essential oil from leaves of *Bidens segetum* Mart. ex Colla (Asteraceae) is reported. Leaves from six individuals of a population were collected in Campos Altos city (MG), Brazil. A voucher specimen was deposited in the herbarium of Embrapa Genetic Resources and Biotechnology (registry: CEN 88412). Fresh leaves were subjected to hydrodistillation in a Clevenger-type apparatus for 2 hours. The oil was analyzed by GC/FID and GC/MS in an Agilent 6890N and an Agilent 5973N systems, both with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1.0 mL/minute. Oven temperature was raised from 60 to 240°C at 3°C/minute. Mass detector was operated in electronic ionization mode at 70eV. The percentage composition was obtained by normalization from FID. Oil components were identified by comparison of both mass spectra and linear retention indices with spectral library and literature (Adams, 2007). Thirty-seven compounds were identified, corresponding to 96.3% of the oil. The leaf oil obtained was rich in monoterpenes (89.8%) with a high content of myrcene (64.5%). Other compounds also detected were limonene (14.4%), *p*-cymene (4.7%) and alpha-pinene (2.7%).

Keywords: *Bidens segetum*, Asteraceae, essential oil, Brazilian Cerrado, biodiversity, myrcene.

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PP-018. Variability of essential oil composition of wormwood (*Artemisia absinthium* L.) affected by harvest date

Vicente Castell^{1*}, Juan-Antonio Llorens-Molina², Sandra Vacas³, and Éva Zámboriné-Németh⁴

¹ School of Agricultural Engineering and Environment, Universitat Politècnica de València (UPV), Camino de Vera, s/n, 46022 Valencia, Spain

² Mediterranean Agroforestry Institute, UPV, same address

³ Centre for Agricultural Chemical Ecology (Mediterranean Agroforestry Institute), UPV, same address

⁴ Corvinus University of Budapest, Department of Medicinal and Aromatic Plants, 1118 Budapest, Villányi str. 29-35, Hungary

*Corresponding author. Email: vcastell@prv.upv.es

A little information is available about seasonal changes of the EO composition in wormwood (*Artemisia absinthium* L.) and it has been obtained from pooled samples of diverse populations (Ariño *et al.* 1999) not reflecting real biochemical changes and chemotaxonomic diversity. In this study, 8 Hungarian and Spanish plants belonging to typical chemotypes were monitored through the vegetation cycle, taking leaf samples from 3 marked individuals (Hungary), belonging to sabinene+myrcene chemotype, and 5 ones (Spain) belonging to (Z)-epoxyocimene and (Z)-epoxyocimene+(Z)-chrysanthemyl-acetate chemotypes (Llorens-Molina *et al.*, 2012). Healthy leaves were sampled (10-20 g) during May and June (vegetative stage), July (budding stage) and August (flowering stage). Simultaneous distillation-extraction (SDE) was performed using a Likens-Nickerson device. Extracts were analysed using GC and GC-MS. Compounds were identified by Kovats retention indexes, NIST MS Search 2.0 library, and available data in literature.

The seasonal variability of the EO composition is low in the Hungarian individuals: monoterpene hydrocarbons are present in 82.4-97.1 % of the oil, being the major components sabinene and myrcene (average values during the studied period: 40.2% and 52.9%, respectively). The fluctuation of sabinene is below 10% and that of myrcene is only 3-4% (in EO) with no firm tendencies. The ratios of both sesquiterpene hydrocarbons and oxygenated sesquiterpenes in EO's remain under 5% through the sampling period. The characteristic spectrum of the Spanish plants proves to be stable through the sampling dates, too. Fluctuations of individual compounds are below 2% (of EO) consistently. However, a significant decrease is found in the ratios of the main compounds (Z) + (E) epoxyocimene in the samples taken in August, compared with the mean value obtained from the data of May, June and July (see table 1).

The main differences between the studied Hungarian and Spanish chemotypes, as well as between the Spanish chemotype A and B, are shown in table 2, making special reference to the oxygenated monoterpene (Z)-chrysanthemyl acetate.

Table 1. The content (% peak areas) of (Z) + (E) epoxyocimene in Spanish *A. absinthium* chemotypes (ChT)

	(E) + (Z)-epoxyocimene		
	Mean May-Jul	Aug.	Decrease (%)
ChT A	78.0	68.0	-12.9
ChT B	75.1	59.4	-20.9

decrease-value differences at 0.05 significance

Table 2. Composition of EO (% peak areas) of Hungarian (HU) and Spanish (E) *Artemisia absinthium* plants

	HU	E - ChTA	E - ChTB
Monoterpenes	89.3	2.1	1.6
Oxygenated monoterpenes	2.7	88.2	88.6
(Z)-chrysanthemyl acetate	0.0	0.3	10.1
Sesquiterpenes	2.9	2.7	3.5
Oxygenated sesquiterpenes	2.2	2.2	2.2
Other	0.6	2.5	1.9
Total identified	97.6	97.7	97.7

ChT A - chemotype A

ChT B - chemotype B

Keywords: *Artemisia absinthium*, essential oil, chemotype, individual variation, seasonal changes.

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PP-020. Synergistic essential oil combinations against *Staphylococcus aureus* and *Propionibacterium acnes*

Fatih Demirci^{1,2*}, Gamze Göger^{1,3}, Betül Demirci¹ and K. Hüsnü Can Başer^{1,4}

¹ Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskişehir, Turkey

² Faculty of Health Sciences, Anadolu University, 26470, Eskişehir, Turkey

³ Graduate School of Health Sciences, Anadolu University, 26470, Eskişehir, Turkey

⁴ College of Science, Department of Botany and Microbiology, King Saud University, 11451 Riyadh, Saudi Arabia

*Corresponding author. Email: fdemirci@anadolu.edu.tr

There are currently more than 30 essential oils in the European Pharmacopoeia (Ph. Eur.). The essential oil of *Salvia lavandulifolia* Vahl. is one of monographs listed in Ph. Eur. It is a plant from the Iberian peninsula. It has an important role in food and cosmetic industry for its flavour and fragrance. *Salviae lavandulifoliae aetheroleum* has been traditionally used for its antioxidant, antiseptic, analgesic, sedative and anesthetic activities (Fu et al., 2013; Porres-Martinez et al., 2013).

In the present study, *Salviae lavandulifoliae aetheroleum* was combined with other commercial Pharmacopoeia Grade essential oils; namely *Salviae sclareae aetheroleum*, *Spicae aetheroleum* and *Citrus reticulatae aetheroleum*, respectively, for the synergistic effect against *Staphylococcus aureus* and *Propionibacterium acnes* standard strains.

The quality of the commercial essential oils were confirmed both by GC-FID and GC-MS, simultaneously. Antibacterial essential oil combinations were evaluated on mixtures of (1:1), (1:5), (1:9), (5:1), (9:1) proportions using *in vitro* microdilution methods.

Initial minimum inhibitory concentrations (MIC) of essential oils were found between 500-2000 µg/mL against *S. aureus* and *P. acnes*, whereas synergic MICs of combinations of the essential oil combinations ranged from 0.125-1000 µg/mL. Chromatographic analysis of essential oils revealed that camphor (30.5 %), 1,8-cineole (24.8 %) and α -pinene (6.5 %) were determined as main components of *S. lavandulifolia*; linalyl acetate (61.9 %) and linalool (20.1 %) for *S. sclarea*; linalool (45.8 %) and 1,8-cineole (25.0 %) for *L. latifolia*; limonene (71.1 %) and γ -terpinene (20.0 %) for *C. reticulata*, respectively.

Keywords: *Staphylococcus aureus*, *Propionibacterium acnes*, synergy, essential oil.

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PP-021. Extracting of limonene from the peels of lemons and limes and the determination of the purity of the obtained compound

Ewa Drewnowska*, Agnieszka Wróblewska* and Alicja Gawarecka

Institute of Organic Chemical Technology, West Pomeranian University of Technology, 10 Pułaskiego Str.,
70-322 Szczecin, Poland

*Corresponding authors. Email: ewa.drewnowska@zut.edu.pl, agnieszka.wroblewska@zut.edu.pl

The industry of fruit juices generates annually a large amounts of waste in the form of the orange peels (biomass component), which can be used as a raw material for the production of limonene. Limonene - 4-isopropenyl-1-methylcyclohexene (carvene), is a natural and cyclic compound belonging to the group of terpenes, which is widely present in nature, especially in fruits, vegetables and spices. This compound has insecticidal properties, and a nice citrus smell and taste. It is often used as an additive in perfumes, cosmetics, cleaning agents and as the flavouring agent for food (EPA 1994; Cagnoli 2005; Matura 2006). The main methods of limonene separation from peels of citrus fruits are: the cold pressing, the solvent extraction and the simple distillation or the steam distillation (Trytek 2007).

During the studies limonene was obtained from citrus peels such as: lemon and lime, by simple distillation and steam distillation. Next, the purity of the obtained compound was tested. Both, the simple distillation and the steam distillation, were carried out with the same amount of raw material (260g of the peels and 600ml of water) and under the same conditions, in order to compare the results of these two distillation methods. The qualitative analysis of the product of distillation was performed by the gas chromatography method with help of a Thermo Focus apparatus equipped with a FID detector. The conditions of the GC analyses were as follows: the capillary column QUADREX 007WAX 0,32 mm, 30m, 0,5 μ m film (polyethylene glycol), detector temperature of 250°C, sample chamber temperature of 250°C, the temperature program of the oven: 60°C for 3 min., the increase of temperature 15°C/min, and next the temperature of 200°C for 5 min, the hydrogen flow 50 kPa, the helium flow 45 kPa, the air flow 70-80 kPa, the sample volume 0,1 μ l.

The study shows that steam distillation is the most effective method of oil obtaining in the comparison to the simple distillation. This conclusion was confirmed by the qualitative analyzes carried out by GC method.

Keywords: limonene, peels of citrus fruit, simple distillation, steam distillation.

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PP-022. Characterization of essential oils and plant extracts secondary metabolites by using Gas Chromatography hyphenated to Carbon Isotope Ratio Mass Spectrometry (GC-C-IRMS)

Paola Dugo^{1,2,3,*}, Luisa Schipilliti¹, Ivana Bonaccorsi¹, Luigi Mondello^{1,2,3}

¹Scienze del Farmaco e Prodotti per la Salute” Department, University of Messina, viale Annunziata, 98168 – Messina, Italy.

²University Campus Bio-Medico of Rome, Via Álvaro del Portillo, 21 - 00128 Rome, Italy.

³Chromaleont s.r.l., c/o “Scienze del Farmaco e Prodotti per la Salute” Department, University of Messina, viale Annunziata, 98168 – Messina, Italy.

*Corresponding author. Email: pdugo@unime.it

The Gas Chromatography hyphenated to Carbon Isotope Ratio Mass Spectrometry (GC-C-IRMS) is considered very useful in quality control of matrices of high economic interest.

Such analytical approach, indeed, is of choice in determining the botanic and geographic origin of the matrices, because carbon isotope ratio is linked to biosynthetic pathways of each plant specie and related to plants CO₂ fixation, subjected to the environmental changes and latitude.

The present research focuses on the characterization of the secondary metabolites coming from the biogenetic pathway linked to the terpenes formation, in *Citrus* and in *Helicrysum italicum* (Roth) G. Don fil. subsp. *italicum* (*H. italicum* subsp. *italicum*) plants, by using the GC-C-IRMS investigations. The obtained $\delta^{13}\text{C}$ values related to several species of genuine *Citrus* peel oils and *Helicrysum italicum* plant extracts, permitted to create a database of authenticity and genuineness, useful in the comparison of the carbon isotope ratio of terpenes, extracted from oils and plants of unknown origin. In order to evaluate the carbon isotope ratio related to secondary metabolites of the samples, the use of an internal standard (i-std), chosen between the common volatile compounds of interest.

The results are applied to identify the origin (geographic and botanical) of side-product and by-products obtained from the investigated matrices.

Keywords: Carbon isotope ratio, essential oils, plant extracts, geographic origin, terpenes.

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PP-023. Traceability of vanilla flavoured food by means Head Space-Solid Phase Micro Extraction (HS-SPME) coupled to Gas Chromatography-Isotope Ratio Mass Spectrometry (GC-IRMS)

Paola Dugo^{1,2,3,*}, Luisa Schipilliti¹, Giorgia Purcaro³, Sara Farnetti⁴, Luigi Mondello^{1,2,3}

¹Scienze del Farmaco e Prodotti per la Salute” Department, University of Messina, viale Annunziata, 98168 – Messina, Italy.

²University Campus Bio-Medico of Rome, Via Álvaro del Portillo, 21 - 00128 Rome, Italy.

³Chromaleont s.r.l., c/o “Scienze del Farmaco e Prodotti per la Salute” Department, University of Messina, viale Annunziata, 98168 – Messina, Italy.

⁴Diabetes Research Institute, Division of Cellular Transplantation of Surgery, University of Miami, 33136 Miami, FL, USA

*Corresponding author. Email: pdugo@unime.it

Vanilla flavour is extracted principally, from the tropical orchid, belonging to the genus *Vanilla*. Two principal species are widely employed as flavorings in food field: *Vanilla planifolia* and *Vanilla tahitensis*. In confectionery industry, vanilla flavouring has gained an important role, improving the sweetness note of bakery products, custards, chocolate and contributing to round out the flavour of ice-creams preparations. Due to the high cost necessary for vanillin extraction from vanilla pods (organic vanillin), vanillin obtained *ex synthetic way* (principally from lignin and guaiacol) was employed in food industry. A third way of vanillin production is represented by biotechnological synthesis, introduced as a viable alternative to around the obstacle of organic vanillin costs and the use of synthetic aroma. The authenticity control of vanilla flavour in foodstuff, was always object of interest in order to reduce the use of hazardous chemicals involved in the chemical synthesis. The recent regulations, concerning the directives about the use and the labelling of additives and flavourings in food industry (Regulation (EC) No 1334/2008 of the European Parliament) have assigned to the right terms to denote natural or synthetic flavorings. In the specific case of vanilla flavoring “vanilla extract” or “vanilla natural flavoring” terminologies refer to the addition of natural vanilla extract, otherwise, “natural flavorings” denotes the biovanillin presence. Finally, if “vanillin” or just “flavorings” are reported on the label, they indicate the addition of synthetic vanillin. Among different approaches the carbon isotope ratio investigation was widely used as a mean to detect the origin of vanillin, being sensible to the biogenetic pathway and the environmental factors that influence the formation of a compound. The present study is focused on the evaluation of vanillin carbon isotope ratio extracted from food products by Head Space-Solid Phase Micro Extraction (HS-SPME). The fibre chosen was Polydimethylsiloxane-Carboxen-Divinylbenzene (PDMS-Car-DVB). The food products label was taken in account to trace the vanillin origin, comparing the $\delta^{13}\text{C}$ results and the qualitative analyses of the samples volatile fraction, obtained by Gas Chromatography coupled to Mass Spectrometry (GC-MS).

Keywords: Carbon isotope ratio, *Vanilla planifolia*, *Vanilla tahitensis*, plant extracts, traceability, flavours.

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PP-024. Quality monitoring of selected essential oils upon storage under real-time conditions

Amina Dzafic*, Claudia Turek, Nadine Kirschmann, Florian C. Stintzing, Dietmar R. Kammerer

WALA Heilmittel GmbH, Department of Analytical Development & Research, Section Phytochemical Research, Dorfstr. 1, D-73087 Bad Boll/Eckwälden, Germany

*Corresponding author. Email: amina.dzafic@wala.de

Essential oils are complex mixtures of volatile, lipophilic components, characterized by a strong and odoriferous scent (Surburg & Panten, 2006). Terpenes and their oxygenated derivatives exhibit a high structural variability and represent the predominant constituents of essential oils (Ashour et al., 2010). Numerous studies have revealed essential oils to be susceptible to chemical alterations upon storage, particularly due to oxygen and light exposure as well as storage at elevated temperatures, which may initiate isomerization, oxidation, dehydrogenation or polymerization reactions (Grassmann & Elstner, 2003). These structural alterations may cause discolouration, a rise in viscosity or the development of off-flavours resulting in quality loss (Pokorný et al., 1998; Grassmann & Elstner, 2003). Such aspects are becoming increasingly important also from a consumer health point of view due to putative allergenicity of essential oil components (Hagvall et al., 2008). However, comprehensive data on quality parameters revealing alterations upon storage under real-time conditions are still scarce (Turek & Stintzing, 2013).

Therefore, the aim of the present study was to evaluate a set of recently established quality parameters (Turek & Stintzing, 2011) for monitoring essential oil alterations upon storage under real-time conditions. For this purpose, aliquots of selected essential oils, e.g. citrus, rosemary and tea tree oil, were stored at room temperature to determine the impact of oxygen and storage time.

Conductivity, pH and peroxide values were monitored as reported (Turek & Stintzing, 2011) and proved to be viable parameters for quality control: all essential oils exhibited a considerable but oil-specific increase in conductivity and peroxide value accompanied by a mostly slight pH value drop. These observations were more pronounced upon prolonged storage in the presence of oxygen showing that storage time is an important factor affecting essential oil quality. Peroxide values as single parameters for monitoring quality proved to be inappropriate, since these may decline again upon extended storage. However, in combination with conductivity, which revealed a steady increase, the aforementioned set of parameters was shown to be suitable to reflect essential oil alteration upon storage under real-time conditions. Preliminary studies suggest a correlation of the above-named observations with the formation of hydrophilic essential oil oxidation products. Further investigations are in progress taking into consideration a more detailed characterisation of essential oil components generated upon storage.

Keywords: Essential oil alteration, storage, peroxide value, conductivity, pH value.

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PP-026. Plant biotechnology in phytonematicidal screening (PlantBioNem)

A. Cristina Figueiredo^{1*}, Jorge M.S. Faria¹, Inês Sena¹, Bruno Ribeiro², Helena Trindade¹, Lia Ascensão¹, José G. Barroso¹, Luis G. Pedro¹ and Manuel Mota²

¹ Centro de Estudos do Ambiente e do Mar Lisboa, Faculdade de Ciências, Universidade de Lisboa, DBV, CBV, 1749-016 Lisboa, Portugal

² NemaLab, ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7002-554 Évora, Portugal

*Corresponding author. Email: acsf@fc.ul.pt

The approaches commonly used to study diseases caused by plant parasitic nematodes seldom evaluate the real-time invasive mechanism. Additionally, the determination of potential phytonematotoxic compounds relies mostly on direct-contact bioassays that do not take into account the plant host response. For this reason, growth of more than one organism or cell type in a combined culture (*in vitro* co-cultures) has the advantage of simulating the host-pathogen interaction and eliminating variables due to environmental *in vivo* conditions. This will contribute to clarify the invasive mechanism of different types of nematodes, and to identify the plant response to nematode and to potential nematicides. Aiming at alternative strategies for nematode control, PlantBioNem project work used *in vitro* plant-nematode co-cultures as testing models that simulate *in vivo* infection conditions. Two types of nematodes that adversely affect worldwide economy have been studied, *Bursaphelenchus xylophilus* (pinewood nematode, PWN), responsible for pine wilt disease (PWD), by affecting the aerial parts of several pine species, and *Meloidogyne chitwoodi* (Columbia root-knot nematode, CRK), causative agent of root galls which damage the root system, in economically important crops such as potato (*Solanum tuberosum*) and tomato (*S. lycopersicum*). Two *in vitro* co-culture systems were established for nematode infection and proliferation studies: a) maritime pine (*Pinus pinaster*) and stone pine (*P. pinea*) shoots with PWN co-cultures (Faria et al. 2015) and b) potato hairy roots with CRK co-cultures (Faria et al. 2014), and their structure, growth and production of volatiles evaluated. After screening several plant species essential oils (EOs) (Faria et al. 2013 2015b), the most active, *Satureja montana* and *Ruta graveolens* EOs, were tested for nematotoxicity and phytotoxicity on both co-culture types. Through semi-quantitative RT-PCRs differential gene expression was evaluated to study changes in terpene synthase genes, in pine shoots with PWN co-cultures, and protease inhibitors for potato hairy roots with CRK co-cultures.

Keywords: Co-culture, essential oils, *Pinus pinaster*, *Pinus pinea*, *Solanum tuberosum*.

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PP-027. α -Pinene synthase gene expression following nematode invasion in *Pinus pinaster* and *P. pinea* with *Bursaphelenchus xylophilus* *in vitro* co-cultures

Inês Sena, Helena Trindade and A. Cristina Figueiredo*

Centro de Estudos do Ambiente e do Mar Lisboa, Faculdade de Ciências, Universidade de Lisboa, DBV, CBV, 1749-016 Lisboa, Portugal

*Corresponding author. Email: acsf@fc.ul.pt

Pinus pinaster (maritime pine) and *P. pinea* (stone or umbrella pine) are the two dominant Portuguese pine species that show different susceptibility to the pine wood nematode, *Bursaphelenchus xylophilus*. Whereas *P. pinea* has been considered from resistant to moderate resistant (Santos et al. 2012), *P. pinaster* plantations are suffering severe damage, showing major pine wilt disease (PWD) symptoms (Mota et al. 1999). Taking into account the different volatile profile and diverse susceptibility of these species to pine wilt disease (PWD), this work investigated the genes involved in monoterpene production in both species. The experiments were run using previously established *in vitro* shoot cultures of both species (controls) and co-cultures of *Pinus* spp. with the pine wilt nematode, *Bursaphelenchus xylophilus* (Faria et al. 2015). Variation in α -pinene gene expression in *in vitro* cultures, following nematode invasion, was evaluated through semi-quantitative RT-PCR, at different periods (2h, 4h, 12h, 24h) after co-culture start. Untreated controls were performed to compare gene expression levels. The results suggested that in *P. pinaster* there is no difference between infected and control cultures, as the same pattern of gene expression was observed throughout the period studied. In *P. pinea*, α -pinene synthase gene was upregulated following invasion, with a peak of expression at 24h that was absent in the control cultures. To what extent this is related to decreased susceptibility remains to be determined. For both species α -pinene synthase gene identification and characterization was performed, revealing that the amino acid sequence of the two α -pinene synthases shared 98.5% pairwise identity. Heterologous expression of α -pinene synthase genes from both species revealed the functionality of both proteins, with the production of α -pinene as the major compound. In *P. pinea* α -pinene synthase activity led to the production of higher amounts of β -pinene in comparison to those of the synthase from *P. pinaster*. The differences in gene expression in *P. pinea* between the control and co-cultures can result in different terpenes produced following nematode infection but this has to be confirmed by essential oil analysis.

Keywords: *Bursaphelenchus xylophilus*, *Pinus pinaster*; *Pinus pinea*, α -pinene synthase gene, heterologous expression.

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PP-028. Chemical composition of the essential oil and some biological effect of *Artemisia ordosica* Krasch. from Mongolian Gobi

Altantsetseg Shatar¹, Daariimaa Khurelbat², Anuu Zorig², Wirginia Kukula-Koch³, Shatar Sandui¹, Kazimierz Glowniak^{3,4}

¹Institute of Chemistry and Chemical Technology Mongolian Academy of Sciences, 13330 Peace avenue, MAS 4th building, Bayanzurkh district, Ulaanbaatar, Mongolia

²Mongolian National University of Medical Sciences, Zorig street, Ulaanbaatar-14210, Mongolia

³Chair and Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1, Chodzki str., 20-059 Lublin, Poland

⁴Department of Cosmetology, University of Information Technology and Management in Rzeszów, Kielnarowa 386a, 36-020 Tyczyn, Poland

*Corresponding author. Email: kglowniak@pharmacognosy.org

The aerial parts of *Artemisia ordosica* Krasch. (Asteraceae) are often utilized in Mongolian folk medicine for the treatment of laryngitis, pharyngitis, hemorrhage and rheumatoid arthritis (Ligaa, 1996). As part of our on-going research on the chemical characterization of the plant collected in Mongolian Southern Gobi (Khurelbat et al., 2010), we have investigated the composition of the essential oils obtained by hydro distillation by GC and GC-MS (Shatar & Altantsetseg, 2011). A total of 14 compounds were identified in the leaf oil, accounting for 92.4% of the total oil composition. The most abundant components of the essential oil of *A. ordosica* were (Z)- β -ocimene (19.14%), β -pinene (18.40%), limonene (8.20%), trans-sabinenehydrate (7.80%), α -pinene (7.10%), sabinene (6.60%), p-cymene (5.74%), γ -terpinene (4.32%), (E)- β -ocimene (3.20%), myrcene (2.90%), cis-sabinene hydrate (2.60%), camphor (1.00%) and camphene (0.80%).

The methanol extract of *Artemisia ordosica* Krasch. was studied towards the free radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The extract showed significant antioxidant activity at tested concentrations of 10 μ g/ml, 50 μ g/ml, 100 μ g/ml. The antioxidant properties of the extract were compared with ascorbic acid. Above 100 μ g/ml, the sample exhibited higher scavenging activity (90%) than in other concentrations.

Moreover, the extract of the plant was dried under reduced pressure and cytotoxic effects of different concentrations (10, 100, 200 μ g/ml) were evaluated by MTT assay against KB (Human epidermoidal carcinoma in mouth cells) cells using ELISA test at 540 nm. The extract showed inhibitory activities against KB cells.

Keywords: *Artemisia ordosica* Krasch., GC-MS analysis, essential oils, antioxidant activity, cytotoxic activity.

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PP-029. Grapefruit peels as a source of limonene - research on isolation and purity of limonene

Alicja Gawarecka*, Agnieszka Wróblewska¹ and Ewa Drewnowska¹

¹Department of Chemical Technology and Engineering, West Pomeranian University of Technology, 10 Pulaskiego Street, 70-322 Szczecin, Poland

*Corresponding author. Email: Alicja.Gawarecka@zut.edu.pl

A significant amount of essential oils is obtained by different kinds of distillation or cold pressing (in case of essential oils - from citrus fruit peels) from natural plant material. In the simply distillation, the plant material has a direct contact with a boiling water. In case of the steam distillation the steam is produced in a separate boiler and then it is directed through a pipe into the flask, where the plant material is placed. The cold pressing is a process in which the oil glands within the peels of citrus fruits are mechanically crushed to release their content. Generally, the steam distillation is a most widely accepted method of the obtaining of the essential oils on a large scale (Baser & Buchbauer, 2010). *Citrus grandis* Grapefruit Oil components have strong bacteriostatic and bactericidal properties. These components exhibit also a potential antiviral, antiprotozoal, anti-dust mite and strong antifungal activity. Moreover, these components are very active against free radicals, and they inhibit aging and degeneration. Grapefruit oil is also recommended for insect bites curing.

Limonene belongs to monoterpenes group, and it is a component of many essential oils for example: orange, lemon, lime, mandarin and grapefruit. The grapefruit peels contain mainly limonene and some myrcene (Gancel et al., 2002). In this work limonene was obtained by the steam and the simple distillation from biomass – waste grapefruit peels. During the studies an appropriate amount of grapefruit peels was subjected to the steam and the simple distillation. Then, the obtained oil underwent freezing and next, after separation of waxes, it was investigated by the GC method on the Focus apparatus. Studies with help of the gas chromatography method showed the presence of large amounts of limonene. Because limonene can be obtained from renewable sources – biomass, therefore it is very interesting for researchers taking into account its transformation to oxygenated products. Especially, the following limonene derivatives: carvone, carveol, α -terpineol, menthol, and perillyl alcohol are very interesting. These compounds are used on a large scale in cosmetic, perfumery and food industry.

Keywords: biomass, citrus peel, grapefruit oil, steam distillation, simple distillation.

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PP-032. Aroma profiles of common Japanese trees: Japanese cypress, camphor tree, Japanese cedar, and hiba arborvitae

Ryo Toriya¹, Toshio Hasegawa^{1*}, Takashi Fujihara¹, Kuniyoshi Shimizu², and Makoto Inagami²

¹ Department of Chemistry, Graduate School of Science and Engineering, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama 338-8570, Japan

² Department of Agro-environmental Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

*Corresponding author. Email: toshihas@mail.saitama-u.ac.jp

Many terpenes have been reported as constituents in the aroma profiles of *Chamaecyparis obtusa* Sieb. et Zucc. (Japanese cypress), *Cinnamomum camphora* Presl. (camphor tree), *Cryptomeria japonica* D. Don (Japanese cedar), and *Thujopsis dolabrata* Sieb. et Zucc (Hiba arborvitae), which are common trees in Japan. However, the key odorants of these trees are unclear. The scents of these trees are constructed from the constituents in the wood and leaves. In this study, we investigated the aroma profiles of both these parts. The odorants in the wood and leaves were obtained by hexane extraction and monolithic material sorptive extraction (MMSE), which is an extraction method for headspace odor. Sensory evaluation for the odor similarity between the materials and their extracts was performed separately by two different groups. In the case of Japanese cypress wood, the odor was woody and refreshing, and the two extraction methods gave extracts that had an odor similar to that of the material. Both the extracts from the leaves also had an odor similar to the material's. However, the odors of both the extracts from the wood were clearly different from those from the leaves. The components of the two extracts were investigated using GC-O and GC-MS. Although the proportions of the two major compounds, 4-terpineol (**1**) and α -terpinyl acetate (**2**), were different between the hexane and MMSE extracts (**1**: 23.8% and 10.4%; **2**: 8.2% and 22.7%, respectively), there was no significant difference in odor between the two extracts. This shows that **1** and **2**, despite being the major constituents, do not strongly affect the odor of Japanese cypress wood. Therefore, we focused on the components with nearly same ratio in the hexane and MMSE extracts (e.g., γ -muurolene: 2.3% and 2.0%, respectively). The results suggest that such components were important in the aroma profile of Japanese cypress wood. The other materials were similarly investigated. We found that minor components rather than the main components were most important in the aroma profiles of all the materials.

Keywords: *Chamaecyparis obtusa*, *Cinnamomum camphora*, *Cryptomeria japonica*, *Thujopsis dolabrata*.

PP-033. Antifungal effect of essential oils against clinically relevant fusaria

Mónika Homa^{1*}, Ildikó Pálma Fekete¹, Andrea Böszörményi², László Kredics¹, Csaba Vágvölgyi¹
and László Galgóczy^{1,3}

¹ Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52., H-6726 Szeged, Hungary

² Institute of Pharmacognosy, Faculty of Pharmacy, Semmelweis University, Üllői út 26., H-1085 Budapest, Hungary

³ Division of Molecular Biology, Biocenter, Innsbruck Medical University, Innrain 80-82, A-6020 Innsbruck, Austria

*Corresponding author. Email: homamoni@gmail.com

Mycotic keratitis due to *Fusarium* species is among the leading causes of visual disability and blindness in South India (Bharathi *et al.* 2007; Srinivasan 2004). The management of this eye infection is still challenging due to the poor susceptibility of the isolates to conventional antifungal drugs (Azor *et al.*, 2007). Hence there is a need for novel antifungals or alternative adjunctive therapeutic agents (such as essential oils (EOs)) to treat these type of infections. However, the antifungal effect of different EOs against filamentous fungi was previously reported in the literature, keratitis-associated fusaria have not been investigated yet in this respect. The present study was carried out to investigate the antifungal effects of *Cinnamomum zeylanicum*, *Citrus limon*, *Juniperus communis*, *Eucalyptus citriodora*, *Gaultheria procumbens*, *Melaleuca alternifolia*, *Origanum majorana*, *Salvia sclarea* and *Thymus vulgaris* essential oils against *Fusarium* keratitis isolates.

Among the other 9 tested EOs *C. zeylanicum* EO (CZEO) proved to be the most effective against all investigated fusaria. The main component of CZEO, trans-cinnamaldehyde (tCA) was also tested and showed similarly strong antifungal activity as the oil. The *in vitro* interaction between tCA and natamycin, which is the first line therapeutic agent of *Fusarium* keratitis, was also investigated. Compared to the single use, an enhanced fungal growth inhibition was observed when these agents were applied in combination. Light and fluorescent microscopic observations revealed that CZEO/tCA reduces the cellular metabolism and inhibits the conidia germination; furthermore necrotic events were significantly more frequent in the presence of these two compounds.

According to our results, CZEO/tCA provides a promising basis to develop a novel strategy for the treatment of *Fusarium* keratitis.

Keywords: *Fusarium* keratitis, antifungal effect, essential oils, *Cinnamomum zeylanicum*, trans-cinnamaldehyde.

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PP-035. Essential oil composition of *Azorella spinosa* of Maule Region, Chile

Adriana Jara^{1,*}, Margarita Gutierrez², Aurelio San Martín³, María E. Cazar⁴

^{1,2} Laboratory of Organic Synthesis, Institute of Chemistry of Natural Resources, University of Talca, Talca, Chile

³ Department of Chemistry, Faculty of Sciences, University of Chile, box 653, Santiago, Chile

⁴ Laboratory of Biotechnology, Faculty of Chemical sciences, University of Cuenca, Cuenca, Ecuador

*Corresponding author. Email: adrianajarabermeo@gmail.com

Azorella spinosa is a species belonging to the genus *Azorella* of the Apiaceae Family, and is widely distributed in the Andean-Patagonian region from Costa Rica to southern Chile and Argentina, and a potential source of bioactive compounds with various diterpenoids structures of type azorellane, yaretane and mulinanes, exhibiting a wide range of biological activities as antibacterial and antihyperglycemic agents for example tea extracts are commonly used in traditional medicine as gastric stimulants, diuretic and analgesics for treatment migraines (Wickens, 1995), (Hoffmann et al., 1992), (Areche et al., 2010), (Loyola et al., 2001), (Delporte et al., 2003), (Fuentes et al., 2005). The essential oils of an genus of *Azorella* have been reported as promising to control insects pest and dermatophyte-related infections (Lopez et al., 2012).

In this study, the plant material was collected in 2015 in the Maule Region, Chile. Essential oil from the leaves of the plant was obtained by hydro-distillation using Clevenger apparatus, their volatiles were determined by gas chromatography with a mass selective detector (GC-MS). The analysis revealed a variety of terpenes, sesquiterpenes, terpenic alcohols, ketones, and aldehydes, in different proportions. This was analyzed the first study of the composition of the essential oil obtained from the leaves of *Azorella*.

Keywords: *Azorella spinosa*, essential oil, GC-MS, Apiaceae.

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PP-036. Antimicrobial activity of the essential oil of cultivated *Micromeria pulegium* (Rochel) Benth. (Lamiaceae)

Srna Jovic^{1*}, Marina Milenkovic¹, Mirjana Kovacevic², Bojan Zlatkovic³, Maria Couladis⁴, Violeta Slavkovska⁵

¹ Department of Microbiology and Immunology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia

² Beo – Lab polyclinic, Resavska 60, 11000 Belgrade, Serbia

³ Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

⁴ Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, Panepistimiopouli Zographou 157 71, University of Athens, Athens, Greece; ⁵ Department of Botany, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia

*Corresponding author. Email: srnajovic@yahoo.com

Micromeria pulegium is an endemic S. Carpathian species distributed in S. W. Romania and E. Serbia. Whereas *M. pulegium* is poorly explored so far this study was undertaken to determine the *in vitro* antimicrobial activity of the essential oils as well as the antimicrobial activity of the combinations of essential oils and antibiotics. The aerial parts of cultivated plant were collected at different developmental stages (vegetative, flowering and fruiting) and the essential oils were obtained by hydrodistillation in a Clevenger-type apparatus and analyzed by GC-MS. Essential oils were investigated for antimicrobial activities on several microorganisms: Gram-positive bacteria (*S. aureus*, *E. faecalis* and *B. subtilis*), Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *S. abony* and *P. aeruginosa*) and yeast (*C. albicans*). The broth microdilution method in 96-microwell plate was used. Minimal inhibitory concentrations (MICs) of essential oils were determined (CLSI, 2007). We also investigated the antimicrobial activity of the combinations of essential oils and antibiotics (ceftriaxone and gentamicin) by cheackboard method in 96-microwell plate. Fractional inhibitory concentration (FIC) indexes were calculated (Hu et al., 2002). Minimal inhibitory concentrations (MICs) of all essential oils were above 500 µg/ml against all of the tested strains. In a combination with ceftriaxone essential oils showed additive effect and in a combination with gentamicin essential oils showed synergistic effect. Gram-positive bacteria were more sensitive to these combinations. Significant differences between the antimicrobial activities of essential oils from different developmental stages were not detected.

Keywords: *Micromeria pulegium*, Lamiaceae, essential oil, antimicrobial activity, antibiotics.

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PP-038. Chemical composition of essential oil of *Helichrysum arenarium* (L.) Moench

Agnieszka Kicel^{1*}, Magdalena Matczak¹, Katarzyna Skowronek¹, Anna Wajs-Bonikowska²
and Monika A. Olszewska¹

¹ Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Łódź, Muszyńskiego 1, 90-151 Łódź, Poland

² Institute of General Food Chemistry, Faculty of Biotechnology and Food Sciences, Technical University of Łódź, Stefanowskiego 4/10, 90-924 Łódź, Poland

*Corresponding author. Email: agnieszka.kicel@umed.lodz.pl

The genus *Helichrysum* Miller (Asteraceae) comprises 16 species native to Europe. In Poland, this genus is represented by one species, *Helichrysum arenarium* (L.) Moench, that is mostly spread on dry and sandy soils of the eastern regions. This perennial herb with yellow to orange flower heads and grayish leaves provides valuable plant material (*Helichrysi inflorescentia*) known in traditional medicine for its choleric, diuretic, anti-inflammatory, hepatoprotective, detoxifying and antiradical activities.

Volatile chemistry of *H. arenarium* inflorescences of Lithuanian, Serbian and Hungarian origin was investigated (Judzentiene & Butkiene, 2006; Lemberkovic et al., 2001; Rančić et al., 2005), but there is large variability of essential oil composition, depending on color of the flowers (from yellow to orange) and the harvest location. Except for one report suggesting that methyl palmitate and capric acid may be the major volatile metabolites of Polish commercial sample of *H. arenarium* (Lemberkovic et al., 2001) there is a dearth of information on volatile chemistry of this species growing in Poland. Therefore, the aim of the present work was to evaluate chemical composition of essential oil of *H. arenarium* inflorescences collected from natural habitats in the Eastern Poland.

The essential oil obtained by hydrodistillation from fresh inflorescences of *H. arenarium* was analyzed by GC-FID-MS. More than ninety volatile compounds were identified, representing over 80% of the total oil composition. The oil is dominated by a sesquiterpene fraction (40.4%), that consisted of sesquiterpene alcohols, oxides and hydrocarbons. The percentage of aliphatic hydrocarbons and oxygenated aliphatics (34.6%) is also considerable. The much smaller monoterpene fraction (8.6%) consists mainly of oxygenated monoterpenes (5.3%). The most abundant ingredients of the oil are decanoic acid (7.7%), caryophyllene oxide (4.8%), decanal (4.5%), β -caryophyllene (4.1%) and γ -cadinene (4.1%).

Keywords: *Helichrysum arenarium*, essential oil composition.

Acknowledgments

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PP-039. Essential oil and proazulene content of 11 different *Achillea collina* Becker accession

Sára Kindlovits*, Péter Rajhárt, Katalin Inotai and Éva Zámbooriné Németh

Department of Medicinal and Aromatic Plants, Corvinus University of Budapest, H-1118 Budapest, Villányi str. 29-35, Hungary

*Corresponding author. Email: sara.kindlovits@uni-corvinus.hu

Yarrow (*Achillea*) species are widely known essential oil bearing medicinal plants all over the world. The drug (*Millefolii herba*) has spasmolytic, analgesic, anti-inflammatory and digestive effects. The quality on the market is however, often inadequate both of the drug from collected sources and cultivated populations as well.

To gather more information about the drug quality of available yarrow populations, 11 different *Achillea collina* Becker taxa were tested including registered cultivars, wild originating populations and some selected strains. The comparative investigation of these accessions was carried out in perennial stands from 2012 to 2014. The experiment was installed in small plots in 3 replications. Phenological and morphological characteristics of the populations were compared, the proportion of useful plant organs were measured in the drug. The essential oil and proazulene contents were detected by the method described in Ph. Eur. VII. The trial was carried out at the Experimental Field and in the Laboratory of the Department.

Morphological features including growth, plant habitus and colour of the foliage showed a large variability. In flowering time a fortnightly shift was observed between the earliest and the latest genotypes. The essential oil content of the flowering horizon (flowers with 30 cm long stems) varied between 0.140 and 0.407 ml/100 g in d.w. Best results were achieved in accessions of wild origin: 'Gb 47' (0.395 and 0.290 ml/100 g) in 2012 and 2013, while in 2014 the highest content was measured in population 'Gyula' (0.407 ml/100 g). The proazulene content exceeded the requirements (0,02 % in the essential oil) of European Pharmacopoeia VII. in each population and showed maximum values in 'Gb 22' (0,174 and 0.122 %) and in 'Gyula' (0.150 %), respectively.

The results of these experiments reflect the large intraspecific variability of *A. collina* from several points of view and show that wild populations may still provide a rich genetic potential for improving drug quality.

Keywords: variability, chemotype, 'Azulenka', chamazulene, genotype, yarrow.

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PP-040. Effect of sowing density and harvesting time on the root production and essential oil content of *Pimpinella peregrina* L.

Sára Kindlovits^{1*}, Claude-Alain Carron², José Vouillamoz², Catherine Baroffio² and Christoph Carlen²

¹ Department of Medicinal and Aromatic Plants, Corvinus University of Budapest, H-1118 Budapest, Villányi str. 29-35

² Research Centre Conthey, Agroscope, CH-1964 Conthey, Route des vergers 18

*Corresponding author. Email: sara.kindlovits@uni-corvinus.hu

Pimpinella peregrina L. is a biennial medicinal plant belonging to Apiaceae family which occurs in alpine and mountainous areas in Europe. Its root, *Pimpinellae radix*, contains essential oil and saponins as active agents. The drug has antitussive and expectorant effect and used for curing diseases of upper-respiratory tract. In the drug production of *P. peregrina*, cultivation seems to achieve an increasing role instead of collection. However, the bases of cultivation are developed, results on economical issues of the production and drug quality are only scarce.

Therefore in this experiments our aim was to explore the effect of sowing density and harvesting time on the root production and essential oil content of the roots. The trial was set at the research field of Agroscope, in Conthey in 2014, the plant material was *Pimpinella peregrina* 'Licora'. As treatments 5 different sowing densities were set between 6 to 30 g/100m², for sowing Sembdner sowing machine was used. The experiment was set in randomized block design. The sampling of the roots were made 8 times from the 1st of September until the 21st of October. The morphological features of the roots (length, diameter, weight, number of roots) were measured in the fresh samples, while root yield was assessed both in the fresh and in the dried samples. The essential oil content of the roots were determined with Clevenger-type hydrodistillation and were calculated for dry matter content.

The fresh and dry root yields showed an increasing tendency during the harvesting dates. With higher sowing densities more plants and higher yields were achieved, with a highest average values (~1200 roots/m² and 211.1 g/m²) reached in 30 g/m² density. The root parameters had a certain connection with sowing density: the biggest root diameter (1.19 cm) and weight (13.8 g) were measured in the lowest density. In essential oil content only slight increase were observed during the vegetation, while the sowing density didn't have an effect on essential oil content (0.134-0.140 ml/100g) of the drug. Based on these results the later harvest results in bigger yields and higher essential oil content, while the sowing density affect only the root yield of *Pimpinella peregrina*.

Keywords: burnet saxifrage, drug quality, *Pimpinellae radix*, volatile oil, yield.

PP-041. Chemical components and variability of the essential oils isolated from inflorescences of *Carduus species*

Małgorzata Kozyra^{1,*}, Marek Mardarowicz²

¹ Department of Pharmacognosy with Medicinal Plant Laboratory, Medical University of Lublin, 1 Chodźki Street, 20-093 Lublin, Poland,

² Analytical Laboratory, Maria Curie-Skłodowska University of Lublin, Poland

*Corresponding author. Email: mkozyra@pharmacognosy.org

Carduus species are plants which belong to the *Asteraceae* family. There are only a few information about essential oils (EO) in *Carduus* sp. The prominent component found in EO obtained from aerial parts of *C. pycnocephalus* L was hexadecanoic acid. (Esmaeili at al., 2005). The major essential oil components from flowers of *C. candidans* ssp. *globifer* were benzaldehyde and palmitic acid. Palmitic acid together with methyl salicylate and benzaldehyde were detected as most characteristic compounds occurring in the EO from *C. thoermeri* (Zhelev at al., 2014). The aim of these studies was qualitative analysis of essential oils hydrodistilled from the inflorescences of four *Carduus* species growing in Poland. These were: *Carduus crispus* L., *C. defloratus* L., *C. nigrescens* Vill., and *C. nutans* L. Essential oils from the inflorescences of investigated species have not been examined so far. Only EO received from aerial parts of *C. nutans* growing in Italy, were investigated and the major component of this EO was hexadecanoic acid (Formisano at al., 2007).

GC-MS method was employed for the analysis of obtained essential oils. Analysis was performed by use of Thermo-Finnigan (USA) GCQ GC-MS apparatus, working in electron impact mode. The identification of individual compounds was based on the calculated retention indices, as well as was made by the comparison of received mass spectra with those of reference compounds, and available in NIST library, MS data from the literature (Adams, 2001) and our library databases. Our study shows the differences in chemical composition of volatile oils obtained from different *Carduus* spp. The major component of the volatile oil received from the investigated inflorescences was palmitic acid. The percentage of this compound in the EO of *C. crispus* was 38.7%, for *C. nigrescens* - 31.9%, and for *C. defloratus* - 30.9%. Another fatty acids with odd number of carbon (linoleic, miristic, lauric, linolenic and their derivatives) were also observed. In the investigated essential oils the presence of small quantities of terpenoids were also detected. These were: thymol, linalool, carvacrol, and β -caryophyllene, among others.

Keywords: *Carduus* spp., essential oil, GC/MS method, *Asteraceae*.

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PP-042. Supercritical carbon dioxide extraction coupled with pressurized liquid extraction in the recovery of terpenes and phenolics from the aerial parts of Cretan barberry

Wirginia Kukula-Koch^{1*}, Nektarios Aligiannis², Maria Halabalaki²,
Alexios-Leandros Skaltsounis², Kazimierz Głowniak^{1,3}, Eleftherios Kalpoutzakis²

¹ Chair and Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1, Chodźki str., 20-093 Lublin, Poland

² Laboratory of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece

³ Department of Cosmetology, University of Information Technology and Management in Rzeszów, Kielnarowa 386a, 36-020 Tyczyn, Poland

*Corresponding author. Email: virginia.kukula@gmail.com

The extraction conditions including extraction techniques, solvent selection, temperature, static extraction time and a number of extraction cycles are important parameters controlling the extraction yield (Jiang et al., 2006; Thurman et al., 2005).

Hyphenated techniques applied in the current study have been proven to be powerful tools for the fast preparation of enriched plant extracts.

The extraction efficiency of supercritical fluid extractor was evaluated towards the recovery of both terpenes and phenolic compounds from aerial parts of *Berberis cretica*. 20 final fractions, whose composition differed significantly, have been collected to well assess the influence of carbon dioxide dosed at different pressure and temperature conditions on terpene composition, and of introduced ethanol gradient - on phenolic content in the SFE extracts.

The following pressurized liquid extraction performed on the same plant material helped to estimate the efficiency of the introduced extraction conditions on the recovery of phenolic content from aerial parts of *Berberis cretica*. GC-MS and LC-MS analyses of the obtained fractions revealed the presence of n-nonacosane, linoleic acid, neophytadiene and isopropyl myristate in the least polar fractions, and several phenolic acids and flavonoids in the carbon dioxide extracts with ethanol gradient.

DPPH and Folin-Ciocalteu tests were applied to estimate the content of phenolic compounds and free radical scavengers in the obtained extracts, depending on the extraction conditions applied.

Keywords: *Berberis cretica*, supercritical carbon dioxide extraction, DPPH, Folin-Ciocalteu, GC-MS, LC-MS.

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PP-043. Influence of digestion and thermal processing on the volatile composition of *Zingiber officinale* and its products

Wirginia Kukula-Koch^{1,2*}, Wojciech Koch^{2,3}, Akemi Umeyama², Marcin Dziedzic⁴, Agnieszka Ludwiczuk¹, Yoshinori Asakawa²

¹ Chair and Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1, Chodźki str., 20-093 Lublin, Poland

² Chair and Department of Food and Nutrition, Medical University of Lublin, 4a, Chodźki str., 20-093 Lublin, Poland

³ Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashirocho, Tokushima, 770-8514, Japan

⁴ Department of Laboratory Diagnostics, Medical University of Lublin, 1, Chodźki str., 20-093 Lublin, Poland

*Corresponding author. Email: virginia.kukula@gmail.com

Zingiber officinale is derived from Asia, however, it has been extensively used as a spice and a medicinal plant characterized by antiemetic, gastrointestinal protective, antispasmodic, antioxidant, antitumor, antiviral and blood pressure lowering properties (Chang et al., 2010; Prakash et al., 2006; Jolad et al., 2004).

The studies on the determination of qualitative and quantitative terpene composition of Japanese ginger diethyl ether extracts performed by GC-MS indicated the highest contribution of zingiberene (30%), β -sesquiphellandrene (10%) and geraniol (7%) into the volatile fraction of the plant.

The aim of the study was to investigate the changes in terpene composition, which occur after the digestion of its rhizomes. All conditions have been reconstructed to well resemble the natural digestion process and several both quantitative and qualitative changes have been observed for the obtained extracts. The results help to understand the behavior of the volatile compounds during the digestion process and help to recognize the actual active ingredients of ginger rhizomes.

Furthermore, a detailed terpene analysis of ginger-containing foods and dietary supplements has been performed to track the temperature affected differences in the volatile composition of the extracts. The undertaken studies showed various significant alternations. The longer the operation of temperature/air/light, the poorer the content and the lower the concentration of terpenes (especially monoterpenes) and phenolic acids in the extracts.

Keywords: *Zingiber officinale*, thermal processing, digestion, GC-MS analysis, volatiles.

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PP-044. A new technique for the measurement of antioxidant activity in essential oils using bicontinuous microemulsions

Eisuke Kuraya^{1,2,*}, Shina Nakada¹, Dai Kato³, Osamu Niwa³ and Masashi Kunitake²

¹ Science and Technology Division, National Institute of Technology, Okinawa College, 905 Henoko, Nago, Okinawa, Japan

² Graduate School of Science, Kumamoto University, 2-39-1 Kurokami, Kumamoto, Japan

³ National Institute of Advanced Industrial Science and Technology, Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan

*Corresponding author. Email: kuraya@okinawa-ct.ac.jp

Essential oils are of great interest because of their potential as alternatives to pharmaceutical medicines. Therefore, the evaluation of natural antioxidants in essential oils to help screen plant materials for possible antioxidant properties has been eagerly anticipated. The 2,2-diphenyl-1-picrylhydrazyl (DPPH·) method is commonly applied to the evaluation of antioxidant activity in essential oils. However, only a few organic solvents are compatible with the DPPH· method owing to the limited solubility of DPPH·. Small amounts of water and the concentration of metal ions in organic solvents frequently influence the antioxidant activity evaluation obtained by the DPPH· method (Dawidowicz et al., 2012). Bicontinuous microemulsions (BMEs), in which water and oil phases coexist bicontinuously on a microscopic scale, can dissolve hydrophilic and lipophilic compounds simultaneously. In our previous research, we proposed a simple electrochemical analysis technique for the evaluation of antioxidant activity using a BME solution. The method allowed us to evaluate hydrophilic, lipophilic and amphiphilic antioxidants individually, without extraction (Kuraya et al., 2012). In our presentation, we would like to introduce an electrochemical method for the evaluation of antioxidant activity in essential oils using a BME solution (BME-EC). We have used the DPPH· method as a standard to compare with the BME-EC method.

BME solutions that comprised a phosphate buffer (pH=7.0), saline, sodium dodecyl sulfate surfactant, 2-butanol cosurfactant, and toluene were prepared for cyclic voltammetry (CV) analysis. A fluorinated nanocarbon film electrode was used as a lipophilic working electrode, and α -tocopherol (TOC) and butylated hydroxyanisole (BHA) were chosen as standard antioxidants. A linear calibration curve was obtained from a quantity of electricity as the integral of the current to 1.5 V.

The antioxidant activities of 20 commercially available essential oils were investigated using the DPPH· and BME-EC methods. Evaluations of the antioxidant activities of TOC and BHA using the BME-EC method compared favourably to those measured using the DPPH· method; the correlation coefficients were above 0.94. These results prove the potential of the BME-EC method, which is simple, rapid, reproducible, easily serviceable and highly reliable.

Keywords: Antioxidant activity, bicontinuous microemulsions, DPPH· method, essential oils.

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PP-045. Chemical and aroma profiles of Northern Limit Yuzu (*Citrus junos*) peel oils from different producing districts

Eisuke Kuraya^{1,*}, Shina Nakada¹, Megumi Kubota¹, Toshio Hasegawa² and Shigeru Itoh³

¹ Science and Technology Division, National Institute of Technology, Okinawa College, 905 Henoko Nago City, Okinawa, Japan

² Graduate School of Science and Engineering, Saitama University, 255 Shimo-Ohkubo, Sakura-ku Saitama, Saitama, Japan

³ National Institute of Technology, Okinawa College, 905 Henoko Nago City, Okinawa, Japan

*Corresponding author. Email: kuraya@okinawa-ct.ac.jp

Citrus fruits, some of the most important commercial crops, are widely cultivated in the regions between the tropical and temperate zones. *Citrus junos* Tanaka (yuzu) has a strong characteristic aroma and yuzu juice is used in Japanese foods. Yuzu produced in Rikuzentakata (Iwate prefecture) is called “Northern Limit Yuzu (NLY)” and has a good aroma. Gas-chromatography-mass spectrometry (GC/MS) revealed that the major volatile component did not vary much between the Rikuzentakata and Kochi juices (Takemoto et al., 2014). Although the juices were identified as being different by a sensory test, the trace compounds, which contribute to the difference in the odour, could not be assigned. In the present study, we evaluated the chemical and aroma profiles of yuzu peel oils from different producing districts using multivariate analysis of GC/MS data.

All yuzu fruits were provided by the producers in Rikuzentakata (NLY), Kochi (KY), Saitama (SY), and Miyazaki (MY). The peel oils of yuzu were extracted using hexane and the volatile compounds were analysed by GC/MS. Principal component analysis (PCA) was performed using SIMCA software (version 13.01; UMETRICS) to determine whether differences in the chemical composition and aroma profiles of peel oils could be correlated to their producing district. About 60 compounds were detected and characterized by comparison with MS libraries. The results were standardized by autoscaling prior to statistical analysis. From the scatter plot of scores for the PCA analysis of the retention indices, PC1, PC2 and PC3 explain 75.5% of the total variances. The peel oils from the NLY, KY and MY groups were statistically similar as shown by the score plot of PC1 and PC2, but the score plot of PC1 and PC3 indicates that NLY was distinct from the others. These results confirm that 1) the chemical composition of the district pairs significantly contributed to the observed clustering, and 2) the aroma profile of yuzu from NLY was distinctly different from the others.

Keywords: *Citrus junos* (yuzu), peel oils, Principal component analysis (PCA), aroma profiles.

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PP-046. Essential oil composition of leaves and berries of *Juniperus oxycedrus* L. growing in siliceous and calcicolous soils from Valencia (Spain)

Juan A. Llorens-Molina^{1,*}, Sandra Vacas González² and Josep Sabater Martínez³

¹ Mediterranean Agroforestry Institute, Univ. Politècnica de València, Camí de Vera s/n, 46022, Valencia, Spain

² Centre for Agricultural Chemical Ecology, (Mediterranean Agroforestry Institute), Univ. Politècnica de València, Spain

³ School of Agricultural Engineering and Environment, Universitat Politècnica de València, Spain

*Corresponding author. Email: juallom2@qim.upv.es

Juniperus oxycedrus ssp. *oxycedrus* L. is a common shrub growing in scrublands of Valencia (Spain). Its essential oil (EO) composition has been studied regarding to plant organ differences, biological activity (Angioni et al., 2003; Loizzo et al., 2007) and seasonal variations (Milos & Radonic, 2000). Nevertheless, no data have been reported with respect to soil influence. In this study, EO composition of leaves and berries of samples coming from siliceous (Segart) and calcicolous (Lliria) soils (Terrasit, 2015) were determined by GC/MS and GC/FID. The main composition is shown below:

	Berries (% peak areas, GC/FID)		Leaves (% peak areas, GC/FID)	
	Segart	Lliria	Segart	Lliria
α -Pinene	65.0 \pm 0.67 ^a	55.7 \pm 3.25 ^b	53.2 \pm 15.92 ^a	42.0 \pm 6.71 ^a
Myrcene	22.6 \pm 0.21 ^a	16.6 \pm 1.11 ^b	3.4 \pm 0.49 ^a	4.4 \pm 0.47 ^a
Monoterpenes	95.4 \pm 0.92 ^a	78.4 \pm 3.03 ^b	72.5 \pm 20.98 ^a	71.4 \pm 10.36 ^a
Oxygenated monoterpenes	1.3 \pm 0.17 ^a	3.6 \pm 0.60 ^b	4.4 \pm 1.96 ^a	3.5 \pm 1.52 ^a
Germacrene-D	0.3 \pm 0.07 ^a	1.3 \pm 0.08 ^b	8.2 \pm 5.20 ^a	7.0 \pm 3.00 ^a
Sesquiterpenes	3.1 \pm 0.77 ^a	12.9 \pm 0.13 ^b	16.8 \pm 9.83 ^a	16.9 \pm 6.55 ^a
Oxygenated sesquiterpenes	-	3.4 \pm 1.62	3.2 \pm 3.12 ^a	3.6 \pm 0.68 ^a

Different superscript letters mean significant differences ($P < 0.05$, Tukey test) between locations for berries and leaves.

Significant differences have been mainly found in berries EO composition. These results lead to pursue this research in order to determine the soil parameters affecting these differences.

Keywords: *Juniperus oxycedrus*, soil, calcicolous, siliceous, essential oil.

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PP-047. Essential oil composition of leaves and berries of *Pistacia lentiscus* L. growing in siliceous and calcicolous soils from Valencia (Spain)

Juan A. Llorens-Molina^{1,*}, Sandra Vacas González² and Josep Sabater Martínez³

¹ Mediterranean Agroforestry Institute, Univ. Politècnica de València, Camí de Vera s/n, 46022, Valencia, Spain

² Centre for Agricultural Chemical Ecology, (Mediterranean Agroforestry Institute), Univ. Politècnica de València, Spain

³ School of Agricultural Engineering and Environment, Universitat Politècnica de València, Spain

*Corresponding author. Email: juallom2@qim.upv.es

Pistacia lentiscus L. grows in a wide diversity of habitats in Valencia (Spain). The essential oil (EO) composition of its aerial parts has been extensively studied (Fernández et al., 2000; Dob et al., 2006) the same way that its antioxidant and antifungal activity (Barra et al., 2007). Nevertheless, no data have been reported with respect to soil influence. In this study, EO composition of leaves and berries of samples coming from siliceous (Segart) and calcicolous (Xeraco) soils (Terrasit, 2015) were determined by GC/MS and GC/FID. The main composition is shown below:

	Berries (% peak areas, GC/FID)		Leaves (% peak areas, GC/FID)	
	Segart	Xeraco	Segart	Xeraco
α -pinene	4.9 \pm 1.8 ^a	24.0 \pm 2.3 ^b	2.4 \pm 1.3 ^a	19.0 \pm 5.7 ^b
Monoterpenes	90.2 \pm 1.9 ^a	74.0 \pm 16.9 ^a	20.4 \pm 16.6 ^a	57.5 \pm 11.3 ^b
Oxygenated monoterpenes	1.5 \pm 0.3 ^a	0.6 \pm 0.9 ^a	11.2 \pm 3.8 ^a	6.7 \pm 0.9 ^b
Germacrene-D	2.0 \pm 1.7 ^a	0.6 \pm 0.2 ^a	17.5 \pm 5.4 ^a	10.8 \pm 3.1 ^a
Sesquiterpenes	6.2 \pm 3.8 ^a	5.2 \pm 1.5 ^a	39.9 \pm 11.5 ^a	32.2 \pm 7.6 ^a
Oxygenated sesquiterpenes	0.3 \pm 0.1 ^a	1.4 \pm 0.7 ^b	15.2 \pm 9.2 ^a	1.6 \pm 0.9 ^b

Different superscript letters mean significant differences ($P < 0.05$, Tukey test) between locations for berries and leaves.

Significant differences have been found in berries and leaves EO composition regarding to type of soil, although it does not affect leaves and berries in the same way. These results lead to pursue this research in order to determine the soil parameters affecting these differences.

Keywords: *Pistacia lentiscus*, soil, calcicolous, siliceous, essential oil.

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PP-048. Chemical composition of SFE CO₂ extracts produced from marigold (*Calendula officinalis* L.), sage (*Salvia officinalis* L.) and chamomile (*Matricaria chamomila* L.)

Agnieszka Maciag^{*}, Radosław Bonikowski, Anna Wajs-Bonikowska, Magdalena Sikora, Agnieszka Stobiecka, Józef Kula

Institute of General Food Chemistry, Faculty of Biotechnology and Food Science, Lodz University of Technology, Stefanowskiego Str. 4/10, 90-924 Lodz, Poland

*Corresponding author. Email: agnieszka.maciag@p.lodz.pl

Calendula officinalis L. (marigold), *Salvia officinalis* L. (sage) and *Matricaria chamomila* L. (chamomile) are medicinal plants frequently cultivated in whole Europe and Poland. Each of those plants are raw material for valuable essential oil production. They also have many biological active constituents and display many rejuvenating properties on human skin. That is why they are used as important cosmetic ingredients. Our research are focused on biological active constituents isolated from plant extracts produced by means of Supercritical Fluid Extraction with CO₂.

Common marigold, common sage and common chamomile herb extracts, were produced by means of Supercritical Fluid Extraction (SFE) with carbon dioxide as a solvent. Production process was provided by New Chemical Synthesis Institute in Pulawy in Poland. In these three products the composition of fatty acids, volatile compounds, tocopherols and phytosterols as well as antioxidant properties were determined.

We observed that marigold and common sage extracts have similar qualitative composition of volatile compounds isolated by means of hydrodistillation in Calvenger type apparatus. Monoterpenes such as α -, β -thujone and camphor were major volatiles in *C. officinalis* and *S. officinalis* SFE extracts. Other compounds such as sesquiterpene hydrocarbons (α -cadinene, δ -cadinene, α -muurolene), sesquiterpene alcohols (α -cadinol, T-muurolol, cubenol) long chain aliphatic hydrocarbons were also detected. The composition of volatiles in SFE extract from common chamomile was qualitatively different. The main identified constituents were sequiterpenes such as chamazulene, bisabolene oxide A and B. The content of tocopherols in SFE extracts were also analyzed. According to obtained results *S. offisinalis* has the highest content of this compounds, that determined very high antioxidant activity of this product.

Keywords: SFE CO₂ plant extracts, marigold, common sage, common chamomile, cosmetic ingredients.

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PP-049. Composition of stinging nettle (*Urtica dioica* L.) hydrolate

Agnieszka Maciąg*, Danuta Kalemba

Institute of General Food Chemistry, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Stefanowskiego Str. 4/10, 90-924 Lodz, Poland

*Corresponding author. E-mail: agnieszka.maciag@p.lodz.pl

Stinging nettle (*Urtica dioica* L.), is well known medicinal plant which can be commonly met in the northern Europe and Asia. It is used as treatment of many diseases such as diabetes, prostatic hypertrophy and inflammation of urinary track. It is also known as cosmetic ingredient. Nettle is used in greasy skin care products, antidandruff shampoos and hair conditioners. *U. dioica* does not contain an essential oil, nevertheless it has very pleasant herbal scent. That is way we found this plant as promising raw material for hydrolate production.

U. dioica fresh herb was collected in 2014 in Poland. Plant material (14.6 kg) was subjected to hydrodistillation and seven fractions of hydrolate (1.5 L each) were produced. Representative sample of the whole nettle hydrolate was prepared by mixing equal volumes of each fraction. It revealed pleasant mild herbal scent. Volatiles from every sample of hydrolate were isolated by liquid-liquid extraction with diethyl ether. The hydrolate volatiles were analyzed by GC-FID-MS.

The total content of volatiles in representative sample of nettle hydrolate amounted to 58.2 mg/L whereas in hydrolate fractions the value varied and changed irregularly from 128 mg/L to 6.2 mg/L.

The dominant volatile constituents in *U. dioica* hydrolate were polar compounds. Aliphatic alcohols (*E*- and *Z*-hex-3-en-1-ol (5.7% and 27.7%, respectively), oxygenated monoterpenes, e.g. eucarvone (10.4%), *p*-cymene-9-ol (3.1%), and sesquiterpene alcohols, e.g. T-muurolol (4.7%), α -cadinol (4.7%), and spathulenol (2.7%), as well as caryophyllene oxide (2.9%) were identified in representative sample of the whole nettle hydrolate. Other compounds like borneol, *p*-cymen-8-ol, oplopanone, linalool, bornyl acetate were also detected.

The qualitative composition of volatiles in hydrolate fractions was similar. However, significant differences were observed in the quantitative composition. The first two fractions were richer in more volatile compounds such as (*E*- and *Z*-hex-3-en-1-ol then other fractions.

According to our results *U. dioica* herb, is non essential oil bearing plant, which can be raw material for valuable hydrolate production.

Keywords: hydrolate, volatile composition, stinging nettle, *Urtica dioica* L.

PP-050. Methods of the isolation of the clove oil (*Oleum Caryophylli*) and identification of its main component (eugenol) by the gas chromatography and infrared spectroscopy methods

Edyta Makuch^{1*}, Agnieszka Wróblewska^{1*}, Łukasz Kucharski² and Adam Klimowicz²

¹ Institute of Organic Chemical Technology, West Pomeranian University of Technology Szczecin, Pulaskiego 10, 70-322 Szczecin, Poland

² Dermatopharmacotherapy Division, Department of Dermatology, Pomeranian Medical University Szczecin, Powstańców Wielkopolskich 72, 70-111 Szczecin, Poland

*Corresponding author. Email: emakuch@zut.edu.pl, agnieszka.wroblewska@zut.edu.pl

The distinctive scent of certain plants since ancient times used as a perfume or spice is caused by the presence of the volatile essential oils found in different parts of plants, for example: flowers, leaves, stems, fruit peels, seeds or bark of trees and shrubs. These oils are usually complex mixtures of various substances (hydrocarbons, ketones, aldehydes, alcohols, ethers or esters). In the case of cloves, however, we are dealing with material, which is particularly rich in one main ingredient - eugenol (4-allyl-2-methoxyphenol). Clove oil (*Oleum Caryophylli*) is obtained mainly from the buds, but much less frequently from clove tree leaves (*Eugenia Caryophyllus*), and its most important component has many practical applications (Chaieb, 2007; Kędzia, 2007).

The present study was conducted with clove oil extracted from the plant material by the following methods: a steam distillation, continuous extraction in the Soxhlet apparatus, shaking and method of using ultrasounds. The obtained oil was then analyzed by the gas chromatography method. Analyses were carried out on FOCUS apparatus equipped with the flame ionization detector (FID), using the capillary column Restek Rtx-WAX filled in polyethylene glycol. Furthermore, identification of the obtained product was performed by the infrared spectroscopy (IR) apparatus NICOLET FT/IR-380 (THERMO ELECTRON CORPORATION).

It was found on the basis of the obtained results that eugenol had the best performance when it was obtained by continuous extraction in the Soxhlet apparatus. IR spectra the obtained oils were consistent with the literature data (NIST library).

Keywords: *Eugenia caryophyllus*, *Oleum Caryophylli*.

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PP-051. Thymol as anti-adhesive and anti-biofilm agent preventing formation of biofilm *in vitro* by *Candida albicans* strains isolated from oral cavity

Anna Malm^{1*}, Agnieszka Wójtowicz² and Jolanta Szymańska³

¹ Department of Pharmaceutical Microbiology with Diagnostic Microbiology Unit, Medical University, Lublin, Poland

² Department of Diagnostic Microbiology, Independent Public Hospital No 1, Lublin, Poland

³ Department of Paedodontics, Medical University, Lublin, Poland

*Corresponding author. Email: anna.malm@umlub.pl

Candida albicans is an important microorganism associated with the development of polymicrobial oral biofilms, including dental plaque. The most popular mouthwashes, preventing formation of dental plaque and other oral biofilms, contain usually essential oils or their active components, e.g. thymol (Bertolini et al., 2011; Vlachojannis et al., 2015).

The objective of this study was to determine the effect of thymol *in vitro* on planktonic cells and biofilm-associated cells of 69 *C. albicans* strains isolated from oral cavity of healthy people. Microtiter Plate (MTP) method and 96-well polystyrene microplates was used, allowing for estimation of MIC (minimal inhibitory concentration). MBIC (minimal biofilm inhibitory concentration) was determined using the same method with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to visualize biofilm formation by an appearance of purple formazan.

It was found that MICs of thymol for planktonic *C. albicans* cells ranged from 0.02-2% with MIC₅₀ = 0.05-0.1% and MIC₉₀ = 0.2%, while MBICs of thymol in case of biofilm-associated *C. albicans* cells ranged from 0.01-0.1% with MBIC₅₀ = 0.02% and MBIC₉₀ = 0.05%. The ratio MBIC₅₀/MIC₅₀ was 0.4 (0.2), while the ratio of MBIC₉₀/MIC₉₀ was 0.25, indicating that biofilm-associated cells showed higher sensitivity to thymol in comparison to that of planktonic cells of *C. albicans*. The obtained results suggest that thymol due to its good anti-adhesive and anti-biofilm activities *in vitro* even at sub-inhibitory concentrations may be used to control oral biofilms *in vivo*, especially those formed by *C. albicans*. These results indicate that thymol should be an important component of mouthwashes widely used in dental prophylaxis.

Keywords: thymol, anti-adhesive activity, anti-biofilm activity, *Candida albicans*, oral cavity.

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PP-052. Investigation of essential oils of decorative herbs

Svitlana Marchyshyn^{*}, Olga Demydyak, Halyna Kozyr, Tetiana Berdei, Iryna Dakhym, Olga Polonets

Department of Pharmacognosy with Medical Botany, I.Ya. Horbachevsky Ternopil State Medical University, 1 Maydan Voli, 46001, Ternopil, Ukraine

^{*}Corresponding author. Email: svitlanafarm@ukr.net

Due to the fact that essential oils possess a wide range of therapeutic actions they have the opportunity to take a significant place in the arsenal of the healing and preventive tools of modern medicine. The most important pharmacological effects of volatile oils are analgesic, anti-inflammatory, antimicrobial and antiviral.

Essential oils of 3 species of genus *Tagetes* herb, *Bellis perennis* herb and *Chrysanthemum morifolium* leaves and flowers were received by steam distillation method. The composition of essential oils was investigated by use of Agilent Technology 6890N gas chromatograph coupled with mass spectrometer 5973N (Chernogorod, 2006, Zhao, 2011).

Results of our investigation have shown that essential oil of *Tagetes patula* consists of 49 ingredients, and 33 of them are identified. Main components are caryophyllene, docosene-1, germacrene D, spatulenol, caryophyllene oxide. In essential oil of *Tagetes tenuifolia* 53 components are found and 31 are identified. *Trans*-ocimene, *cis*-ocimene, dihydrotagetone, *cis*-tagetone, *cis*-ocimene, *trans*-tagetone are main ingredients of this volatile oil. Composition of *Tagetes erecta* essential oil lists 50 substances, and 37 of them are identified. This ether oil contains caryophyllene, piperitone, caryophyllene oxide. According to the investigation we state that essential oils of all 3 types of Marigold contain: caryophyllene, caryophyllene oxide, germacrene D, α -terpinylacetate, bicyclogermacrene and neophytadiene.

54 components were found in wild common daisy herb (*Bellis perennis*) and 47 were identified. In cultivated common daisy herb all 28 components were identified. Essential oil of cultivated common daisy herb contains in high amounts geranyl acetate, geraniol, γ -himachalene, and volatile oil of cultivated common daisy herb - hexahydrofarnesyl acetone, viridiflorol, squalene, γ -himachalene, α -bisabolol. Similar components for both objects are geraniol, geranyl acetate, γ -himachalene, hexahydrofarnesyl acetone, eicosane, phytol, pentacosane, heptacosane, nonacosane, and untriacontane, but they differ in quantitative content.

The investigation of *Chrysanthemum morifolium* essential oils has shown the presence of 49 components in leaves and 24 in flowers. Common components for two objects are decane, *cis*-*para*-ment-2-en-1-ol, *trans*-*para*-ment-2-en-1-ol, β -caryophyllene, α -farnesene, nerolidol, spathulene, caryophyllene oxide and squalene. Essential oil of *Chrysanthemum* leaves contains in high amounts caryophyllene oxide, epi- α -cadinol, thymol, spatulenol; in flowers – pentacosane, germacrene D, dehydrosabinaketone.

Keywords: essential oils, genus *Tagetes*, *Bellis perennis*, *Chrysanthemum morifolium*.

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PP-054. Chemical composition and biological activities of hydrosol extract of *Micromeria inodora* (Desf.) Benth. from Algeria

Ali Medbouhi^{1,*}, Fatima Zohra Benomari¹, Rania Belabbes¹, Nassim Djabou¹, Alain Muselli², Mohammed El Amine Dib¹, Jean Costa²

¹University of Tlemcen, Laboratory of Natural and Bioactive Substances (LASNABIO) Chemistry Department, Faculty of Science, PO Box 119, 13000 Tlemcen, Algeria.

²University of Corsica, UMR 6134, Laboratoire Chemistry of Natural Products (CPN), Campus Grimaldi, BP 52, 20250 Corte, France

*Corresponding author. Email: sm-ali13@hotmail.fr

Chemical composition of hydrosol extract of North Algerian *Micromeria inodora* (Desf.) Benth. has been investigated, from the first time, using GC/RI and GC-MS. Antioxidant activity of hydrosol extract was established by four methods (TLC, DPPH, FRAP and the bleaching test β -carotene). Antifungal activity was established in vivo and in vitro against four phytopathogenic fungi causing the deterioration of *Citrus sinensis* fruits. GC and GC/MS analysis of *M. inodora* hydrosol extract allowed the identification of 49 compounds accounting for 93.1 % of hydrosol extract. The main components were β -bisabolol (10.7%), (Z)-verbenol (8.7%), α -terpinyl acetate (7.3%) and (E)-sesquisabinene hydrate (6.7%). Antioxidant activity of Algerian hydrosol extract exhibited very strong RSCs, reducing the DPPH radical formation with $IC_{50}=35.07\mu\text{g/mL}$. In addition, the hydrosol extracts present good capacity to slow the rate of oxidation of linoleic acid and β -carotene with $IC_{50}=0.92\mu\text{g/mL}$. In the other hand, hydrosol extract showed strong in vitro antifungal activity based on the inhibition zone and minimal inhibitory concentration values against the pathogen (*Aspergillus niger*, *Aspergillus oryza*, *Penicillium italicum* and *Fusarium solani*). *Citrus sinensis* fruits (Orange) infected by *Penicillium italicum* pathogen were treated in vivo with hydrosol extract and hydrosol. Very low concentrations were needed for the absence of orange infection and very low disease incidence. This activity can be correlated with chemical composition of hydrosol extract and hydrosol which are rich in oxygenated components. Hydrosol of *Micromeria inodora* could be used for management of this pathogen as alternative source of ecological fungicides or an ideal alternative to control *P. italicum* infection during oranges storage.

Keywords: *Micromeria inodora* (Desf.) Benth., hydrosol extract, antioxidant and antifungal activities.

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PP-058. Investigation of essential oils of *Schizandra chinensis* Baill.

Olena Mykhaliuk^{1*}, Svitlana Marchyshyn²

¹ Department of Management and Economy of Pharmacy with Drugs Technology, I.Ya. Horbachevsky Ternopil State Medical University, 1 Maydan Voli, 46001, Ternopil, Ukraine

² Department of Pharmacognosy with Medical Botany, I.Ya. Horbachevsky Ternopil State Medical University, 1 Maydan Voli, 46001, Ternopil, Ukraine

*Corresponding author. Email: kalushka84@mail.ru

Essential oils (EO) are multicomponent mixtures of volatile organic compounds which are formed in plants and have inherent aroma. Due to this multicomponent structure, EO's are widely used as components of medicinal remedies as well as in perfumery and food industry. Essential oils as multicomponent compounds of natural biologically active substances have wide specter of therapeutical means that is why the study of essential oils in vegetative objects is very urgent.

We have conducted the research of essential oils of plant introduced into Ukraine - *Schizandra chinensis* Baill. Genus *Schizandra* first was discovered in North America in 1803 by a scientist of French origin André Michaux. He referred to this genus the only one North American species. Modern scientists count 7 species in this genus. The commonest species is *Schizandra chinensis* Baill. It is a ornamental, valuable fruity and medicinal plant. It is known because it manifests tonic and stimulating action on central nervous system, stimulates reflex excitation, improves cognitive ability and memory.

Essential oils from the leaves and fruit of *Schizandra chinensis* Baill. were hydrodistilled in Chromatograph *Agilent Technology 6890N* with mass spectrometer 5973N (Chernogorod, 2006, Zhao, 2011). The composition of the essential oils was conducted by use of *Agilent Technology 6890N* gas chromatograph coupled with mass-spectrometric detector 5973N (Chernogorod, 2006).

The results of the study showed that essential oils of *Schizandra* fruit consist of 58 components, 40 of which were identified. The main components of essential oil, received from fruit, are the following identified ones: α -muurolol (2,15 % from general amount of essential oils), epi- α -muurolol (2,13 %), oplopenone (2,08 %), salvial-4(14)-en-1-one (2,06 %), spathulenol (2,04 %), nerolidol (2,03 %), valencene (1,98 %), δ -cadinene (1,97 %), γ -cadinene (1,95 %), α -selinene (1,92 %), δ -selinene (1,91 %). Among 69 components present in EO of *Schizandra* leaves 56 were identified. The main components of this EO were untriacontan (2,45 %), nonacosane (2,35 %), squalene (2,31 %), heptacosane (2,25 %), hexacosane (2,19 %), pentacosane (2,13 %), tetracosane (2,08 %), tricosane (2,01 %), docosane (1,95 %). Such a large amount of components of essential oil arranges conditions for further study of *Schizandra chinensis* Baill. as perspective vegetative source of essential oils.

Keywords: essential oils, *Schizandra chinensis* Baill.

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PP-059. Composition and biological activity of essential oils from the leaves and stems of stevia (*Stevia rebaudiana* Bertoni) from eastern Poland

Agnieszka Najda*

Department of Vegetable Crops and Medicinal Plants, University of Life Sciences in Lublin, 20-068 Lublin, 58 Leszczyńskiego Street, Poland

*Corresponding author. Email: agnieszka.najda@up.lublin.pl

Over the past few years, one of the raw materials of great interest and demand, both in international markets and domestic, is *Stevia rebaudiana* Bertoni. Numerous studies on stevia focus on growth parameters of plants and their adaptation to different climate zones. Whereas systematic study of phytochemicals allowed to explore and document the healing and functional properties, resulting mainly from the presence of secondary metabolites in leaves: steviol glycosides, amino acids and phenols. There is currently no data on changes in the content of volatile compounds present in various parts of the anatomy of this plant depending on the growth phase. Therefore, in this paper the first comprehensive analysis of the content of essential oils (EO) distilled from aerial parts of the plant (leaves and stems) *Stevia* grown under conditions of Eastern Poland was carried out. Qualitative and quantitative composition of oils and their antioxidant activity in different stages of plant growth were defined. The essential oil was obtained from air-dried leaves and stems of *Stevia rebaudiana* Bertoni by steam distillation with the addition of m-xylene in Clevenger type apparatus. Analyses were carried out using gas chromatography (GC) and gas chromatography - mass spectrometry (GC-MS), simultaneously.

The yield of oil was variable and dependent on the growth phase of the plant, a greater amount was obtained from the leaves (0.34% - 0.67% v/v), significantly less from stems (0.03% - 0.11% v/v). Altogether 72 compounds (43 volatile components in the oils from leaves and 29 from stems), which represents more than 98.7%, and 99.2% of the tested oils composition were identified. The main components of EO derived from studied raw material were isoprenoids and among them monoterpenes and sesquiterpenes. The predominant compounds in all the samples were: spathulenol (11.34 – 32.72%), α -pinene (6.13-18.58%), (Z) -caryophyllene (4.19 – 17.10%), caryophyllene oxide (5.83 – 14.57%), α -cadinol (0.91 – 6.16%) and β -guaiene (0.08 – 4.32%). The results obtained indicate that essential oils and their components are invaluable source inhibiting free radical DPPH.

Keywords: Asteraceae, *Stevia rebaudiana*, essential oil composition, GC, GC/MS, DPPH.

PP-061. Changes in yield and essential oil compounds of four Lamiaceae species due to different water supply

Éva Németh-Zámoriné^{1*}, Katalin Inotai¹, Krisztina Szabó, Zsuzsanna Pluhár¹, Mahmoud Malekzadeh¹, Péter Radácsi¹, Szilvia Sárosi¹, Katarzyna Seidler-Lozykowska²

¹ Department of Medicinal and Aromatic Plants, Corvinus University of Budapest, H-1518 Budapest, P.O. Box 53., Hungary

² Institute of Natural Fibres and Medicinal Plants, ul. Wojska Polskiego 71B; 60-630, Poznan, Poland

*Corresponding author. Email: eva.nemeth@uni-corvinus.hu

The effect of water supply on medicinal and aromatic plants has not been studied satisfactory until today. Finding the optimum for dry matter production and that for the accumulation of secondary compounds is an important task in the practice and has been the goal of our recent study. In 2014 a pot experiment was conducted under controlled conditions. The plants were grown in 40% (S: stress) and 70% (C: control) saturation of soil water capacity (SWC%). Five cultivars of *Melissa officinalis* ('Lorelei', 'Lemona', 'Soroksári', 'Quedlinburger Niederliegende' and 'Gold Leaf'), four accessions of *Thymus vulgaris* ('Varico 3' and different chemotypes such as TV17- thymol, TV115- geraniol, TV143- alpha-terpineol), the variety 'Mexian' of *Mentha piperita* and the cultivar 'Magyar' of *Majorana hortensis* were investigated. Shoot mass, essential oil (EO) content and composition were tested in 3-10 replications. The dried herb was cleaned and the leaf fraction hydrodistilled. The oil was analyzed by GC-MS and the components identified by MS libraries and their LRIs.

Stress conditions reduced the biomass formation of each species. However, in lemon balm and peppermint the rate of this decrease reached 4.4 and 4.0-6.7 fold respectively, while the reduction was less in thyme (2.06-3.95 fold) and much lower in marjoram (1.6 fold). The lower water supply resulted also in a lower EO accumulation in each case but the reduction was less characteristic for the species and showed 1.10-1.30 fold decreases. As for the characteristic components of the oil, peppermint proved to be the most stable with only insignificant changes. Similar stability could be established for lemon balm, with slight differences among the cultivars. On the contrary, marjoram and thyme showed characteristic quantitative changes of the EO profile: in marjoram oil the ratios of γ -terpinene, trans-sabinene-hydrate acetate, terpinene-4-ol and the overall ratio of monoterpenes decreased. In thyme each chemotype showed specific reactions: in TV115, geraniol and geranyl acetate decreased while linalool increased, in TV143 α -terpinyl acetate sharply increased with a parallel decrease of thymol. Thymol decreased also in the thymol chemotypes while the ratios of γ -terpinene and p-cymol appeared in higher percentages. The findings show that quantitative and qualitative changes of EO compounds due to water supply might be highly specific for each species and not necessarily parallel with biomass production.

Keywords: lemon balm, thyme, peppermint, marjoram, drought stress, biomass.

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PP-062. Evaluation of different *Perilla frutescens* (L.) Britt. origins in Hungary

Péter Radácsi^{1,*}, Katalin Inotai¹, Bálint Medgyesi² and Éva Németh¹

¹ Department of Medicinal and Aromatic Plants, Corvinus University of Budapest, H-1118 Budapest Villányi str. 29-43., Hungary

² Biomed Ltd., H- 3043Egyházasadengeleg, Sport str. 39.

*Corresponding author. Email: peter.radacsi@uni-corvinus.hu

Perilla frutescens L. ('Chinese basil') is used traditionally in the Far East as a medicinal and aromatic plant. The Chinese Medicine uses the leaves against bronchitis or other problems of the respiratory system as well as for skin allergy (Habegger et al., 2006). In the last decades its strong antiallergic, anticancer and immune-stimulant effects have been proved. Seeds are rich in unsaturated fatty acids therefore and consumed in dietary food products.

In our research work five *Perilla frutescens* L. accessions (Pf1, Pf2, Pf3, Pf4 and Pf5) of different origin were evaluated in an open-field experiment in 2014. Morphological characteristics and production as well as content and composition of essential oil were studied. Samples were taken before flowering in 3 replications. The dried herb was hydrodistilled and the oil analysed by GC-MS. The components were identified by MS libraries and their LRIs.

The height of the plants varied between 88.0 cm and 108.3 cm. Highest fresh weight was produced by the accession Pf1 (401.0 g·plant⁻¹) while the lowest fresh weight was measured in the population of Pf2 (334.1 g·plant⁻¹). Parallely, dry mass of the studied populations varied between 124.0 g·plant⁻¹ (Pf1) and 108,7 g·plant⁻¹ (Pf2). The highest essential oil content was detected in the population Pf3 (1.252 ml·100 g d.w.⁻¹) while the lowest result were detected in Pf4 (0.591 ml·100 g d.w.⁻¹). Based on the essential oil composition the investigated populations represent different chemotypes. In Pf3, Pf4, Pf5 the main components of the essential oil were perillaldehyde (app. 65-71 %) and limonene (16-18%) that is, they are belong to the perillaldehyde (PA) chemotype (He-Chi et al., 1997). The main component of the essential oil in Pf1 population was perillaketone (99 %) (PK – perillaketone chemotype). In the essential oil samples of Pf2, Pf3, Pf5 accessions perillaketone was not detectable at all, however, in the essential oil of Pf4 perillaketone was present in low percentages (below 3 % of EO).

Keywords: chemotypes, perillaaldehyde, perillaketone, essential oil, *Lamiaceae*.

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PP-063. Fingerprint analysis of essential oils from *Achillea sp.* by high-performance liquid chromatography

Małgorzata Niemiec^{1,*}, Mirosław Hawrył¹, Monika Waksmundzka-Hajnos¹, Mykhaylo Chernetsky²

¹Department of Inorganic Chemistry, Medical University of Lublin, Chodźki 4A St., 20-093 Lublin, Poland

²Botanical Garden of Maria Curie-Skłodowska University in Lublin, Sławinkowska 3, 20-810 Lublin, Poland

*Corresponding author. Email: malgorzata.niemiec@op.pl

A number of herbs used traditionally exhibit pharmacological activity with great potential in therapeutic applications. The genus *Achillea* is one of the most important genera of the Asteraceae family and is represented by 85 species, mostly found in Europe and Asia and a handful in North America (Abd-Alla et al., 2015; Candan et al., 2003). *Achillea sp.* are widely distributed medicinal plants that have been used for over 3000 years as folk medicine for gastrointestinal disturbances (Cavalcanti et al., 2006), anxiety, and insomnia (Baretta et al., 2012). It is used as an anti-inflammatory (Benedek et al., 2007), and an astringent, as well as in the treatment of fever, diarrhea, hemorrhoids, cancer, bacterial infections, hypertension, as diuretic (de Souza et al., 2013) and as an emmenagogue (Innocenti et al., 2007). Strong antioxidant properties of *Achillea sp.* essential oils have been reported (Candan et al., 2003; Dias et al., 2013).

Phytochemical screenings revealed that chemical constituents of *Achillea sp.* are presented by several secondary metabolites, including essential oil components (cineol, borneol, pinenes, camphor, menthol, eugenol, azulene, and chamazulene), sesquiterpenes, steroids, triterpenes and flavonoids. A number of these substances had beneficial effects evidenced in several pathological conditions (de Souza et al., 2011).

High-performance liquid chromatography (HPLC) was developed for fingerprint analysis of 9 essential oils from several species of *Achillea*. Essential oils were obtained from herbs by the most commonly used hydrodistillation in Deryng apparatus. Gradient elution HPLC was applied to develop fingerprints of 9 essential oils obtained from selected *Achillea sp.* Two column types Kinetex RP8 and Kinetex phenyl-hexyl (Phenomenex, Torrance, USA) at flow of 1ml/min were used. Temperature of columns oven was set at 30°C. Solvent – acetonitrile (ACN) gradient grade from Merck (Darmstadt, Germany) and bidistilled water were used. Solutions of test substances were placed in vials and 10 µl each of them were applied into the column. Linear gradient elution of all test compounds was performed as follows: 5 – 100% (V/V) in 60 min using acetonitrile-water as mobile phase.

The chemotaxonomic value of the essential-oil composition was discussed according to results of principal-component analysis (PCA). By comparing the chromatograms obtained by HPLC and PCA analysis similarity measures and identify of the species and varieties of *Achillea* can be distinguished.

The aim of our work was analysis terpenes and other compounds occurred in essential oils of *Achillea sp.*, popularly known and used in medicine, by use of HPLC and by construction of fingerprints of them to facilitate the identification of *Achillea* species and varieties. Our investigations can also be helpful for chemotaxonomic research.

Keywords: *Achillea sp.*, HPLC, essential oils, fingerprint.

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PP-064. Central nervous system activities and chemical composition of essential oil of fresh fruits of *Piper guineense* (Piperaceae) in mice

Idris Oyemitan^{1,2,*}, Christianah Elusiyan³, Adebola Oyedeji²

¹Department of Chemistry, Walter Sisulu University, Nelson Mandela Drive Campus, Mthatha, 5117, South Africa

²Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, 220005, Nigeria

³Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, 220005, Nigeria

*Corresponding author. Email: ioyemitan@wsu.ac.za; oyemitan@oauife.edu.ng; oyemix@yahoo.com

The aim of this study was to determine the chemical composition and some central nervous system (CNS) activities of the essential oil obtained from the fresh fruits of *Piper guineense* Schum & Thonn (Piperaceae) in order to validate its traditional use. *P. guineense* is one of the several medicinal plants commonly used in managing mental ailments and related disorders. Preliminary acute toxicity study of the essential oil indicates its LD₅₀ to be 693 intraperitoneally (Oyemitan *et al.*, 2014).

Essential oil of *P. guineense* (EOPG) obtained by hydrodistillation was analyzed by GC/MS. EOPG (50-200 mg/kg, i.p.) was evaluated for behavioural, hypothermic, sedative, muscle relaxant, anti-psychotic and anticonvulsant activities.

Behavioural effect was assessed on the open field test and parameters considered were rearing, locomotion and head-dipping; effect on rectal temperature was tested using digital thermometer; sedative activity was assessed on ketamine-induced hypnosis; muscle relaxant activity was assessed using hind-limb grip, sliding board and rota rod; antipsychotic effect was assessed with catalepsy, apomorphine-induced locomotion, apomorphine-induced climbing, apomorphine-induced grooming and apomorphine-induced hypothermia models; while the anticonvulsant activity was determined on pentylenetetrazole-induced convulsion model.

Analysis of the oil revealed 44 compounds of which 30 compounds constituting 84.7% were identified. The oil was composed mostly of sesquiterpenoids (64.4%) while four were monoterpenoids (21.3%) were found present in the oil. Major compounds identified were β -sesquiphellandrene (20.9%), linalool (6.1%) and limonene (5.8%). The EOPG (50-200 mg/kg, i.p.) caused significant ($p < 0.01$) inhibition on rearing and locomotor activity but decreased head dips suggesting CNS depressant effect; decreased rectal temperature, signifying hypothermic activity; decreased ketamine-induced sleep latency and prolonged total sleeping time, indicating sedative effect; reduced muscular tone on the hind-limb grip test, inclined board and rota rod implying muscle relaxant activity; induced catalepsy, inhibited apomorphine-induced climbing behaviour and inhibited apomorphine-induced locomotor activity, suggesting anti-psychotic effect. Finally, it protected mice against pentylenetetrazole-induced convulsions, indicating anticonvulsant potential (Oyemitan *et al.*, 2015).

The most abundant component of the oil was identified to be β -sesquiphellandrene (20.9%). Furthermore, the oil exhibited significant CNS depressant, hypothermic, sedative, muscle relaxant, antipsychotic and anticonvulsant activities. Results obtained here justify ethnomedicinal use of the plant.

Keywords: *Piper guineense*, volatile oil, β -sesquiphellandrene, sedative, anti-psychotic.

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PP-065. Essential oil composition and insecticidal activity of *Inula viscosa* (L.) Aiton. from Cyprus

Kaan Polatođlu^{1,*}, Yasemin Yücel Yücel², Ömer Cem Karakoç³, Salih Gücel⁴, Betül Demirci⁵, Kemal Hüsnü Can Başer⁶

¹ Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul Kemerburgaz University, 34217, İstanbul, Turkey

² Department of Biochemistry, Faculty of Pharmacy, Istanbul Kemerburgaz University, 34217, İstanbul, Turkey

³ Department of Crop and Animal Protection, Yapraklı Vocational School, Cankiri Karatekin University, 18100, Cankiri, Turkey

⁴ Institute of Environmental Science, Near East University, 10, Nicosia, Cyprus

⁵ Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskişehir, Turkey

⁶ Department of Botany & Microbiology, Faculty of Science, King Saud University, 11451, Riyadh, Saudi Arabia

*Corresponding author. Email: kaan.polatoglu@kemerburgaz.edu.tr

The essential oil of *Inula viscosa* (L.) Aiton [Syn.: *Dittrichia viscosa* (L.) Greuter] (Asteraceae) from Cyprus was investigated for the first time. The aerial parts of the individual plant sample (100g) afforded 0.01 mL of essential oil. The composition of the oil was identified by GC, GC-MS analysis. Fifty two compounds were identified in the oil corresponding to 71.6% of the oil. The main components of the oil were (*E*)-Nerolidol 26.6%, Selina-6-en-4-ol 8.5%, fokienol 6.8% and caryophyllene oxide 5.1%. Fumigant insecticidal activity of the essential oil was evaluated against *Sitophilus granarius* at different concentrations. The 0.01 mL/1mL (in *n*-hexane) oil concentration further diluted to 5%, 2.5% 1% with acetone which afforded 100.00±0.00%; 95.47±3.41 and 4.53±3.41 mortality against *S. granarius* respectively (*n*=3). Additionally in order to provide a further proof to the observed insecticidal activity AChE inhibitory activity of the oil was also evaluated (Lopez & Pascual-Villalobos, 2010). Pure oil and 10 mg/mL oil solution afforded 24.42±6.81% and 17.50±0.39% AChE inhibition (*n*=3). The AChE inhibition was concentration dependent. The low AChE inhibition indicates observed insecticidal activity mechanism was not related to the inhibition of AChE.

Keywords: *Inula viscosa*, Asteraceae, AChE inhibition, *Sitophilus granarius*, insecticidal activity.

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PP-066. Acetylcholinesterase inhibitory and PRAP activities of the essential oils on selected *Tanacetum* species

Kaan Polatoğlu^{1,*}, Yasemin Yücel Yücel², Hüseyin Servi³, Cem Kaya¹, Betül Demirci⁴, Nezhun Gören⁵

¹ Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul Kemerburgaz University, 34217, İstanbul, Turkey

² Department of Biochemistry, Faculty of Pharmacy, Istanbul Kemerburgaz University, 34217, İstanbul, Turkey

³ Department of Chemistry, Graduate School of Science & Technology, Yildiz Technical University, 34210, İstanbul, Turkey

⁴ Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskişehir, Turkey

⁵ Department of Molecular Biology, Faculty of Science and Letters, Yildiz Technical University, 34210, İstanbul, Turkey

*Corresponding author. Email: kaan.polatoglu@kemerburgaz.edu.tr

Essential oils obtained from flowers of *Tanacetum mucroniferum* Hub. - Mor. & Grierson, leaves of *Tanacetum densum* (Labill.) Heywood ssp. *eginense* Heywood, stems of *Tanacetum densum* (Labill.) Heywood ssp. *sivasicum* Hub. - Mor. & Grierson were investigated for their AChE inhibitory and PRAP activities. We have previously investigated chemical composition of these oils (Polatoglu et al. 2009, 2011, 2012, 2015) however we have also suspected that these oils also do have strong AChE inhibitory activities due to the insecticidal activities we have reported for the other members of this genus. Highest activities were observed for pure oils: *T. mucroniferum*, *T. densum* ssp. *sivasicum* and *T. densum* ssp. *eginense* were 100.00±0.0%; 100.00±0.0% and 91.31±3.30% respectively ($n=3$). The observed activities were concentration dependent. All of the investigated oils produced low PRAP activity at the highest concentration (10 mg/mL) studied when compared with the positive control α -tocopherol (3.735±0.063 AU; $n=3$) at the same concentration. Highest activity was observed for *T. mucroniferum* (1.319±0.017 AU; $n=3$) at 10 mg/mL concentration.

Keywords: AChE Inhibition, PRAP Activity, *T. mucroniferum*, *T. densum* ssp. *sivasicum*, *T. densum* ssp. *eginense*.

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PP-067. Content and composition of essential oil in *Betula* spp. leaves growing naturally in Estonia

Ain Raal^{1,*}, Anne Orav², Tatjana Boikova¹ and Elmar Arak¹

¹ Department of Pharmacy, University of Tartu, Nooruse 1, Tartu 50411, Estonia

² Institute of Chemistry, Tallinn University of Technology, Akadeemia tee 15, Tallinn 12618, Estonia

*Corresponding author. Email: ain.raal@ut.ee

The content of essential oils and dynamics of polyphenols in the leaves of four birch species (*Betula pendula* Roth, *B. pubescens* Ehrh. *B. humilis* Schrank and *B. nana* L.) growing in Estonia were determined in our previous studies (Orav et al., 2011; Raal et al., 2015).

The aim of the study was to determine seasonal variation in the content and chemical composition of the essential oils in Birch leaves collected in the middle of June, August and October. The dried plant material (20 g) was hydrodistilled for 2 h using a Ginsburg-tube by U.S.S.R. Pharmacopoeia (1990) and essential oils were analyzed by GC and GC-MS (Orav et al., 2011).

The yield of essential oils was the highest in the leaves of *B. humilis* collected in June and in October (both 0.27%). The leaves of the other Birch species studied had very low concentrations of oil: 0.11% in the *B. pendula* and 0.05% in the *B. pubescens* leaves collected in spring. Later the exact yield of essential oils, as well as in *B. nana* from June to October, was not determinable.

A total of 47, 41, 56, and 35 compounds were identified in the essential oils of *B. pendula*, *B. pubescens*, *B. humilis* and *B. nana* leaves, respectively, representing over 92% of the total volume of oil. α -betulenol (0.9-33.1%), α -betulenol acetate (2.9-31.9%), β -betulenol (2.5-7.6%) and β -betulenol (0.3-2.0-5.8%) were identified as the main constituents of the essential oils hydrodistilled from *B. pendula*, *B. pubescens* and *B. humilis* leaves. The content of bicyclic sesquiterpenoids (birkenal, δ -cadinene, τ -cadinol, α -betulenol, β -betulenol, β -betulenol, α -cadinol, 6-hydroxy- β -caryophyllene acetate, α -betulenol acetate) decreased from June to October. The amount of the aliphatic compounds (*n*-heptadecane, *n*-hexadecanal, *n*-nonadecane, palmitic acid, *n*-eicosane, *n*-octadecanal, *n*-heneicosane, *n*-tricosane, and *n*-pentacosane) contrariwise, showed the tendency of increasing from spring to autumn. A strong negative correlation ($r=-0.903$; $p<0.01$) between the total content of aliphatic compounds and bicyclic sesquiterpenoids was found.

In conclusion, the total amounts of bicyclic sesquiterpenoids showed the tendency of decreasing, and aliphatic compound increased from spring to autumn.

Keywords: *Betula pendula*, *Betula pubescens*, *Betula humilis*, *Betula nana*, essential oil.

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PP-068. Antioxidant activity and chemical composition of essential oils from two *Guatteria elliptica* R. E. Fries (Annonaceae) specimens

Agnieszka K. Rajca-Ferreira^{1*}, Felipe Rebello Lourenço¹, André Rolim Baby¹, Edna T. Myiake Kato¹, Maria Cláudia Marx Young², Marcos E. Leite Lima², Inês Cordeiro² and Paulo Roberto H. Moreno^{1,3}

¹ Faculty of Pharmaceutical Sciences, University of Sao Paulo, C.E.P. 66083, 05389-970, Sao Paulo, SP, Brazil

² Sao Paulo Botanical Institute, CP 4005, 01061-970, Sao Paulo, SP, Brazil

³ Institute of Chemistry, University of Sao Paulo, PO Box 26077, 05513-970, Sao Paulo, SP, Brazil

*Corresponding author. Email: arajcaferreira@gmail.com

Guatteria elliptica R. E. Fries (Annonaceae) is a native Brazilian plant from the Atlantic Rainforest and belongs to a genus known for its pharmacological properties. However, essential oils of many *Guatteria* spp. have not yet been studied. Thus, the purpose of this work was to evaluate the antioxidant activity and chemical composition of essential oils from two *G. elliptica* specimens.

Plants were collected in Natural Reservation areas at Paranapiacaba and Caraguatatuba in Sao Paulo. Collected plants were taxonomically confirmed and deposited in the herbarium of the Botanical Institute of Sao Paulo (CARREIRA 104 and CORD 2924). The essential oils were obtained by hydrodistillation in a Clevenger type apparatus for 4 hours. The transverse histological cuts were made and dyed, using appropriate stains (Rio et al, 2014), in order to identify secretory structures in the leaves. Later the cuts were observed under optical microscope and photographed. The GC-MS analyses were carried out on Agilent 5973 equipment. The samples (1.0 µL) were dissolved in acetone (0.1 %) and injected in splitless mode, using helium as a carrier gas. The compound identification was performed by comparing the obtained mass spectra with Wiley 275 and Adams libraries, literature data and its retention indices (Kóvats Index, KI), determined relatively to the retention times of a series of *n*-alkanes (Adams, 2007). The antioxidant activity evaluation was performed by the DPPH scavenging method in 96-well plates (Sharma and Bhat, 2009). The results were statistically processed with Origin 9.1®.

The volatile oils yields for the Caraguatatuba and Paranapiacaba individuals were 0.11 and 0.21 %, respectively. The main compound class for both oils was oxygenated sesquiterpenes. The oil from Paranapiacaba specimen contained spathulenol (53.9 %) as a major component, while that from Caraguatatuba – caryophyllene oxide (40.9 %). The structures responsible for oil storage in the leaves were secretory canals, neighbouring epidermal cells. Antioxidant EC₅₀ for the Paranapiacaba specimen was 7.24±2.57 mg/mL and for that collected in Caraguatatuba - 28.68±14.36 mg/mL. In comparison, the EC₅₀ for the positive control quercetin was 3.61±0.27 µg/mL.

The essential oils yield, chemical composition and their antioxidant activity differ for two *G. elliptica* specimens collected in different location. Paranapiacaba specimen higher activity could be due to the higher spathulenol contents in these essential oils.

Keywords: antioxidant activity, essential oils, GC-MS, *Guatteria elliptica*.

Acknowledgments

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PP-069. Nematicidal activity of essential oils against the potato cyst nematode *Globodera rostochiensis*

Marek Renčo^{1*}, Andrea Čerevková¹, Nicola Sasanelli², Ion Toderas³

¹ Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 040 01, Košice, Slovak Republic

² Institute for Sustainable Plant Protection, CNR, G. Amendola 122/D, 70126 Bari, Italy

³ Institute of Zoology, Academy of Sciences of R. Moldova, Street Academiei 1, MD-2028, Chisinau, R. Moldova

*Corresponding author. Email: renco@saske.sk

Phytoparasitic nematodes can cause severe yield losses to numerous vegetables therefore it is necessary their control. To evaluate the efficacy of two essential oils (EOs) isolated from *Thymus vulgaris* and *Cinnamomum camphora* in the control of the potato cyst nematode *Globodera rostochiensis* an *in vitro* experiment (hatching test) was carried out.

Batches of 50 *G. rostochiensis* cysts, of similar size, extracted by the Fenwick can from infested soil, were placed in Eppendorf tubes (2mL) in 1 mL of distilled water before treatments with EOs. The two EOs solutions (10% ethanol, v/v) in appropriate amount were diluted in distilled water containing 0.3% polysorbate surfactant 20 (Tween-20) (v/v) to obtain 4, 8 and 16 $\mu\text{L mL}^{-1}$ solutions. The EOs were then used to treat *G. rostochiensis* cysts adding to each Eppendorf tube 1 mL of each solution. Then, tubes were closed and cysts exposed to the EOs treatment for 48 hrs. Untreated cysts were used as control. Four replications for each treatment were considered. After treatments cysts were removed from the Eos solutions, rinsed in distilled water and put in 2 cm diam sieves (215 μm aperture) placed in 3.5 cm diam Petri dishes. In the hatching test potato root leachate was used as natural hatching agent and the test was carried out in a growth cabinet at 21°C. Emerged juveniles were removed and counted every week over a 6 week period. The hatching agent was renewed at each observation time. Numbers of second stage juveniles emerging every week were expressed as cumulative percentages of the total egg content of the cysts.

Percentage hatch of *G. rostochiensis* eggs was significantly reduced ($P < 0.05$) by the different concentrations of both Eos, although significantly higher eggs mortality was observed in cysts immersed in thyme oil. Our results suggest that essential oils of *Thymus* and *Cinnamon* could be favourably considered in a sustainable control of the potato cyst nematode, although further studies are needed.

Keywords: Hatching test, *Thymus vulgaris*, *Cinnamomum camphora*. (10P).

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PP-070. *In vitro* anti-herpes activity of *Salvia desoleana* Atzei & V. Picci essential oil

Valeria Cagno¹, Barbara Sgorbini², Cinzia Sanna³, Cecilia Cagliari², Mauro Ballero³, Carlo Bicchi², David Lembo¹, Patrizia Rubiolo^{2*}

¹Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via Pietro Giuria 9, I-10125 Torino, Italy

²Laboratory of Molecular Virology, Department of Clinical and Biological Sciences, Università degli Studi di Torino, I-10043, Torino, Italy

³Dipartimento di Scienze della Vita e dell'Ambiente, Università degli Studi di Cagliari, Viale S. Ignazio 13, I-09123 Cagliari, Italy

*Corresponding author. Email: patrizia.rubiolo@unito.it

Salvia desoleana Atzei & V. Picci is an indigenous species in Sardinia island used in folk medicine to treat menstrual, digestive and central nervous system disease. Nowadays, it is widely cultivated for the pleasant smell of its essential oil, whose antimicrobial and antifungal activities have been already screened (Peano et al. 1999, Sokovic et al. 2009).

Within a project aiming to investigate the potential antiviral activity of endemic plants from Sardinia, this study evaluated the *in vitro* anti-Herpes Simplex Virus (HSV-2) activity of *Salvia desoleana* essential oil (EO) and its main components: linalyl acetate (25%), alpha terpinyl acetate (16%) and germacrene D (18%).

The results showed that *S. desoleana* EO inhibits both acyclovir sensitive and acyclovir resistant HSV-2 strains with IC₅₀ values of 23.72 µg/ml for the former and 28.57 µg/ml for the latter. Moreover, a significant suppression of HSV-2 replication was observed with an EC₅₀ value of 33.01 µg/ml (95% CI: 26.26 to 41.49) when the EO was added post-infection.

A bioassay-guided fractionation procedure was therefore adopted to identify the active fraction(s) and/or compounds in *S. desoleana* EO. Among the fractions resulting from flash column chromatography on Silica gel, that containing 84% of germacrene D showed a similar spectrum of activity of *S. desoleana* EO although increased because of the germacrene D enrichment (EC₅₀ of 10.19 µg/ml against HSV-2 and 6.58 µg/ml against HSV-2 acyclovir resistant) and with a stronger suppression in post-infection stage.

In conclusion, *S. desoleana* EO and germacrene D can be of interest to develop new and alternative anti-HSV-2 products also active against acyclovir-resistant HSV-2 strains.

Keywords: *Salvia desoleana*, germacrene D, antiviral activity, HSV-2.

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PP-071. *In vitro* and *in vivo* control of *Fusarium oxysporum* and *F. proliferatum* by commercial *Laurus nobilis* L. and *Syzygium aromaticum* L. Merr. & Perry essential oils

Josefa Roselló¹, David García¹, Mercedes Verdeguer¹, M. Amparo Blázquez² and M. Pilar Santamarina^{1*}

¹ Departamento de Ecosistemas Agroforestales, Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural. Universitat Politècnica de València. Camino de Vera s/n, 46022 Valencia, Spain

² Departament de Farmacologia, Facultat de Farmàcia, Universitat de València. Vicent Andrés Estellés s/n 46100 Burjasot, Valencia, Spain

*Corresponding author. Email: mpsantam@eaf.upv.es

Fungal contamination is a chronic problem in food products and has a negative effect on their quality and quantity (Sumalan et al., 2013). Pathogenic fungi can reduce yields of major foods and cash crops by nearly 20%. New methods to control spoilage fungi in food are being investigated because the application of synthetic fungicides has led to a number of environmental and health problems. In line with public demand, it is necessary to study natural compounds (Tabassum & Vidyasagar, 2013). In recent years, numerous studies have documented the antifungal effects of essential oils of plants to control food spoilage fungi *in vitro* (Hsuan et al., 2010; Blázquez, 2014; Roselló et al., 2015).

In the present study chemical composition of commercial *Laurus nobilis* L. and *Syzygium aromaticum* L. Merr. & Perry essential oils and antifungal activity against two pathogenic fungi have been investigated. Thirty-seven compounds accounting for more than 99.5% of the total essential oil were identified by GC and GC-MS. 1,8-Cineole (51.95%), α -terpinyl acetate (12.93%) and the monoterpene hydrocarbon sabinene (9.56%) were the main compounds in bay leaf essential oil, while the phenylpropanoid eugenol (88.58%), and the sesquiterpene hydrocarbons β -caryophyllene (8.13%) and α -humulene (2.35%) were found in clove essential oil. Clove essential oils at 300 μ g/mL showed more antifungal effect than bay leaf essential oil against both tested strains. *S. aromaticum* essential oil showed the best antifungal activity towards *Fusarium* species and similar antifungal activity than pure eugenol. In inoculated rice-grain significantly reduced the fungal infection *in vivo*, so *S. aromaticum* essential oil could be a good alternative as preservative in stored products.

This research showed that clove essential oil has a great potential to control both fungal pathogens. In the *in vitro* test, the essential oil reduced fungal growth by 90% and almost 100%.

Keywords: Essential oils, bay leaf, clove, *Fusarium oxysporum*, *Fusarium proliferatum*, antifungal activity.

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PP-072. Antifungal activity and potential use of commercial essential oils against *Curvularia hawaiiensis* isolated from rice

Josefa Roselló¹, Silvia Giménez¹, M. Amparo Blázquez² and M. Pilar Santamarina^{1*}

¹ Departamento de Ecosistemas Agroforestales, Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural. Universitat Politècnica de València. Camino de Vera s/n, 46022 Valencia, Spain

² Departament de Farmacologia, Facultat de Farmàcia, Universitat de València. Vicent Andrés Estellés s/n 46100 Burjasot, Valencia, Spain

*Corresponding author. Email: mpsantam@eaf.upv.es

Rice is the second most cultivated crop worldwide in cultivated areas after wheat, and the first most important in the human diet. This crop is a primary food source for more than one third of the world population. Rice is an important crop in the Valencian autonomous region (east Spain), with an origin denomination Rice of Valencia, and is grown according to environmental conditions and characteristics in the la Albufera Natural Park. The incidence of fungi *Curvularia hawaiiensis*. on stored rice samples is high. These fungi cause considerable grain and seed loss.

Essential oils of bay leaf, cinnamon, clove and oregano were tested *in vitro* and oregano essential oil *in vivo*, against *Curvularia hawaiiensis* isolated from stored rice. Chemical composition was identified by gas chromatography-mass spectrometry. Essential oils presented a high percentage of oxygenated components: 78.8% in bay leaf (eucalyptol 51%); 90.3% in clove (eugenol 89.8%); 92% in cinnamon (eugenol 60% and eugenyl acetate 18.3%); 71.8% in oregano (carvacrol 49.6% and thymol 21.2%). Monoterpenes and sesquiterpenes were: 18% in bay leaf, 9% in clove, 5% in cinnamon, 25% in oregano. This research showed that essential oils (Dayan et al., 2009; Antunes & Cavaco, 2010) have a great potential to control the fungal pathogen. In the *in vitro* test, the essential oils of cinnamon, clove and oregano reduced fungal growth by 90% and almost 100%, being oregano the most effective essential oil to inhibit fungal growth. The effect of the oregano essential oil on fungal development in inoculated rice grains was tested *in vivo* by exposing inoculated and control rice grains (kernels) to essential oil vapour. The obtained results demonstrated its effectiveness in rice grain conservation. The essential oils of oregano, clove and cinnamon provide an alternative for controlling *Curvularia hawaiiensis* in rice, cereals and stored products.

Keywords: Essential oils, bay leaf, clove, cinnamon, oregano, *Curvularia hawaiiensis*, antifungal activity.

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PP-073. Effect of essential oils on the hatch of eggs of cyst-forming phytoparasitic nematodes

Sebastiano Laquale¹, [Nicola Sasanelli](#)^{2*}, Trifone D'Addabbo²

¹School of Agricultural, Forest, Food and Environmental Sciences - University of Basilicata, Viale dell'Ateneo Lucano 10, Potenza, 85100, Italy

²Institute for Sustainable Plant Protection, CNR, G. Amendola 122/D, 70126 Bari, Italy

*Corresponding author. Email: nicola.sasanelli@ipspp.cnr.it

Essential oils (EOs) have been largely investigated for their biocidal activity on root-knot nematodes, *Meloidogyne* species, the most economically relevant group of phytoparasitic nematodes. Adversely, experimental activity on the effect of EOs on cyst-forming nematode species is very poor, though sustainable management of these nematode species should need new control tools alternative to chemicals, such as EOs-based formulates. EOs of *Eugenia caryophyllata* (L.) Merr. & L.M. Perry and *Schinus molle* L. are reported for a wide spectre of biological activities, among which also a biological activity against plant insect pests and fungal pathogens. The major component of the EO of *E. caryophyllata* is usually considered to be eugenol, with β -caryophyllene and lower amounts of other components such as benzyl alcohol. The major constituents in *S. molle* EO were α -phellandrene and β -phellandrene, with variable amounts of β -pinene, p-cymene and α -pinene.

An experimental activity was carried out to assess the *in vitro* effect of treatments with EOs on egg hatch of different cyst nematodes. Results of a hatching test with cysts of the potato and carrot cyst nematodes, *Globodera rostochiensis* and *Heterodera carotae*, respectively, treated with EOs of *E. caryophyllata* and *S. molle* are reported in this work. Batches of 100 cysts of each nematode species were exposed to 125, 250 and 500 $\mu\text{l L}^{-1}$ solutions of each EO for 12, 24 and 48 hours. There were 4 replications for each concentration x exposure time. Non-treated cysts were used as control. After treatments, hatching test of *G. rostochiensis* and *H. carotae* continued over 7 weeks in sodium metavanadate (0.6 mM) or zinc chloride (10 mM) solutions, respectively, counting emerged juveniles at weekly intervals. At the end of the experiment, unhatched eggs were determined and final cumulative percentage hatch was calculated. The EO of *E. caryophyllata* significantly reduced hatch of eggs of *G. rostochiensis* only after a 24 or 48 hour exposure to the highest concentration, whereas cyst treatment with the 125 $\mu\text{l L}^{-1}$ solution resulted in a significant increase of hatched eggs compared to the non-treated control. Adversely, all treatments with the same EO significantly reduced the percentage hatch of *H. carotae* eggs, with the exception of the 12 hour exposure to the 125 $\mu\text{l L}^{-1}$ solution. Compared to non-treated cysts, all treatments with the EO of *S. molle* did not significantly affect the hatch of *G. rostochiensis*, whereas significantly reduced the percentage hatch of *H. carotae*.

Keywords: *Eugenia caryophyllata*, *Globodera rostochiensis*, *Heterodera carotae*, *Schinus molle*, sustainable control.

PP-075. Chemical composition of the essential oils of the chemotypes *Mentha spicata* L.

Olga Shelepova*

Laboratory of Plant Physiology, Main Botanical Garden name after N.V. Tsitsin RAS, 127272, Moscow, Russia

*Corresponding author. Email: shelepova-olga@mail.ru

Mentha spicata L. is one of the *Mentha* species commonly known as spearmint. It is native species of Europe, North Africa, in Asia Minor and near East and India. The flowering aerial parts of *M. spicata* has been traditionally used for its antiseptic for treatment of sinusitis and bronchitis and also as antitussive, expectorant and diuretic. Some pharmacological effect of *M. spicata* essential oil such as anticarcinogenic, antimutagenic and cytotoxic activity against different human cell lines and its antioxidant effect were confirmed. The ingredients of essential oil of this species have been subjected to a number of studies which have shown a difference in its constituents depending on the region of cultivation and there have been some variations in the constituents from different zones. This study aimed to investigate the chemical composition of essential oils of *M. spicata* from different zones Russia (Rostov (MS-1), Irkutsk (MS-2) and Voronezh (MS-3) region) and Belarus (Grodno (MS-4) region).

Plants were collected during the flowering stage in 2013-2014. Essential oil was extracted from air-dried aerial parts of plants using Clevenger-type apparatus for 3.0 h and analyzed. Qualitative analysis of the essential oil was performed using chromatography-mass spectrometry on the analytical complex: GC analyses - a Shimadzu GC 2010; GC-MC – a Shimadzu GCMS-QP 2010 (in Analytical Group INBI RAS – RFMEFI62114XX0002).

In the essential oil MS-1, MS-2 and MS-3 42 compounds were analyzed by GC-MS, representing 96.1-97.2% of the total essential oil mass. The major component was carvone (33.2-58.6%), other components present in appreciable contents were: limonene (2.1-21.5%); 1,8-cineol (1.4-6.8%); germacrene-D (1.5-2.7%), β -caryophyllene (2.4-4.2%); rotundifolone (MS-2) (6.1%); dihydro-carveol (MS-3) (11.3%); dihydro-carvon (MS-3) (14.0%). The data presented here are in agreement with those reported in previous studies. In other studies (Chauhan et al., 2009; Znini et al., 2011), carvone (29.4-76.7%) is also the major constituent.

In contrast, the essential oil of MS-4 26 compounds was analyzed by GC-MS, representing 95.7% of the total essential oil mass from which 17 compounds were elucidated. The major component was rotundifolone (69.8%), other components present in appreciable contents were: germacrene-D (13.9%); γ -elemene (3.4%); β -caryophyllene (3.3%); 1,8-cineol (1.9%); limonene (1.5%). In the study of Venskutonis (1996), rotundifolone (44.2-57.2%) is the major constituent of the essential oil of *M. longifolia* L. from Lithuania. Rotundifolone is a constituent of several essential oils and known to have antinociceptive activity.

The antimicrobial activities of essential oils MS-1, MS-2, MS-3 and MS-4 were assessed against a panel of plant pathogenic fungi. The results from the disc diffusion method indicated that chemotype *M. spicata* from Grodno region of Belarus showed maximum antimicrobial activity. Such results could be used for the treatment of infectious diseases plants, leading to synthesis of new plant drugs from this plant.

Keywords: *Mentha spicata* L., essential oil composition, chemotype.

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PP-076. Improving functional ingredients of seed oil by underwater shock wave

Ken Shimojima^{1,*}, Zoran Ren², Matej Vesenj², Osamu Higa¹, Ayumi Takemoto¹, Athushi Yasuda³ and Shigeru Itoh¹

¹ Okinawa National College of Technology, Henoko 905 Nago Okinawa 905-2192 Japan

² University of Maribor, Smetanova 17, 2000 Maribor, Slovenia

³ Osaka Sanitary Co., 1-7-46 Torikaihonmachi, Settsu, Osaka 566-0052, Japan

*Corresponding author. Email: k_shimo@okinawa-ct.ac.jp

The food processing device by the underwater shock wave has been developed at the Okinawa National College of Technology (Takemoto et al. 2011; Shimojima et al. 2012). The improvement of juice extraction and scents is achieved with this device, because the shock wave is able to destroy the cell wall of the plant (Kanel et al., 2004). In this study, the improvement effect on the juice extraction and scents is investigated. Additionally, juice extractions are evaluated. For this research, widely available pumpkin seeds have been used. Accordingly, food processing device by the shock wave has been developed. The device is composed of the water filtration device, the disintegrator (pressure vessel) and the power supply for shock wave generation. Water is filled in the pressure vessel and two opposing electrodes are fixed with a small gap between each other. A high voltage is impressed to the electrode with the power supply circuit, generating the shock wave. The seeds are packed within a silicone tube. The seeds are crushed by the spalling destruction of the shock wave. The pumpkin seeds were crushed by the shock wave and the following results are obtained:

1. Quantity improvement of oil extraction.
2. The most suitable number of shock wave repetitions – relation between number of shock wave processing and the amount of the oil extraction.
3. Comparison of scent before and after crushing.
4. Comparison functional ingredients.

Keywords: Seed oil, Under Water Shock Wave, Explosion.

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PP-077. The evaluation of the cytotoxicity of carrot seed essential oil and HPLC separation of its main constituents

Elwira Sieniawska^{1*}, Łukasz Świątek², Ewelina Koziol¹, Barbara Rajtar², Małgorzata Polz-Dacewicz² and Krystyna Skalicka-Woźniak¹

¹ Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

² Department of Virology, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

*Corresponding author. Email: elwira.sieniawska@gmail.com

Carrot seed essential oil (CSEO) is a common fragrance component in soaps, detergents, creams, lotions, and perfumes, as well as a flavour ingredient in different categories of food products, including alcoholic and non-alcoholic beverages, frozen dairy desserts, candy, baked goods, gelatins and puddings, meat products and soups, usually in rather low use levels (<0.003%). According to literature data it is non-toxic (Opdyke, 1976; Khan et al., 2010).

The objective of presented study was to evaluate the chemical composition of commercially available carrot seed essential oil (*Daucus carota* L.) and its cytotoxicity on VERO (green monkey kidney) and FaDu (human pharynx squamous cell carcinoma) cell lines using the MTT method. The high performance counter current chromatography (HPLC) separation of CSEO was carried out to obtain individual components for further biological testing.

The GC/MS analysis on the ZB5 column revealed that carotol is the main constituent amounting 33% in the sum of compounds. The other compounds exceeding 1% in the sum were: α -pinene, sabinene, myrcene, limonene, geranyl acetate, bisabolene, caryophyllene oxide and daucol.

HPLC separation with the use of mixture of hexane/acetonitrile/t-butyl methyl ether (1/1/0.1v/v/v) in the reversed phase mode yielded α -pinene, sabinene, limonene, geranyl acetate, caryophyllene oxide, carotol and daucol with the purity higher than 95%. The applied method enabled isolation of all main constituents of CSEO in the single run.

The results of cytotoxicity testing are shown in the table. IC₅₀ and IC₁₀ are the concentrations inhibiting the cell proliferation by 50% and 10%, respectively.

	VERO		FaDu	
	IC ₅₀ (µg/ml)	IC ₁₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₁₀ (µg/ml)
CSEO	35,3	8,2	35,4	14,8

Results indicate that CSEO exhibits cytotoxic effect on both normal and cancer cell lines, without any selectivity. Because of extensive use of CSEO, it is crucial to further assess the cytotoxic potential of this essential oil and its isolated main constituents, especially carotol amounting 33% in the sum of compounds.

Keywords: Carrot seed essential oil, carotol, cytotoxicity, high-performance counter-current chromatography.

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PP-078. *In vitro* antibacterial activity of essential oils on pathogenic and probiotic microbiota of poultry and swine

Carmen M. Sinche Ambrosio¹, Eduardo Micotti Da Glória^{1,*}

¹ Department of Agroindustry, Food and Nutrition-College of Agriculture "Luiz de Queiroz", University of São Paulo, Piracicaba-São Paulo, Brazil

*Corresponding author. Email: emgloria@usp.br

Antibiotic growth promoters have been used in animal diet for a long time to reduce mortality and increase animal performance. However, disadvantages emerged from the use of antibiotics, such as the development of bacteria resistant to antibiotics and the possible transmission of this bacterial resistance to humans. As a result, in 2006 the European Community banned the use of antimicrobials in animal nutrition. Studies with essential oils have shown their high antibacterial activity, which can make them a good alternative as growth promoters. The objective of this research was to evaluate the antibacterial activity *in vitro* of different essential oils on pathogenic and probiotic bacteria of occurrence in gastrointestinal tract of poultry and swine. An initial screening of twenty-eight essential oils (EO) for their antibacterial activity was performed by disk diffusion method on *Salmonella* serotype *Enteritidis* and *Lactobacillus plantarum*. Each essential oil solution was prepared at 90% (v/v) using acetone as solvent. Analysis of variance (ANOVA) showed a significant antibacterial activity difference between the twenty-eight essential oils, and Tukey's test ($p \leq 0.05$) allowed for differentiating each oil. Thereby, EO's obtained from *Eucalyptus globulus*, *E. exserta*, *Pimenta pseudocaryophyllus*, and also two EO's which are by-products of orange juice production, orange oil phase essence, and citrus terpens were selected based on their greatest activity on pathogenic bacterium (*S. Enteritidis*) and their lowest activity on probiotic bacterium (*L. plantarum*). These five essential oils were also tested on other four pathogenic bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* and *Listeria innocua*, and on two probiotic bacteria: *Lactobacillus rhamnosus* and *Bacillus subtilis*. Orange oil phase essence and citrus terpens were oils that showed greater activity on pathogenic bacteria and lower activity on probiotic bacteria ($p \leq 0.05$). Limonene was detected as a major compound in orange oil phase essence (87.2%) and citrus terpens (28.7%) by GC/MS. Due to the same antibacterial potential and different quantity of Limonene in those two oils, it is possible to conclude that the antibacterial activity was a result of the presence of minor compounds.

Keywords: Antibacterial activity, essential oil, pathogenic bacteria, probiotic bacteria, poultry and swine.

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PP-079. Essential oil content in thyme (*Thymus vulgaris* L.) depending on potassium and calcium carbonate fertilization

Natalia Skubij*, Katarzyna Dzida

Department of Horticultural Plants Cultivation and Nutrition, Faculty of Horticulture and Landscape Architecture, University of Life Sciences in Lublin, Leszczyńskiego 58, 20-068 Lublin, Poland

*Corresponding author. Email: natalia.skubij@gardenc.pl

The main active component of thyme is essential oil, widely used in pharmacy, medicine, perfumery-cosmetic and food industries. Thyme (*Thymus vulgaris* L.) is a pleasant smelling perennial shrub, which grows in several regions of the world. Its natural habitat is the Western Mediterranean region and southern Italy (Hendawy et al., 2010, Marzec et al., 2010). The aim of this study was to determine the influence of different potassium and calcium carbonate fertilization levels on the chemical composition of thyme essential oil. The empiric experiments were done with two factor schema. The doses of potassium (0,6; 0,9; 1,2; 1,5 g·dm⁻³) and calcium carbonate (7,5 and 15 g·dm⁻³) were the factors of the experiment. The content of essential oil in an air-dry herb was determined in accordance with Polish Pharmacopoeia VIII (2008). The quantitative and qualitative composition of thyme essential oil was examined with the use of gas chromatography method and mass spectrometry (GC/MS). The qualitative analysis was performed on the basis of MS spectra, which were compared with the ones from NIST and LIBR (TR) libraries (Adams, 2004). The identity of compounds was also confirmed by measuring the retention indices.

The content of the essential oil of thyme ranged from 2.34 to 3.35%, its content depended on of diversified potassium and calcium fertilization. In the investigated plants the content of oil increased significantly, after applying the dose of potassium from 0.6 to 0.9 g dm⁻³ substrate. 42 components were identified in the essential oil of thyme. The dominant compound was thymol, its content ranged from 42.8 to 51.7%. The calcium fertilization did not influence substantially the quantity and quality of the essential oil of thyme. The plants fed with potassium in an amount of 0.9 g dm⁻³ with a lower dose of calcium carbonate (7.5 g dm⁻³ substrate) involved the highest content of essential oil, and the greatest amount of thymol in the oil. The use of higher doses of potassium resulted in decreasing the accumulation of essential oil in the raw material and thymol in the oil. Apart from thymol, the main compounds in the essential oil of thyme were γ -terpinene, p-cymene, and α -terpinene. The obtained results have the cognitive and applicative significance for the manufacturers of herbs.

Keywords: *Thymus vulgaris* L., fertilization, essential oil.

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PP-080. Chemical Differentiation of *Boswellia sacra* and *Boswellia carterii* Essential oils by Gas Chromatography and Chiral Gas Chromatography-Mass Spectroscopy

Cole Woolley*¹, Brett Smith**¹, Karen Boren¹, and D Gary Young²

¹ Department of Research and Discovery, Young Living Essential Oils, 3125 W. Executive Pkwy, Lehi, UT, USA

² Founder, Young Living Essential Oils, 3125 W. Executive Pkwy, Lehi, UT, USA

* Corresponding author. Email: cwoolley@youngliving.com

** Presenting author. Email: btsmith@youngliving.com

Major botanical and scientific references currently identify two species of frankincense, *Boswellia carterii* and *Boswellia sacra*, as being synonymous or the same species. Essential oil samples were used of *B. sacra* (n=88), resin originating from the Dhofar region of Oman and Hadhramaut region of Yemen (hydrodistilled in Salalah, Oman), and *B. carterii* (n=39), resin originating from Somalia (hydrodistilled in Nairobi, Kenya). We evaluated the Somalian (*B. carterii*) and Omani/Yemeni (*B. sacra*) species by GC-MS, GC-FID, Chiral GC-MS, specific gravity, refractive index, and optical rotation to determine if there were any minor or major differences between the two species of frankincense. Components identified with their average percent for *B. sacra* (n=88) are α -thujene (0.6%), α -pinene (68.2%), camphene (2.1%), sabinene (2.9%), β -pinene (2.0%), myrcene (0.7%), limonene + β -phellandrene (6.2%). Components identified with their average percent for *B. carterii* (n=39) are α -thujene (7.9%), α -pinene (37.3%), camphene (0.8%), sabinene (4.9%), β -pinene (1.8%), myrcene (7.3%), limonene + β -phellandrene (14.4%). Initially, GC-MS analysis did not reveal major statistical differences. However, optical rotation values, *B. Sacra* (+30.1°) and *B. carterii* (-13.3°), demonstrated a greater significant difference (P-Value <0.0001). Enantiomeric ratio (+)/(-) values were determined by GC-MS using a BetaDex 325 column. Enantiomeric ratio (+)/(-) values of α -pinene for *B. sacra* (n=5) and *B. carterii* (n=9) are 8.24 and 0.68, respectively, were also calculated aiding our conclusion that *B. sacra* and *B. carterii* are not synonymous but rather two distinct and individual frankincense species.

Keywords: chirality; *Boswellia sacra*; *Boswellia carterii*; frankincense; GC-MS; optical rotation.

Acknowledgments

Mahmoud Suhail, Lindsey Taylor, Marc Schreuder, Jeramiah Chai, Herve Casabianca, S Haq, HK Lin, AA Al-Shahri, S Al-Hatmi.

PP-081. Antinociceptive activity of *Amorpha fruticosa* L. (Fabaceae) essential oil

Nikola M. Stojanović^{1,*}, Niko S. Radulović^{2,*}, Pavle J. Randjelović³, Miljana Djordjević²

¹ Faculty of Medicine, University of Niš, Zorana Djindjića 81, 18000 Niš, Serbia

² Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

³ Department of Physiology, Faculty of Medicine, University of Niš, Zorana Djindjića 81, 18000 Niš, Serbia

* Corresponding authors. Email: nikola.st90@yahoo.com; nikoradulovic@yahoo.com

Inflammation and pain are two kinds of defense reactions of living systems in reply to any invasive factor. Acetic acid-induced writhing test represents a model that can reveal both antinociceptive and anti-inflammatory properties of substances. The essential oil of *Amorpha fruticosa* L. (Fabaceae) was evaluated, at the doses of 400, 200 and 100 mg/kg, for its antinociceptive activity using this test in BALB/c mice (n=6), alongside aspirin (200 mg/kg) and olive oil (negative control). The tested essential oil was isolated by hydrodistillation from ripe dry fruits. The analyses by GC and GC/MS revealed that the major constituents of the oil were: germacrene D (24.5%), δ -amorphene (11.4%) and α -pinene (10.6%). All tested essential-oil doses reduced the number of writhings induced by an *i.p.* injection of acetic acid. The calculated percent of inhibition for the 400 mg/kg dose of the oil (54.4%) statistically differed from the positive control, aspirin (90.2%) applied at a dose of 200 mg/kg. As the injection of acetic acid produces the release of prostaglandins, such as PGE_{2 α} and PGF_{2 α} , and sympathetic nervous system mediators in peritoneal fluids, the results suggest that the inhibition of prostaglandin release might represent one of the possible mechanisms of action exerted by the oil.

Keywords: Antinociception, *Amorpha fruticosa*, Aspirin, essential oil.

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PP-082. Influence of *Hypericum perforatum* essential oil on the mice central nervous system

Nikola M. Stojanović^{1,*}, Pavle J. Randjelović², Milena Živković³, Milan Nešić³, Marija Genčić³, Niko S. Radulović^{3,*}

¹ Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000 Niš, Serbia

² Department of Physiology, Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000 Niš, Serbia

³ Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

* Corresponding authors. Email: nikola.st90@yahoo.com; nikoradulovic@yahoo.com

The usage of St. John's worth, *Hypericum perforatum* L., Hypericaceae, (abbreviated as *HP*), more specifically of the non-volatile constituents of the plant, in the treatment of a variety of health disorders, is well known, while its predominant usage for the management of depression is best documented. The aim of this study was to evaluate the effect of the essential oil of *HP* on the behaviour of mice in several standard models. The essential oil used in this study was isolated from fresh aerial parts of the plant in the flowering stage, using standard procedures, whereas its chemical composition was analysed using GC/MS and GC. The analyses revealed that the major constituents of the tested oil were: 2-methyloctane, nonane, α -pinene, 3-methylnonane, β -pinene and myrcene. The animals used in the study were female BALB/c mice that were, after the appropriate treatment with the vehicle – olive oil (at 10 ml/kg), diazepam (at 2 mg/kg) and the essential oil of *HP* (at 12.5, 25, 50, 100, 150 and 200 mg/kg), subjected to the open field, light/dark, hole board, rota-rod and wire grasp tests. The behavioural tests revealed an upside parabolic shaped dependence between the applied doses of *HP* oil and the response of the animals, i.e. the lowest and the highest doses produced a very similar effect. This type of dosage-effect dependence was previously observed for other known activities of *HP* extracts in different pharmacological tests (e.g. nociception, depression). Our experiments provided direct proof that the volatiles of *HP* also significantly contribute to the overall effect of this plant species on the central nervous system of mice.

Keywords: *Hypericum perforatum*, essential oil, mouse, anxiety, behaviour.

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PP-083. Biotechnological potential of ionic liquids containing components of essential oils

Urszula Świerczek^{1,*}, Joanna Feder-Kubis² and Jolanta Bryjak¹

¹ Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wrocław, Poland

² Department of Chemical Engineering, Faculty of Chemistry, Wrocław University of Technology, Wrocław, Poland

* Corresponding author. Email: urszula.swierczek@pwr.edu.pl

Essential oils are one of the most popular vegetable substances at the whole world. So far they are a renewable natural source of valuable therapeutic, protective and fragrances compounds, for example: terpenes (Kołodziejczyk 2013). Their high biological activity makes them increasingly used by scientists in organic synthesis. It is expected that the obtained new compounds will exhibit interesting properties (Matos, Andrade 2008).

The best example are ionic liquids ILs – organic salts which remain liquid at room temperature or melt below 100°C. In the literature it is shown that quaternary ammonium chlorides with essential oil component: (–)-menthol are characterized by very strong antimicrobial effect (Feder-Kubis, Tomczuk 2013). On the other hand bis(trifluoromethanesulfonyl)imide ionic liquids with (–)-menthol derivative tend to be perfect medium for enzymatic reactions (Feder-Kubis, Bryjak 2013) (Fig. 1.).

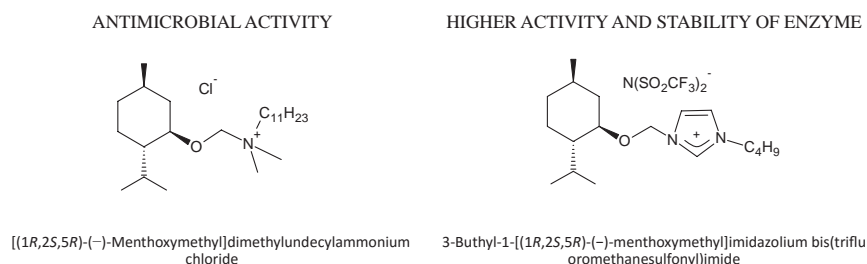


Fig. 1. Ionic liquids containing (–)-menthol derivative

Keywords: antimicrobial activity, enzyme activity and stability, organic salts, biotechnological applications.

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PP-084. New chemotype of spearmint, *Mentha spicata* L. from Slovakia

Attila Szkukalek^{1,*}, Tekeľová Daniela¹, Anton Ťažký^{2,3} and Daniel Grančai¹

¹ Department of Pharmacognosy, Faculty of Pharmacy, Comenius University, Odbojárov 10, P.O. Box 95, 820 05 Bratislava, Slovakia

² Department of Department of Pharmaceutical analysis and nuclear pharmacy, Comenius University, Odbojárov 10, P.O. Box 95, 820 05 Bratislava, Slovakia

³ Toxicological and anti-doping center, Comenius University, Odbojárov 10, P.O. Box 95, 820 05 Bratislava, Slovakia

*Corresponding author. Email: szkuki@gmail.com

Mentha spicata L. is one of the mint species which shows great chemical variability. Tucker and coworkers (1991) described five basic chemotypes of spearmint. These are: chemotype A, which is rich in 2-oxygenated monoterpenes (e.g. carvone and dihydrocarvone), chemotype B - rich in 3-oxygenated monoterpenes (e.g. piperitone, pulegone, menthone), chemotype C - rich in 3-oxygenated monoterpene oxides (e.g. piperitenone-oxide, piperitone-oxide), chemotype D - rich in both 2- and 3-oxygenated monoterpenes (menthol, carvone, menthone, dihydrocarveol) and chemotype E, which is rich in linalool. The aim of our work was the chemical analysis of the new spearmint chemotype from Slovakia. This mint species (*Mentha spicata* L., ssp. *serpylliodora*) is naturally growing in High Tatras in part of Horný Smokovec, and has been introduced into agroclimatic conditions of Southern Slovakia in Komárno (112 m a.s.l., 47°45'48"N 18°07'42"E). This species is characterized by odour and taste reminiscent of thyme, which is very different from all known spearmint chemotypes.

GC/MS analysis of hydrodistilled essential oils showed that 1,8-cineole (24%), carvacrol (25%) and thymol (13%) are the major components. Due to the content of p-cymene derivatives, usually occurring in mint volatile oils in traces only because of the presence of thymol and carvacrol, the spearmint growing in Slovakia seems to be a new chemotype. Brian M. Lawrence confirmed our results in private communication: "Carvacrol and thymol have been found as small to trace constituents in a number of mint oils but to the best of my knowledge not in levels of 5% or more".

Keywords: spearmint, *Mentha spicata* L., thymol, carvacrol, p-cymene derivatives.

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PP-085. Comparison of high efficiency of essential oil extraction using instantaneous high pressure process in hydrodistillation method and microwave method

Ayumi Takemoto^{1,*}, Taisuke Hamada, Yasutomo Tamaki and Shigeru Itoh²

¹ Department of Bioresources Engineering, National Institute of Technology, Okinawa College, 905 Henoko, Nago, Okinawa, Japan

² National Institute of Technology, Okinawa College, 905 Henoko, Nago, Okinawa, Japan

*Corresponding author. Email: tkmt@okinawa-ct.ac.jp

The instantaneous high pressure of the order of a few microseconds which was brought by shock wave, partially destroys cell walls and tissues of the plant by the action of the spalling destruction phenomenon (Takemoto et.al., 2008). In other words, the extraction path of biosynthesis component is formed in the plant. By using this effect, it is able to extract the sap of conifers (Takemoto & Itoh, 2007), and raise the coffee extraction efficiency (Takemoto & Itoh, 2008). Similar effect is obtained on the essential oil extraction by the steam distillation (Takemoto & Itoh, 2010, 2011). Thus, the instantaneous high pressure is expected to be utilized as a preprocessing for efficient essential oil extraction. Meanwhile, other essential oil extraction methods include hydrodistillation method and microwave method. These methods have not been made verification of extraction efficiency by instantaneous high-pressure processing. In this study, authors report effect of the instantaneous high pressure preprocessing on hydrodistillation method, microwave method and steam distillation method. Dried leaves of lemongrass (*Cymbopogon citratus*) is used for sample, and analyzed for differences in the amount of extraction and composition of aroma components. The instantaneous high pressure is caused by two methods, gap discharge method and wire explosion method.

Keywords: shock wave, instantaneous high pressure, spalling destruction.

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PP-086. Essential oil composition of wild growing and *in vitro* cultivated *Inula britannica* L.

Antoaneta Trendafilova^{1,*}, Milka Todorova¹, Roza Dimitrova¹, Dimitar Dimitrov², Evelyn Wolfram³ and Kalina Danova¹

¹ Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

² National Museum of Natural History, Bulgarian Academy of Sciences, 1000 Sofia, Bulgaria

³ Zurich University of Applied Sciences, Institute of Biotechnology, 8820 Wädenswil, Switzerland

*Corresponding author. Email: trendaf@orgchm.bas.bg

Inula britannica L. is well known medicinal plant used as an antibacterial, carminative, diuretic remedy and for treating asthma, hepatitis and tumors (Khan et al., 2010). The presence of sesquiterpene lactones, main bioactive components of this species in *in vitro* cultivated plants was reported previously (Todorova et al., 2014). Here we compare the volatile components of *in vitro* cultivated and wild collected material. The plant was collected from a native population in Bulgaria and leaves and flowers were analyzed separately. *In vitro* culture was initiated from steam segments of the plant in 0.5 benzyl adenine supplemented medium. The essential oils were obtained by micro steam distillation-extraction. Identification and quantification were performed by GC/MS. Eighty-three components were registered as chromatographic peaks in concentration more than 0.2% at least in one of the samples. Insignificant difference in the qualitative composition of flower (**F**) and leaf (**L**) volatiles was observed. Both oils were rich in oxygenated components reaching 74.56% in **L**. Higher terpenoid content in comparison with other compound types was also characteristic for the analyzed samples. It should be noted that the ratio terpenoids:others was 1.48 in **F** and 3.97 in **L**. The results revealed that the sesquiterpenoids exceeded monoterpenoids. The amount of monoterpenoids in **F** and **L** was almost equal, while sesquiterpenoids in **L** were 20% higher than in **F**. β -Pinene (1.71% in **F** and 1.21% in **L**) and 1,8-cineole (2.00% in **F** and 1.06% in **L**) were the main monoterpenoids. Principal sesquiterpenoids were viridiflorol (7.17% in **F** and 8.20% in **L**) and himachalol (3.45% in **F** and 8.71% in **L**) followed by β -selinene, intermedeol, caryophyllene oxide, T-cadinol, and T-muurolol. Aliphatic hydrocarbons (C₂₃-C₂₉) dominated in **F** (17.03%), while their content in **L** was 3.64%. The composition of the volatiles of *in vitro* culture was very similar to the ones from leaves (mainly sesquiterpenoids with viridiflorol and himachalol as major components). The obtained results confirmed our previous study that the *in vitro* culture system can be utilized as a biotechnological source for targeted delivery of phytopharmaceuticals, characteristic for *I. britannica*.

Keywords: *Inula britannica* L., essential oils, sesquiterpenoids, *in vitro* culture.

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PP-087. Are relations between essential oil profile and endogenous cytokinins independent of rooting in *Artemisia alba* *in vitro* model?

Milka Todorova¹, Antoaneta Trendafilova^{1,*}, Vaclav Motyka², Petre Dobrev² and Kalina Danova¹

¹ Institute of Organic Chemistry with Centre of Phytochemistry, BAS, 1113 Sofia, Bulgaria

² Institute of Experimental Botany AS CR, 165 02 Prague, Czech Republic

*Corresponding author. Email: trendaf@orgchm.bas.bg

Artemisia alba Turra is a fragrant shrub, characterized by a marked variability of its essential oils depending on the origin of collection of the wild growing plant. Previously we established that inhibition of rooting caused by combination of low concentration of benzyl adenine (BA) combined with different indole-3-butyric acid (IBA) supplementations significantly lowered monoterpene/ sesquiterpene ratio in the essential oils of the aerial parts of the plant *in vitro* (Danova et al., 2012), which was also related to a substantial drop of the endogenous cytokinins in these samples (Krumova et al., 2013). The observed effect was logically attributed to a drop of endogenous cytokinins biogenesis due to the inhibited rooting, as it is widely accepted that roots are the main spot of endogenous cytokinins synthesis in the plant. In the present work we further developed the *A. alba* shoot cultures model by inhibiting rooting and stimulation of callusogenesis by treatments of BA alone, as well as with different combinations of BA and IBA. Interestingly, though BA treatments completely inhibited rooting, they did not affect monoterpene levels to such extent, as the BA and IBA combinations. Noteworthy, while IBA decreased the ratio of irregular towards regular oxygenated monoterpenes in the oils, on the contrary, BA increased this parameter. In addition, it was established that in the BA treatments, bioactive cytokinins levels were also not as strongly reduced, comparing with non-treated control as, were the IBA and BA combinations. Little is still known on the interplay between endogenous hormonal status and the biosynthetic capacity of plants. A recent work sheds light on this problem, showing that for the five plant secondary metabolic gene clusters reported so far, the enzymes for the first committed steps all appear to have been recruited directly or indirectly from primary metabolic pathways involved in phytohormone synthesis (Chu et al., 2011). Further research is in progress for the elucidation of physiologic factors affecting terpenoid biogenesis in *A. alba* *in vitro* model system.

Keywords: *Artemisia alba* Turra, *in vitro* culture, terpenoid biogenesis, phytohormone treatment, cytokinins.

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PP-088. Investigation of some yield and quality characteristics of wild *Salvia fruticosa* Mill. populations

Kenan Turgut^{1,*}, Fatma Uysal²

¹ Department of Field Crops, Faculty of Agriculture, Akdeniz University, 07058 Antalya, Turkey

² Batı Akdeniz Agricultural Research Institute, Aksu, 07100 Antalya, Turkey

*Corresponding author. Email: kturgut@akdeniz.edu.tr

Salvia fruticosa is intensively collected from the wild flora of Antalya and commercially marketed in Turkey. Dried leaves of this species are used as herbal tea and for essential oil production. Therefore, natural populations have been decreased year after year. Cultivation of *S. fruticosa* seems to be the most convenient way for conservation of wild populations. In this study, some yield and quality components of different wild genotypes were determined. These genotypes were collected from 15 wild populations grown in the flora of Antalya province. As a result, dried herbal yields varied between 1490.24 kg/ha and 22880.73 kg/ha; dried leaf yields varied between 720.77 kg/ha and 10620.63 kg/ha; leaf / stem ratio varied between %29.55 and %82.61; essential oil yields varied between 1.00% and 3.75%. Essential oils obtained by hydrodistillation of the aerial parts of *S. fruticosa* were analysed by GC-MS and 14 different components were identified. In all genotypes, 1,8-cineole was found be a major component of the essential oil and its ratio was varied between 34.51% and 73.49%. It is concluded that significant variations were exist among the wild populations of *S. fruticosa*. These findings could be very useful for breeding studies.

Keywords: *Salvia fruticosa*, wild populations, yield, essential oil.

PP-089. The quest of productive variety of carvacrol chemotype of *Thymus pulegioides*: metabolomic analysis and effects of meteorological conditions

Vaida Vaičiulytė*, Kristina Ložienė

Nature Research Centre, Institute of Botany, Vilnius, LT-08406, Lithuania

*Corresponding author. Email: vaiciulyte.vaida@gmail.com

The species of genus *Thymus* are essential oils (EO) bearing medicinal plants. The monoterpene phenol carvacrol (C) often is the main compound of essential oils of species of this genus; it has wide spectrum of antimicrobial and antioxidant activity, therefore is widely usable in food and pharmaceutical industry (Baser, 2008). *Thymus pulegioides* growing wild in Lithuania also accumulates C (to 31.0%) and is suitable for cultivation in the Baltic States region, because not winterkills and is enough for mechanical gathering (Mockutė & Bernotienė, 1999; Ložienė et al., 2003). As distinct from collection in natural habitats, the cultivation enables to grow the chemically homogeneous and standardized raw material. Therefore the purpose of study was: 1) to select the productive variety of C chemotype of *T. pulegioides*, 2) to evaluate the stability of amount of EO, C and its precursors (p-cymene and γ -terpinene) under different meteorological conditions. The individual plants of C chemotype of *T. pulegioides* were moved from natural habitats into field collection of the Nature Research Centre (Vilnius, Lithuania). Selection of productive variety carried out by yield of total biomass, amount of EO and C. The EO were isolated by hydrodistillation in a European Pharmacopoeia apparatus. The analysis of EO and identification of C and precursors was carried out using a FOCUS GC (Thermo Scientific) gas chromatograph with a flame ionisation detector (FID) and analytical standards (Sigma-Aldrich). The influence of meteorological conditions (temperature, precipitation, photosynthetically active solar radiation (PAR) and sunshine duration) was investigated six years.

The study showed that the amount of EO in selected C chemotype varied from 0.72% to 0.98% (CV=12%) across years and correlated with PAR ($r=0.89$, $p<0.05$) and temperature ($r=0.83$, $p<0.05$). The amount of C varied from 16.9% to 29.29%, however, significant connections with meteorological factors not established. The increasing of rainfall was escorted by increasing in p-cymene ($r=0.94$, $p<0.05$). The selected C chemotype of *T. pulegioides* can be source of natural carvacrol.

Keywords: *Thymus pulegioides*, essential oils, carvacrol, meteorological conditions.

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PP-090. Inhibition of bacterial attachment and biofilm formation on food industry surfaces using essential oils

Anita Vidács^{1*}, Csaba Vágvölgyi¹, Erika Kerekes¹, Judit Krisch²

¹ Department of Microbiology, Faculty of Science and Informatics, University of Szeged, 6726, 52 Közép fasor, Szeged, Hungary

² Institute of Food Engineering, Faculty of Engineering, University of Szeged, 6724, Szeged, Hungary

*Corresponding author. E-mail address: anitavidacs@gmail.com

Biofilm formation is a serious hygiene problem in the food industry (Nostro et al., 2012) and can lead to outbreaks of food borne illnesses (Simoes et al., 2010). The first step in biofilm formation is the attachment of microbes to different surfaces. Bacteria in the biofilm are more resistant to sanitizers than planktonic cells (Van Houdet & Michiels, 2010). Thus there is a continuous need to find new solutions for efficient industrial disinfection. Essential oils (EOs) have antimicrobial, antiviral and antifungal effect and could represent alternatives to traditional disinfectants (Burt, 2004).

In our study the inhibition effect of cinnamon, marjoram and thyme EOs were investigated against attachment and biofilm formation of *Escherichia coli*, *Pseudomonas putida*, *Bacillus cereus*, *Bacillus subtilis*, *L. monocytogenes*, *Staphylococcus aureus* and MRSA on industrial surfaces (stainless steel and polypropylene). Bacterial attachment to polypropylene surfaces was monitored after 4 hours pre-incubation in broth/saline with and without EOs. Attached cells were swabbed from the surface and number of viable cells was evaluated with plating. Effect on biofilm formation was determined after 24 h incubation of the attached cells. Formed biofilms were treated with different concentrations of the EOs. The concentration and disinfection time of EOs were determined in previous studies.

Most of the used EOs prevent attachment of the investigated bacteria and/or were able to eliminate the formed biofilms from the surfaces. Best results were achieved by marjoram EO inhibiting both processes. Best biofilm removers were marjoram and thyme EOs. In this study the attachment and biofilm formation of *Bacillus subtilis* was very weak.

The investigated EOs had the potential to be used for disinfection on food industry surfaces and it is an alternative solution to the eco-food industry too.

Keywords: essential oils, attachment, biofilm, disinfection.

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PP-091. A high-performance counter-current chromatography (HPCCC) in the isolation of the compounds from *Thymus vulgaris* L. essential oils

Magdalena Walasek* and Krystyna Skalicka-Woźniak

Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodzki St.,
20-093 Lublin, Poland

*Corresponding author. Email: m.walasek@interia.pl

In this study, a high-performance counter-current chromatography (HPCCC) has been successfully used for the semi-preparative isolation and purification of the compounds from the essential oil from the *Thymus vulgaris* L.

Thymus vulgaris L. is a plant very commonly occurring in the environment. The main component of this herb is an essential oil in the amount of from 0.75 to 3.5%. The parent compounds in the essential oil are thymol and carvacrol, which are responsible for the taste and pharmacological properties of the plant. The thyme oil is also antibacterial, antifungal and antiviral.

During the studies the appropriate conditions for the separation of both major and minor compounds with the use of the modern HPCCC method were developed. For proper isolation a number of two-phase systems, mixtures of n-hexane, ethyl acetate, methanol and water (HEMWat) were tested. Considering the results of the partition coefficients for thyme oil, mixtures composed of 5: 2: 5: 2 (v/v/v/v) and 4: 1: 4: 1 (v/v/v/v) were chosen. The isolation was performed in the reversed phase mode. The collected fractions were analyzed by GC-MS. HEMWat system composed of 5: 2: 5: 2 let to obtain seven pure compounds such as thymol, p-cymene, β -caryophyllene, linalool, borneol, terpinen-4-ol and caryophyllene oxide, whereas solvent mixture in a ratio of 4: 1: 4: 1 allowed to isolate the pure camphor and terpinen-4-ol.

HPCCC is a modern method that allows to carry out the separation process in a fast, selective and repetitive way. The advantage is also a quick transfer of the analytical-scale conditions to semi-preparative scale, which was also demonstrated in the present study.

Keywords: high-performance counter-current chromatography, *Thymus vulgaris* L., terpenoids.

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PP-092. Volatile compounds from the fruits of *Heracleum mantegazzianum* and their purification by the high-performance counter-current chromatography (HPCCC)

Magdalena Walasek^{1,*}, Hosam O. Elansary^{2,3} and Krystyna Skalicka-Woźniak¹

¹ Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodzki St., 20-093 Lublin, Poland

² Biodiversity Institute of Ontario, University of Guelph, Guelph, ON N1G 2W1, Canada

³ Department of Floriculture, Ornamental Horticulture and Garden Design, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt

*Corresponding author. Email: m.walasek@interia.pl

Heracleum mantegazzianum is an invasive plant naturally growing in Western Europe and North America. The dominant component of the plant is an essential oil, which includes alpha-pinene, beta-pinene, myrcene, limonene and cymene. The plant is mostly known from phototoxic properties.

The volatile compounds from the fruits of *Heracleum mantegazzianum*, wild-growing in Poland, were obtained by hydrodistillation (HD) and analyzed using GC-MS. A total of 35 components were identified and the most abundant compounds were hexyl-2-methylbutanoate (10.8%) and 1-octanol (10.6%).

Additionally, for the first time, the appropriate conditions for the separation of active ingredients from the essential oil using the high-performance counter-current chromatography (HPCCC) were developed. The method allows to carry out the separation process in a fast, selective and repetitive way, which is considered as simple device transferring conditions from the analytical scale to the semi-preparative and industrial scale. For proper isolation a number of two-phase systems, being a mixture of n-hexane, acetonitrile, tert-butylmethylether or n-heptane, ethyl acetate, methanol, water were tested. Finally, the mixture of n-hexane, acetonitrile and tert-butylmethylether in a ratio of 1: 1: 0.1 (v/v/v/v) was selected. 5 pure compounds including n-octanol, n-octyl acetate, psolaren, octyl hexanoate, hexyl-2-methylbutyrate as well as their mixtures were purified and identified by the GC-MS.

Keywords: high-performance counter-current chromatography, *Heracleum mantegazzianum*, essential oil.

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PP-093. Effect of essential oils of three cedar wood species on humans

Iris Stappen¹, Sanja Milovac¹, Dinka Gazibegovic¹, Sladjana Banika¹, Jürgen Wanner^{2,*}, Erich Schmidt¹

¹ Department of Pharmaceutical Chemistry, Division of Clinical Pharmacy and Diagnostics, University of Vienna, Althanstraße 14, 1090 Vienna, Austria

² Kurt Kitzing GmbH, Hinterturm alten Schloss 21, 86757 Wallerstein, Germany

*Corresponding author. Email: juergen.wanner@kurtkitzing.de

The essential cedar wood oils used in this study derived from three different species: *Juniperus virginiana* L. (red cedar, Juniperaceae), *Cedrus atlantica* Endl. Manetti (Atlas cedar, Pinaceae) and *Chamaecyparis funebris* Endl. (Chinese weeping cypress, Cupressaceae). With thujopsene (25% and 23.6%, respectively), α -cedrene (22.7% and 22%, respectively) and cedrol (20.8% and 10.4%, respectively) the oils of red cedar and Chinese weeping cypress showed strong similarities in their compositions. The character impact compounds of Atlas cedar oil were β -himalachene (44%), α -himalachene (16%) and γ -himalachene (9.5%). Despite these major differences in constituents the odor of red cedar oil bears great resemblance to Atlas cedar oil: both smell typical woody and green whereas the odor of the EO of Chinese weeping cypress can be described as harsh smoky and dry. Given the great semantic and hedonic influence of odors on humans (Jelinek, 1997) we would expect similar effects of red cedar and Atlas cedar.

In two within subjects-effect experiments with repeated measures design, 64 participants (32 men) aged between 18 and 35 years were tested. In one experiment the odors were inhaled (aroma lamp, water as control) in the second experiment the oils were administered on the skin (20% in peanut oil, pure peanut oil as control). Blood pressure, puls rate and well being were determined in time. Hedonic valence and familiarity of the oils were rated. Smoking behavior as well as sex-specific differences (Doty & Cameron, 2009) were also included.

Differences were found between men and women in the dermal condition for blood pressure and calmness. Smokers felt significantly less tired after inhalation of Chinese weeping cypress oil. Red cedar oil was rated significantly more pleasant in all subjects compared to the other oils.

Keywords: *Juniperus virginiana*, *Cedrus atlantica*, *Chamaecyparis funebris*, psychophysiology, inhalation, dermal.

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PP-094. Some rectifications on the composition of costus root oil (*Saussurea costus* (Falc.) Lipsch) and absolute and GC-MS-FID analyses thereof

Juergen K.R. Wanner^{1,*}, Alin Bosilcov²

¹ Kurt Kitzing GmbH, Hinterm Alten Schloss 21, 86757 Wallerstein, Germany

² Brueder Unterweger GmbH, Thal-Aue 13, 9911 Assling, Austria

*Corresponding author. Email: juergen.wanner@kurtkitzing.de

Even though costus root oil is now prohibited for use in fragrances and cosmetics in the EU (Off. J. EU L 342/59, 2009) due to the presence of allergenic and cytotoxic α,β -unsaturated sesquiterpene γ -lactones (costunolide, dehydrocostuslactone) (Steglich et al., 2000) it is still used and produced today since it has a millennia-long history for its aromatic, spicy and alleged medicinal values (Pandey et al., 2007).

Confronted with this small but existing demand we analyzed this oil and absolute by GC-MS and GC-FID and for comparison searched the literature for a mostly complete analysis. But even though there were plenty of papers on *Saussurea costus* we did not find what we were looking for but came up with incomplete and partly questionable analytical results.

To identify doubtful substances we took the oil apart through a simple separation in a polar and apolar fraction and could thus assign substances to a chemical substance group or confirm the identity by simple derivatization reactions. Doing this we could determine about 90% of all substances in the essential oil and absolute.

Keywords: *Saussurea lappa*, essential oil, absolute, GC-MS-FID.

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PP-095. Differences in composition of volatile compounds in cryptic species B of the *Aneura pinguis* complex (Marchantiophyta, Metzgeriidae)

Rafał Wawrzyniak^{1,*}, Wiesław Wasiak¹, Beata Jasiewicz¹, Alina Bączkiewicz² and Katarzyna Buczkowska²

¹ Department of Analytical Chemistry, Faculty of Chemistry, Adam Mickiewicz University in Poznań, Umultowska 89b, 61-614 Poznań, Poland

² Institute of Experimental Biology, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61-614 Poznań, Poland

*Corresponding author. Email: rafwawrz@amu.edu.pl

Aneura pinguis (L.) Dumort. is a thallose liverwort with simple morphological structure. Genetic studies revealed that *A. pinguis* is a complex of cryptic species i.e. species completely isolated reproductively and unambiguously distinct genetically, but not differentiated morphologically. Up to now, five cryptic species tentatively called A, B, C, D and E were distinguished within the *A. pinguis* complex (Bączkiewicz et al., 2008)(Wachwiak et al., 2007). The combination of chemical and genetic studies of the *A. pinguis* complex revealed that detected volatile compounds allow to distinguish cryptic species (Wawrzyniak et al., 2014). Further studies of the *A. pinguis* complex based on analysis of DNA sequence revealed a greater differentiation of the species than it was previously detected only on the basis of isozyme markers (Bączkiewicz & Buczkowska, 2005). Sequences of four DNA regions (*rbcL*, *matK*, *trnL-F* and *trnH-psbA*) from chloroplast and one region (ITS1-5.8S-ITS2) from nuclear genome revealed that the cryptic species B initially discovered on the basis of isozyme markers is composed of three genetically distinct groups (B₁, B₂ and B₃). Studied samples were collected from different regions in Poland in summer and autumn season. The HS-SPME technique coupled to GC/MS analysis has been applied. The fiber coated with DVB/CAR/ PDMS have been used. The results obtained revealed differences in the composition of the volatile compounds between the studied groups. Detected compounds are mainly from the group of terpenoids. The statistical methods (cluster analysis and principal component) showed that results obtained from chemical analysis of the studied groups of the *A. pinguis* complex are consistent with the grouping on the basis of DNA sequences.

Keywords: *Aneura pinguis*, cryptic species B, terpenoids, GC/MS.

Acknowledgments

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PP-096. Analysis of essential oils from three species from the genus *Ferula* (Apiaceae)

Jarosław Widelski^{1,*}, Katarzyna Bobel¹ and Tomasz Mroczek¹

¹ Department Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodźki Street, 20-093, Lublin, Poland

*Corresponding author. Email: jwidelski@pharmacognosy.org

The genus *Ferula*, belonging to the Apiaceae family, comprises about 160 species. This genus is well-known in folk medicine and use in folk medicine of Arabian countries as anticonvulsant, carminative, diuretic, aphrodisiac, tonic and laxative for the treatment of various disorders.

Most of *Ferula* species possess strong aromatic that is due to the presence of essential oil or oleoresin in their different organs.

Essential oils were obtained by hydrodistillation in Deryng apparatus from fruits of three species *Ferula assa-foetida* L., *F. narthex* Boiss., *F. tingitana* L.

The plant material was collected in Pharmacognostic Gardem of Department of Pharmacognosy with Medicinal Plants Laboratory, Medical University of Lublin.

GC-MS analysis showed β -pinene, β -cymene and limonene in fruits of *Ferula assa-foetida* L.; α -pinene, β -pinene, p-cymene and β -phellandrene in fruits of *Ferula narthex* Boiss.; α -phellandrene, α -pinene, β -phellandrene and γ -terpinene in fruits of *Ferula tingitana* L. as dominant compounds.

Keywords: essential oils, *Ferula assa-foetida*, *Ferula narthex*, *Ferula tingitana*.

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PP-097. Volatiles from herb and fruits of *Peucedanum luxurians* and their antimicrobial activity

Jarosław Widelski^{1,*}, Agnieszka Grzegorzczak³, Anna Malm³, Ioanna Chinou² and Kazimierz Głowniak^{1,4}

¹ Department Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, 1 Chodźki Street, 20-093, Lublin, Poland

² Department of Pharmacognosy and Chemistry of Natural Product, Faculty of Pharmacy, University of Athens, Greece

³ Department Pharmaceutical Microbiology with Laboratory of Microbiological Diagnostics, Medical University of Lublin, 1 Chodźki Street, 20-093, Lublin, Poland

⁴ Department of Cosmetology, University of Information Technology and Management in Rzeszów, Kielnarowa 386a, 36-020 Tyczyn, Poland

*Corresponding author. Email: jwidelski@pharmacognosy.org

Plants of Apiaceae family usually contain coumarins and essential oils and are used worldwide in traditional medicine as well as in modern therapeutics. *Peucedanum luxurians* Tamamsch. is an endemic plant of Armenia (it grows near mount Ararat). The herb and fruits of above plant was collected from the Botanical Garden of University of AMU of Poznań, Poland.

The plant material was extracted with petroleum ether and composition of ether extract was analysed using GC-MS. In the case of *Peucedanum luxurians* herba trans- α -farnesene, germacrene D, α -zingiberene and β -caryophyllene were appeared as the most abundant ones. The fruits of investigated plant was rich in α -phellandrene, γ -elemene, β -curcumene.

The antimicrobial activity of extract containing volatiles, against six bacterial strains (*S. aureus*, *S. epidermidis*, *E. coli*, *E. cloacae*, *P. aeruginosa*, *K. pneumoniae*), oral pathogens (*S. mutans*, *S. viridans*) and three fungi (*Candida albicans*, *C. tropicalis*, *C. glabrata*) is under investigations, but preliminary results show special activity against *Staphylococcus aureus*.

Keywords: essential oils, *Peucedanum luxurians*, antimicrobial activity.

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PP-098. Fungistatic properties of selected essential oils for controlling Fusarium wilt in flax

Katarzyna Wielgusz*, Małgorzata Byczyńska and Krzysztof Heller

Breeding and Agronomy of Fibrous and Energy Plants Department, Institute of Natural Fibres And Medicinal Plants, Wojska Polskiego 71 B str., 60-630 Poznań, Poland

*Corresponding author. Email: katarzyna.wielgusz@iwnirz.pl

For a few years researchers at the Institute of Natural Fibres and Medicinal Plants in Poznan have been carrying out studies on development of non-chemical protection methods for flax against fungal diseases (Wielgusz & all 2010, Wielgusz e& all. 2012). One of the methods is using fungistatic properties of essential oils. In 2012-2013 efficiency of essential oils from the following plants: wormwood (*Artemisia absinthium* L.), thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare*), angelica (*Angelica archangelica* L.), lavender (*Lavendula* L.), fennel (*Foeniculum vulgare* Mill.) and geranium (*Geranium* L) was tested in laboratory, pot and field experiments. The laboratory tests focused on the effect of the essential oils on linear growth of mycelium of *F. oxysporum* f. sp. *lini*. Also, the influence of the oils on germination capacity of linseed was tested.

The pot experiment assessed the effect of developed seed dressing formulations with specific essential oils (in two concentrations - 0,5 % and 1,0 %) on control of infestation of flax with *F. oxysporum* f. sp. *lini*. The experiment was conducted at the Experimental Farm of the Institute in Petkowo. In the pot experiment sterilized wheat grains overgrown with monosporous cultures of *Fusarium oxysporum* f. sp. *lini* served as inoculum.

In the field experiment the same combinations were used as in the vegetative hall, with the exception of the combination without inoculum. The source of infestation in the field experiments was population of *Fusarium oxysporum* present on the field, where 3 – 4 years earlier flax was cultivated (a provocative field). During the vegetation period the number of healthy and infested plants was calculated at four developmental stages of flax. Additionally, the seed and straw yields obtained from specific combinations were assessed. The results of laboratory experiments indicated that among the tested essential oils those from wormwood, angelica and oregano had the strongest fungistatic properties. The essential oils from wormwood, thyme and angelica improved germination capacity of linseed by a few percent as compared with the control combination. The results of pot and field experiments allowed for concluding that some of the oils had effect on controlling (inhibiting?) flax infestation with Fusarium wilt. In the combination where flaxseed was treated with 1 % formulation of wormwood essential oil, the percent of infested plants, as compared with the control (no seed dressing used) was lower by 8,5 %. Treatment of seeds with 1 % formulation of angelica essential oil lowered the infestation by 7 %. Other essential oils controlled infestation of flax with fusarium wilt to even lower degrees. The combinations where seeds were dressed with 1 % essential oil from wormwood and angelica gave the highest yields of seeds and straw as compared with the control combination.

Keywords: essential oils from medicinal plants, flax, plant protection, *Fusarium oxysporum*.

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PP-099. Investigation of the volatile oil in modified roots, flowers and leaves of *Hemerocallis* species

Olena Zarichanska^{1,*}, Svitlana Marchyshyn², Myroslava Garnyk¹

¹Department of Pharmaceutical Chemistry, M.I. Pirogov Memorial Vinnytsya National Medical University, 56 Pirogov str., 21018 Vinnytsya, Ukraine

²Department of Pharmacognosy with Medical Botany, I.Ya. Horbachevsky Ternopil State Medical University, 1 Maydan Voli, 46001 Ternopil, Ukraine

*Corresponding author. Email: lena.zarichanska@yandex.ua

Species of *Hemerocallis* genus are prospective for application in medicine as antioxidant, wound healing, hypnotic, sedative, anti-depressing and anti-inflammatory remedies (Que et al., 2007). Actually, extracts from modified roots, flowers and leaves of daylilies are used only in folk medicine in China, Japan and USA and require systemic chemical and pharmacological studies. We have investigated volatile oil in *Hemerocallis fulva* L. flowers (HFF), leaves (HFL), modified roots (HFR) and *Hemerocallis hybrida* var. 'Stella De Oro' flowers (HHF), leaves (HHL), modified roots (HHR).

The aim of our research was to obtain volatile oil from plant raw materials of *Hemerocallis fulva* L. and *Hemerocallis hybrida* var. 'Stella De Oro', to determine their organoleptic properties, quantitative content and qualitative composition (constituents of volatile oils). Quantitative content of volatile oil in plant raw materials have been determined by hydro distillation with Clevenger trap (Sepahvand et al., 2014). Color, transparency and odor of the obtained oils have been analyzed organoleptically. Qualitative composition of the volatile oil's samples was analyzed by GC/MS on mass spectrometric system Agilent 6890N/5973 inert (Agilent Technologies, USA) (Zhao et al., 2011).

The results of the experiments showed that dried HFF contain 0.24% of volatile oil, HHF – 0.29%, HFL – 0.08%, HHL – 0.09%, HHR – 0.49%, HHR – 0.46%. All listed volatile oils occur as transparent colorless viscous liquids with aromatic odor. By GC/MS there were determined that volatile oil of HFF includes 36 components, HHF – 18, HFL – 43, HHL – 61, HFR – 32, HHR – 33. Among the chemical constituents of both flowers' oils docosane, tricosane, tetracosane, pentacosane, hexacosane, eicosane, and nonacosane were identified as major components. In addition, terpenes have been identified. HFF contain bergamotol and caryophyllene oxide. Hydrocarbons are also major components of leaves' oils. Besides alkanes, both leaves' oils contain phytol and some other terpenes. HHL oil includes squalene and terpenes. The chemical profile of modified roots' oils is characterized by the presence of some long chain alkanes and terpenes.

Keywords: daylily, volatile oil, hydro distillation, gas chromatography, mass spectrometry.

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PP-100. Histopathological effects of eucalyptol on the midgut of larvae *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae)

Beata Zawitowska*, Maryla Szczepanik¹, Czesław Wawrzeńczyk² and Antoni Szumny²

¹ Department of Invertebrate Zoology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Lwowska 1, 87-100 Toruń, Poland

² Department of Chemistry, Wrocław University of Environmental and Life Sciences, C.K. Norwida 25/27, 50-375 Wrocław, Poland

*Corresponding author. Email: beatamalina87@wp.pl

Essential oils (EOs) and their components show a broad spectrum of insecticidal activity ranging from repellent, antifeedant, oviposition deterrent and growth regulatory. They can also disturb digestion and nutrient absorption, growth and development of insects. Such a wide variety of effects EOs and their main constituents provides the possibility to use them as an alternative for the use of chemical insecticides (Bakkali et al., 2008; Arun et al., 2009).

In the present work, the effect of eucalyptol from *Eucalyptus globulus* essential oil on the growth and histological structure of the midgut of the lesser mealworm (*Alphitobius diaperinus* Panzer) larvae was studied. Toxicity activity of eucalyptol was studied by incorporating it into the diet of this pest. The chemical identification of the components of essential oil was performed using gas chromatography (GC) coupled to a mass spectrometer (MS) performed on a Saturn 2000 MS Varian Chrompack. The main components of *E. globulus* oil, were: eucalyptol (68.27%), limonene (12.83%) and *p*-cymene (10.15%). The pure eucalyptol was isolated *via* column chromatography. For separation silica gel 60 (63-220 mm) Merck and as eluent mixture hexane-diethyl ether (gradient from 40:1 to 2:1) were used.

After application of eucalyptol the growth inhibitory effect and histopathological changes in the midgut of young (10-day old) larvae of the lesser mealworm were observed. The tested component incorporated into the diet clearly inhibited the growth of larvae of *A. diaperinus* and extended a development life cycle in comparison to control. The changes in the midgut structure observed in larvae after a 14-day treatment with the substance were: disintegration the columnar cells of epithelium, strong vacuolisation of the epithelium cells and damage to epithelium cell nuclei. The absorption of digested food was impossible due to pathological changes in the midgut.

Keywords: *Eucalyptus globulus*, *Alphitobius diaperinus*, histology, biopesticide.

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PP-101. Historical and ethnobotanical review of labdanum resin usage

Klaudyna Hebda*

Institute of Anthropology and Cultural Ethnology, Jagiellonian University, Cracow, Poland

*Corresponding author. Email: klaudyna.hebda@uj.edu.pl

The aim of the study was to examine traditional and historical uses of labdanum (also known as labdanum, ladanum) - highly aromatic resin obtained from *Cistus* spp. (eg. from *Cistus creticus*, *Cistus ladanifer*, *Cistus salviifolius*) – a genus growing in the Mediterranean region. Although technically not an essential oil, labdanum, throughout the centuries, was highly regarded as a perfumery and medicinal ingredient.

The main purpose of the research was to explore different ways of labdanum usage, especially those listed in British medicinal books and journals from the 16th up to 19th century. The analysis revealed that labdanum was used in a number of ways: both externally (it was recommended to hold labdanum in hand and smell it during epidemics) and internally (for example it was mixed with quince marmalade and recommended as a remedy for upset stomach). The analysed books and journals often put references to classical Greek medicine (mainly Dioscorides) and quote older texts, only some authors offer their own recipes for labdanum usage. The texts also discuss the quality of labdanum (depending of species, place of origin and/or organoleptic characteristic).

It appears that labdanum was valued both from aromatic and medicinal point of view and it was considered appropriate for oral intake which dramatically differs from modern recommendations not to take labdanum internally in a pure form.

Keywords: labdanum, ladanum, cistus, resin.

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Manuscripts must be written in English. Authors whose native language is not English are recommended to seek the advice of a native English speaker, if possible, before submitting their manuscripts. The pages in the manuscript should not be numbered and in the text no reference should be made to page numbers; if necessary, one may refer to sections. Try to avoid excessive use of italics and bold face. These instructions also contain the necessary information for APA reference style editing.

Materials and Methods

Heading 2

Text

Heading 3

Text

Results and Discussion

Results and Discussion Text

Heading 2 (optional)

Results and Discussion Text

Tables: The number of the tables should be indicated as Table 1., Table 2. *etc.*, and refer to all of them in the text. Number all tables with Arabic numerals sequentially. Do not use suffix letters (e.g. Table 3a, 3b, 3c); instead, combine the related tables. Column headings should be brief, but sufficiently explanatory. Maximum table width of 16.8 cm. The minimum *font size* for characters in tables is 9 points. SI units should be used.

Table 1. Each table should have a brief and self-explanatory title.

Heading 1	Heading 2	Heading 3
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^aAny explanations essential to the understanding of the table should be given in footnotes at the bottom of the table. To indicate specific notes, use superscript lowercase letters (e.g. ^a, ^b, ^c), and order the superscripts from left to right, top to bottom. ^bEach table's first footnote must be the superscript.

Figures and Schemes: It is possible to have figures printed in colour. Do not use illustrations taken from the Internet. Figures should be have a minimum resolution of 600 dpi, save as EPS or TIFF. Grayscale should have a minimum resolution of 300 dpi or 500 dpi and save as tiff. Do not save figures as JPEG. scheme or figure with a wide of 16.8 cm (max.)

Scheme 1. Description... or

Figure 1. Description...

Any explanations should be given in footnotes at the bottom of the scheme or figure.

Acknowledgments

Generally the last paragraph of the paper is the place to acknowledge people (dedications), places, and financing (you may state grant numbers and sponsors here).

References

Calfee, R. C., & Valencia, R. R. (1991). *APA guide to preparing manuscripts for journal publication*. Washington, DC: American Psychological Association.

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Moller, G. (2002, August). Ripples versus rumbles [Letter to the editor]. *Scientific American*, 287(2), 12.

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Scruton, R. (1996). The eclipse of listening. *The New Criterion*, 15(3), 5-13.

REFERENCES (APA STYLE)

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In subsequent citations, only use the first author's last name followed by "et al." in the signal phrase or in parentheses.

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„SHIM-POL A. M. BORZYMOWSKI” E. Borzymowska-Reszka,
A. Reszka, Spółka Jawna
ul. Lubomirskiego 5, 05 - 080 Izabelin.
fax: (22) 722-70-51 e-mail: biuro@shim-pol.pl