Final Programme
& Book of Abstracts

48th International Symposium on Essential Oils (ISEO2017)
Final Programme & Book of Abstracts
10-13 September 2017, Pécs, Hungary

The professional and grammatical level of the materials is the authors' responsibility.

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Welcome

It is our great pleasure to welcome you to the 48th International Symposium on Essential Oils (ISEO 2017) in one of the most significant Hungarian cities, Pécs. Pécs received the title of the European Capital of Culture in 2010, it is the fifth largest city of Hungary located on the slopes of the Mecsek Mountains in the south-west region of the country close to Croatia. In Hungary, our city is the richest in terms of Turkish architectures, additionally, the Roman Paleochristian cemetery is included in the UNESCO World Heritage list. The Zsolnay Porcelain Manufacture, the company that introduced the famous eosin glazing process and pyrogranite ceramics, can also be found here. The Medieval University (Hungary's first university founded in 1367) can be visited behind the monumental Cathedral of St. Peter and Paul. Kodály Centre, the concert hall of the city, was one of the most important and most spectacular investments of Pécs 2010 European Capital of Culture project and now it is one of the best and increasingly recognized music venues of Hungary and Central Europe.

ISEO symposia have been organised annually in Europe since 1969. Each year, this Symposium provides an excellent possibility for academic and industrial scientists for a discussion of the latest and novel research findings focusing on essential oils and volatiles. Several representatives of the essential oil industry were also invited to join this scientific congress. The ISEO attracts the attention of participants not only from European countries but also from the broader scientific world focusing on essential oils, e.g. South Africa, Turkey, United States, Brazil, Chile, Japan, Israel, Egypt, among others.

We offer you highly scientific topics, stimulating presentations and deep discussions combing with colorful touristic activities in the city of the culture.

Abstracts of the ISEO 2017 are published in the Special Issue of Natural Volatiles and Essential Oils (NVEO). This journal will be pleased to accept reviews and original research papers in related fields.

We wish all of the ISEO 2017 participants a highly fruitful and successful symposium and many unforgettable memories of a very pleasant stay in Pécs, Hungary. Thank you for joining this meeting.

Györgyi Horváth,
President of the ISEO 2017 Local Organising Committee

Committees

ISEO 2017 Organising Committee

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György Horváth

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Éva Németh Zámboriné (Budapest, Hungary)

Young scientist fellowships

We are really grateful to the International Federation of Essential Oils and Aroma Trade (IFEAT) Executive Committee, who agreed to give financial support for the participation of 20 selected young scientists in the 48th International Symposium of Essential Oils (ISEO) 2017. The Young Scientist Fellowship award consists of the registration fee reimbursement.
Because of this generosity, the supported young scientists are now able to contribute to the development of the essential oils and natural volatiles research field. There is also an excellent possibility to bring together the young generation of scientists, to receive new experiences and to develop new ideas.
After an intense selection procedure, the Local Organising Committee of ISEO 2017 could accept twenty contributions. Six were chosen for oral presentation and fourteen were accepted for poster presentation.

The IFEAT registration fellowships awardees are

Nadire Pelin Bahadirli (Mustafa Kemal Univerity, Turkey): poster presentation
Enikő Détár (Szent István University, Hungary): poster presentation
Miljana Djordjevic (University of Niš, Serbia): poster presentation
Andreas Douros (National and Kapodistrian University of Athens, Greece): poster presentation
Barbara Horváth (University of Pécs, Hungary): oral presentation
Erika Beáta Kerekes (University of Szeged, Hungary): oral presentation
Gergő Kovács (Szent István University, Hungary): poster presentation
Erika Martins (Universidade Paulista, Brazil): poster presentation
Cynthia Murakami (Instituto de Botânica de São Paulo, Brazil): poster presentation
Milan Nešić (University of Niš, Serbia): poster presentation
Thi Huong Nguyen (Szent István University, Budapest): oral presentation
Branimir Pavlić (University of Novi Sad, Serbia): poster presentation
Marko Pešić (University of Niš, Serbia): poster presentation
Elwira Sieniawska (Medical University of Lublin, Poland): oral presentation
Nikola Stojanović (University of Niš, Serbia): poster presentation
Daniel Strub (Wroclaw University of Science and Technology, Poland): poster presentation
Jérémie Topin (Université Côte d'Azur, France): oral presentation
Mami Tsuchiyama (Fukuoka University, Japan): poster presentation
Musa Turkmen (Mustafa Kemal Univerity, Turkey): poster presentation
Margita Utczás (Chromaleont s.r.l, Italy): oral presentation

General information

Conference venue

Hotel Palatinus City Center***
H-7621 Pécs, Király utca 5.
+36-72-889-400
pecs.reservation@danubiushotels.com
GPS: N 46°4'35" E 18°13'46"

Website

You will find the latest details on the website at http://iseo2017.hu/

Opening hours of the registration

In the lobby of Hotel Palatinus:

Sunday, 10 September 2017	-	16.00 – 19.00
Monday, 11 September 2017	-	08.00 – 18.00
Tuesday, 12 September 2017	-	08.00 – 18.00
Wednesday, 13 September 2017	-	08.30 – 13.00

Badges

All participants and accompanying persons will receive a personal badge upon registration. Delegates are kindly requested to wear their name badge when attending the meetings or social events.

Official language

Official language of the Symposium is English. No translation service is to be provided.

Exhibiton

In accordance with the conventions of the symposium, parallel to the scientific sessions a professional exhibition is to be organised in Hotel Palatinus. The exhibition will be open during the whole scientific programme.

Meals

Every participant will be privded lunches at the venue hotel. Lunches will be served in Hotel Palatinus. Extra consumption, which is not included in the menus are kindly requested to settle prior to departure.

Liability and insurance

The organisers cannot accept liability for any personal accidents, loss of belongings or damage to private property of participants and accompanying persons that may occur during the symposium.

Social programmes

Welcome reception

Sunday, 10 September, 19.00 – 21.00
Venue: Hotel Palatinus
Music will be provided by Shisha Cafe Band.
Included in the registration fee.

Gala dinner

Tuesday, 12 September, 19.00-24.00
Venue: Bock Wine Cellar, 7773 Villány, Batthyány Lajos u. 15.
Gathering: In the hotel lobby at 18.30
Bus transportation is provided.
Price: 60 EUR/person.

Golden Age of Zsolnay – Gyugyi Collection

Wednesday, 13 September, 14.45
Venue: 7630 Pécs, Zsolnay Kulturális Negyed
Price: 1000 HUF/person. Advanced application is required.

Zsolnay Glove Manufactory

Wednesday, 13 September, 14.45
Venue: Pécs 7626, Major utca 21., Zsolnay Kulturális Negyed.
Included in the registration fee. Advanced application is required.

Oral presentations (PL-01 – PL-06, OP-01 – OP-18, YS-01 – YS-06)

The length of oral presentations is either 45 or 20 minutes. The organisers suggest leaving 5 minutes for questions and discussion. The organisers kindly ask a strict adherence to the agreed time, as the session chairs will be asked to rigorously maintain the time schedule. During the presentation the lecturer will have a laptop (Windows PC) and a microphone at their disposal. The presentation may be prepared in any available presentation format (MS, OpenOffice, LibreOffice, Prezi, pdf, etc.). Please bring your presentation on a USB key. Transferring individual presentations to the laptops will take place before the start of the symposium each day and during coffee or lunch breaks. 30 min. prior to your session at the latest.

Poster presentations

Poster session I; P-01 – P-55
Monday, 11 September 2017, 17.00-18.30
Room Nádor I-II.

Poster session II; P-56 – P-104
Tuesday, 12 September 2017, 16.30-18.00
Room Nádor I-II.

The useful area of the poster board is 90 cm at width and 125 cm at height. The recommended size for your poster is about the standing A0 standard (cca. 84 x 119 cm). Pins will be provided to fix the posters. Posters will be identified by posters numbers, which are printed in the final programme. Poster presenters are kindly requested to hang up their poster on Monday morning and remove their posters on Wednesday afternoon.
Authors of posters should stand at their posters and be available to discuss their research during the Poster Sessions on Monday or Tuesday according to the schedule in the final programme.

Final programme

Sunday, 10 September 2017

16.00 – 19.00 **Registration** - *Hotel Palatinus*

19:00 – 21:00 **Welcome reception** - *Hotel Palatinus*

Monday, 11 September 2017

8.00 – 9.30 **Registration**

9.30 – 10.10	**Opening**
10.10 – 10.30	**Coffee break**
10.30 – 12.35	**Session I.** **Chairs:** *Agnieszka Ludwiczuk, Hüsnü Can Baser*
10.30 – 11.15	**PL-01** **Ana Cristina Figueiredo** (Portugal): Biological properties of essentials oils and volatiles *p33*
11.15 – 11.35	**OP-01** **Sandy van Vuuren** (South Africa): Commercial essential oils against acne: chemistry and antimicrobial efficacy *p40*
11.35 – 11.55	**OP-02** **Adam Feyaerts** (Belgium): Detection, quantification and differential analysis of the vapour-phase-mediated antimicrobial activity of essential oil volatiles in standard multi-well plates *p41*
11.55 – 12.15	**OP-03** **Valtcho D. Zheljazkov** (United States): Essential oil, composition, and antimicrobial activity of the galbuli of six juniper species in Bulgaria *p43*
12.15 – 12.35	**OP-04** **Marketa Houdkova** (Czech Republic): New broth microdilution volatilization method for evaluation of antibacterial potential of plant volatile compounds *p45*
12.35 – 14.15	**Lunch**

14.15 – 16.20	**Session II.** **Chairs:** *Stanislaw Lochynski, Nicolas Baldovini*
14.15 – 15.00	**PL-02** **Luigi Mondello** (Italy): Isolation and characterization of the oxygen heterocyclic compounds (coumarins, psoralens, and polymethoxylated flavones) in cosmetic products *p33*
15.00 – 15.20	**OP-05** **Jennifer Bufalo** (Brazil): Volatile fraction from passionfruit: a fragrant water recovery of industrial process sidestream *p47*
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15.40 – 16.00	**OP-07** **Andreas Klingberg** (Germany): ChromIdent – towards a comprehensive utilization of aroma fingerprints for sample comparison and quality control *p49*
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17.00 – 18.30	**Poster session I. (P1-P54)**
19.00 –	**Meeting time for ISEO Permanent Scientific Committee**

Tuesday, 12 September 2017

9.00 – 10.45	**Session III.** **Chairs:** *Ana Cristina Figueiredo, Luigi Mondello*
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10.05 – 10.25	**YS-02** **Thi Huong Nguyen** (Hungary): Phytochemical and molecular characterization of intraspecific variability of wormwood (*Artemisia absinthium* L.) *p52*
10.25 – 10.45	**YS-03** **Elwira Sieniawska** (Poland*):* *Nigella damascena* L. essential oil – a source of β-elemene for antimycobacterial testing *p53*
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11.05 – 12.50	**Session IV.** **Chairs:** *Sandy van Vuuren, Niko Radulovic*
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Young Scientists Lectures (IFEAT support)	
11.50 – 12.10	**YS-04** **Barbara Horváth** (Hungary): Theoretical and practical aspects of essential oil emulsions stabilization with solid nanoparticles *p54*
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Oral presentations

PL-01 Biological properties of essentials oils

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Abstract

Plants use volatiles as a part of their strategy to adapt to the environmental biotic and abiotic factors (Figueiredo and Barroso 2015), and man has learned to use both volatiles and essential oils for their diverse array of properties. In addition to the well-known therapeutic actions and positive effect on health and well-being, essential oils find application in other fields such as food, beverages, cleaning, textile, perfume and cosmetic industries, to name a few.

In the great majority of the cases it is impossible to differentiate in an essential oil its diverse biological activities, and so its action, in a certain circumstance, is the result of the interaction of several of its components properties and, many times, of the way and amount used. But being bioproducts, essential oils are also prone to variability, thus requiring assurance of constancy and quality, to guarantee efficacy. On the other hand, the use of essential oils is not devoid of undesired side effects which are, in most cases, related with their misuse. Knowledge on the essential oil chemical composition is essential for understanding possible tolerance mechanisms and/or adverse effects.

Some selected examples of essential oils biological properties will be surveyed and discussed in light of their chemical composition and methods of biological activity evaluation.

Keywords: Essential oils, volatiles, biological properties, chemical composition.

Acknowledgments

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PL-02 Isolation and characterization of the oxygen heterocyclic compounds (coumarins, psoralens, and polymethoxylated flavones) in cosmetic products

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Abstract

The oxygen heterocyclic compounds (coumarins, psoralens and polymethoxylated flavones) present in citrus essential oils (Lemon, Mandarin, Sweet Orange, Bitter Orange, Bergamot, Grapefruit and Lime) were isolated by semipreparative HPLC-HPLC system. The isolated components, after their characterization, have been injected into a HPLC-PDA/MS/MS system equipped with an APCI source. The mass spectra obtained at a defined collision energy have been used to build an MS/MS library. For reliable identification MS similarity search has been used interactively with Linear Retention Indices (LRI) and UV spectrum with an unified software. This method allowed the rapid identification of the oxygen heterocyclic components of citrus oils, and the detection of authenticity and possible adulterations.

The use of an MS/MS system allowed to achieve limit of detection and quantification (LOD and LOQ) in the ppb range, thus making the method very useful for cosmetic industries that must respect the law limits on the presence of allergene compounds, in this case psoralens, in cosmetic products.

Furthermore, the goal of the present research was to propose an easier to use method for the characterization of the oxygen heterocyclic compunds in essential oils, by using a simpler HPLC-PDA instrument and the internal standard/response factor approach. The method was validated for all the compounds according to the International Fragrance Association (IFRA) regulation allowing the quantification of the 15 most common furocoumarins with a LOQ of 10 ppm. The novel LRI identification system played a key role for discriminating between components having similar or identical UV spectra.

Keywords: Oxygen Heterocyclic Compounds, Coumarins, Furocoumarins, flavour, Polymethoxylated Flavones, Citrus Oils.

Acknowledgments

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PL-03 Production of mono- and sesquiterpenes in *Camelina sativa* oilseed

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Abstract

Plants provide mankind with a vast array of phytochemicals that have a wide-ranging industrial and pharmacological application. Large-scale availability of phytochemicals can limit their use. Microbial production systems such as *Escherichia coli* and *Saccharomyces cerevisiae* are well established biotechnological platforms that can often be successfully bioengineered to serve as alternative sources for natural compounds. In addition to microbes, plant cell cultures have been exploited as potential biotechnological production platforms for phytochemicals. Despite the advantages of such cell-based production systems over the native producer, a common drawback is the requirement for specialized fermentation facilities, energy input and a continuous supply of macro- and micronutrients. Bioengineering of low-input crop plants to synthesize high value compounds would allow production of phytochemicals on farmland. However, whereas production of pharmacological proteins in plants has recently significantly advanced, suitable plant feedstocks for production of small molecules remain under-explored. Many plant-derived compounds of high value for industrial or pharmaceutical applications originate from plant species that are not amenable to cultivation. Biotechnological production in low-input organisms is, therefore, an attractive alternative. Here we explore whether *Camelina sativa*, an emerging low-input non-foodstuff Brassicaceae oilseed crop grown on marginal lands or as a rotation crop on fallow land, can successfully be refactored to produce and store novel compounds in seed. As proof-of-concept, we use the cyclic monoterpene hydrocarbon (4*S*)-limonene and the bicyclic sesquiterpene hydrocarbon (+)-δ-cadinene, which have potential biofuel and industrial solvent applications. Posttranslational translocation of the recombinant enzymes to the plastid with concurrent overexpression of genes of the MEP pathway resulted in the accumulation of (4*S*)-limonene **1** and (+)-δ-cadinene **2** up to 14 mg g^{-1} seed. This study presents the framework for rapid engineering of camelina oilseed production platforms for terpene-based high value compounds.

(4*S*)-Limonene **1**

(+)-d-Cadenine **2**

Keywords: Terpene, limonene, cadenene, Camelina, genetic engineering.

PL-04 Cyclodextrin-based molecular coating for the protection of sensitive essential oils

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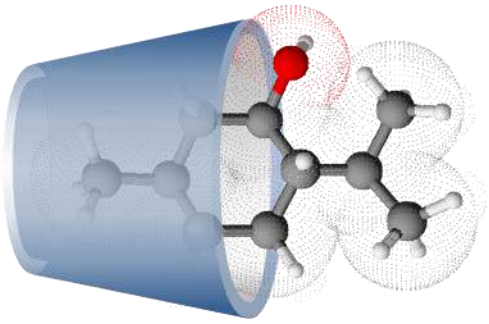
Abstract

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of α-1,4-glucopyranose units. The main representatives are alpha-, beta- and gamma-CDs (native CDs) with 6-8 glucose units, respectively. These macrocycles are produced *via* an enzymatic process and thus considered natural compounds. Due to their relatively lipophilic central cavity, they can physically include apolar molecules (e.g. oils). Native CDs have favourable toxicology profiles and their use is approved in food, cosmetic and pharmaceutical products.

The inclusion complex formation creates well-defined nanostructures leading to molecular dispersion in solution. This phenomenon improves the physico-chemical properties of the included substance enabling to overcome typical formulation and handling challenges associated with volatile and/or sensitive material. CDs increase aqueous solubility, decrease the volatility of the included guests and stabilize them against oxidation reactions and microbiological deterioration. CDs are effectively applied for masking unpleasant taste and smell through host-guest interactions.

A brief overview on the CDs regulatory background is going to be presented. Representative examples of essential oil inclusion complexes (e.g. thyme, oregano and citrus oils) with various CDs are going to be discussed with experimental data of CycloLab through comparison with the non-complexed oils. Future perspectives are going to be evaluated. Marketed products containing essential oil/CD complexes are going to be shown and their formulations will be explained in detail.

Keywords: Cyclodextrin, CD, cyclodextrin complex.



PL-05 Odors mechanics

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Abstract

The sense of smell is too often neglected in humans. It however plays a crucial role in memory, emotions, food intake and more generally within our social sphere. How does our nose work? How is it wired within our brain? Are we all equal in front of smells? And by the way, what is an odorant molecule?

Let's dive into chemistry, biology and psychology of the perception of smells.

Keywords: Odorant, odors, olfaction, receptors, emotions.

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PL-06 Shall we stress the plants to produce more volatiles?

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Abstract

It seems to be a widespread opinion that plants growing under unfavourable conditions may produce a higher level of secondary metabolites among others volatile compounds (Selmar and Kleinwächter, 2013). There are several research reports on very different species ascertaining this view (e.g. Simon et al., 1990; Bahreininejad et al., 2014). On the other side, publications on decreasing concentration of volatiles in consequence of stress are also available in a large number (e.g. Razmjoo et al., 2008; Németh-Zámbori et al., 2016). Data on compositional changes of the obtained essential oil due to stress circumstances show an even more variable picture.

For a well established conclusion on the available information it would be necessary to consider at least the type of stress factor, the applied dosage (severity) and duration of the effect, the ontogenetic stage of the plant furthermore the used methods and treatments. The meaning of *high* or *low* dosage may vary on a large scale if the optimum is not known and the basis of comparison is not adequately determined. The behaviour of the plants is also depending to a large extent on the possible acclimatization period before the disadvantageous changes in growth conditions. While this issue is well studied in case of the biomass, almost no information exists on it for the accumulation of volatiles. Discrepancies might arise also of the fact that intraspecific taxa of the same species react differently but this issue has very rarely been considered.

Results of stress factors may be either direct changes in metabolic processes or alterations caused through their indirect effects. This latter may include shift in ontogenesis, changed ratio and/or size of plant organs, modifications in the number or concentration of essential oil ducts, etc. Direct changes in metabolic processes could be revealed only if the research includes studies on expression of the relevant genes and/or on changes of enzyme activity. Unfortunately, this kind of knowledge in connection with stress and volatiles is very limited.

Well oriented and comprehensive studies including the mentioned biological and genomic aspects are necessary to form a sophisticated answer on the question in the title.

Keywords: Drought, salinity, chilling, gene expression, essential oil, composition.

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OP-01 Commercial essential oils against Acne: chemistry and antimicrobial efficacy

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Abstract

Pathogens associated with acne, such as *Propionibacterium acnes* and *Staphylococcus epidermidis,* have become problematic micro-organisms to treat in recent years due to antimicrobial resistance. Essential oils have thus gained popularity in treating these infections which are not only chronic but also have adverse social implications. A number of essential oils have been recommended for acne treatment, yet scientific studies on acne pathogens have been sorely neglected.

This study looks at the antimicrobial activity of essential oils (singularly, in oil combinations, with carrier oils and conventional acne treatments) recommended as a treatment for acne. The minimum inhibitory concentration (MIC) assay was used with fractional inhibitory concentrations (FIC) as well as isobolograms to demonstrate interactive profiles. Chemometrics coupled to mass spectrometry is used to analyse the chemical composition and correlation with antimicrobial activity. Best antimicrobial activity against *P. acnes* was observed for *Allium sativum* (0.09 mg/ml), and best antimicrobial activity against *S. epidermidis* was observed for *Santalum album* and *Vetiveria zizanioides* (0.13 mg/ml). Chemometric biomarkers identified nerol and eugenol with good activity against both pathogens. These finding were confirmed *in vitro* with MIC values ranging from 0.75-1.00 mg/ml. Synergy (ΣFIC = 0.5) was observed with the combination of *Leptospermum scoparium* and *Commiphora myrrha* against *S. epidermidis*. When carrier oils were combined with oils recommended for the treatment of acne, three synergistic interactions were observed against *P. acnes* and two against *S. epidermidis*. Some essential oils (*Melaleuca alternifolia*, *Eucalyptus globulus*, *Pogostemon patchouli*, *Syzygium aromaticum*) demonstrated antagonism when combined with conventional acne treatments.

Chemometric algorithms made it possible to detect the connection between chemistry and antimicrobial activity of essential oils. This may be adopted as a useful tool to identify compounds responsible for antimicrobial activities. The antimicrobial efficacy has demonstrated a number of essential oils with noteworthy activity. Selected essential oil combinations demonstrate synergy, yet when combined with conventional treatments may act antagonistically. This comprehensive study on essential oils used to treat acne provides a valuable scientific basis for aromatherapeutic practises.

Keywords: Antagonism, chemometrics, interactions, minimum inhibitory concentration, Propionibacterium acnes, Staphylococcus epidermidis, synergy

OP-02 Detection, quantification and differential analysis of the vapour-phase-mediated antimicrobial activity of essential oil volatiles in standard multi-well plates

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Abstract

In the search for antimicrobials, *in vitro* standardized bioassays are indispensable and commonly a first step in a long investigative process. To quantify their antimicrobial potential, the minimal inhibitory concentration, *i.e.* the lowest concentration of a (mixture of) chemical(s) that prevents visible growth of a microorganism, is typically determined using a broth microdilution method. However, some of these antimicrobials have a relatively high vapour pressure at room temperature *e.g.* essential oil components, which may allow them to also exert their antimicrobial activity (AA) over a distance. The AA can be a *direct* result of the vapour-phase of the antimicrobial as quantified with *e.g.* the disc volatilization assay, a method derived from the commonly used agar diffusion assay. However, the AA can also be an indirect result of the vapour-phase of the antimicrobial dissolving at a distance, which we named the vapour-phase-mediated antimicrobial activity (VMAA).

We introduced novel assays to evaluate the VMAA using a standard 96-well microtitre plate and a procedure based on a standardized protocol of a broth microdilution assay. To characterize the assay, we determined the VMAA of a large collection of essential oil(s) (components) and antifungals against two pathogenic human *Candida* species. We showed that there was no correlation between the VMAA and the minimal inhibitory concentration of the essential oil(s) (components), indicating that these are complementary measures. Furthermore, we showed that *C. glabrata* was on average more susceptible to essential oil(s) (components) than *C. albicans* and identified the essential oil(s) (components) with a significantly differential VMAA. As such, this is the first detailed characterization of a novel approach to qualitatively and quantitatively assess the VMAA of molecules using standard multi-well plates.

Keywords: Vapour-phase-mediated antimicrobial activity, essential oil, volatility, multi-well plate, *Candida.*

Acknowledgments
This work was supported by a bilateral grant from FWO and CNPq (G0D4813N; BIL11/19T)

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OP-03 Essential oil, composition, and antimicrobial activity of the galbuli of six juniper species in Bulgaria

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Abstract

Junipers are some of the most widespread and adapted species in the world. In the European flora there are 10 juniper species, six of which are also found in the Bulgarian flora; *Juniperus communis* L. (common juniper), *J. oxycedrus* L. (cade juniper, red juniper), *J. sibirica* Burgsd (Siberian juniper), *J. sabina* L. (savin, or Cossack juniper), *and J. excelsa* M. Bieb. (forest or Gracian juniper). Of these, *J. communis*, *J. oxycedrus*, *J. sibirica* are widely dispersed, whereas *J. sabina* and *J. exelsa* have limited distribution range. Juniper trees, juniper galbuli and their extracts have wide applications in the folk medicine, pharmaceuticals, aromatherapy, alcoholic beverages and in cooking. Chemical composition of the juniper galbuli (known as cones or berries) may play a role in the species distribution. Some wildlife species feed on juniper galbuli, which foster spreading of the junipers species. The objective of this study was to evaluate the essential oil, composition, antimicrobial and antioxidant activity of the galbuli of the six juniper species found in the Bulgarian flora. The galbuli were extracted via hydrodistillation and the essential oil analyzed. The essential oil content of the galbuli of the six juniper species varied from 0.47 % (in *J. sibirica*) up to 1.6% (in *J. sabina*). The oil profile of the galbuli was also different among species. The three oil constituents with the highest concentration (in descending order) in the galbuli of each of the species were as follows:

- β-Myrcene, α-Pinene, and Germacrene D, in *J. oxycedrus*;
- α-Pinene, Germacrene D, β-Myrcene in *J. communis;*
- α-Pinene, α-Cedrol, Germacrene D in *J. excelsa*;
- α-Pinene, β-Myrcene, Germacrene D *in J. sibirica*;

- α-Pinene, Sabinene, β-Myrcene, in *J. pigmea*;
- Sabinene, α-Pinene, Terpinene-4-ol, *in J. sabina*.

Overall, the antioxidant capacity of the six oils were as follows: *J. sibirica* > *J. excelsa* = *J. communis*. The results from this study may contribute to further understanding of juniper ecology in Bulgaria, and may also be used by industry utilizing juniper products.

Keywords: Juniper, galbuli, essential oil, antioxidant capacity, antimicrobial activity.

Acknowledgments
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OP-04 New broth microdilution volatilization method for evaluation of antibacterial potential of plant volatile compounds

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Abstract

Several methods for testing of antimicrobial effects of essential oils have been developed with aim to study the potential of their volatile constituent. In contrast to well established methods for antimicrobial testing in liquid media, there are no standardized assays for determination of microbial sensitivity to volatile compounds in vapour phase. For this reason the antimicrobial activity of volatile essential oils has been studied intensively e.g. by the methods based on disc volatilization [1] and using special apparatus [2]. However, they are not applicable for a high-throughput screening, some of them allow evaluating only one concentration of samples, some needs special equipment which is not commonly available, and they determine antimicrobial activity of volatile compounds either in liquid or gaseous phase. We designed a new broth microdilution volatilization method [3] for simple and rapid simultaneous determination of antibacterial potential of plant volatiles in the liquid and the vapour phase at different concentrations. Using this method, an antibacterial activity of carvacrol, cinnamaldehyde, eugenol, 8-hydroxyquinoline, thymol and thymoquinone was determined against bacteria causing respiratory infections, namely *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. The most effective antibacterial agents were 8-hydroxyquinoline and thymoquinone with the lowest minimum inhibitory concentrations (MICs) ranging from 2 to 32 μg/mL in liquid medium and from 8 to 128 μg/mL in vapour phase. The results demonstrate validity of new broth microdilution volatilization method for development of novel inhalation therapeutics. It allows cost and labour effective high-throughput antimicrobial screening of volatile agents without the need of special apparatus. In our opinion, this assay can potentially be used for development of various medicinal, agricultural, and food applications that are based on volatile antimicrobials such as disinfection gases in healthcare facilities, fumigants for controlled-atmosphere storage of agricultural products, and preservation agents for active or smart packaging of food products.

Keywords: Antibacterial activity; colorimetric assay; cytotoxicity; essential oil; vapour; volatilization method

Acknowledgments

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OP-05 Volatile fraction from passionfruit: a fragrant water recovery of industrial process sidestream

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Abstract

Symrise designed a technology to create aromatic products from industrial food processing waste. In general, during a fruit processing pulp, seeds and shells are generated and they have different destinations such as juice, frozen pulp, seed oil, feed and fertilizer. Specifically during the seed processing, the industry makes a washing process before the oil extraction production and this washing water is considered as a waste and is discarded. Symrise shimmered an opportunity to use this fruit enriched wastestream to create a new aromatic ingredient. The company has developed a recovery process for concentrating and converting waste water with volatile compounds into an added value product with application in flavors and fragrance called Symtrap®. This patented technology is composed of a polymeric resin which concentrates polar organic compounds dispersed in aqueous phase at very low concentrations. Symrise has mapped local Brazilian communities which produces passion fruit pulps and collaborated with them mapping the production process and investigated opportunities on uses of wastes. For the seed washing process we standardized it and developed a process to generate a raw material with highly concentrated natural material and authentic natural scent. The process is gentle, without thermal degradation and the product works as longer lasting of top notes, giving intense characteristic for fragrances. Also, cleaner water is returned to the environment, since the diluted compounds are removed. This project allowed exploring sidestreams and wastestreams from fruit processing and generation of an added value product. This technology represents a fragrance tool for inspiration and is an opportunity for developing new aromatic ingredients from natural sources. The whole project also allows sustainable sourcing and connection with communities.

Keywords: Fragrances, wastestream, sustainability, Passiflora *edulis*.

OP-06 The volatile components of the liverwort *Leptolejeunea elliptica*

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Abstract

The volatile components of the liverwort *Leptolejeunea elliptica* grown on the leaves of tea (*Camelia sinensis*), collected in Tokushima, Japan, were analysed using headspace solid phase micro extraction (SPME) and GC-MS. In the previous papar[1)], three phenolic components, such as 1-ethyl-4-methoxybenzene (**1**), 1-ethyl-4-hydroxybenzene (**2**), and 1-acetoxy-4-ethylbenzene (**3**), were detected from this liverwort *L.elliptica*. Among them, compound (**1**) was the major component while the content of compounds (**2**) and (**3**) were very low. Then, these two compounds were prepared from compound (**1**), and determined as one of the volatiles. In the other report [2)], the monoterpene hydrocarbons, such as α-pinene, β-pinene, and camphene, were identified in the same species.

In this study, we also determined 1-ethyl-4-methoxybenzene (**1**) (51 %) having the characteristic floral, faint sweet anisic spicy note. In addition, compound (**2**) (14 %) and compound (**3**) (4%) were also identified as the major components. Then, several other phenolic components and terpene alcohols such as menthol, nerolidol with floral note, and sesquiterpene hydrocarbons, such as selinene, elemene, bisabolene and caryophyllene, were identified but monoterpene hydrocarbons were not.

The identified phenolic components were enzymatically induced by decarboxylation of free phenolic acids (ferric acid, *p*-coumaric acid and cinnamic acid) to their corresponding vinyl aromatics. The antibacterial and antifungal activities of these phenolic components are now under progress.

Keywords: volatile, *Leptolejeunea elliptica*, 1-ethyl-4-methoxybenzene, 1-ethyl-4-hydroxybenzene, 1-acetoxy-4-ethylbenzene

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OP-07 ChromIdent – towards a comprehensive utilization of aroma fingerprints for sample comparison and quality control

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Abstract

Gas chromatography/mass spectrometry (GC/MS) is regarded as a valuable fingerprinting technique for essential oils. The intriguing fact that GC/MS is capable to provide highly reproducible fingerprints with a potentially large amount of compositional information is, in many cases, opposed by the considerable complexity of the chromatograms, which may contain more than hundred components. Many of the peaks are overlapping and demand deconvolution processing or can not be identified by commercially available mass spectral libraries. Consequently, the analyst is often faced with a tedious and time-consuming analysis of the generated data – in particular when long measurement series were acquired and the complex fingerprints are to be compared against each other or against reference measurements.

It is this tremendous drawback which motivated the development of ChromIdent®, a software extension for the chromatography software OpenChrom® [1]. ChromIdent has been designed for the comprehensive comparison of fingerprints generated by GC or GC/MS. After peak detection and deconvolution, peak identification and other common processing steps in OpenChrom, ChromIdent allows to build reference libraries on the basis of chromatographic fingerprints characteristic for each sample. By the library building process a global peak table valid for all considered samples is constructed, where next to mass spectral match quality (in case of GC/MS data) also the retention time or retention index may be utilized to ensure a correct peak matching. This resulting peak matrix is the basis for subsequent analysis of unknown samples or, alternatively, may be exported to perform e.g. chemometric approaches with other software solutions.

In order to build useful libraries the user may decide to utilize all detected peaks or just a subgroup within the measurement, e.g. the terpenes only. Thereafter unknown fingerprints, e.g. composite materials, can be compared against the libraries to find similarities with reference fingerprints, or sub-fingerprints, and, additionally, elucidate whether there are unique marker components hinting towards particular references.

In the present study the applicability of ChromIdent for essential oil authentication and marker component detection is verified on two GC/MS datasets.

Keywords: GC/MS, chromatography, fingerprint, quality control, authentication.

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OP-08 Assessment of volatile and non-volatile fractions of two *Galatella* species for biological activities and chemical profiles

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Abstract

In the present work, the essential oils of *G. villosa* Rchb.f. and *G. tatarica* (Less.) Novopokr were obtained by hydrodistillation, as well as non-volatile constituents were obtained with methanol. The essential oils (EOs) and the methanol extracts (MEs) were analyzed with GC-FID, GC/MS and LC-MS/MS techniques. The antioxidant activities of the oils and extracts were assessed by both chemical and enzymatic methods against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals, linoleic acid hydroperoxides and superoxide anion radical (O^{2-}) generated by xanthine - xanthine oxidase (XO) system. Also, inhibitory activities of the EOs and MEs were evaluated *in vitro* against α-amylase, tyrosinase and lipoxygenase enzymes. In the oil of *G. tatarica*, β–pinene (23.6 %) and α-pinene (14.4 %) were the main constituents, while the oil of *G. villosa* was characterized not only with monoterpene α–pinene (9.0 %), but also fatty acid, hexadecanoic acid (10.2%). The sesquiterpenes were presented in the both oils in scarce amount with caryophyllene oxide (2.1% and 4.6%) and spathulenol (4.7% and 4.4%) in *G. tatarica* and *G. villosa*, respectively. 3-, 5-, 1,5- and 3,4-caffeoylquinic acids, 3-, 4- and 5-feruloylquinic acids, quercetin and its different glycosides were detected in MEs according to mass spectra. *G. tatarica* (IC_{50}=74.3 μg/mL) and *G. villosa* (IC_{50}=78.5 μg/mL) extracts showed an antioxidant activity significantly higher than the essential oils. In β-carotene bleaching test, the both extracts demonstrated quite noteworthy activity (%Inh 44.46 and 41.14). In TEAC assay, the extracts scavenged $ABTS^{\bullet+}$ radical as 1.85 mM and 1.27 mM of Trolox, while the oils were lower in activity. In xanthine-xanthine oxidase model system the extracts demonstrated inhibitory potent ranged between 43% and 39%, while the EOs were found inactive at concentration 1 mg/mL. In concentration at 0.5 mg/mL the oils showed 89% and 85% inhibition percentage of α–amylase, while the extracts showed 86% and 78% inhibition percentage at 2 mg/mL concentration, respectively. In LOX experiment, the EOs demonstrated noteworthy potent enzyme inhibition ranged between 45% and 57%. The present work is the first contribution into chemistry and biological activities of *G. tatarica* and *G. villosa*.

Keywords: *Galatella*, oil, extract, antioxidant, α-amylase, tyrosinase, lipoxygenase, xanthine oxidase.

YS-01 Essential oils as food preservatives: from lab experiments to use in real foods

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Abstract

Food spoilage is caused by microorganisms represents a serious problem by lowering the shelf-life of foods and causing food safety problems (Hyldgaard et al. 2012). Application of essential oils (EOs) in the food industry as natural antimicrobials for extension of shelf-life seems to be a good approach (Burt, 2004; Bakkali et al. 2008). EOs or their components are used in the food industry as aroma compounds and, in the recent years, intensive research has started to use them as natural preservatives. EOs are generally regarded as safe (GRAS) and are accepted by consumers (Burt, 2004). Following previous antimicrobial experiments *in vitro* we investigated the effect of thyme (*Thymus vulgaris*), thymol (major component of thyme EO), marjoram (*Origanum marjorana*), and lemon (*Citrus lemon*) EO *in vivo* on the shelf-life of meat, fruits and vegetables. Chicken breast fillets were marinated with marjoram and thyme EOs, and thymol. Sensory evaluations showed that for chicken meat we could determine a dose of thyme EO which combines antimicrobial efficiency with a pleasant flavor (1 mg/mL for thyme and 0.2 mg/mL for thymol). Thyme EO (20 µl/L) in vapour phase also elongated the shelf-life of cherry tomatoes with 4 days and lemon EO (20 µl/L) prevented mold growth on strawberries 6-8 days longer compared to the untreated samples. Sensory evaluations showed that using EOs in low concentrations a spicy, harmonizing taste was achieved. According to our findings the tested EOs could be used as potential preservatives in real foods.

Keywords: Food spoilage, essential oils, sensory evaluation, shelf-life, food preservation.

Acknowledgments

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YS-02 Phytochemical and molecular characterization of intraspecific variability of wormwood (*Artemisia absinthium* L.)

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Abstract

A trial with nine wormwood accessions was installed to carry out a systematic evaluation of intraspecific chemical diversity. The experiment was carried out in open field in 2016. Essential oil (EO) yield and composition were measured, besides, 11 RAPD and 15 ISSR molecular markers were tested to determine the genetic diversity of the accessions. The essential oils were obtained by hydrodistillation of dried leaf samples and analyzed by gas chromatography-mass spectrometry.

Based on EO yield, the studied accessions were divided into three significantly diverse groups. Highest yield was produced by "Spanish" accession (3.215ml/100g) while "Norwegen" and "Belgien" produced medium values (1.569-1.892 ml/100g) and the other six accessions showed EO yields below 1% (0.349-0.832 ml/100g DW). 69 compounds which were present in higher than 1% of GC area percentage were identified representing 62.9-100% of total oil compositions, among them 30 monoterpenes and 39 sequiterpenes. Major components of these oils over 30% were both isomeric forms of thujone, α-thujone (0%-51.7%) and β-thujone (0%-89.8%), cis-epoxy-ocimene (0%-75.7%), trans-sabinyl acetate (0%-94.5%), sabinene (0%-38.5%) and β-myrcene (0%-68.4%). Some of the individuals of the investigated *A.absinthium* accessions represented new chemotypes. Considering the EO composition of the individuzals the accessions from Spain and Belgium were evaluated as homogenous ones while some others, including wild growing populations from Hungary and an accession from commercial seed sample in England presented a large variability among their individuals. High polymorphism was found among the wormwood accessions also by molecular markers: 81.15% for RAPD and 73.10% for ISSR primers. The three groups of genotypes based on the Nei's genetic distances were identical to those based on EO yield.

The study confirmed the large intraspecific variability of wormwood but revealed that it is not definitely connected to geographical origin of the populations.

Keywords: Wormwood, essential oil, GC-MS, thujone; RADP; ISSR; DNA.

Acknowledgments

The work was supported by the Stipendium Hungaricum Scolarship. Special thanks for prof. Antonio Llorens-Molina (Valencia) for providing us the Spanish accession.

YS-03 *Nigella damascena* L. essential oil – a source of β-elemene for antimycobacterial testing

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Abstract

β-elemene is a broad spectrum antieoplastic agent which reverses drug resistance against other antieoplastic compounds and enhances the immunity of cancer sufferers (Wang, 2012). The major source of β-elemene is *Curcuma wenyujin* Y.H.Chen & C.Ling (syn. of *Curcuma aromatica* Salisb.).

In this work we aimed to isolate β-elemene from the other rich source – the *Nigella damascena* L. essential oil, in which β-elemene can account for up to 70% (Malhotra, 2012). The essential oil and purified target compound were tested for its activity against *Mycobacterium tuberculosis* H37Ra.

High performance countercurrent chromatography (HPCCC) was used for β-elemene isolation. The application of the mixture of petroleum ether, acetonitrile and acetone in the ratio 2:1.5:0.5 (v/v) in the reversed phase mode yielded β-elemene with 93 % purity in 50 minutes. The values of the minimal inhibitory concentration (MIC) were determined by $\log^2$ dilution method. The antimycobacterial activity of *N. damascena* essential oil expressed as MIC was 128 µg/mL, while for isolated β-elemene this value equaled to 32µg/mL. β-elemene also enhanced the activity of first line antimycobacterial antibiotics decreasing the MIC values for rifampicin, ethambutol and isoniazid from 1, 4, 0.125 µg/mL to 0.062, 1, 0.062 µg/mL, respectively, when applied in sub-minimal inhibitory concentration (1/2 MIC).

Our study revealed that β-elemene sensitizes mycobacreria to the tuberculostatic antibiotics.

Keywords: β-elemene, *Nigella damascena* L. essential oil, *Mycobacterium tuberculosis* H37Ra, HPCCC, MIC.

Acknowledgments

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YS-04 Theoretical and practical aspects of essential oil emulsions stabilization WITH solid nanoparticles

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Abstract

The low water solubility limits the use of essential oils in pharmaceutics. We can overcome this problem, by preparing emulsions, but the materials used for stabilization of emulsions like surfactants and cosolvents, can cause irritation or other side effects.[1] They can even influence the role and the effect of active components which then leads to misinterpretation of experimental data. To avoid the use of these materials we can use solid particles as stabilizing agents (e.g. $CaCO_3$, clay minerals, silica) to prepare stabile emulsions.

The aim of our work is to prepare Pickering emulsions of essential oils with well defined properties. Beside the emulsion stability, for the pharmaceutical experiments two important parameters are size and density of emulsion droplets. Both of them can influence the essential oil adsorption in target cells or tissues. To prepare emulsions with desired properties we must consider the theoretical aspects of Pickering emulsion formation. We have performed calculations to optimize the composition of emulsions. The parameter that were taken in to the calculations are size-, volume-, surface-, density- and mass of stabilizing solid nanoparticle, the size and the density of essential oil droplet and the volume of the oil phase. The calculations were performed assuming that the contact angle of the solid particles with liquid phases is 90° which leads to formation of stabile emulsion. This condition can be achieved by systematic modification of hydrophilic/lipophilic surface properties of solid nanoparticles. Silica nanoparticles were synthesized by one-step synthesis based on the Stöber, Fink and Bohn method[2]. The surface modification of silica nanoparticles was performed using methyltriethoxysilane, ethyltriethoxysilane in post modification process. The synthesized nanoparticles were characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM) and preferential adsorption to determine their hydrophilic/lipophilic character. They were used as emulsifier to prepare Pickering emulsions with thyme volatile oil. The emulsions were characterized regarding their stability and droplet size.

Keywords: Pickering emulsion, essential oil, silica nanoparticles, drug delivery

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YS-05 Analysis of potentially harmful volatiles in e-liquids by HS-GC-MS

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Abstract

Electronic cigarettes were proposed as a healthier alternative to conventional cigarettes. Nevertheless, there is a lack of study about the long-term effect of its use, they have become increasingly popular. The main components of e-liquids are propylene glycol and glycerin, in addition they may contain nicotine and fruity, tobacco, spicy or other flavourings. The flavour and fragrance compounds used by vaping industries can be harmless applying them via ingestion, but not necessarily also via aspiration. Inhalation of some flavouring substances present in e-liquids, such as diacetyl or acetoin, was found as a cause of serious lung diseases or allergic reactions. This is the reason why in 2016 the U.S. Food and Drug Administration (FDA)'s regulation was extended to all tobacco products, including e-liquids, on the purpose to label the exact list of e-liquid ingredients.

A simple, fast and solvent-free head-space method followed by GC-qMS determination was developed for the qualitative analysis of flavour and fragrance compounds in e-liquids. The extraction and the analytical method were optimized, obtaining good performance characteristics, and applied to analyse fourteen e-liquid samples. The volatile compounds were identified using mainly a unique MS spectral library with embedded linear retention indices (LRI), namely Flavour and Fragrance Natural and Synthetic Compounds (FFNSC). Using a dual-filter search, namely MS similarity (>90%) and LRI (±5 LRI unit), 170 individual aroma compounds were identified in the analysed e-liquids. Some of them are well-known harmful or allergen substances, such as acetoin, vanillin or *E*-anethol. Among the various flavourings the most frequently used were limonene, decanal, α-pinene and short-chain fatty acid methyl esters. It is noteworthy that the presence of nicotine was not always in accordance with producer's label.

The presented method could be applicable for the determination of e-liquid composition and the identification of harmful flavour and fragrance compounds. The FFNSC database demonstrated an excellent coverage (>95% of the detected compounds) to the applied aroma substances in e-liquids.

Keywords: HS-GC-MS, e-liquid, Flavour and Fragrance Natural and Synthetic Compounds (FFNSC) MS database, flavour, fragrance.

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YS-06 Olfactory adaptation

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Abstract

Our sense of smell is triggered by the activation of odorant receptors (ORs) expressed at the surface of olfactory sensory neurons. The perception of an odor by the brain relies on a so-called combinatorial code of OR activations. Each OR can recognize multiple odorants, while one odorant can activate multiple types of ORs. The variability and combinatorial activation of our ORs endows us with spectacular discriminatory power.

Olfactory adaptation consists in the decrease of an odor perception as a result to its regularly exposure. However, this loss of sensitivity is not permanent and the normal perception is recovered after a time without stimulation. At a physiological point of view, the adaptation induces a decrease in the neural response.

In this study, we assess the effect of the adaptation to different odorants, from essential oils to isolated molecules. Essential oils are complex mixture of odorant compounds. Their inhalation activates a large panel of olfactory receptors while single molecules activate fewer receptors. Adaptation and recovery time to these odorants was examined. A discrimination test was used as a metric to evaluate the diminution and recovery of the olfactory perception. These experiments will allow gaining knowledge on adaptation time during sensory analysis.

Keywords: Odor stimulation, physiological parameters, emotion.

Acknowledgments

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OP-09 The effect of caraway essential oil on the microbial stability and sensory properties of cooked pork sausages

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Abstract

The purpose of this study was to evaluate the effect of caraway essential oil (CO) on the microbial stability and sensory properties of cooked pork sausages. Pork cooked sausages with different concentrations of CO (1.0, 2.5 and 5.0 µl/g) and control (without CO) were prepared. pH and instrumental parameters of colour (*CIEL*a*b**) have been assessed.

The addition of CO had no significantly ($p>0.05$) effect on pH values of pork cooked sausages. All three concentrations of CO positively affected the colour of the product by reducing lightness (CIE *L**) and yellowness (CIE *b**) and increasing redness (CIE *a**). The addition of CO had significantly reduced ($p<0.05$) the total number of aerobic mesophilic bacteria compared with control. It was, probably, the consequence of antimicrobial properties of caraway essential oil. The flavour of sausages produced with the addition of 1.0 µl/g CO were moderate/large difference ($p<0.05$) compared to control.

This study demonstrates that the caraway essential oil could be used in processing of cooked pork sausages in order to improve microbial stability as well as to enhance their colour characteristics.

Keywords: Caraway essential oil, cooked sausage, microbial stability, flavour.

Acknowledgments

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OP-10 Aroma profile of Thai tea leaves (Chinese and Assam varieties)

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Abstract

Tea is a very popular beverage worldwide, and its fascinating, unique aroma is an important characteristic point in tea quality control. The three types of tea—green, black, and oolong—are well known and poses explicitly different odor characters. It is generally recognized that the differences in their odors are due to degree of fermentation: green tea is unfermented, oolong tea is semi-fermented, and black tea is fermented. However, there is another important factor; tea is made from *Camellia sinensis* var. *sinensis* (Chinese variety) or *Camellia sinensis* var. *assamica* (Assam variety), with *sinensis* mainly used for green and oolong tea and *assamica* mainly for black tea. Because the Assam variety is weaker in cold climates, the Chinese variety is mainly used in Japan. However, in Thailand, both varieties are used, yielding two types of tea (green and black teas). In this study, we investigated the effect of tea plant variety on the odor of green tea using our proposed method.

Two fractions with different odor were obtained by fractional distillation of hexane extracts. One group (distillate) was composed of compounds with relatively low boiling point, and the other group (residue), relatively high-boiling point compounds. The characteristic odor of black tea (Assam) was strongly due to the distillate. The distillate of Thai green tea and black tea prepared from Assam had similar odor characteristics: sweet, roasted, and sour. However, the residue of the Thai green tea prepared from Assam had a sweet, roasted, and sour odor in addition to weak green notes, which differed from the matcha-like odor of Japanese green tea that we have reported previously. Thai green tea prepared from Chinese variety had an intermediate character between Japanese green tea from Chinese variety and Thai green tea from Assam variety. These results indicate that tea plant variety is an important factor in determining the odor of the green tea produced. The compounds of these obtained fractions were analyzed by gas chromatography–olfactometry and gas chromatography–mass spectrometry. Several compounds were found to affect the characteristic odors of both varieties in Thai tea leaves.

Keywords: Thai tea leaves, Chinese variety, Assam variety, green tea, black tea.

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OP-11 Subjective assessments of a lavender essential oil-based supplement on sleep: A randomized, double-blind, placebo-controlled, crossover study

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Abstract

Background: Insomnia, or difficulty sleeping, is a common sleep disorder and is associated with elevated risk of various diseases. Targeted supplementation with well-studied botanical ingredients may alleviate insomnia. This study evaluated a softgel supplement containing primarily lavender essential oil, along with l-theanine and extracts of lemon balm, passion flower, and chamomile.

Methods: Fifty-one adult participants completed a 6-week crossover study divided into placebo and treatment groups. The treatment and placebo softgels were designed so they could not be distinguished by the participants. Participants filled out the Leeds Sleep Evaluation Questionnaire (LSEQ) survey weekly and kept a sleep diary daily. Mixed model analysis was used to determine statistical significance.

Results: Compared to the placebo, participants taking the sleep supplement showed highly significant improvement in overall LSEQ scores ($p<0.01$) as well as significant improvement in several individual LSEQ measures. No significant differences emerged when comparing the sleep diary data. Participants' age, BMI, gender, and ethnicity did not significantly interact with any dependent variables. There was no significant difference in adverse events.

Conclusion: The lavender oil-based supplement was effective in improving a range of subjective sleep parameters in adults suffering from insomnia.

Keywords: lavender essential oil, insomnia, lemon balm, passion flower, chamomile, l-theanine, LSEQ, sleep, dietary supplement

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OP-12 Volatile components of the liverworts growing under different climatic and geographical conditions

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Liverworts are spore-forming plants that can grow in almost every available habitat, although most often in humid locations. Many liverwort species demonstrate wide geographical distribution, grow under diverse ecological conditions, and often are pioneers in extreme habitats. One of the outstanding feature of the liverworts is their chemistry. They produce a wide array of secondary metabolites, mainly terpenoids and aromatic compounds. Many of these compounds are characterized by unprecedented structures, and some have not been found in any other plants, fungi or marine organisms (Asakawa et al., 2013; Ludwiczuk & Asakawa, 2014, 2015). The aim of this study was to demonstrate both the similarities and differences in chemical composition of terpenoids and volatile aromatic compounds detected in liverwort species growing under different climatic and geographical conditions. GC/MS fingerprinting of the volatile extracts obtained from liverworts growing in climatic conditions of the islands, Tahiti, Ua Huka, Borneo and Yakushima was performed. The received data was compared with our own and literature data concerning liverwort species collected in different places within the northern and southern hemisphere. The results indicated that the liverworts growing in different geographical conditions can be divided into two groups. The first one is composed of the liverwort species which biosynthesize the compounds unique to a place where they live, but they also produced compounds which are chemical markers of a given species or genus. An example would be the presence of neryl acetate only in *Wiesnerella denudata* from Borneo, and guaianolides that are the chemical markers of this liverwort species. The second group consisted of the liverworts which have a completely different chemical composition depending on the collection place. Good examples of this situation were *Conocepephalum conicum*, *Dumortiera hirsuta* and *Reboulia hemisphaerica.* Both liverwort species were characterized by the occurrence of chemotypes with very different chemical profiles.

Keywords: Liverworts, terpenoids, aromatic compounds, chemical markers, chemotaxonomy.

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OP-13 Changes in essential oil properties of different *Thymus vulgaris* varieties influenced by developmental phases, growing locations and seasons

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Abstract

In open field studies, conducted in two countries (Budapest/Hungary & Poznan/Poland) involved, four thyme (*Thymus vulgaris* L.) varieties have been investigated for three years (2014-2016) in different developmental phases. The F1 hybrid cultivar 'Varico 3' (V3) and further three standard varieties ('Sloneczko'-SL, 'Standard Winter'-SW and 'French Summer'-FS), all belonging to the thymol chemotype were applied. Our aim was to obtain data on general performance and essential oil (EO) producing ability of these varieties available for thyme growers, conducting parallel investigations established in two different growing locations. Sampling was carried out from vegetative phase, during budding, in full bloom till overblown period (from mid May to late June).

Summarizing the data obtained during 3 years of the experiment we could conclude that significant differences have been found among cultivars concerning essential oil content and composition, influenced by the growing site, by the plant age/year as well as by the ontogenetical phases. When comparing varieties involved, the EO accumulating power of 'Varico 3' was outstanding in both growing sites, especially in the first year ($Poznan_{2014}$: 5.78 ml/100g DW; $Budapest_{2014}$: 3.97 ml/100g DW; $Budapest_{2014\text{-}2016}$: 2.34 ml/100g DW), while Slonecko was also highly productive ($Budapest_{2014\text{-}2016}$: 2.06 ml/100g DW). However, decreasing values have been observed in the second and third years of age in each cultivars and also in the average of them (e.g. in Budapest: 2014 (1): 2.78 ml/100g; 2015 (2): 1.59 ml/100g; 2016 (3): 1.09 ml/100g DW). Concerning growing sites, Budapest was proven to be more advantageous for all the varieties included, and also in the EO average of the years and varieties ($Poznan_{2016}$: 0.846 ml/100g DW; $Budapest_{2016}$: 1.099 ml/100g DW). In general, thymol percentage was appropriate in all cases, showing the highest mean value in full bloom (71.09 %) followed by the vegetative phase (67.09 %) concerning the means of cultivars and years (2015-2016). Nevertheless, there were differences among values (V3: 75.44%→FS: 67.08 %) and accumulation tendencies of varieties involved with one (V3, SW) or two (FS, SL) peaks of timol %. These features of varieties should be considered during cultivation, in particular from the third year.

Keywords: Essential oil content, thymol percentage, growing site, plant age, thyme varieties.

Acknowledgments

Our work has been suppoted by the OTKA National Scientific Fund under the project No. NN108633 and we are also very thankful to Agroscope and Mediseed, Switzerland supplying seeds of 'Varico 3' cultivar

OP-14 Analysis of cosmetic allergens using Ultra Performance Convergence Chromatography (UPC^2) with MS detection

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Abstract

Fragrances are complex combinations of natural and/or man-made substances that are added to many consumer products (for example, perfumes, shampoos, conditioners, moisturizers, facial cosmetics, and deodorants) to give them a distinctive smell, impart a pleasant odor, to mask the inherent smell of some ingredients, but ultimately to enhance the experience of the user of the product.

In most types of cosmetics and skin products, including perfumes, shampoos, conditioners, moisturizers, facial cosmetics, and deodorants, there are more than 5000 different fragrances present. Many people suffer from allergies, which are due to an abnormal reaction of the body to a previously encountered allergen which can be introduced in many ways, such as by inhalation, ingestion, injection, or skin contact. Allergies are often manifested by itchy eyes, a runny nose, wheezing, skin rashes (including dermatitis), or diarrhea.

In the current EU Cosmetic Regulations (1223/2009), there are 'currently' 26 fragrance ingredients, 24 volatile chemicals and 2 natural extracts (oak moss and tree moss), that are considered more likely to cause reactions in susceptible people. These 26 fragrance ingredients must be indicated in the list of ingredients of the final product, if the concentration exceeds 0.001% in leave-on products (10 mg/kg) (e.g. a moisturizer), or 0.01% (100 mg/kg) in rinse-off products (e.g. a shampoo). The 24 current regulated allergens contain compounds from different classes, (phenols, cyclic hydrocarbons, alcohols, carbonyl compounds, esters and lactones) with different polarities, similar structures, many are small molecules (ions with low m/z) and many are also isobaric, which results in nonspecific fragment.

This presentation will consider how hyphenating Ultra Performance Convergence Chromatography (UPC^2) with MS detection can be used to achieve specificity, selectivity and sensitivity for the analysis of fragrance allergens in perfume and personal care products in a fast 7 minute run.

Keywords: Cosmetic allergens, perfume, regulations, convergence chromatography, supercritical fluid chromatography, personal care products, mass spectrometry.

OP-15 Essential oils yield and composition of Myrtaceae species from Atlantic Forest of South Brazil

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Abstract

Myrtaceae is one of the largest botanical families occurring in Brazil mainly in the Atlantic Forest and it is recognized by its great potential for volatile oil production of economic interest. This work aimed to evaluate the essential oil yield and chemical composition he of Myrtaceae species with occurrence in a preservation area of the Atlantic Forest in Parana stae, Southe of Brazil. The essential oil extraction was carried out by hydrodistillation of fresh and dried leaves and the chemical composition was determined by gas phase chromatography coupled with flame ionization and mass detectors. The following species were evaluated: *Myrciaria delicatula, Campomanesia xantocarpha, Campomanesia aurea, Calyptranthes clusiifolia, Myrcia splendens, Eugenia osoriana, Myrciaria tenella, Myrceugenia reitzii, Calyptranthes concinna and Myrcia arborensis*. The *Myrceugenia reitzii* showed essential oil yield of 1.59%, being superior to the other species. The average essential oil yield for each species studied was higher when using dried instead of fresh plant material. The chemical composition of showed sesquiterpenes in a high percentage. The drying process affected the chemical composition of the essential oil for most species.

Keywords: Atlantic Forest, medicinal and aromatic plants, terpenes, spathulenol.

OP-16 Non-pharmacological analgesia - based on epigraphic experiences - in the field of obstetrics with the help of essential oils

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Abstract

It is well known that essential oils have diverse biological activities, some of them possess analgesic effect. Essential oils may have supportive role in the field of gynaecology. In our study we focused on the analgesic activity of essential oils and their mixture during delivery. Our experiences were obtained from a Hungarian Hospital (Imre Saint Hospital, Budapest) involving pregnant women, obstetrician-gynaecologists and midwives. Our presentation will focus on:

1. The supporting role of essential oils during pregnancy: Solving of problems and symptoms during pregnancy (e.g.: stress, anxiety, fear, bloating, vomiting, oedema) with essential oils. The regular, gentle massage will help to the mothers to get uncomplicated births.

2. Effective analgesia during childbirth: Essential oils during delivery can alleviate the fear, the tension, help inward rotation, prepare the body and the patient's soul for birth, thereby reducing pain.

3. Essential oils can restore physical and mental balance after birth: they provide unmatched help in post-natal uncertainty, problems that appear during this period, such as low breast milk, painful breasts, slowly regenerating perineum, anxiety, and partner tensions.

According to our observation it is concluded that aromatherapy has supportive role in the obstetrics-gynaecology, mainly in reducing pain and symptoms during and after delivery.

Keywords: Pregnancy, childbirth, essential oils.

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OP-17 Essential oil blends as preventive feed supplement for animal health

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Abstract

Essential oils (EO) are natural extracts possessing numerous biological properties for human health and are potential candidates for the improvement of animal health[1]. The objective is the preparation of optimized essential oil blends (EOB) with enlarged ranges of activities, according to previous results obtained on single EO.

Three EOB have been tested *in vitro* for their antibacterial and anti-biofilm activities against three bacteria involved in bovine mastitis *Streptococcus uberis*, *Streptococcus agalactiae* and *Streptococcus disgalactiae*. Minimal inhibitory and bactericidal concentrations (MIC – MBC) were established using a broth microdilution assay. A tetrazolium salt (MTT) assay was then performed on biofilms formed by each strains to determine their susceptibility. EOB1 was the most performant blend, reducing the amount of viable biomass at sub-MIC concentrations. Concentrations of 625 µg/ml, 500 µg/ml and 25 µg/ml led respectively to an inhibition of biofilm formation of 72.58 ± 5.14 % for *S. uberis,* 66.03 ± 2.92% for *S. agalactiae* and 81.35 ± 5.95% for *S. dysgalactiae*. This activity holds on the presence of cinnamaldehyde, as it was previously observed for EOB2, who demonstrated its effectiveness against the biofilm of *Pseudomonas aeruginosa*[2]. Antioxidant properties were evaluated for each blend using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and *β*-carotene bleaching assay. EOB3 developed the highest DPPH· scavenging activity with an IC_{50} = 10.03 ± 0.1 µg/ml compared to Clove EO (*Syzygium aromaticum* (L.) Merrill & Perry, Myrtaceae) (IC_{50} = 5.3 ± 0.1µg/ml). According to the *β*-carotene bleaching assay, each blend developed equivalent lipid protective activities, inhibiting lipid peroxidation with values ranging from 69.22 ± 0.84% to 77.01 ± 6.28%. EOB2 developed the highest anti-inflammatory potential by inhibiting 15-lipoxygenase with an IC_{50} = 5.82 ± 1.23 µg/ml, close to IC_{50} of clove EO (5.25 ± 0.62 µg/ml).

According to these results, EOB1 seems to be the most adapted blend, efficient in different biological area and products based on these mixtures of EO could allow animals to face common pathologies. Blending essential oils permit to obtain active natural ingredients cumulating antimicrobial, antioxidant and anti-inflammatory activities, without significant loss of the active concentration when compared to the single EO.

Keywords: essential oil blends, antibacterial, anti-biofilm, antioxidant, anti-inflammatory.

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OP-18 Determination of slow released essential oil components from nanoparticles applied in citrus postharvest disease management

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Abstract

Essential oils (EOs) isolated from aromatic plants are biodegradable and their complex compositions impede the development of fungal resistance. They have been proven to inhibit or reduce green mould (*Penicillium digitatum*) and sour rot (*Geotrichum candidum*) on citrus fruits. The *in vitro* and *in vivo* uses of their components for postharvest control have been investigated. Since EOs are highly volatile, encapsulation protects them from degradation that is stimulated by environmental factors and allows for controlled release of the active volatile components of the oils [2, 3]. However, reliable quantitative techniques to measure the concentrations of active ingredients in the headspace are lacking. Studies to determine the efficacies of EOs in packhouse environments rely on accurate quantitative data.

In this study, lemongrass, spearmint and thyme oil were encapsulated using various substrates, to produce essential oil nanoparticles (EO-NP). These encapsulated oils were characterized and their efficacy against *Penicillium digitatum* and *Geotrichum candidum* (*in-vivo* and *in-vitro*) was investigated. A mobile gas chromatography (GC) system for headspace analysis was designed and constructed. This system comprises a flame ionisation detector, multi-port gas sampling valve with loop, temperature controlled water-bath and gas-coupled ports for thermal desorption. Fruit can be placed inside a temperature-controlled, gas-tight holder, permitting headspace samples to be taken after specified intervals for analysis. The temperature can be reduced to below zero and the gasflow through the sample vessel can be regulated to mimic various packhouse scenarios. Three options are available for headspace analysis i.e. direct headspace, solid phase microextraction and the use of thermal desorption traps. Essential oil volatiles from the oil-encapsulates were analysed using this GC modular system, to determine the quantity of active volatile components released over time.

Keywords: *Penicillium digitatum*, *Geotrichum candidum,* essential oil-nanoparticles, mobile gas chromatography system.

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Poster presentations

P-01 Comparison of *Foeniculum vulgare* MILLER essential oil and commercial oil samples from Turkey

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Abstract

The *Foeniculum vulgare* MILLER oil is used for many purposes including the treatment of colic babies (Alexandrovich et al., 2003). The *F. vulgare* fixed and essential oils are being sold in pharmacies as well as herb markets in Turkey. Unfortunately, in many cases *F. vulgare* is regarded as an edible plant and pharmaceutical products derived from this plant such as essential oils are not monitored effectively. In the present study essential oil contents of fruits of *F. vulgare* obtained from two different herb markets (FV1, FV2) and three samples of essential oil obtained from the pharmacies (S1, S2, S3) were compared in terms of their essential oil composition. According to the results FV1, S1, S2, S3 oils were characterized with (*E*)-anethole (60.9-42.8%), limonene (10.6-4.4%) and methyl chavicol (9.7-4.9%) respectively. Additionally, FV1, S2, S3 oils contained fenchone (17.6-3.6%) and α-pinene (8.3-0%) respectively. Also, S1 oil contained pulegone (8.9%) and S2 oil contained α-phellandrene (6.5%) in considerable amount different than other oils. Unlike others, FV2 oil contained methyl chavicol (58.7%), limonene (15.2%) and fenchone (7.8%) as major components.

The present study clearly points out that chemical composition of *F. vulgare* commercial oil samples obtained from the pharmacies and from herb markets have considerable differences in their composition. One of the oils (FV2) contained methyl chavicol in a large quantity and another (S1) contained pulegone in large quantity, which are considered to be carcinogenic and hepatotoxic compounds respectively (Smith et al., 2002; Sulivan et al., 2002).

Keywords: *Foeniculum vulgare*, essential oil, commercial oils, (*E*)-anethole, methyl chavicol.

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P-02 Variations of essential oil contents and components of 16 wild *Salvia* species and propagated clones

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Abstract

In the study 16 different *Salvia* species were collected from 23 locations in flora of Hatay, Turkey. These species are; *S. pilifera* Montbret et Aucher Ex Benth, *S. aucheri* Benth., *S. glutinosa*, *S. verbenaca*, *S. verticillata* subsp. *amasiaca*, *S. tomentosa* Miller, *S. microstegia* Boiss. & Amp; Heldr., *S. multicaulis* Vahl., *S. aramiensis* Rech. Fil., *S. sericeo-tomentosa* Rech. *S. fruticosa* Miller, S. cassia Samuelss Ex Rech Fil., *S. palaestina* Benth., *S. sclarea* L., *S. virgata* and *S. viridis* L.. *Salvia officinalis* L. were also added for field experiments as a control group. Essential oil contents and components of species were determined during two years both with cultivated clones and wild species from natural flora.

The highest essential oil content among the species from natural flora were found in *S. fruticosa* and *S. aramiensis* with 3.65% and 3.06%, respectively. The highest essential oil content from cultivated species were found in the same species with 2.50% and 2.30%, respectively. In general essential oil contents were found lower in cultivated plants.

Considering the essential oils components in general, main components found to be as eucalyptol, β-pinene and α-pinene. Highest Eucalyptol content found in *S. aramiensis* and *S. fruticosa* species. However, different main components were identified in different sage species.

Keywords: Sage, essential oils, eucalyptol, *S. fruticosa*, sage, *S. aramiensis*.

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P-03 Essential oils and antioxidant activity of hemp (*Cannabis sativa* L.) cultivated in Lithuania

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Abstract

Essential oils of industrial hemp possess analgesic, anti-inflammatory, sedative, antidepressant, antimicrobial, muscle relaxant and anti-carcinogenic properties. The oils significantly inhibit the growth of some food-borne pathogens and phytopathogens, thus can be applied to control food spoilage (Nissen, L. 2010).

Cannabis essential oils were obtained by hydro-distillation method (2.5h) from different plant parts during various vegetative stages. GC-MS analyses were performed on a chromatograph Shimadzu GC-2010 PLIUS interfaced to Shimadzu GC-MS-QP2010 ULTRA mass spectrometer and fitted with Rxi-5MS (30mx0.25mmx0.25 mm) capillary column. Three major constituents were found to be: *α*-pinene, *trans*-caryophyllene and *α*-humulene in inflorescences and leaves essential oils. Insignificant amounts of cannabidiol were found in all oil samples. Radical scavenging activity and potential antioxidant properties of the oils were evaluated by DPPH assay and cyclic voltammetry, respectively (Sochor, J. 2013).

Table 1. Essential oil antioxidant activity

EO nr.	DPPH, TEAC mM
1	11,144 ± 0,103
2	14,033 ± 0,136
3	19,227 ± 0,103
4	20,311 ± 0,171

1-inflorescences eo,
2-leaves eo at blooming,
3,4- leaves eo at seed stage

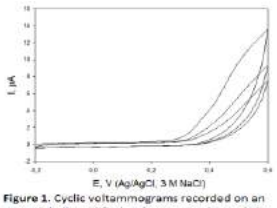

Figure 1. Cyclic voltammograms recorded on an essential oil-modified carbon paste electrode in phosphate buffer at pH 7.2. Potential scan rate 50mV/s.

Keywords: *Cannabis sativa* L., essential oils, antioxidant activity, DPPH, cyclic wave voltammetry.

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P-04 Essential oils from the plant parts of *Peucedanum oreoselinum* and *Peucedanum austriacum* collected in Austria

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Abstract

Peucedanum oreoselinum and *P. austriacum* are two umbelliferous plants occurring in the low mountain range of Central and Eastern Europe (Tutin, 1968). The plants were collected in Carinthia and Lower Austria, respectively. The essential oils from the different dried plant part (stems, leaves, inflorescences and fruits) were isolated by hydrodistillation and analysed by GC/MS and GC/FID. *P. oreoselinum* oils of all plant parts were characterized by α-pinene (15.9-32.8%), β-pinene (18.0-21.1%), limonene (8.4-23.1%) and sabinene (3.0-16.2%) and were low in sesquiterpenes. Fruits contained also γ-terpinene (25.6%). Terpinolene and myrcene were present in lower amounts. In contrast fruits of the same species collected at various sites near Vilnius (Lithuania) had 44.1-82.4% limonene as main oil compound (Motskute and Nivinskene, 1999). The main oil compounds α-pinene, sabinene, β-pinene and limonene were also present in the oils from the closely related *P. cervaria* (Chizzola, 2012).

P. austriacum stem oil had caryophyllene oxide (23.3%) and α-pinene (18.9%) as main compound followed by *Z*-β-ocimene (7.8%), β-caryophyllene (6.6%) and myrcene (4.7%), while in the leaves caryophyllene oxide (19.9%), germacrene D (19.8%) and β-caryophyllene (16.3%) prevailed. The fruit oil, finally, had 75.0% β-phellandrene and low amounts of α-phellandrene (3.6%), α-pinene (2.6%), β-caryophyllene (2.7%), germacrene D (2.2%) and spathulenol (2.2%). A fruit oil from Serbia had 45.2% β-phellandrene and 10.1% β-pinene while the leaf oil from plants of the same site displayed caryophyllene oxide (23.3%), germacrene D (12.2%) and ß-caryophyllene (10.2%) as main compounds (Jovanovic et al. 2013).

Keywords: *Peucedanum oreoselinum*, *Peucedanum austriacum*, limonene, β-phellandrene, essential oil.

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P-05 Essential oil composition from hop cultivated at Southern Brazil

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Abstract

Hop has economic importance around the world mainly because of the use bythe brewery companies. Althoughthe hop production in Brazil is incipient, the cultivation has grown and the evaluation of genetic materials adapted to the Brazilian environmental conditions is needed.The main objective of this work was to evaluate the cone aromatic profiles of hopgrown in Southern Brazil. Mature cones of hop cultivated in two regions of São Paulo state were harvested and the essential oil samples extracted by hydrodestillation and analyzed by gas chromatography-mass spectrometry. The results of the volatile fraction of all samples showed myrcene (10.79-16.29%) and α-humulene (22.7-29.47%) as the main constituents. β-selinene (10.79-11.99%)andγ-muurolene (9.96-10.73%) were also identified in allof the evaluated samples.The results showed thatphytochemical variability among the samples according to the different environmental conditions. The improvement of the variability of the genetic materials adapted to different regions could result on increase of cultivated areas in the country.

Keywords: Humulus lupulus L., chemical diversity, aromatic profile, myrcene, humulene, β-selinene, γ-muurolene.

P-06 Evaluation of the essential oil properties of lavender (*L. angustifolia* and *L.* x *intermedia*) cultivars originating from different growing areas of Hungary

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Abstract

Lavandula species are valuable and widely cultivated essential oil (EO) producing medicinal plants, mainly applied in the households and by the perfumery industry. The appearance of inflorescences in *Lavandula* plantations in autumn is not common in our region and the quality of the drug has not been investigated thoroughly. Second flowering is likely to originate in the rainy summer season of 2016. In our recent research we have focused on the effects of the growing areas, cultivars, and different plant organs (leaves, flowers) on the EO content and composition. Seven cultivars of *L. angustifolia* and three ones of *L. x intermedia* were involved in the study. Samples were collected at four distinct locations in Hungary (Budapest, Tihany, Dörgicse, Szomód) in the autumn period of 2016. Essential oil was isolated by hydrodistillation in a Clevenger type apparatus and analyzed by GC/MS, using dried flower and leaf samples separately. Concerning EO content, the highest overall value has been found in flower samples of *L. x intermedia* 'Grosso' originating from Dörgicse (4.562 ml/100g). When comparing the leaf samples of different origin, the Hungarian hybrid lavender variety 'Judit' (Budapest) was proven to be the best (0.958 ml/100g). In Szomód the EO obtained from flowers of 'Hidcote' lavender outstanding percentage of linalyl acetate (50.09 %) was measured. Leaf volatiles from the naturalized plantation of the Tihany Peninsula showed special composition, including higher ratios of 1,8-cineole, caryophyllene-oxid, p-cimole and o-cimole, in comparison to *L. angustifolia* leaf samples collected in Budapest. Moreover, elevated levels of 1,8-cineole, p-cimol and tau-cadinol were measured in the leaves of cultivars grown in Szomód. In Dörgicse several French cultivars showed unusual leaf EO composition as well: linalool and linalyl acetate percentages were detected at 'Mailette' (6.01 and 8.39%) 'Beate' (2.18 and 3.01%), 'Aromatico Silver' (1.0 and 0.6%) and at 'Hidcote Blue' (1.68 and 4.56%), respectively. We continue our studies in the full bloom period and our work will be completed by the analyses of soil and weather conditions.

Keywords: *Lavandula* species, *Lavandula* cultivars, growing area, essential oil composition, GC-MS.

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P-07 Variation in the volatile oils of *P. atriplicifolia* Benth. and its two cultivars

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Abstract

Perovskia atriplicifolia Bent. grown in Afghanistan and its two cultivars, 'Little Spire' and 'Blue Spire' grown in Japan were evaluated for their essential oils chemical composition difference. The essential oils obtained in mother plant and its two cultivars were analyzed by gas chromatography-mass spectroscopy. Methyl n-decanoate was used as internal standard. The main component of *P. atriplicifolia* Benth. were *α*-myrcene (16.57%), 1,8-cineole (10.69%), borneol (8.52%), *β*-caryophyllene (8.30%) and *α*-caryophyllene (7.42%). The essential oil from 'Little Spire' were rich in 1,8-cineole (17.79%), camphor (14.28%), D-limonene (10.93%), *δ*-3-carene (6.76%) and *α*-pinene (6.64%). In the essential oil from 'Blue Spire', however, the main components were 1,8-cineole (15.72%), *β*-caryophyllene (10.32%), *α*-caryophyllene (9.35%), borneol (9.32%), camphor (7.3%) and *δ*-3-carene (7.10%). In addition, *trans*-longipinocarveol (0.18%) was only present in the essential oil of 'Blue Spire', while *α*-myrcene (16.57%) was present in the essential oil from the mother plant. The result indicated that cultivar and environmental condition had significant effect on the quality of the essential oil of *P. atriplicifolia*.

Keywords: *P. atriplicifolia*, Little Spire, Blue Spire, compositional difference.

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P-08 The essential oil of *Myrtus communis* L. from Montenegro contains a cyclic polymethylated polyketide

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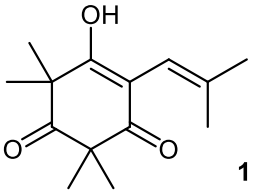
Abstract

Myrtus is a genus of three recognized species from the Myrtaceae plant family.[1] *Myrtus communis* L., known as true myrtle, grows spontaneously in the Mediterranean area and is widely distributed (Spain, France, Morocco, Tunisia, Montenegro, Italy etc.). Up to now a number of researchers have analyzed the composition of the essential oil of this evergreen shrub, and these studies have revealed a great variation in the content and identity of its volatile secondary metabolites. Herein we present the results of detailed analyses of the essential-oil constituents obtained from *M. communis* leaves, collected during the summer period from rocky coasts of the peninsula Luštica, Montenegro (the second report ever). Plant material yielded a transparent, yellowish fragrant essential oil (2.2%, w/w). Subsequent detailed GC and GC-MS analyses enabled the identification of more than 60 constituents, among which 1,8-cineole (28.4%), linalool (18.3%), α-pinene (16.6%), geranyl acetate (6.6%), α-terpineol (6.3%) and linalyl acetate (4.2%) were the major ones. Among the minor contributors, a constituent identified as 5-hydroxy-2,2,6,6-tetramethyl-4-(2-methylprop-1-en-1-yl)cyclohex-4-ene-1,3-dione (**1**) caught our attention. The mentioned compound and the related tautomers were previously reported on as synthetic intermediates in the synthesis of (±)-calliviminones A and B, G-factors (natural cyclic peroxides extracted from various Myrtaceae), and myrtucommulones A and B[2]. This is the first report of this cyclic polymethylated polyketide from a natural source.



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Keywords: *Myrtus communis* L., Myrtaceae, essential oil, polyketide, natural occurrence.

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P-09 Essential oil characterization of the *Thymus vulgaris* L. vegetative collection from the Plant Germplasm Bank of Cuenca (CIAF Albaladejito-IRIAF)

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Abstract

Thymus vulgaris L. is an essential oil bearing species distributed in the Mediterranean region. In Spain it is particularly present in the calcareous soils of the eastern half of the Iberian Peninsula. It has been broadly studied by its essential oil production and bioactivities. In these studies, wide chemical polymorphism has been described. Its different chemotypes were classified by Llorens-Molina & Vacas (2017) as phenolic (thymol and carvacrol) and non-phenolic (geraniol, α-terpineol, thujan-4-ol, linalool and 1,8-cineole). Another non-phenolic chemotype (camphor/camphene) was described in Eastern-Morocco populations (Imelouane et al., 2009). 1,8-cineole type has been reported to frequently occur in Spanish wild populations (Stahl-Biskup & Sáez, 2002; Jordán et al., 2006; Torras et al., 2007; Llorens-Molina & Vacas, 2016). Furthermore, a mixed 1,8-cineole/camphor chemotype was also documented (Stahl-Biskup & Sáez, 2002). In our study, 57 populations of *T. vulgaris* from Castilla-La Mancha region (Central Spain) were propagated from seeds collected in their original locations and cultivated in the experimental fields of the Bank of Plant Germplasm of Cuenca (vegetative collection) in the context of an ex-situ conservation programme of native aromatic species. Aerial parts from these cultivated materials (in advance fruit maturation phenological stage) were subjected to hydrodistillation and GC-MS analysis of the essential oils. 1,8-cineole was a major compound in most of the samples but showing great variability among them (4.8-46.4%). The concentration of this monoterpene showed a frequency distribution very close to a normal Gaussian distribution unimodal type, which difficults genotypical discrimination based on this factor**.** Multivariate statistical analyses were performed to avoid arbitrariness in genotypes definition. As a result, populations were classified into three different genotypes: 1,8-cineole; 1,8-cineole/camphor and camphor/camphene. These genotypes did not present a clear geographical distribution pattern regarding the original locations of the populations. On the contrary, they appeared interspersed throughout the prospected area.

Keywords: *Thymus vulgaris*, essential oil, chemotype, 1,8-cineole, camphor.

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P-10 Variability in the essential oil composition of cultivated populations of *Rosmarinus officinalis* L.

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Abstract

Rosmarinus officinalis L. is a Mediterranean species which has been grown around the world as ornamental and aromatic plant. In Spain, it is mainly cultivated in the Mediterranean coast and Southern Spain but wild collection still having an important contribution to the essential oil production both in these regions and Central Spain. The lack of selected and well characterised plant material for cultivation together with the existence of different chemo/eco/genotypes (Salido et al., 2003; Angioni et al., 2004; Jordán et al., 2013) leads to heterogeneous productions in quantity and quality. In this sense, as a result of a prospection of 331 Spanish wild populations of *R. officinalis,* 13 populations were selected on the basis of the variability of composition of their essential oils. These selected populations were vegetatively propagated and cultivated in two experimental fields under different soil and climate conditions. Essential oils from the cultivated material in full-bloom phenological stage were extracted and characterised by gas chromatography during two years. Multivariate statistical techniques were performed to study the variability of the data set. The populations showed great stability regarding essential oil composition independently of the cultivation year and the location of the experimental field and maintained the main features of the original wild populations. They were grouped in three different clusters which are clearly related to the geographic origin of the population: C1 included seven populations prospected in the Eastern area of the Iberian Peninsula and characterized by an oil rich in 1,8-cineole, α-pinene, camphene and borneol. C2 comprised four populations from the mountain regions of Central Spain and whose essential oils were the richest in camphor and limonene. Finally, C3 included two populations, chemically very different from the rest in which β-pinene + myrcene clearly appeared as the main compounds and that could be considered as a different chemotype. This characterization of populations of *R. officinalis* serves as a basis for a future program of plant breeding and selection with the goal to offer a more homogeneous plant material to the farmers.

Keywords: *Rosmarinus officinalis,* essential oil, 1,8-cineole, camphor, population.

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P-11 Intraspecific variability of sweet basil (*Ocimum basilicum* L.) in special respect of essential oil content and composition

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Abstract

Sweet basil is a one-year-old plant from *Lamiaceae* family. The herb of this plant, rich in essential oil, is used as a spice in a fresh form and as dried raw material in food and cosmetics industry. The herb of this plant reveals different pharmacological activities, e.g. spasmolytic, antimicrobial, antioxidant and diuretic. Thus, it can be applied in gastrointestinal disorders and loss of appetite. The aim of the study was to determine the intraspecific variability of the species in terms of total content and composition of essential oil, as well as selected developmental traits. Objects of work were 6 sweet basil populations originated from Romania and cultivated in experimental field of WULS-SGGW, Poland. Developmental characteristic (i.a. plant height, number of shoots, shape, length and width of leaf, fresh and dry mass of herb) was carried out according to descriptors evaluated in Department of Vegetables and Medicinal Plants, WULS-SGGW. The total content of essential oil was determined according to EP8th, while its composition was performed by GC-MS and GC-FID. Morphological observations and harvest of raw material was carried out at the beginning of plants blooming.

Obtained results indicate that investigated populations differed significantly both in respect of developmental and chemical traits. The height of plants ranged from 51.0 to 71.6 cm, the fresh mass of herb from 420.74 to 635.11 g per plant. Populations differed in other morphological features, as well. The total content of essential oil varied from 1.25 to 2.00%. Among identified compounds of essential oil, the dominants were: linalool (42.89-72.48%), estragol (0.41-25.11%) and 1.8 cineole (0.60-7.01%). Eugenol, α-terpineol and γ-terpinen were also present in considerable amounts. Such variable plant material may be used in future investigations to provide interesting, cultivated forms of sweet basil.

Keywords: sweet basil, biodiversity, essential oil, linalool, estragol

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P-12 TLC-GC/MS Method for identifying and selecting valuable essential oil chemotypes from wild populations of *Mentha longifolia* L

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Abstract

Wild populations can be considered resources of natural biodiversity for selecting valuable genotypes in order to obtain well-characterized cultivars adapted to changing market demands of essential oils (Bernath, 2001). Often, their high intrapopulational variability requires preliminary screenings based on a significant number of individuals and, therefore, simple and fast methods to identify EO profiles. For this purpose, a TLC-GC/MS method was proposed and applied, according the following steps: (1) Stolons of 50 individuals coming from a wild population located in Calamocha (Spain) were sown and bred in greenhouse conditions before growing in experimental plots; (2) Individual sampling and extraction of leaves were performed to determinate TLC individual profiles according Wagner & Bladt, (2002); (3) TLC identification was validated by preparative TLC and GC/MS analysis of discriminant spots; (4) The fresh material belonging to each defined TLC profile collected in full flowering stage was subjected to SDE extraction and GC/MS analysis. Five chemotypes were characterized (Sharopov *et al*., 2012; Llorens-Molina *et al*., 2015): A (piperitone and piperitenone oxides); B (piperitone oxide + pulegone); C (□-terpineol acetate + carvone acetate); D ((*E*) – dihydrocarvone) and E; (pulegone + isomenthone + menthol). (5) This method was applied to individuals coming from another population located in Tuéjar (Spain). All of them could be clearly identified as belonging to last chemotype, which was afterwards confirmed by GC/MS analysis.

Keywords: TLC, *Mentha longifolia*, essential oil, chemotype, biodiversity.

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P-13 Chemical composition of the essential oils from the aboveground parts of *Erigeron annuus* (L.) Pers. and *Erigeron canadensis* L. growing in Serbia

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Abstract

Erigeron annuus (L.) Pers. (daisy fleabane) and *Erigeron canadensis* L. (Canadian fleabane) are widespread herbaceous annual plant species from the family *Asteraceae*, that originate from North America, and represent adventive invasive weed species in Serbia. They have been used in traditional medicine as diuretics, as well as for the treatment for gastro-intestinal problems, such as diarrhoea and dysentery [1]. Both taxa had been the subject of several phytochemical studies, but up to this point only a few studies dealt with the composition of their essential oils [2,3]. Herein, we analyzed (by GC and GC-MS) the composition of the essential oils from fresh aboveground parts of *Erigeron annuus* (L.) Pers. and *Erigeron canadensis* L. in the end of the flowering phase from wild-growng populations in Serbia (Oblačina lake, near Niš). A total of 61 and 122 components were identified in *E. annuus* and *E. canadensis* respectively. The small amount of a greenish essential oil (0.105%) obtained by hydrodistillation of *E. annuus* had polyacetylenes ((*Z*)-lachnophyllum ester (8-dihydromatricaria ester; 25.2%) and (2*Z*,8*Z*)-matricaria ester (3.7%)) as the major constituents, along with germacrene D (38.6%). Hydrodistillation of *E. canadensis* yielded a somewhat larger amount of a yellow essential oil (0.21%) with a higher content of polyacetylenic compounds. The major constituents were identified as (2*Z*,8*Z*)-matricaria ester (22.29%), (*Z*)-lachnophyllum ester (8-dihydromatricaria ester; 7.04%) and (*E*)-α-bergamotene (5.87%). C_{10} polyacetylenes seem to be typical of Astereae, with matricaria ester-related compounds as the most widespread ones.

Keywords: *Erigeron annuus* (L.) Pers., *Erigeron canadensis* L., matricaria ester, lachnophyllum ester, polyacetylenes.

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P-14 Variability of *Artemisia alba* Turra volatile profile

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Abstract

Artemisia alba Turra, Asteraceae, is a sub-Mediterranean species, widespread in South-Eastern Europe, Italy and Spain, at altitudes between sea level and 1400 m [1]. Its aerial parts have been traditionally utilized in folk medicine as a stomach digestive and tonic in the form of a decoction [2]. When compared to some other representatives of the same plant genus, the essential oil of this taxon was only occasionally phytochemically studied [3]. Nonetheless, *A. alba* volatile profile appears to be susceptible to changes in environmental conditions [1]. Thus, to get a better insight into the external-factor-related variability of *A. alba* volatile profile, we have studied the chemical composition of the essential oils obtained from aerial parts of two different populations of these species. Plant material was collected during anthesis, from two different locations (I – Srećkovo vrelo ravine, 422 m above sea level: EO-I; II – Devojački grob pass, Suva planina mountain, 1317 m above sea level: EO-II), and during three different years (2008, 2014 and 2016). The essential oils were isolated by hydrodistillation in a Clevenger-type apparatus and were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The yields of oils ranged from 0.14% to 0.33% (w/w). The analyses showed that the two populations differed not only in the relative amount, but also in the identity of the dominant volatiles: EO-I – artemisia ketone (13.2-25.8%), camphor (11.8-15.4%), 1,8-cineole (3.5-9.0%), eremophilone (2.6-5.2%) and artemisia alcohol (4.6-5.0%); EO-II – *cis*-pinocamphone (17.0-20.2%), camphor (15.1-19.6%), 1,8-cineole (8.7-9.6%) and *cis*-chrysanthenyl acetate (4.5-6.9%). Although the volatile profiles were investigated over a 8-year long period, intrapopulational differences (plant material harvested during different years) in the chemical profile of *A. alba* essential oil were much less pronounced than the interpopulational ones. This suggests that *A. alba* volatile profile is highly dependable on external factors and that the two analyzed populations correspond to two distinct chemotypes of the studied taxa.

Keywords *Artemisia alba* Turra, Asteraceae, essential oil, environmental conditions.

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

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P-15 Volatile constituents of extracts from selected Brassicaceae plants

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Abstract

This study represents the continuation of our research on chemistry of volatile compounds from Croatian wild-growing Brassicaceae plants. Brassicaceae plants are known as a source of sulfur-containing secondary metabolites, glucosinolates. During the extraction of glucosinolates their decomposition often occurs resulting with the isolation of volatile sulfur- and nitrogen-containing compounds. So, volatile sulfur- and nitrogen-containing compounds are not naturally present in intact plant but are formed mostly by enzymatic breakdown or thermal degradation of naturally occurring glucosinolates. These volatile compounds have been assigned for various biological activities.

The volatile constituents of extracts from four plants, namely *Aurinia leucadea*, *Cardaria draba*, *Capsella rubella* and *Calepina irregularis*, were investigated. All extracts were prepared by enzymatic hydrolysis achived by endogenous enzyme myrosinase, *i.e.* autolysis, followed by solvent extraction at room temperature. The analyses of the extracts were performed by GC-FID/MS.

The identification of volatile compounds revealed that sulfur- and nitrogen-containing compounds were qualitatively and quantiatively dominating constituents in all extracts. The major compound in *Aurinia leucadea* extract was 4,5-epithiopentanenitrile (50%), followed by 5,6-epithiohexanenitrile (18.5%) and but-3-enyl isothiocyanate (16.2%). *Cardaria draba* extract was characterized by a high percentage of 4-(methylsulfinyl)butyl isothiocyanate (57.3%), known by its trivial name sulforaphane, and ethyl isothiocyanate (26.5%). The main compounds among volatiles of *Capsella rubella* extract were 3,4-epithiobutanenitrile (44.1%) and ethyl isothiocyanate (29.4%). Two sulfur-containing volatile compounds, 3-(methylthio)propyl isothiocyanate, known as iberverin, and 3-(methylsulfynyl)propyl isothiocyanate, known as iberin, were present in almost the same amounts in *Calepina irregularis* extract, 41.0% and 40.0%, respectively.

Keywords: Volatiles, *Aurinia leucadea*, *Cardaria draba*, *Capsella rubella*, *Calepina irregularis*.

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P-16 Chemotaxonomy of the genus *Tordylium* L. based on the essential-oil chemical composition: the case of *Tordylium maximum* (Apiaceae)

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Abstract

Tordylium L. (syn.: *Hasselquistia* L., *Condylocarpus* Hoffm., *Ainsworthia* Boiss., *Synelcosciadium* Boiss.) is a medium-sized genus of the family Apiaceae, with an unresolved infrageneric taxonomy; the taxa are characterized by an annual habit, 1-3 pinnate leaves, dorsally compressed mericarps, and thickened mericarp margins.[1,2] In this study we analyzed the chemical composition of the essential oil obtained from the schizocarps of *Tordylium maximum* L., growing in Serbia, and used these results and those previously published for other *Tordylium* spp. to assess the applicability of such data in chemotaxonomic purposes by means of multivariate statistical analyses. The plant material of *T. maximum* was collected in June 2016, in the village Podrimce, near Leskovac, Serbia. The essential oil of *T. maximum* was characterized by the presence of 1-octanol (4.1%), octyl isobutanoate (18.0%) and octyl 2-methylbutanoate (47.9%). Multivariate statistical analyses included the data from 24 different samples of the genus *Tordylium* L.: *T. pustulosum* (four different habitats), *T. aegyptiacum* (two different habitats), *T. lanatum* (two different habitats), *T. syriacum* (four different habitats), *T. pestalozzae, T. trachycarpum* (two different habitats), *T. hasselquistiae, T. elegans* (two different habitats), *T. ketenoglui, T. apulum* (four different habitats) and *T. maximum*. All mentioned essential oils represented complex mixtures of monoterpenoids, sesquiterpenoids, diterpenoids, and fatty-acid/carotenoid derived compounds. Most species contained large relative amounts of *α*-bisabolene, *β*-caryophyllene, caryophyllene oxide, germacrene D, *α*-humulene, hexadecanoic acid, 1-octanol and *n*-octyl esters (isobutanoate, 2-methylbutanoate, hexanoate and octanoate). The results of the performed statistical analyses are in general agreement with the currently accepted taxonomical status (section circumscription) of the mentioned species within the genus *Tordylium* L. *Tordylium maximum* from Serbia is a typical representative of this genus with regard to its volatiles.

Keywords: Essential oil, multivariate statistical analyses, *Tordylium maximum*, chemotaxonomy.

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P-17 Chemical composition of the essential oils of Portuguese *Lavandula pedunculata* (Miller) Cav. flowers

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Abstract

Lavandula genus were being used for centuries either dried or as essential oils for a variety of therapeutic, cosmetic, fragrance and condiment proposes[1]. The existence of eight species and subspecies in the Iberian Peninsula is referenced by Morales 2010 [2]. Some of them found in Portugal such as *Lavandula pedunculata* (Miller) Cav.

In this work flowering aerial parts from *L. pedunculata* wild populations collected at ten different places of Portugal (09838-Vila Viçosa; 09845-Évora; 10369-Elvas; 10372-Aronches; 10378-Marvão; 10391-Évora; 10400-Portalegre; 10412-Castelo de Vide; 10418-Nisa and 11290-Bragança) were investigated. The essential oils were isolated by hydrodistillation according the method of pharmacopeia. The essential oils yields ranged between 0.5-0.8% (v/w). Composition of essential oils was established by Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. A total of 22 compounds were identified. All the samples were characterized by the dominance of fenchone (18.9 – 53.7%) and camphor (8.7 – 33.8%) and belong to the binary chemotype fenchone/camphor, but two of the accessions (09838-Vila Viçosa and 09845-Évora present camphor as main component (32.9 and 32.3%) and the otheraccessions present fenchone as the main component (34.7-68.8%).

Keywords: Essential oils, chromatography, *Lavandula pedunculata*, hydrodistilation, chemotype.

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P-18 The essential oil of *Melissa officinalis* L. (Lamiaceae): composition, structural elucidation and synthesis of new constituents

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Abstract

The perennial herb *Melissa officinalis* L. (Lamiaceae), commonly known as lemon balm, has been extensively phytochemically studied. Lemon balm, native to southern Europe, the Mediterranean region and western Asia, has been traditionally used to reduce stress and anxiety, promote sleep, improve appetite, and ease pain and discomfort from indigestion. Detailed GC, GC-MS and GC-FID analyses of *M. officinalis* essential-oil samples (isolated from the aerial parts of a wild-growing lemon balm population (MO-1) and a commercial essential-oil sample (MO-2), purchased from a local producer, Siempreviva, Niš, Serbia) allowed a successful identification of, in total, 143 essential-oil constituents that represented 98.7 – 99.1% of the total GC peak areas. Terpenoids comprised 93.6 – 94.8% of the essential oils with (oxygenated) monoterpenoids as the most abundant compound class (82.1 – 82.8%). The oils were mainly comprised of acyclic monoterpenoids with citronellal (21.2 – 21.8%), neral (17.8 – 18.4%), and geranial (22.9 – 23.5%) as the major constituents. Interestingly, monoterpene hydrocarbons contributed less than 1% of the essential-oil samples. The remaining part of MO samples was comprised of sesquiterpenoids, fatty acid-related and carotenoid-derived compounds 10.8 – 12.7%, 1.0 – 1.4%, and 2.0 – 2.9%, respectively. According to the results, there were, in general, only small quantitative differences observed between the analysed essential-oil samples. Variations of the content were in the range 0.05 – 1.3% (e.g. the content of citronellal, neral, geranial and (*E*)-caryophyllene, as the main essential-oil constituents, varied 0.6 – 1.3% from one essential-oil sample to the other). More than fifty compounds are reported here for the first time as constituents of *M. officinalis* essential oil, including six acetals of two diastereotopic 8-hydroxymenthols with citronellal, neral and geranial (four of these are completely new natural products). The identity of these new acetals was unambiguously confirmed by co-injection of the essential oil samples with synthesized standards and their structure was elucidated by NMR (1D and 2D), IR and MS.

Keywords: *Melissa officinalis*, Lamiaceae, essential oil, acetals, structure elucidation.

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P-19 Essential oil composition of aerial parts and roots of *Artemisia herba-alba* Asso coming from Teruel (Spain)

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Abstract

Essential Oil composition of aerial parts and roots of *Artemisia herba-alba* Asso coming from a wild population located in Teruel (Spain) was studied in full flowering stage. Samples from ten randomly selected individuals were extracted separately for aerial parts and roots by simultaneous extraction distillation (SDE) and analysed by GC/MS and GC/FID. Monoterpenic fraction was found the major one both in aerial parts and roots. Camphor (23.1-67.7 %), $\alpha + \beta$ thujone (1.2-53.9 %), 1,8-cineole (4.2-17.4 %) and camphene (2.5-8.9 %) were found the main compounds in aerial parts showing a high intrapopulational variability. These results are relatively similar to those found in some Spanish samples (Feuerstein, 1988; Salido, 2004) and Tunisia and Morocco populations as well (Boukrich, 2008; Imelouane, 2010; Bellili, 2016).

Unlike previous reports (Bellili, 2016), roots oil were found composed in general by a high amount of bornyl acetate (49.1-88.5 %) and myrtenal (1.4-8.1 %, and 29.3 % in one sample). This predominance of monoterpenic esters agrees with roots oil composition of *Artemisia absinthium* L from nearby populations sharing habitat (Llorens-Molina, 2015). On the other hand, some individuals show divergences because the relative high occurrence of sesquiterpene and diterpene compounds. No evident relationships were found when comparing individual profiles of aerial parts and roots.

Keywords: *Artemisia herba-alba*, aerial parts, roots, essential oil.

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P-20 Effects on growth, essential oil content and composition of *Cymbopogon flexuosus* (DC) Stapf cultivated in solution systems deficient in macro-elements

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Abstract

Essential oils of the species *Cymbopogon* (Poaceae) are of great commercial value as flavors, and fragrances in perfumes and cosmetics. However, knowledge about the agronomic aspects is still incomplete. The aim of the study was to determine which elements effect the growth, essential oil content and composition of the essential oil of *Cymbopogon flexuosus* (DC) Stapf. Acclimated scions were transferred into pots containing either complete nutrient solution or a solution of equivalent composition but lacking one of the elements N, P, K, Ca, Mg or S. The experiment was of completely randomized design, and each of the seven treatments was replicated four times with each replicate comprising three plants. Plants were cultured in the greenhouse under natural light for 90 days, during which time air was supplied to the nutrient system and the nutrient solution was changed each week. Plants were harvested at the end of the culture period and growth parameters (shoot number, leaf, root and total dry matter), contents of essential oils and compositions of leaf were evaluated. The results showed that the composition of the nutrient solution exerted a significant effect on all of the growth parameters and essential oil. With regard to total dry matter, the order of limiting nutrients was K= Mg=N>Ca=P>S for macro-elements. Omission of S induced increases in essential oil content. The major components of the volatile fractions were identified as neral, geraniol, geranial and geranyl acetate, and the proportions of these compounds were affected substantially by the omission of macro-elements.

Keywords: Medicinal plant, hydroponic system, nutrient omission, fertilization.

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P-21 Essential oil content of *Thymus* sp. cultivated in Northern Greece

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Abstract

Thymus cherlerioides Vis., *T. longicaulis*, C. Presl, *T. serratus* Opiz and *T. sibthorpii* Benth. were cultivated at the Laboratory of Conservation and Evaluation of the Native and Floricultural Species (North Greece). After one year of cultivation the essential oils of their aerial parts were analysed on the basis of their yield and quality. GC-MS analyses were performed on a Hewlett-Packard 5973-6890 system operating in EI mode (70eV) using a fused silica HP-5 MS capillary column. Retention indices for all compounds were determined according to the Van den Dool approach (Van den Dool and Kratz, 1963), using n-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries (Massada, 1976) and those described by Adams (2007), as well as by comparison of their retention indices with literature data (Adams, 2007). The main compound of *T. cherlerioides* and *T. sibthorpii* was thymol (ca. 60%, 50% respectively), while of *T. longicaulis* was linalool (ca. 80%) and the major constituents of *T. serratus* were citral (ca.28%) and geranial (ca.38%). The essential oils yields and contents did not show a negative response to cultivation. The results obtained are an important indication of the potential economic utility of cultivated *Thymus* spp. as a raw material and source of useful industrial oil compounds.

Keywords: *Thymus cherlerioides*, *T. longicaulis*, *T. serratus*, *sibthorpii*, cultivation, essential oils.

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P-22 Effect of plant age on the root drug yield and quality of lovage (*Levisticum officinale* W.D.J.Koch)

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Abstract

1-, 2- and 3-year-old lovage populations established from the same genetic material were compared in 2014 cultivating in two growing place (in Soroksár – Pest county and in Nagydorog – Tolna county) in order to determine the influencing role of plant age on the root drug production and quality. Roots were harvested at the beginning of November (5 individuals/plot) and after natural drying we determined their root drug mass, the essential oil content by water distillation according to PhEur and the component spectrum by GC-MS method based on standard compounds and retention indices. The histological examination of roots was carried out by light microscopy where sections were prepared with rotating microtome after ethanol fixation and paraffin embedding.

1-year-old plants had the lowest average root drug mass significantly in both growing site (in Soroksár: 23.5 g/plant, in Nagydorog: 25.5 g/plant) and the highest yields were measured in case of 3-year-old populations (in Soroksár: 93.2 g/plant, in Nagydorog: 125.7 g/plant). 3-year-old plants had higher yields than 2-year-old ones but we couldn't prove the difference statistically in every case. Evaluating our results it can be established that plant age affects the root drug mass significantly: there is a strong, linear, positive correlation between them.

Evaluating the essential oil content of roots we can also ascertain that youngest roots had the lowest average essential oil accumulation (in Soroksár: 0.26 ml/100g, in Nagydorog: 0.18 ml/100g) significantly too, while 3-year-old populations could be characterised by the highest accumulation levels (in Soroksár: 0.55 ml/100g, in Nagydorog: 0.46 ml/100g). 3-year-old individuals had higher root essential oil content than 2-year-old ones but differences didn't prove to be significant. Thus it was established that the essential oil content of roots increases linearly with the age of lovage plants. According to our histological investigations the main reason of this is that in older roots the number of primary and secondary oil ducts increases significantly.

In the essential oil of roots (Z)-ligustilide was detected as main constituent (74.9-91.1%). Further minor compounds were 1-pentyl-cyclohexa-1,3-diene, β-phellandrene, (E)-ligustilide and (Z)-3-butylidenephthalide. In both growing place 1-year-old plants contained the most (Z)-ligustilide in the essential oil, and it's ratio decreased linearly with plant age, but not significantly.

Keywords: Essential oil, histology, lovage, plant age, root, yield, Z-ligustilide.

P-23 Effect of low pressure cold plasma treatment on lemon verbena (*Lippia citriodora* Kunth.) essential oil

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Abstract

Cold plasma is a novel non-thermal method for potential decontamination of medicinal and aromatic plants, however there is not sufficient information about its effects on their active ingredients. Therefore, in the present study, the influence of low pressure cold plasma (LPCP) treatments (1, 3 and 5 min) on the essential oil content of lemon verbena leaves as well as its composition were evaluated on the basis of a completely randomized design with three replications. The essential oil content was determined using hydro-distillation (Clevenger apparatus) and the composition of the extracted essential oils was quantified by using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) techniques. The results showed that essential oil content was reduced by increasing the LPCP treatment duration from 1.2 to 0.9 (% w/w) after 1 and 5 min, respectively. The highest content of monoterpene hydrocarbons (*e.g.,* limonene) and oxygenated sesquiterpenes (*e.g.,* spathulenol and globulol) were also determined in LPCP treated ones, whereas the oxygenated monoterpenes (*e.g.,* citral) content of control was reasonably higher than LCPC. Our findings confirmed that LPCP could significantly affect the essential oil content and composition of lemon verbena.

Keywords: Lemon verbena, Aromatic plant, Cold Plasma, Citral, Essential oil.

Acknowledgments

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Pignata, C., D'Angelo, D., Fea, E. & Gilli, G. (2017). A review on microbiological decontamination of fresh produce with non-thermal plasma. *Journal of Applied Microbiology, 122,* 1-18.

P-24 Effect of growth regulators, light intensity and LED on plant growth and volatiles compounds of *Lippia rotundifolia Cham.*

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Abstract

Lippia rotundifolia Cham. (Verbenaceae) is an endemic plant of the Cerrado, aromatic, characterized by the presence of glandular trichomes in its leaves, rich in monoterpenes. *In vitro* propagation has been used in the multiplication of several species with medicinal properties with difficulty in the conventional propagation and to obtain a homogeneous material. Micropropagation is an alternative to production of medicinal plants; however some physics and chemistry factors can affect plantlet growth and its compounds. The light generally used for in vitro propagation is fluorescent lamps. However, these lamps contain very spread wavelengths that are of low quality for growth and development of plantlets. The influence of growth regulators, different light spectra and intensities were evaluated in an *in vitro* culture and volatiles compound of *L. rotundifolia*. The treatments were: a) different concentrations of benzylaminopurine (BAP), naphthalene acetic acid (NAA), thidiazuron (TDZ) alone or in combination, b) use of light emitting diode (LED) lamps in the white, red, blue, red/blue, 2red/1blue and 1red/2blue wavelengths, c) photosynthetic photon flux of 20, 54, 78, 88 and 110 µmol $m^{-2}s^{-1}$. The growth regulators, quality and intensity of light significantly influenced the in vitro growth and volatiles compounds of *L. rotundifolia*. Concentrations of 1.5 and 2.0 mg L^{-1} of TDZ obtained higher number of shoots. The type and concentration of the growth regulator influenced the content of the compounds *in vitro*. The lowest light intensities (20 and 54 µmol $m^{-2}s^{-1}$) and 2red/1blue presented better results in plant growth. Analysis by HS-GC-MS detected presence of myrcene, limonene and myrcenone in the plantlets developed in different growth regulator, intensity and quality of light. The production of volatiles constituents is highly influenced by the type of growing environment.

Keywords: Volatile fraction, Cytokinins, Auxins, Photon flux densities, Spectral qualities.

Acknowledgments

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P-25 Production and essential oil related parameters of four *Perilla frutescens* (L.) Britt. accessions

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Abstract

Perilla frutescens (L.) Britt. is an aromatic, annual plant from the *Laniaceae* plant family. It is usually used as a medicinal plant in China, Japan and Korea. The species raises more and more interest, mainly because its anti-allergic effect, there is still a lack of experiences about the cultivation of different accessions in Europe.

The aim of the current study is to provide information on behaviour, production, essential oil content and composition of two purple (J3, PS3) and two green (203P, 465P) coloured accessions. Small scale open field experiment was carried out. The plants were harvested at the beginning of flowering in September 2015. As production related parameters, the plant height, fresh biomass, dry mass and leaf ratio were recorded. The essential oil content was measured with a Clevenger-type apparatus according to the VII. Hungarian Pharmacopoeia.

Highest plants were registered in PS3 (81 cm) while the smallest in 203P (65 cm). 203P produced the highest fresh (305.7 g plant^{-1}) and dry mass (81.4 g plant^{-1}) while J3 the smallest (fresh mass: 216.0 g plant^{-1}, dry mass: 59.2 g plant^{-1}). 465P had the highest measured leaf ratio (58.39 %). Generally, it can be concluded that the accessions with green leaves had higher biomass production. Similar tendency was detected in the essential oil content (EOC): the purple accessions had lower EOC that the green ones (465P=0.933 ml 100 g^{-1} dry mass (DM); 203P: 1.246 ml 100 g^{-1} DM; J3= 0.144 ml 100 g^{-1} DM; PS3 0.359 ml 100 g^{-1} DM).

The essential oil composition was analysed by GC-MS. The mass spectra and linear retention indices (LRI) were compared with commercial (NIST, Wiley) and home-made mass spectra libraries. In the essential oil of 203P and J3 the *perillaldehyde* was the main component (60-70 area %). In the PS3 the *ß-dehydro-elsholtziaketone* reached 76% respectively. 465P contains mainly *perillaketone* which area % was over 90% in the essential oil. *Perillaketone* was formerly classified as a potent lung toxin (Müller-Walldeck et al., 2010) that is why this latest accession is not recommend for human consumption.

Keywords: Cultivation, *Lamiaceae*, leaf ratio, perillaldehyde, perillaketone.

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P-26 Influence of growing location on the essential oil production of garden thyme (*Thymus vulgaris* L.)

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Abstract

Garden thyme (*Thymus vulgaris* L.) is a well-known and a worldwide cultivated medicinal and aromatic plant with increasing economic importance. Its drug, *Thymi herba* contains essential oil (0.5-4.0 ml/100 g) which is considered as the main active constituent, being responsible for the typical odour and taste as well as for several therapeutic effects of thyme. Our aim was to test the influence of the growing location on the quality of essential oil of 'Varico 3' variety. The experiment was carried out at two parallel sites (Budapest, Hungary and Poznan, Poland) using samples cut from two-year-old plantations in 3 ontogenetic phases (vegetative, full bloom and overblown) in 2015. For determination of the essential oil (EO) content (mL/100 g DW), 50 g of dried thyme leaves was measured and hydro-distilled in a Clevenger-type apparatus for 3 hours in 3 replications. The composition of the essential oil was analysed by GC/MS (HP 6890 N, connected to HP 5975 mass selective detector, Agilent Technologies). The EO content was in the range, which is known to be characteristic for garden thyme in each cases. However, we detected differences between the two growing locations concerning essential oil accumulation in the amounts and in the tendencies as well. With the exception of the vegetative phase, the plots in Hungary assured higher EO content (full bloom: 3.06 mL/100g; overblown: 1.18mL/100g). The percentage composition of the main components (p-cymene, γ-terpinene, thymol, carvacrol and β-caryophyllen) were similar in Budapest and in Poznan. Although the amounts were different, we detected thymol (Poland: 66.18%, Hungary: 59.94%) and p-cymene (Poland: 15.43%, Hungary: 14.61%) in the largest amounts in both growing locations. Differences were found in the accumulation dynamics of thymol and carvacrol, however, the other compounds showed similar tendencies. Our results demonstrated, that there can be slight differences between growing sites in the essential oil content and composition as well, but a properly selected variety may assure a good drug quality even in the Northern regions of Europe.

Keywords: Garden thyme, environmental conditions, plant ontogenesis, thymol, essential oil content.

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P-27 Aroma of Luchu Pine (*Pinus luchuensis* Mayr) cones and examination of essential oil extraction by steam distillation

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Abstract

Luchu pine (*Pinus luchuensis* Mayr) is distributed in the Southwestern Islands south of Amami Oshima Island (Shidei and Moromizato, 1971), and only Luchu pine is a pine tree species native to Okinawa (Nakamura *et. al.*, 2010). Its cone has a sweet aroma. As with other pinaceous plants, Luchu pine has possibility as an essential oil material. However, Luchu pine has little past research reports due to the narrowness of the distribution area.

GCMS analysis was performed by directly concentrating the aroma component from the cones, a total of 23 peaks were obtained except for internal standard substances. α-Pinene, β-Pinene, β-Phellandrene, d-Limonene, Caryophyllene and Camphene were confirmed in descending order of peak area%, accounting for 62% in total.

Steam distillation was attempted with crushing the cones and under the following conditions prescribed by The Japanese Pharmacopoeia, Seventeenth Edition (JP 17). 500 g weight of the sample, heat the water of the container in an oil bath 140 ^{o}C to make steam, temperature of coolant water is 4 ^{o}C and continued heating by steam for 1 hour. During the extraction there was a sweet, refreshing aroma, however it was not be able to get an oil layer. It is considered that one of the reasons why the oil layer could not be obtained is probably because the amount of the raw material, Luchu pine cones was too small. In 2017, Luchu pine cones will try to extract essential oils by increasing the sample used for one extraction to kg units.

Keywords: *Pinus luchuensis* Mayr, Luchu pine.

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P-28 Distillation time affects chemical profile of lemongrass and palmarosa

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Abstract

Lemongrass [*Cymbopogon flexuosus* (Steud.) Wats, (syn. *Andropogon nardus* var. *flexuosus* Hack; *A. flexuosus* Nees)] and palmarosa [*Cymbopogon martini* (Roxb.) Wats. var *martinii* (syn. C*. martini* Sapg var. *motia*)] are well-known essential oil plants in the subtropical regions. Our recent studies in Northern and Southern Mississippi showed that the two crops can be successfully grown in this region. Both crops produce significant amount of biomass containing essential oil with specific aroma. The essential oil of these plants is traditionally extracted via steam distillation from either fresh or dried biomass. There is no consensus in the literature regarding the optimal distillation time (DT), especially for dried biomass. We hypothesized that the duration of the DT will significantly alter essential oil yield and essential oil composition. The objective was to test 7 DT (2.5, 5, 10, 20, 40, 80, and 160 min) on essential oil yield and composition of the dried biomass of the two plants. Maximum oil yields were achieved at 20 min DT in lemongrass and 40 min in palmarosa; further duration of DT did not increase oil yields. **In lemongrass**, the concentration of methyl heptenone, limonene, 4-nonanone, linalool, verbenol-cis and verbenol-trans were highest in the oil from 2.5 min DT. The concentration of neral and geranial was less affected by the DT, although they were lowest in oil from 160 min DT. Conversely, the concentrations of geranyl acetate, gamma-cadinene, and caryophyllene-oxide were higher in the longest DT and lower in the shorter than 10 min DT. **In palmarosa**, the concentration of linalool was highest in the oil from the 2.5 min DT and lowest in the oil obtained at 160 min DT. The concentration of beta-caryophyllene reached maximum in the oil at 80 min DT, while the concentrations of farnesal and farnesol reached maximum at 160 min DT. DT did not affect the concentrations of geraniol, geranial, and geranic acid in palmarosa. The duration of DT can be used to modify the essential oil composition of lemongrass and palmarosa, and it may be used to target specific composition. The duration of the DT needs to be reported in all papers reporting the oil content and composition of the two species. This will facilitate comparison of values in different reports.

Keywords: Palmarosa, lemongrass, essential oil, distillation time.

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P-29 Essential oil composition from *Xylopia brasiliensis* Spreng. (Annonaceae) leaves and flowers

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Abstract

Several Annonaceae species are known for their distinctive, penetrating floral aromas. *Xylopia* L. is the most important and numerous genus in this family, producing a wide variety of secondary metabolites, including essential oils. The aim of this work was to analyse the essential oil composition from *X. brasiliensis* leaves and flowers and their relationship with the plant pollination and breeding system. The flower and leaf oils were obtained by hydrodistillation for 3 hours, affording yellow-coloured essential oils with yields of 0.08% for the flowers and 0.17% for the leaves. The GC-MS/GC-FID analysis indicated as the main components for the flower oil were bicyclogermacrene (16.96%), spathulenol (13.76%), germacrene D (9.83%), globulol (5.47%) and α-cadinol (5.20%), while for the leaf oil spathulenol (47.5%) was the most abundant compound. *X. brasiliensis* presents a beetle-pollination syndrome, with a floral scent defined as "fruity" (Andrade et al., 1996). The major flower essential oil compounds are also known to occur in fruits, like germacrene D and bicyclogermacrene, suggesting that pollinators might be attracted to the flowers by these scents mimicking fruits (Ratnayake et al., 2007). Spathulenol might also be involved in the attraction due to its odour, herbal and mouldy, and increased amounts during the flowering stage (Lago et al., 2003)

Keywords: *Xylopia brasiliensis*, Annonaceae, GC-MS, essential oil, ecological function.

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P-30 The volatile components of the Liverwort *Cyathodium foetidissimum*

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Abstract

The volatile components of the liverwort *Cyathodium foetidissimum* were analyzed using headspace solid phase micro-extraction (SPME) and GC-MS. From this liverwort *C. foetidissimum* collected on the wet soil at the cave in Tahichi island in 2016 November, 4-methoxyvinylbenzene (same as 4-methoxystyrene) (**1**) and 3,4-dimethoxystyrene (**2**) and skatole (**3**) were detected as the major components.

In the previous papar[1)], Ludwiczuk *et al* reported that 4-methoxystyrene and skatole were identified as the major volatile components from the same speceis, collected in the different place in the same island. As this liverwort *C.foetidissimum* showed the characteristic aging odor reminiscent the damp smell from old chest of drawers, we expected the aldehydes and ketones might be existing in the volatiles.

At the result, C8～C10 aldehydes (octanl, nonanal and decanal) were detected but *trans*-2-nonenal recognized aged malodor was not identified. By the way, the smell of *C.foetidissimum* was characteristic to utilize for the fragrance creation.

The compound (**1**) which had already been identified from rose flower and compound (**2**) showed the strong inhibitory effect on melanin *in vitro*.[2)] Now, the physiological experiments, such as whitening, antimicrobial, antifungal effect of these phenolic components are under progress.

Keywords: *Cyathodium foetidissimum*, 4-methoxystyrene, 3,4-dimethoxystyrene, skatole, aging odor.

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P-31 Molecular cloning and functional characterization of a novel monoterpene synthase isolated from the aromatic wild shrub *Thymus albicans*

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Abstract

The essential oil of *Thymus albicans* Hoffmanns. & Link, a native shrub from the Iberian Peninsula, is mainly composed of monoterpenes. In this study, a 1,8-cineole synthase was isolated from the 1,8-cineole chemotype. A partial sequence that lacked the complete plastid transit peptide but contained an extended C-terminal when compared to other related terpene synthases was generated by PCR and Rapid Amplification of cDNA Ends (RACE). The predicted mature polypeptide was 593 amino acids in length and shared 78% and 77% sequence similarity with the homologue 1,8-cineole synthase from *Rosmarinus officinalis* and *Salvia officinalis*, respectively. The putative protein possessed the characteristic conserved motifs of plant monoterpene synthases including the RRx_8W and DDxxD motifs and phylogenetic analysis indicated that the amplified 1,8-cineole synthase bears greater sequence similarity with other 1,8-cineole synthases from Lamiaceae family relatively to the terpene synthases from the genus *Thymus*. Functional activity of the expressed recombinant protein in *Escherichia coli* revealed that in the presence of geranyl diphosphate (GPP) 1,8-cineole was the major product, but that its production was low. Other minor conversion products included α-pinene, β-pinene, sabinene and β-myrcene, suggesting the isolated 1,8-cineole synthase may be a multi-product enzyme. To our knowledge, this is the first report of a functionally characterized monoterpene synthase from *Thymus albicans.*

Keywords: Thyme, heterologous expression, 1,8-cineole, terpene synthase, geranyl diphosphate.

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P-32 Methyl 3-(5-(prop-1-yn-1-yl)thiophen-2-yl)propanoate: a rare secondary metabolite from *Artemisia absinthium* L. essential oil

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Abstract

Essential oils (EOs) of plant species from the genus *Artemisia* (Asteraceae) were previously extensively studied. However, cases of "unidentified" *Artemisia* EO constituents are still frequent in the literature [1]. Thus, as a part of our ongoing interest in the volatile profiles of *Artemisia* species, and as there is only scarce data [2] on the EO of underground parts of wormwood (*Artemisia absinthium* L.), we performed a GC-MS analysis of the EO of *A. absinthium* roots. This enabled us to detect a minor constituent (0.7% of the total detected GC peaks), eluting in the RI range of oxygenated sesquiterpenes (**1**; RI on DB-5 MS column: 1694), with little clues on its identity available from its MS (MS (EI, 70 eV) *m/z* (%) = 208([$M^{\cdot+}$] 46), 148(37), 135(100), 115(6), 102(3), 91(13), 83(2), 77(5), 69(4), 63(3), 51(4), 45(3), 39(3)). Dry flash chromatography of the EO sample at hand afforded 3.8 mg of pure **1**, while a combination of MS and 1D/2D-NMR (^{1}H and ^{13}C, DEPT90/135, selective ^{1}H-^{1}H homonuclear decoupling, HSQC, HMBC, NOESY, ^{1}H-^{1}H COSY) established its structure as that of methyl 3-(5-(prop-1-yn-1-yl)thiophen-2-yl)propanoate (biosynthetically related to dehydromatricaria ester). The NMR-based identification of **1** was corroborated by a simulation of its ^{1}H and ^{13}C NMR spectra using GIAO method (DFT level of theory). This is the second record on this compound ever (it was previously reported on only once, in *A. absinthium* solvent extract [3]), and the very first one regarding **1** as an EO constituent. The restricted natural occurrence of **1** suggests it might be used as potentially valuable chemotaxonomic marker, and could even provide new insights into the taxonomic relationships between polyacetylene producing Asteraceae/*Artemisia* species.

Keywords: *Artemisia absinthium* L., Asteraceae, root essential oil, polyacetylene secondary metabolites, NMR, GIAO-DFT.

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P-33 A new bioactive presilphiperfolane diol from *Pulicaria vulgaris* Gaertn. (Asteraceae) essential oil

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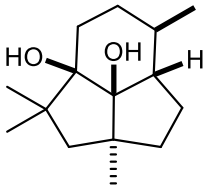
Abstract

A chromatographic separation of the essential oil from the aerial parts of *Pulicaria vulgaris* Gaertn. (Asteraceae) led to the isolation of a new compound belonging to presilphiperfolane sesquiterpenes. A combination of spectral evidence (extensive 1D- and 2D-NMR) and chemical transformations enabled us to establish the structure of the presilphiperfolane diol as that of *rel*-(2a*R*,2a^1*R*,4a*S*,7*S*,7a*R*)-2a,4,4,7-tetramethyldecahydro-1*H*-cyclopenta[*cd*]indene-2a^1,4a-diol.

Presilphiperfolanes were extensively studied since they represent structural precursors of angular and propellane triquinane sesquiterpenes. However, there are no previous reports regarding compounds with a 7,8-dioxygenated presilphiperfolane skeleton, and there are no known presilphiperfolane diols. The presilphiperfolane diol was found to be rather toxic to *Artemia salina* (LD_{50} 0.04 and 0.03 mmol/L after 24 and 48 h, respectively; for comparison sake, the toxicity of strychnine sulfate in the same model was LD_{50} 0.2 mmol/L).



Keywords: *Pulicaria vulgaris*, presilphiperfolane diol, triquinane, essential oil.

Acknowledgments

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

P-34 Development of antifungal liquid soap containing microparticle from blended essential oils

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Abstract

From the previous study, It has been found that the blended essential oils (BEOs) that compose of lavender oil, clary sage oil, and ylang ylang oil in the ratio of 4:4:2 (v/v) have an activity against *Candida albicans* but the blended essential oils can be easily evaporated and easily degraded. The microparticles may protect the BEOs. The objective of the study is to develop the liquid soap containing BEOs for antifungal activity against *C. albicans* by prepared 2 formulae of liquid soap which have a different in a ratio of SLES (major surfactant) and Cocamidopropyl betaine (minor surfactant) (1:1 = formula 1 liquid soap, 2:1 = formula 2 liquid soap). The BEOs was encapsulated in microparticles by ionic gelation method using 7 %w/v of sodium alginate solution and 10 %w/v of calcium chloride solution. And separated each formula into 2 parts for mixing with BEOs (BEO 1, BEO 2) and mixing with microparticles containing BEOs (Bead 1, Bead 2). Agar well diffusion method was used to test the antifungal activity against *C. albicans* in the incubator at 28 ºC for 14 days. The positive control is ketoconazole solution 10 µg/ml (MIC))(+) ive) and using each formula of liquid soap as negative controls ((-) ive1, (-) ive2). The results showed that Bead 1 and Bead 2 had significantly longer activity against *C. albicans* than BEO 1 and BEO 2 *(p-value < 0.05)* from the second day of incubation (day 2). Moreover the study showed that Bead 1 and Bead 2 had significantly longer activity against *C. albicans* than ketoconazole. The microparticles which prepared by ionic gelation method can protect the evaporation and degradation of BEOs. It can improve the activity and duration of activity against *C. albicans* of BEOs.

Keywords: Essential oils, ionic gelation, microparticles, liquid soap, anti-fungal.

Acknowledgments

The authors would like to thanks Faculty of Pharmacy Srinakharinwirot University for the grant.

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P-35 Comparison of microwave-assisted and conventional hydrodistillation in the extraction of essential oils from Algerian *Melissa officinalis* L leaves

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Abstract

Microwave-assisted hydrodistillation (MWHD) is an advanced hydrodistillation (HD) technique, in which a microwave oven is used in the extraction process. MWHD and HD methods have been compared and evaluated for their effectiveness in the isolation of essential oils from fresh leaves of *Melissa officinalis* L. MWHD offers important advantages over HD in terms of energy savings and extraction time (30 min against 3 h). The composition of the extracted essential oils was investigated by GC-FID and GC-MS. A total of 62 compounds, constituting 92.17% of the oil, were identified in the oil obtained by HD, whereas 54 compounds, representing 94.76% of the oil, were characterized in the MWHD oil. MWHD-distilled oil is richer in lightly oxygenated monoterpenes (83.14%) than HD oil (69.81%). It also has a higher amount of hydrocarbon sesquiterpenes HD (11.70%) than MWHD (4.03%).

Results indicate that the use of microwave irradiation did not adversely influence the composition of the essential oils. MWHD was also found to be a green technology.

Keywords: *Melissa officinalis,* Lamiaceae, essential oil composition, Microwave-Assisted Hydrodistillation, Hydrodistillation, GC, GC/MS.

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P-36 Qualitative, quantitative analysis and enantiomeric distribution of some monoterpenoid components of *Juniperus communis* L.

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Abstract

Essential oil (EO) of *Juniperus commuis* growing in Algeria was obtained by hydrodistillation extraction method. The composition of EO was analysed by means of GC-MS and GC-FID, using the internal standard method and relative response factors.

Around 74 compounds were determined in total, representing 88.28g/100 g of the oil were identified. The needles volatile oil was dominated by oxygenated monoterpene (43.68g/100 g) and terpenic hydrocarbon fractions (31.14g/100g). The main components from oil were terpinene 4-ol (19.9g/100g) followed by *p*-cymene (9.54g/100g), α-pinene (7.65g/100g) sabinene (7.35g/100g) and α-terpinene (4.73g/100 g). Also, the enantiomeric distribution of some terpenes chiral has been assessed by enantioselective monodimensional gas chromatography (enatio-GC) through the use of a chiral β-cyclodextrin based stationary phase was determined.

Keywords: *Juniperus communis*; Cupressaceae; GC quantitative analysis; enantio-GC-FID; GC-MS.

P-37 Introducing a new and automated spectral deconvolution software for perfume analysis with GC coupled with single quadrupole mass spectrometry

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Abstract

Chromatograms and mass spectra of compounds in perfume can be both convoluted and many of the mass spectra are very similar coming from a class of compounds like for instance the terpenoids.

Being able to have clean mass spectra is key to identify the individual compounds correctly in any fragrance analysis.

This newly developed deconvolution tool performs spectral clean up by monitoring the peak apexes of each mass followed by a library search using the NIST MS search algorithm and checking for retention time indices.

The software is also capable of comparing two or more chromatograms and a heat map is depicted to quickly identify the main differences.

In this poster a perfume sample analyzed by the ThermoFisher Trace1310 GC and ISQ LT mass spectrometer is submitted to the deconvolution software and the compounds are identified by using a dedicated perfume library and the NIST lbrary.

P-38 The essential oil of *Zosima absinthifolia* Link (Apiaceae) from Iran: a rich source of lavandulyl esters

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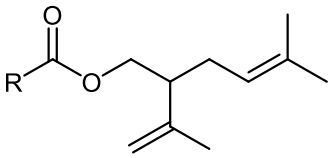
Abstract

Zosima absinthifolia Link belongs to the family Apiaceae (Umbelliferae) distributed in many countries (Iran, Turkey, Iraq) of eastern and central Asia. The aerial parts of the plant are consumed as a vegetable; *Z. absinthifolia* fruits are used as a food flavoring in Iran and Turkey. The fruits have also been used in folk medicine for their digestive/carminative and anti-inflammatory properties (Razavi et al., 2013). Previous reports on the essential oils of *Z. absinthifolia* revealed the presence of octyl esters as the main components of the oils (up to 87.5%; Javidnia et al., 2008; Başer et al., 2000). Our current investigation of the essential oil of *Z. absinthifolia* from Iran showed that its major components were lavandulyl acetate (23.9%, ester of the irregular monoterpenol), bornyl acetate (12.0%), octyl octanoate (11.7%), lavandulol (5.0%), octyl hexanoate (4.2%) and lavandulyl octanoate (3.1%). A detailed GC/MS analysis of the oil (one hundred-sixty identified constituents, in total) revealed the presence of the series of lavandulyl, neryl, geranyl and linalyl esters. Lavandulyl esters of octanoic, nonanoic and decanoic acids represent new natural products. The structures of all esters were confirmed by synthesis and full spectral characterization (^{1}H and ^{13}C NMR, IR, and MS).



R = C_7H_{15}, C_8H_{17}, C_9H_{19}

Keywords: *Zosima absinthifolia*, essential oil, lavandulyl octanoate, irregular monoterpenol.

Acknowledgments

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

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P-39 Chemical composition of the root essential oil from *Conium maculatum* L. (Apiaceae)

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Abstract

Conium maculatum L. (Apiaceae; poison hemlock) is a perennial herbaceous flowering plant. Poison hemlock is a very common and one of the most renowned poisonous plant species of European origin found throughout many parts of the world (Vetter, 2004). The fresh leaves, fruits and flowers contain a volatile alkaloid, coniine, historically associated with the death of Socrates (mixture of an extract from *C. maculatum* and opium was reported to be the lethal poison for Socrates). The whole plant has a bitter taste and unpleasant odor when mechanically damaged.

Strangely, the composition and the associated activities of its essential oils of hemlock were the subject of only several previous studies. Recently, the chemical compositions of the leaf and flower essential oils of *C. maculatum* from Serbia, as well as the essential oil of Iranian hemlock, were published (Radulović et al., 2008; Masoudi et al., 2006). We decided to perform the first analysis by GC and GC-MS of the hydrodistilled essential oil of *C. maculatum* L. roots. The yield of the root essential oil was rather low (0.08%, based on fresh root weight, collected near the city of Leskovac, Serbia). Eighty-six constituents were successfully identified, representing 97% of the total detected GC-peak areas. The main constituents of the essential oil were (*Z*)-*β*-ocimene (25.3%), *β*-pinene (17.8%), (*Z*)-falcarinol (17.2%), myrcene (13.1%), *β*-sesquiphellandrene (3.2%) and elemicin (2.4%). No traces of piperidine alkaloids were detected in the roots.

Keywords: *Conium maculatum*, essential oil, root, (*Z*)-*β*-ocimene, piperidine alkaloids.

Acknowledgments

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

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P-40 Essential oil composition and fatty acid Profile of *Cota tinctoria* subsp. *euxina* (Boiss.) Oberpr. & Greuter and Endemic *Anthemis pauciloba* Boiss. var. *sieheana* (Eig) Grierson from Turkey

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Abstract

Anthemis L. species are member of Asteraceae family and represented with 83 taxa in Turkey, 29 of which are endemic to Turkey (Hamzaoğlu et al., 2011). In the past, *Cota* was one of three sections of genus *Anthemis* L. in Flora of Turkey. As a result of recent researches, taxa belonging to *Cota* section have been raised to genus level and *Cota* was accepted as a genus name as *Cota* J. Gay (Özbek, M.U., 2010). Lastly, *Cota* J. Gay has been represented with totally 22 species and subspecies in Turkey, 9 of which are endemic to Turkey. Previously the essential oil constituents and antimicrobial activity of *Anthemis aciphylla* Boiss. var. *discoidea* Boiss. were investigated. The main components of the essential oil from dried aerial parts, leaves and flowers were α-pinene (49.4, 9.4, 39.0 %) and terpinen-4-ol (21.8, 24.3, 32.1 %), respectively (Baser et. al., 2006). Chemical composition and antimicrobial acitivity of the essential oils of *Anthemis xylopoda* O. Schwarz from Turkey were also investigated. The main components of the oils from flower were borneol (31.8 %), carvacrol (12.67 %), 1,8-cineole (5.45 %) and 2,5,5-trimethyl-3,6-heptadien-2-ol (5.1 %). The main components of the oils from leaves were borneol (30.15 %), 1,8-cineole (16.74 %), α,β-thujone (12.08 %), 2,2,5-trimethyl-3,6-heptadien-2-ol (8.5 %) and carvacrol (5.21 %) (Uzel et. al., 2004). In this research, essential oil composition and fatty acid profile of *Cota tinctoria* subsp. *euxina* and *Anthemis pauciloba* var. *sieheana* were analyzed by means of gas chromatography-mass spectrometry (GC-MS). The major components of the essential oil of *Cota tinctoria* subsp. *euxina* were β-pinene (24.22 %), α-pinene (5.84 %), limonene (3.66 %), 1,8-cineol (3.48 %) and vulgarone B (3.47 %). The major components of the fatty acid of *Cota tinctoria* subsp. *euxina* were 9,12-octadecadienoic acid methyl ester (56.35 %), 9-octadecanoic acid methyl ester (16.0 %), and hexadecanoic acid methyl ester (15.4 %). The major components of the essential oil of *Anthemis pauciloba* var. *sieheana* were 1,8-cineol (8.27 %), β-pinene (4.97 %), spathulenol (3.16 %) and caryophyllene oxide (3.17 %). The major components of the fatty acid of *Anthemis pauciloba* var. *sieheana* were 9,12-octadecadienoic acid methyl ester (48.46 %), 9-octadecanoic acid methyl ester (16.17 %) and hexadecanoic acid methyl ester (13.3 %).

Keywords: *Cota tinctoria* subsp. *euxina, Anthemis pauciloba* var. *sieheana, essential* oil, fatty acid.

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P-41 Essential oil composition of two endemic *Gypsophila* species from Turkey

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Abstract

Gypsophila L. species are member of Caryophyllaceae family and distributed mainly in Mediterranean and Iran-Turan areas In Turkey. *Gypsophila* has 56 species in 10 sections and 33 species are endemic (Chandra et. al., 2015). There were very few reports on the phytochemistry of the *Gypsophila* L. species in the literature. Previously antimicrobial activity and chemical constituents of the essential oils from flower, leaf and stem of *Gypsophila bicolor* from Iran were investigated. The main components of the essential oil from flower were germacrene-D (21.2 %), *p*-cymene (20.6 %), bicyclogermacrene (17.6 %), γ-dodecadienolactone (13.7 %) and terpinolene (9.4 %). The main components of the essential oil from leaves were germacrene-D (23.4 %), terpinolene (14.5 %), bicyclogermacrene (7.5 %), γ-dodecadienolactone (6.8 %), *p*-cymene (6.7 %) and *cis*-β-ocimene (6.3 %). The main components of the essential oil from stems were γ-dodecadienolactone (28.5 %), bicyclogermacrene (14.8 %), germacrene-D (12.6 %), *p*-cymene (12.5 %), terpinolene (11.6 %) and *trans*-β-ocimene (4.2 %) (Shafagha et. al., 2011). To the best of our knowledge this is the first report on the essential oil composition of *Gypsophila turcica* and *Gypsophila pinifolia*. Essential oil composition of *Gypsophila turcica* Hamzaoğlu and *Gypsophila pinifolia* Boiss. Et Hausskn. were analyzed by means of gas chromatography-mass spectrometry (GC-MS). The major components of the essential oil of *G. turcica* were hentriacontane (12.943 %), 1-octadecanol (8.955 %), hexahydrofarnesyl acetone (6.881 %), pentacosane (6.621 %), diisobutyl phthalate (4.483 %), 1-eicosanol (3.626 %), triacontane (2.406 %), phytol (2.710 %) and tricosane (2.212). The major components of the essential oil of *G. pinifolia* were hexadecanoic acid (17.404 %), 1-tetradecanol (7.52 %), phytol (5.54 %), diisobutyl phthalate (2.882 %), hexahydrofarnesyl acetone (2.7 %), undecanal (2.53 %), decanal (2.57 %), tridecanal (2.23 %), farnesyl acetone (2.03 %) and linalool (1.977 %).

Keywords: *Gypsophila turcica, Gypsophila pinifolia,* essential oil.

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P-42 Tackling the extended list of fragrance allergens by flow-modulated GC×GC with parallel FID/TOF MS detection

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Abstract

In 2003, an EU Directive restricting the use of allergenic compounds in fragrances was released. The Directive named a total of 27 allergens, stating that they should be labelled if present at >100 ppm in 'wash-off' products (such as shower gels), or >10 ppm in 'leave-on' products (such as perfumes).

Compliance with this Directive therefore requires that these compounds are identified and quantified accurately - a considerable challenge due to the complex matrix and wide concentration ranges involved. In addition, it has been proposed to expand the list of monitored allergens to over 80 different compounds, making the process even more demanding.

Comprehensive two-dimensional GC coupled with time-of flight mass spectrometry (GCxGC-TOF MS) is the ideal choice to tackle this issue. The enhanced separation capacity copes with the most complex of matrices, while the commercialization of simple, consumable-free flow modulation devices has made routine use more feasible.

This study focuses on the use of parallel detection by flame ionisation detection (FID) and TOF MS – for robust quantitation and confident identification in a single run.

Furthermore, the TOF mass spectrometer used in this study also incorporates a novel ion source design enabling both hard and soft ionisation spectra to be acquired simultaneously – in a technique deemed Tandem Ionisation – for two complementary datasets in a single analysis.

Keywords: GCxGC, allergens, soft ionisation, time-of-flight mass spectrometry.

P-43 Fast Quality Control method for aromatic waters: identification of roses hydrosols spoiled by microorganisms based on detection of associated volatiles

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Abstract

Hydrosols are hydrodistillation products of aromatic plants. They contain less than 1 g/L of dispersed essential oils giving organoleptic properties. Hydrosols are subjected to microbial proliferation. Reasons for spoilage have to be found in the nature of substrates supporting growth and of microbiological contaminants. Non-volatile compounds were likely carried over during distillation by a priming and foaming effect, and could be used as nutrients by microorganisms. A microbial proliferation at ambient temperature is communly observed in hydrosols when stored in a non-sterile container. In a recent study, Labadie and all reported that some strains contaminating roses hydrosols during process operations have an impact on volatile profile. The production of 6-methyl-5-hepten-2-one in hydrosols was reported as specific to the presence of Novosphingobium capsulatum.

With the objective to propose a fast quality control method for evaluation of spoilage of commercial roses hydrosols using this chemical marker as a proof of contamination, a method based on an headspace analysis of more than 20 samples originated from France, India, Tunisia and Bulgaria was developped. Optimisation of experimental conditions of gas phase SPME and liquid phase SPME (equilibration time, fiber adsoprtion time) were performed before injection in GC-MS. 6-methyl-5-hepten-2-one was identified in more 30% of the commercial samples studied despite storage in colored glass bottles. In addition the method allowed to identifiy 3 samples as not natural hydrosols but synthetically flavored ones

Keywords: Hydrosols, spoliage marker, SPME-GC-MS-2O.

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P-44 GC-MS analysis of various ratios of blended essential oils

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Abstract

This study aimed to investigate the similar and different chemical compounds in various ratios of the blended essential oils. The blended essential oils consisted of three top note oils (eucalyptus oil, pine oil and lime oil), one middle note oil (rosemary oil), using mineral oil as the carrier oil. The single and combination essential oils were characterized by gas chromatography-mass spectrometry (GC-MS). The data revealed that several chemical compounds exist in the mixture oils. Two important compounds of blended essential oils were eucalyptol and (+)-2-bornanone. Eucalyptol was mainly found in eucalyptus oil. (+)-2-bornanone was mainly found in rosemary oil. α-terpineol was mainly found in both pine oil and lime oil. The present study provided the scientific support for the potential application of blended essential oils to be used as an aromatherapy recipe. In addition, GC-MS identification of the main compounds afforded the fingerprints for the qualitative analysis of different contents of blended essential oils.

Keywords: Blended essential oil, Eucalyptus oil, Rosemary oil, Pine oil, Lime oil.

P-45 Effect of distillation time on composition of essential oil of lemon thyme (*Thymus* × *citriodorus*)

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Abstract

Genus *Thymus* (Lamiaceae) is one from commercially important genus integrating about 350 essential oils bearing species. The essential oils (and/or their chemical compounds) of this genus are used in perfume and cosmetic industry as aromatizers, in food industry as food flavouring, spices and natural antioxidants, in pharmaceutical industry for manufacturing of pharmaceuticals. Lemon thyme (*Thymus* × *citriodorus* (Pers.) Schreb.), interspecific hybrid between *T. vulgaris* and *T. pulegioides*, is an attractive perennial aromatic subshrub with a delightful rose-lemon-like flavor. Geraniol, the main compound of *T.* × *citriodorus* essential oil, is the commercially important monoterpene alcohol and characterized by wide spectrum of antibacterial activity (Pauli & Schilcher, 2004). Therefore *T.* × *citriodorus* is good source for essential oil with high percentage of natural geraniol. The main method to obtain essential oils from plant matrix is hydrodistillation. Distillation time can both to optimize the production and to engineer the composition of essential oil in essential oil bearing plants. According to standards of European Pharmacopoeia the distillation time of essential oils from *Thymi herba* (i. e. leaves and flowers of *T. vulgaris* or *T. zygis* or a mixture of both species) is two hours (European Pharmacopoeia, 2008). The chemical composition of *T.* × *citriodorus* very differs from *T. vulgaris* or *T. zygis* which accumulate phenols, therefore the evaluation of duration of essential oil distillation from lemon thyme is important for optimization of essential oil yield and/or qualitative composition. The objective of the present study was to evaluate the effect of duration of water distillation on quantitative and qualitative composition of essential oil of *T.* × *citriodorus*.

Essential oils were isolated by water distillation using different distillation times and analysed by GC/MS. Increase in percentage of essential oil during all distillation time gradient was uneven. Elongation of distillation time decreased percentage of monoterpenes but increased percentage of sesquiterpenes in essential oil. Trend of percentage of geraniol during investigated distillation time gradient was slightly rising. Results showed that the water distillation of essential oil from lemon thyme longer than 60 min is useless.

Keywords: *Thymus* × *citriodorus*, geraniol, water distillation time.

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P-46 Creation of nature – identical and non allergen Geranium and Jasmin oils

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Abstract

Contact allergy to fragrances is a common and significant problem around the world. The studies from the SCCNFP opinion on fragrance allergy in consumers in 1999 (SCCNFP/0017/98) (SCCNFP 1999) have confirmed that the 2 natural extracts; EVERNIA FURFURACEA EXTRACT* and EVERNIA PRUNASTRI EXTRACT*, identified by the SCCNFP, are fragrance allergens for consumers because of their exposure from cosmetic products. [1] The Annex III of Cosmetics Directive states that these natural extracts must be indicated in the list of ingredients, in addition to the word 'parfum', if their concentration exceeds 0.001% in leave-on products (e.g. a moisturiser) and 0.01% in rinse-off products (e.g. a shampoo) [2]. The Scientific Committee on Consumer Safety (SCCS) of European Comission has stated on its opinion SCCS/1459/11 adapted on 26-27 June 2017 includes 26 new natural extracts as established contact allergens in humans , making a total of 28 [3].Two of these extracts which are widely used in fragrance industry PELARGONIUM GRAVEOLENS (Geranium Oil) and JASMINUM GRANDIFLORUM / OFFICINALE (Jasmin Oil Egypt) were analyzed by GC/MS. Studies were made in order to develop nature - identical of the essential oils mentioned above that will not be classified as contact allergens. The derived nature - identicals were examined and compared by GC/MS. Although the results showed differences in some constituents, only trace amounts (< 0.1%) of these contact allergens were observed. In conclusion, nature - identicals resembling essential oils olfactively, which can be used without any restrictions, were created.

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P-47 Composition and antioxidant activities of essential oil of aerial part of *Oliveria decumbens* Vent growing in Iran

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Abstract

Oliveria decumbens Vent belong to the Umbelliferae family, which grows in south and south west parts of Iran, with the common local names of “Mooshkorok”, “Den” and “Denak”. This herb is used as a folk medicine to treat several diseases such as diarrhea, indigestion, abdominal pain and feverish conditions (Mahboubi et al., 2008; Sereshti et al., 2011). The aim of the present study was to determine chemical compound and antioxidant activity *Oliveria decumbens* essential oil. The essential oil of aerial part obtained by hydrodistillation was analyzed by gas chromatography (GC). The main components of essential oil are thymol (24.8%), carvacrol (23.3%), γ-terpinene (21.4%) and p-cymene (18.4%). This essential oil exhibited strong antioxidant activity. 50% inhibitory concentration value (IC_{50}) was evaluated by DPPH radical scavenging activity using a Perkin-Elmer Lambda EZ-210 spectrophotometer at 517 nm. The value IC_{50} for *Oliveria decumbens* essential oil was obtained 84.4 µg/ml. Also, ferric reducing antioxidant power (FRAP) method was used to measure the antioxidant activity (Yassa et al., 2015). This strong antioxidant properties of essential oil can be attributed to phenolic compounds (carvacrol and thymol).

Keywords: *Oliveria decumbens* Vent, essential oil, gas chromatography, antioxidant activities.

Acknowledgments

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P-48 Essential-oil components of *Bupleurum praealtum* L. synthesis and spectral characterization of a rare perillyl ester

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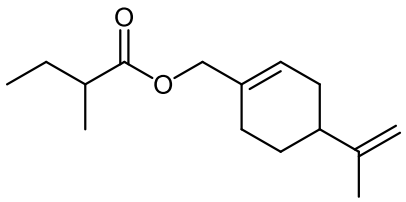
Abstract

Bupleurum L. is one of the largest genera belonging to the family Apiaceae (Umbelliferae).[1] The genus is almost exclusively native to Europe and eastern Asia. Some of the species such as *B. falcatum* and *B. chinense* are well-known for their over 2000-year long use in traditional Chinese medicine: "liver tonics", the treatment of fever producing infections, etc.

Prompted by the rich ethnopharmacological usage of taxa belonging to this genus, and the lack of data on the secondary metabolites of *Bupleurum praealtum* L., we decided to investigate the essential-oil composition of this taxon wild-growing in Serbia. Dried umbels with unripe fruits subjected to hydrodistillation yielded a small amount of the essential oil (0.06%, w/w). GC and GC-MS analyses revealed an unusual composition of the oil, with undecane (20.1%) and germacrene D (17.8%) as the major contributors. Additonally, our attention was drawn to several minor constituents which seemed to be rare esters of perilla alcohol. As there are no spectral data available in the literature for higher esters, we decided to prepare them by synthesis. A reduction of the commercially available perilla aldehyde, followed by esterification with an appropriate acid gave the desired target esters. A co-injection experiment verified the occurrence of one of the diastereoisomeric perillyl 2-methylbutanoates in the essential oil of *B. praealtum*. This ester, as well as perillyl isovalerate and isobutanoate, were fully spectrally characterized (1D- and 2D-NMR, MS, IR, UV) and all NMR data assigned.



Keywords: *Bupleurum praealtum*, essential oil, perillyl esters, synthesis, NMR assignment.

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P-49 The first report on the chemical composition of the inflorescence essential oil of *Eupatorium cannabinum* L. from Serbia

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Abstract

The genus *Eupatorium* belongs to the tribe of Eupatorieae, one of the 13 tribes of the Asteraceae, and comprises 44 species with an almost cosmopolitan distribution.[1] The type species, *Eupatorium cannabinum* L., commonly known as hemp-agrimony or holy rope, is the only *Eupatorium* taxon native to Europe, but it also populates wide regions in North America and Eastern Asia. *E. cannabinum* is a medicinal and aromatic herb with antibacterial, immunological, cytostatic, antiinflammatory, fungicidal properties, etc.[2]

The purpose of this work was to investigate the essential-oil composition of *E. cannabinum* from Serbia for the first time. The plant material, fresh inflorescence collected from the slopes of Suva Planina Mountain (near Niš, SE Serbia), produced upon hydrodistillation, a moderate yield of the essential oil (0.39%, w/w). The essential-oil composition was analyzed by GC and GC/MS and these analyses resulted in a successful identification of over one hundred compounds, accounting for 91.3% of the detected GC peak areas. Thymol methyl ether (17.3%), germacrene D (12.7%) and α-farnesene (8.4%) were the most abundant components with appreciable amounts of δ-carene (6.0%), α-phellandrene (3.4%), *p*-cymene (3.2%) and bicyclogemacrene (2.6%). The majority of the monoterpenic constituents originated from a *p*-menthane pathway usually giving rise to aromatic compounds, i.e. derivatives of *p*-cymene. Among the less abundant compounds, a number of interesting thymol derivatives was detected. A positive identification of these constituents required a synthetic preparation of appropriate standards which are not commercially available. This also enabled us to ascertain certain structure-retention index relations that could be of importance for further investigations.

Keywords: *Eupatorium cannabinum*, essential oil, thymol derivatives, synthesis.

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

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P-50 Utilization of sage (*Salvia officinalis* L.) herbal dust as raw material for essential oil recovery

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Abstract

During the production of filter tea, particularly during grinding of plant material with hammer mill, certain amount (approx. 20%) is being grinded in fine powder, i.e. herbal dust. This fraction has particle size <0.315 mm, therefore it could not be used for filter tea packing. Even though, certain loss of valuable bioactive compounds is being observed during processing in filter tea factory (cutting, grinding, vibro-sifting, etc.), this material still possesses significant amount of bioactive compounds and could be suitable for solid-liquid extraction due to low mass transfer limitations. Therefore, the aim of this work was utilization of sage (*Salvia officinalis* L.) herbal dust as raw material for essential oil recovery. Conventional (hydrodistillation and Soxhlet extraction with methylene chloride and hexane as extraction solvents) and novel (supercritical fluid extraction; SFE) extraction techniques were applied for that purpose. SFE experiments were performed using Box-Behnken experimental design with pressure (100, 200 and 300 bar), temperature (40, 50 and 60 ˚C) and CO2 flow rate (0.2, 0.3 and 0.4 kg/h) as independent variables. Total extraction yield (Y) and chemical profile of terpenoid compounds, determined by GC-MS and GC-FID was observed in all samples. It could be observed that Y obtained by SFE was from 1.81 to 8.83% (Figure 1). The most abundant terpenoids in these samples were oxygenated monoterpenes (α-thujone and camphor), oxygenated sesquiterpenes (virdiflorol) and diterpene polyphenols (epirosmanol).

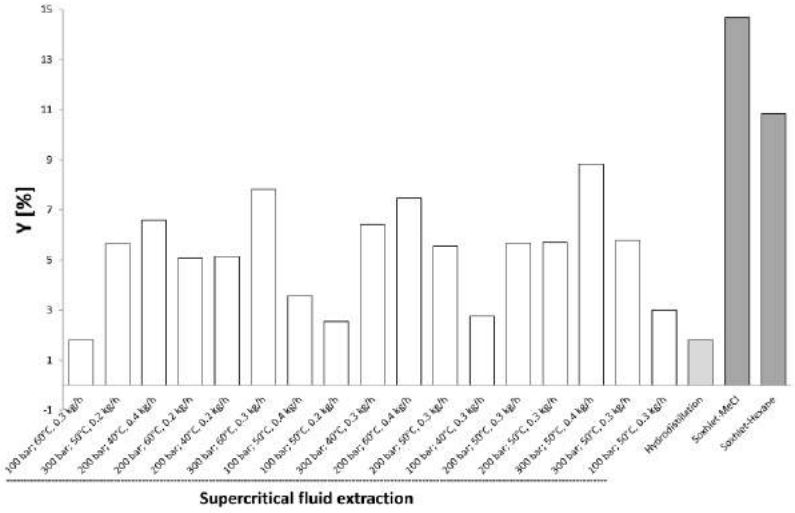

Figure 1. Total extraction yield (Y) obtained by SFE, hydrodistillation and Soxhlet extraction

Keywords: Sage herbal dust, supercritical fluid extraction, essential oil, terpenoids.

P-51 Subcritical water extraction of sage (*Salvia officinalis* L.) herbal dust lipids

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Abstract

Nowadays, herbal tea is rapidly becoming more and more popular beverage worldwide. During the production of filter tea, plant material is being processed by various unit operations, such as drying, cutting, grinding, fractionation, etc. Grinding of plant material with hammer mill causes the production of certain amount of fine powder (approx. 20%), which has been recognized as herbal dust and it is discarded as by-product. The aim of this work was valorization of sage herbal dust as raw material for production of lipophilic bioactive compounds. Subcritical water extraction (SWE) of sage lipids was optimized by response surface methodology (RSM). Three level, three variables, Box-Behnken experimental design (BBD) with temperature (X_1: 120 – 220°C), extraction time (X_2: 10 – 30 min) and HCl concentration in extraction solvent (X_3: 0 – 1.5%) were investigated as independent variables. Experimentally obtained values of investigated responses were fitted to a second-order polynomial model, and multiple regression analysis and analysis of variance (ANOVA) were used to determine fitness of the model and optimal conditions. Moreover, 3D surface plots which visualize interactive influence of independent variables on responses, were generated from mathematical models. Total yield of lipid compounds (Y) obtained by SWE (0.75 – 2.98%) were compared by Y obtained by conventional techniques (hydrodistillation and Soxhlet extraction). Chemical profile of all samples was determined by GC-MS and GC-FID analysis.

Keywords: Sage herbal dust, subcritical water extraction, terpenoids, optimization, response surface methodology.

P-52 Seasonal variation in essential oil composition and distillation yield of Grand Fir (*Abies grandis* ssp *idahoensis*)

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Abstract

In the essential oil industry, yield and quality of oil, as well as optimal harvest time, are vital in producing an economical product. The intent of this research was to evaluate the composition of the essential oil from *Abies grandis ssp idahoensis* (Grand Fir) at different time points during the year and to compare yield differences in relation to temperature at the time of harvest.

Harvest and distillation occurred approximately once per week for twenty-four weeks and is still ongoing. Approximate age of trees at harvest was 12-15+ years. Temperature measurements of the outside air were recorded every half hour for the duration of the study. Essential oil was steam distilled from the branches and needles. To date, a total of 24 samples of essential oil have been analysed by GC-MS, and tested for optical rotation, refractive index, and specific gravity.

After 24 weeks of sampling, a trend in the data shows that harvesting during cooler temperatures produces a greater yield of essential oil. The average temperature at harvest during August was 58.8°F, and the average yield was 0.41% (n=4). The average temperature at harvest during December and January decreased to 22.3°F, and the average yield increased to 0.61% (n=8).

The data show small changes in the composition of essential oil samples. The average bornyl acetate content in August was 18.2% (n=4), and this increased to an average of 19.6% in December and January (n=8). The average beta-pinene content in August was 27.9% (n=4), and decreased to an average of 20.2% in December and January. There was little to no variation in optical rotation, refractive index, or specific gravity.

These results show that harvesting during cooler temperatures producing a greater yield; however, year round harvest is possible without considerable changes to essential oil composition.

Keywords: *Abies grandis ssp idahoensis,* Grand Fir, steam distillation, temperature, yield, GC-MS.

Acknowledgments

Young Living Highland Flats Farm

P-53 Characterization of limonoids in Citrus essential oils by means of supercritical fluid chromatography tandem mass spectrometry (SFC-QqQ MS)

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Abstract

Limonoids are a class of bioactive compounds, naturally occurring both as aglycones and glucosides, widely known for their biological activity, which include antiviral, antibacterial, antifungal and antioxidant properties; moreover, several recent studies refer to the anti-proliferative effects, which they have shown in many cancer cells lines. Plants of Rutaceae and Meliaceae families are the main natural sources of limonoids and many data are available concerning their characterization in *Citrus* fruits. However, to the best of our knowledge, there are no previous reports about the composition of limonoids in *Citrus* essential oils.

Nowadays, supercritical fluid chromatography (SFC) is considered a powerful analytical approach, suitable to achieve fast and green analyses, due to the diffusivity and the solvation power of CO_2 above its critical point, which is the main solvent utilized.

In this study, SFC coupled with a triple quadrupole mass spectrometer was employed to investigate the content of limonoids in different cold pressed *Citrus* essential oils. The separation was carried out on a C18 column, 250 x 4.6 mm I.D. with particle size of 5 □m, using low amount of methanol as co-solvent to elute all the targets compounds in fast way.

Due to the lack of available standards of limonoids, the Multiple Reaction Monitoring (MRM) parameters of each compound were optimized using the sample which showed the most abundant content. Quantification of limonin was carried out by constructing its calibration curve in MRM transition mode, under the same chromatographic conditions optimized for the samples, using the standard previously isolated in our laboratory (M. Russo, *et al.* 2015).

This work provides a qualitative profile of limonoid aglycones in different *Citrus* essential oils, by means of an innovative SFC-QqQ MS system, in a rapid and environmental friendly way. Among the samples analysed, differences in the composition of limonoids were observed, adding further data to the characterization of *Citrus* species.

Keywords: Limonoids, essential oils, citrus, supercritical fluid chromatography, mass spectrometry.

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P-54 Essential-oil composition and chemotypification of *Achillea atrata* L. subsp. multifida from Bulgaria

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Abstract

Achillea atrata L. (Asteraceae), commonly known as black yarrow or Dark Stemmed Sneezewort, is a European species of herbaceous perennial flowering plants native to the Alpine region and the Balkans (Radulović et al., 2006). Several reports on the pharmacological properties of this taxon can be found in the literature. Flavones isolated from *A. atrata* subsp. multifida extract demonstrated *in vitro* inhibitory activity against *Candida albicans* and *Bacillus subtilis* (Aljančić et al., 1999); the essential oil of this taxon exhibited strong antifungal action against 18 tested micromycetes (Ristić et al., 2004). From the time when the analysis of the essential oil by Ristić and co-workers (2004) was published, no one dealt with this plant taxon. We decided to reinvestigate, in more detail, the chemical composition of the hydrodistilled essential oil of *A. atrata* subsp. multifida collected in Bulgaria (Stara planina mountain). In total, two hundred forty constituents were identified by GC and GC-MS that accounted for 98.1% of the total GC peaks detected. The major volatile constituents were found to be: α-thujone (20.4%), camphor (14.0%) and 1,8-cineole (11.7%). In order to establish the chemotaxonomic status of *A. atrata* subsp. multifida within the genus *Achillea*, multivariate statistical analysis (principal component analysis and agglomerative hierarchical cluster analysis) was performed on the compositional data at hand and 23 additional taxa of Balkan *Achillea* species. The analyses revealed that *A. atrata* was most closely related to other taxa of the section *Anthemoideae*.

Keywords: *Achillea atrata* subsp. *multifida*, Asteraceae, chemotaxonomy, multivariate statistical analysis, α-thujone.

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P-55 Photochemical irradiation of verbenone as a source of natural product diversification

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Abstract

In this work, photochemical irradiation of the monoterpene verbenone, a common essential oil constituent, was investigated. A cyclohexane solution of verbenone was exposed to ultraviolet radiation for 24 h and the obtained product mixture was analysed by GC, GC-MS and NMR. Chrysanthenone was identified as the overwhelming major product (>90% of the reaction mixture calculated from the GC peak area) which was in accordance with previously published results (Erman, 1967). However, a number of naturally occurring monoterpenes were also detected as minor products. Among others, we were able to identify (*E*)- and (*Z*)-ocimenone, carvone, piperitenone, isopiperitenone and thymol. The possibility that under certain circumstances these known volatiles occur not as the direct products of secondary metabolism but as in fact possible artefacts (i.e. products of verbenone isomerisation by exposure to sunlight) would be discussed. Also, possible mechanistic pathways for the side-product formation are proposed. This reaction represents a good example of a green-oriented transformation that starts from a natural product and provides access to a diversity of other useful chemicals.

Keywords: Chrysanthenone, verbenone, photochemistry.

Acknowledgments

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

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P-56 Effects of essential oils of *Cuminum cyminum* and *Artemisia dracunculus* on biological activity of *Sitophilus oryzae* (Coleoptera: Curculionidae)

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Abstract

Now a days, extensive researches are performed on plant material in order to achive a safe and effective alternative to chemical insecticides for pest control. Effects of essential oils of *Cuminum cyminum* and *Artemisia dracunculus* were tested against first instar larvae, egg hatching and oviposition rate of Rice weevil (*Sitophilus oryzae*). Experiment was carried out at 27 ± 2 ˚C and 60 ± 5% R. H. under dark condition adopting a complete randomized block design. For each essential oil, six concentrations with six replications were tested. Increasing the essential oil concentration increased the effects of essential oils on eggs; first instar larvae and oviposition rate. The maximum concentration of essential oils (0.56 µl/cm3) of *C. cyminum* and *A. dracunculus* caused, 100 and 95.5% mortality of eggs and 100 and 96.66% mortality of first instar larvae, respectively.All the three tested plant essential oils reduced the oviposition rate of adults significantly. The highest concentration (0.37 µl/cm3) of essential oils of *C. cyminum* and *A. dracunculus* lead to oviposition deterrency, with a deterrence of 100 and 98.3%, respectively. LC50 values indicated that essential oil of *C. cyminum* with 0.048 and 0.041 µl/cm3 against eggs and first instar larvae was more effective than the essential oil of *A. dracunculus*. It was found that plant essential oils particulary *C. cyminum* could be used as either a safe pesticide or model for new synthetic pesticides to control store pests.

Keywords: *Sitophilus oryzae*, Plant essential oil, Oviposition deterrence, Ovicide, Bioactivity.

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P-57 Patterns of essential oil use among the Slovenian population: an online survey

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Abstract

Essential oils (EO) are gaining in popularity not only in pharmaceutical, food, cosmetic and chemical industry but also in their home use among the general population. Surprisingly, very little is known about their everyday use.

With the aim of investigating the habits of EO use at homes, an online survey was conducted on a sample of Slovenian population of EO users (n=350). The survey was active from mid-December 2016 to mid-January 2017 and consisted of 29 questions, focussed on four main areas: (1) general habits of EO use (use frequency and purposes, sources of information, frequently used EO), (2) application habits (modes, application sites, dilution rates), (3) individual cases of targeted use and potential side effects, and (4) use of EO with kids (how, when and for what purpose).

The results confirmed the rapid growth of EO users in Slovenia, as more than half (53 %) of participants have used them for 3 years or less. The vast majority of EO users are women; almost 75 % of users are younger than 45 years, and 63 % use EO with their kids. Nearly half (48 %) of all users are in daily contact with EO, mostly to relieve or prevent various respiratory and skin conditions or to influence the mood. The most frequently used EO are lavender (*Lavandula angustifolia*), tea tree (*Melaleuca alternifolia*), eucalyptus (*Eucalyptus* sp.) and frankincense (*Boswellia* sp.) that are, besides inhalation, applied most frequently to the chest and soles. Interestingly, almost 20 % of participants have experience with at least one type of internal use.

The results also show that the users rely on potentially unreliable sources of information: popular aromatherapeutic books, online sources and EO suppliers. As the long-term risks of frequent EO use are not well understood, awareness should be raised about the safety of EO use and the habits of home users monitored more systematically.

Keywords: Essential oils, aromatherapy, home users, safety.

P-58 Antioxidant capacity of zahtar, myrtus, fennel, bay laurel, coriander and lemongrass essential oils

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Abstract

Natural antioxidants have a very growing interest because of some toxic effects of synthetic antioxidants. Essential oils have been used centuries for their antimicrobial, antiseptic, anti-inflammatory and antioxidant benefits. In this study the essential oils (EOs) of *Thymbra spicata* L., *Myrtus communis* L., *Foeniculum vulgare* Mill., *Laurus nobilis* L., *Coriandrum sativum* L. and *Cymbopogon citrates* Stapf. were tested by the ferric reducing antioxidant power (FRAP) assay to determine possible antioxidant capacity. Essential oils were obtained with hydro-distillation method and components were determined with gas chromatography/mass spectrometry.

The antioxidant capacity of essential oils was found to be highest in zahtar with 26,36 mmol.Fe^{+2}/kg, followed by bay laurel (13,77 mmol.Fe^{+2}/kg), fennel (11,53 mmol.Fe^{+2}/kg), myrtus (8,21 mmol.Fe^{+2}/kg), lemon grass (3,82 mmol.Fe^{+2}/kg) and coriander (1,06 mmol.Fe^{+2}/kg). Essential oil components were found; in zahtar EO carvacrol and o-cymene (55,30-13,51%), in bay laurel EO eucalyptol and sabinene (49,10-12,68%), in fennel EO anethol and α-pinene (66,86-7,19%), in myrtus EO α-pinene and eucalyptol (37,25-32,76%), in lemon grass EO citral and z-citral (50,20-27,66%) and in coriander EO linalool and α-pinene (88,92-1,61%), respectively.

It was concluded that zahtar (*Thymbra spicata* L.) essential oils could be utilized as food additives. Additionally further studies should be done to obtain antioxidant properties of the components specified.

Keywords: Antioxidant capacity, thymbra, coriander, lemongrass, bay laurel, zahtar, fennel.

P-59 Olfacto Chemical Finger Printing (OCFP) of odorous volatiles: application to differentiation of Pansies varieties

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Abstract

Differentiation of flowers varieties based on emitted volatile profiling was extensively applied in the past 10 years in combination with metabolomics and genotyping but aromagrams were poorly used for differentiation despite to their potentialities.

In the framework of a study on the Viola genus (including Violas and Pansies), an innovative method called Olfacto Chemical Finger Printing (OCFP) was developped in order to combined both chromatograms and aromagrams for differentiation based on a home designed SPME-GC-MS-2O (dual parallel olfactometry quantification). In the present paper, we report the analysis of 20 certified samples of pansies provided by Municipal Green Houses of Toulouse with various flowers colors (red, yellow, purple, pink). Flowers were immediately analyzed after collection in the field in order to capture the naturally emitted volatiles. Optimisation of experimental conditions of gas phase SPME (equilibration time, fiber adsoprtion time) were performed. Training of panelists is set up under the supervision of our Resident Perfumer. Preliminary results reported that olfacto finger printing could be more efficient for differentiation of Panola pansies in comparison with chemical finger printing due to the low concentration of emitted volatiles but with low odorous threshold.

Keywords: Pansies, Olfacto Chemical Finger Printing, SPME-GC-MS-2O.

P-60 Recognition of *kawaii* fragrance by sensory evaluation

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Abstract

The concept of the Japanese adjective “kawaii” which is closer in meaning to “cute” is spreading rapidly around the world since 2010 through the values of women and young people towards fashion, goods, art and others. Then, we predicted that kawaii fragrance might be present, and analyzed the perception of kawaii fragrance from impression evaluations against 20 essential oils (EO) by 18 Japanese men and women within the age group 20-23.

Fig.1 shows a ten-scale evaluation value of kawaii and disgust towards EO without informing them about the contents. As an example, citrus fruits and Ylang Ylang showed high kawaii values. On the whole, there was a high correlation of R = -0.89 between kawaii and disgust.

Next, the typical recognition test for each fragrance was conducted and results classified into six. Table 1 shows the results of multiple regression analysis on kawaii values. EO recognized as flowers or fruits, but not wood, showed high kawaii values. Although, Rosemary is recognized as flower, its kawaii value was low as shown in Fig.1.

Further, to understand the depth recognition of kawaii fragrance, impression evaluation by Semantic Differential (SD) method was investigated. As shown in Table 2, the impression terms of “feminine” and “sweet” were derived to the depth recognition on high kawaii fragrance. On the other hand, for preference, “comfortable” and “tenderly” were derived to high influencing factors. In comparing the preference and kawaii, the evaluation terms affecting them were different. From this, the feelings of preference and kawaii are considered to be by different causes in spite of the high correlation between them.

Keywords: Kawaii fragrance, essential oil, semantic differential, sensory evaluation.

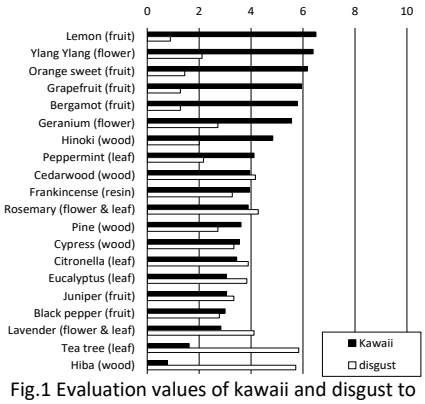

Fig.1 Evaluation values of kawaii and disgust to various EO

Table 1 Degree of influence for each type with regards to kawaii fragrance derived by multiple regression analysis.

Type	t
Flower	8.53
Fruit	5.55
Wood	- 4.07
Animal	- 1.98
Food	1.94
Medicine	- 1.84

t: degree of influence

Table 2 Impression terms influencing kawaii and preferred fragrances derived by multiple regression analysis.

on “kawaii”		on “preference”	
Term	t	Term	t
feminine	7.45	comfortable	12.93
sweet	6.02	tendrly	3.30
tendrly	2.50	t < 2	
comfortable	2.28	sharp	thick
warm	2.07	mildly	cold
t < 2		apparent	dirty
light (ant. heavy)	light (ant. dark)	sweet	anonymous
blunt	anonymous	light (ant. dark)	heavy
strong	dirty	feminine	
intense	clear	soft	
apparent	thin	clear	
soft		strong	

t: degree of influence

P-61 Does olfactory adaptation influence the psychophysiological effect of orange absolue on men and women?

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Abstract

In aromatherapy essential oils (EO) are either used by dermal application or by inhalation. While a pharmacological effect of an EO can be expected after topic use, the effect after inhalation is rather based on the great hedonic and semantic impact of the oil's odor. However, exposure to an odor over a longer period of time leads to a decrease of odor perception, which is called olfactory adaptation. This well-known phenomenon acts as a short-term filter that allows us to respond to new or changing olfactory stimuli. The question that arose under this aspect was whether the permanent exposure to an odor, as used in aromatherapy, changes its psychophysiological effect on humans compared to an intermitted odor confrontation.

In continuation of our investigations on the influence of olfactory adaptation on the psychophysiological effect of EOs, orange absolue, derived from *Citrus sinensis* (L.) OSBECK, was tested in the present study. Ninety healthy nonsmokers, 45 men and 45 women, volunteered for this study. They were randomly divided into three groups ("permanent odor", "intermitted odor", control = no odor), each group consisted of 15 males and 15 females, respectively. To access the adaptation-effect, an intensity rating was performed every five minutes within each session of 30 minutes. Blood pressure, heart rate and mood were determined in time (prior to and after odor exposure). Hedonic valence, familiarity and expected effect of presented odors (orange or control) were rated at the end of each session.

Keywords: Orange absolue, human, mood, olfactory adaptation, psychophysiology.

Acknowledgments

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P-62 Scents from Brazilian Cerrado: The essential oil from *Aldama bracteata* (Gardner) E.E.Schill. & Panero (Asteraceae)

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Abstract

The genus *Aldama* (Asteraceae) is found exclusively in the Americas. In Brazil, 35 species are known for this genus, 17 of them endemic (Bombo et al., 2017). Essential oils have been distilled from *Aldama* plants (Bombo et al., 2017, Oliveira et al., 2016). *Aldama bracteata* is an endemic, intensily aromatic herb from the Cerrado, a savannah-like biome with more than 12,000 botanical species in Central Brazil. Samples from a population (n>5) were collected from Chapada dos Veadeiros National Park, in Goiás State, Brazil. A voucher was deposited at the Embrapa Genetic Resources herbarium (CEN 84459) and the essential oil obtained by hydrodistillation for 2 h, using a Clevenger-type apparatus. It was analyzed by GC/MS and CG-FID using an Agilent 6890 GC coupled to an Agilent 5973N mass selective detector, using a HP5-MS capillary column. Identification was performed by both mass spectra and linear retention indices.

Oil yield was 0.3 %. Thirty-eight components were found in the oil, and 97.1 % of its composition was identified. The major compounds found were myrcene (72.1 %), β-pinene (8.4 %) and α-pinene (4.4 %). All the other components were present individually in less than 2 %. This composition is quite different from other oils of *Aldama* species described in the literature. *A. bakeriana* and *A. grandiflora*, for example, are rich in sesquiterpenes such as germacrene D, bicyclogermacrene and β-caryophyllene (Bombo et al., 2017). To the best of our knowledge, this is the first analysis on the essential oil from *A. bracteata*.

Keywords: Essential oil composition, *Aldama bracteata*, Asteraceae, Cerrado, myrcene.

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P-63 Scents from Brazilian Cerrado: The essential oil from *Calea teucriifolia* (Gardner) Baker (Asteraceae)

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Abstract

The Brazilian Cerrado is a savannah-like biome with more than 12,000 botanical species in Central Brazil. It is an endangerous biome, considered a biodiverisity hotspot (Myers et al., 2000). *Calea teucriifolia* (*syn Meyeria teucrifolia*) is a shrub native from the Cerrado. Samples from a population (n>5) were collected from a rupestrian field in the Serra dos Pirineus National Park, in Goiás State, Brazil. A voucher was deposited at the Embrapa Genetic Resources herbarium (CEN 84468) and the essential oil obtained from the leaves by hydrodistillation for 2 h, using a Clevenger-type apparatus. It was analyzed by GC-MS and GC-FID using an Agilent 6890 GC coupled to an Agilent 5973N mass selective detector, fitted with a HP5-MS capillary column. Identification was performed by both mass spectra and linear retention indices.

Oil yield was 0.3 %. The major compounds found were p-cymene (15.2 %), myrcene (13.5 %), caryophyllene oxide (9.7 %) and α-phellandrene (9.5 %). The oil composition is quite different from other *Calea*, like *C. clematidea*, rich in clematerol, a terpenic epoxide (Flach et al., 2002). A previous phytochemical study on the hexane extract of *C. teucriifolia* identified two nerolidol derivatives, together with some sesquiterpenes and a furanoheliangolide (Bohlmann et al., 1981). To the best of our knowledge, this is the first analysis on the essential oil from *Calea teucriifolia*.

Keywords: Essential oil composition, *Calea teucriifolia*, Asteraceae, Cerrado.

Acknowledgments

The authors thank Capes, CNPq and FAPERJ for financial support.

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P-64 Essential oils from Amazonian herbs: a business opportunity for local communities

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Abstract

The Amazonian herbs are considered an important economic product for local communities which trade these plants in street markets. These plants have been cultivated in small scale on farms around the Amazon forest and they contain essential oils that are locally described in the literature as raw materials for fragrances. However, a few or none of these species seem to be available in the necessary scale for the fragrance industry. The artisanal form in which they are cultivated and collected limits the market, since the essential oils have a poor quality and low volume to satisfy the demands of the fragrance industry. On the other hand, the sustainable management is possible like what happened with priprioca species, which is produced in a large scale to attend the market (Potiguara, 2008). In the street markets in Belém and Manaus many aromatic herbs species were identified and documented as essential oil sources (Alves et al. 2008; Zoghbi et al. 2014). Generally, the plants are sold for culinary and medicinal uses and the prices rate around EUR 0.15 to 1.15 for each pack. These prices are common during the peak season of each species but out-of-season prices may increase to EUR 1.72 or 2.00. These plants are also used by the local people as perfumes, such as scented baths, or in religious ceremonies. Among the herbs traditionally used in the Amazon as perfume are patchoulli, rosemary, pataqueira, estoraque, basil, wild mint, vetiver and capitiú, which are placed in an infusion to perfume the body. Some of these plants provide essential oil rich in linalool (Zoghbi et al. 2014). As most aromatic herbs have a short life cycle (three to six months), it is possible for the fragrance industry to establish a sustainable supply chain and help the communities develop, generating employment and also income for them giving.

Keywords: Fragrances, aromatic plants, supply chain, sustainable.

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P-65 Olfacto Chemical Finger Printing (OCFP) of odorous volatiles: application to differentiation of Lavenders varieties

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Abstract

Differentiation of essential oils of lavender vs lavendins by chemical finger printing are widelly used by aromatic industry for Quality Control but olfacto finger printing is not so communly used desptte its potentiliaties due to presence of odorous markers with low threshold. If Bulgaria and France are the country leaders in production, Croatia has developped since 20 years a production of lavenders/lavendin essential oils for tourism purpose but with badly controlled labelling.

In the framework of project associating Croatia and France, an innovative method called Olfacto Chemical Finger Printing (OCFP) was developped in order to combined both chromatograms and aromagrams for differentiation based on a home designed SPME-GC-MS-2O (dual parallel olfactometry quantification). In the present paper, we report first the analysis of 10 certified essential oils (Lavandula angustifolia, Lavendula officinalis, Lavandula latifolia, Lavendula bumatii, Lavendula hybrida grosso, Lavendula hybrida reydovan, Lavendual hybrida suniam, Lavendula stoechas, Lavendula dentata, Lavendula burnati briquet) originated from Bulgaria, France and Spain in order to create a database associating chromatograms and aromagrams. Then 10 samples of croatians lavenders originated from the different areas production were analyzed in order to determine the genuine appellation.

Keywords: lavender, lavendin, essential oil, GC-MS-2O

P-66 Citrus essential oil cause higher disturbance on growth kinetic of a pathogenic bacterium than a beneficial bacterium

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Abstract

The antibiotics use as Antimicrobial Growth Promoters (AGP) in livestock was banned in 2006 by European Community due to increasing bacterial resistance to antibiotics, considered a serious problem of public health. Antibiotics were added in animal diets due to their effects on animal gut microbiota as well as in animal immune system. Several studies searching for alternatives to substitute antibiotics have focused in evaluate essential oils (EOs) due to their recognized antimicrobial properties. Thus, the aim of this work was evaluate the antibacterial activity of a commercial citrus EO (Brazilian Orange Terpens) and its effect on growth kinetic of a pathogenic bacterium and a beneficial bacterium that can occur in pig gut microbiota. The pathogenic bacterium was *E. coli* U21 (K88 LT/STb/F18/STa) isolated from pig gut and the beneficial bacterium was *L. rhamnosus* ATCC 7469. Minimal Inhibitory Concentration (MIC) was determined by microdilution method and Minimal Bactericidal Concentration (MBC) by plating. Bacterial growth kinetic was studied by absorbance reading in each hour at several EO concentrations (from 0.116 to 14.8 mg/ml) and without EO (control). All curves were modeled using the modified Gomperzt Model (Zwietering *et al.*, 1990). MIC and MBC results showed that *E. coli* was more sensitive than *L. rhamnosus* to BOT. Modified Gomperzt Model enabled to determine maximal bacterial population (*A*), maximum growth rate (μ_{max}) and Lag phase duration (λ). To *E. coli*, *A* and λ parameters were greatly affected as EO concentration was increased, *A* was reduced in 55.9% at the higher effective concentration in contrast to control, and λ increased approximately eight times than control. To *L. rhamnosus*, only λ suffered a significant increase as EO concentration increased (1.6 times). The μ_{max} for both bacteria was not affected. Finally, both bacteria were subjected to sequential doses of BOT (0.925 mg/ml) every 3h until 9h. After the third dose, *E. coli* suffered a severe higher reduction on its population than *L. rhamnosus* even when both bacteria were subjected to a single dose. Therefore, these results showed that BOT has a selective antibacterial activity with higher effect on the pathogenic bacterium. Limonene (81.37%), cis-limonene oxide (2.73%) and myrcene (2.61%) were the major compounds detected in BOT by CG/MS analysis.

Keywords: Citrus essential oil, growth kinetic, *E. coli*, *Lactobacillus rhamnosus*, antibacterial activity.

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P-67 Evaluation of the chemical composition and antibacterial activities of the essential oils of *Lavandula angustifolia* Mill. obtained by hydrodistillation and steam distillation

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Abstract

Lavandula angustifolia Mill. is a perennial herb that belongs to the *Lamiaceae* family and has a traditional use in many industries. It is known for its healing and antimicrobial effects in herbal medicine. In aromatherapy it is used as a relaxing agent, but also forms an integral part of perfumes, soaps or dishes in the kitchen. Essential oils (EOs) were obtained from flowers of this plant by hydrodistillation (HD) and steam distillation (SD). The total yield of the volatile fractions obtained through HD and SD was 2.23% and 2.82%, respectively. EOs were characterized by gas chromatography with mass spectrometry (GC-MS) and by gas chromatography with a flame ionisation detector (GC-FID). In both oils, main components were linalool, linalyl acetate and 1,8-cineole, but in various amounts. Higher amount of linalool (48.6%) was presented in the oil of HD in comparison with SD (40.0%). However, SD oil contained more linalyl acetate (26.1%) than HD oil (13.4%). Amount of 1,8-cineole was similar in both oils. The EOs obtained by both distillation methods were tested against *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Candida albicans* by disc diffusion test method. Both EOs were active against all the bacteria tested except *Pseudomonas aeruginosa*. Higher antimicrobial activity against the bacteria and yeast tested generally showed the essential oil obtained by HD, when higher inhibitory effects were observed at *Escherichia coli* and *Staphylococcus aureus*. The most sensitive was *Staphylococcus aureus*. Both essential oils had the same inhibitory effect on *Enterococcus faecalis* and *Candida albicans*.

Keywords: Lavander, essential oil, hydrodistillation, steam distillation, antibacterial properties.

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P-68 Antibacterial evaluation of clove, peppermint and thyme essential oil against *Haemophilus* species

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Respiratory tract infections (RTIs) are frequently caused by *Haemophilus* species especially in patients with suppressed immune system. In case of RTIs the narrowing possibilities in antibiotic therapy and growing antibacterial resistance indicate the discovery of new alternative treatments which could support the medical therapy. Our previous experiments showed that essential oil (EO) of peppermint, clove and thyme have strong antibacterial activity against several respiratory tract pathogens (e.g. *Streptococcus pneumoniae, S. pyogenes* and *Moraxella catarrhalis*). Thus, the antimicrobial investigation of these EOs should be reasonable against other causative pathogens as well.

Therefore, the aim of our study was the microbiological evaluation of clove, thyme and peppermint oils against *H. influenzae* (DSM 4690), and *H. parainfluenzae* (DSM 8978) by different *in vitro* techniques.

The EOs were obtained from a Hungarian company (Aromax Ltd.) and were analysed with GC-MS. In case of broth dilution method (BDM) 5% emulsion was prepared from each oil containing 10% solution of Tween 80 and *Haemophilus* culture. The MIC and MBC values were expressed in mg/mL. The antimicrobal effect of EOs and their individual components was determined with TLC-direct bioauthography (TLC-DB). The visual detection of inhibition zones was made with an aqueous MTT solution. The antibacterial activity was expressed as the diameter (mm) of inhibition zones and measured with Motic Images Plus 2.0 program. Effect of detergent, solvent and reference antibiotics was also detected in our test systems. All measurements were made in triplicate.

Our results revealed that EO of thyme was the most effective (MIC: 0.1 mg/mL), while peppermint and clove showed similar activity in BDM. In comparison with reference antibiotics EOs produced antibacterial activity in higher concentrations. The TLC-DB results demonstrated that both bacteria were the most sensitive to thyme EO (12.5 mm) and thymol (11.9 mm). The EO of clove was also effective with 11 mm diameter of inhibition zone. Peppermint oil showed weaker activity (8 mm). To the best of our knowledge, we performed TLC-DB with *Haemophilus* species firstly.

In our future study, we would like to focus on the mode of actions of these EOs and involve other respiratory tract pathogens in our experiments.

Keywords: Essential oil, antibacterial activity, bioautography, broth dilution, *Haemophilus.*

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P-69 Examination of changes in quality of selected essential oils stored at room temperature and under refrigerated conditions

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Introduction: Essential oils (EOs) are susceptible for chemical alterations during storage mainly due to oxygen and light exposure [1] resulting in quality impairment. Therefore, the pertinence of their storage conditions may be questionable in pharmacies. The aim of our work was to follow the alterations of EOs, which are most frequently used in Hungarian officinal practice, under different storage conditions.

Materials and methods: Pharmacists were personally interviewed to estimate the storage conditions and frequency of application of EOs in magistral preparations in the area of Baranya and Tolna counties. The five most common EOs were purchased from three different pharmacies. Half of each sample was stored at room temperature (at 25°C) and the other half was stored in a refrigerator (at 4°C). Peroxide value was measured by the „A" method of the European Pharmacopoeia (a biphasic titration). Quality of the samples was also monitored by thin layer chromatography. These tests have been repeated in six-month periods.

Results: The five most common EOs were eucalyptus, lavender, lemon, peppermint and rosemary. The presence of the main EO components (eucalyptol, linalool, linalyl acetate, citral and menthol) did not change within the investigated time frame (1.5 year). An increase in peroxide value was detected in all samples obviously indicating oxidative processes, but in the EOs of eucalyptus, rosemary and peppermint stored in refrigerator (at 4°C) a significantly lower peroxide value could be measured.

Conclusion: Our experiments showed that EOs undergo oxidative damage, which was confirmed by the increase in their peroxide value. According to our results, storage in pharmacies' refrigerator may be reasonable in the case of eucalyptus, peppermint and rosemary oils. The evaluation of GC-MS results is in progress.

Keywords: Essential oil, storage, magistral preparation, peroxide value, TLC.

Acknowledgments

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P-70 Chemical composition and anti-cholinesterase activity of essential oils of *Daucus carota* L. subsp. *carota* from two different localities

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Abstract

Daucus L. belongs to essential oil content rich Apiaceae family. *D. carota* (carrot) is the best known species of the genus are cultured widely around the world. Carrot root is used as food, essential oil derived from fruits is used in perfumery. Carrots contain substances that are diuretic effective, helping to resist cancer and mitigating digestive system irritations. In folk medicine, carrots are used as a carminative, emmenagogue and diuretic in the treatment of tonsillitis, intestinal parasites and constipation (Ozçelik et al, 2004; Radulovic et al,2011).

In tis study, the essential oils of fruits of *D. carota* subsp. *carota* collected from two different localities between Bodrum and Güvercinlik in Muğla, Turkey, were obtained by hydrodistillation. The essential oils were coded as EO1 and EO2. All samples were analysed by GC-FID and GC-MS systems, simultaneously. Trans-methyl isoeugenol (45.70%), 1-α-H-himachal-4en-1-β-ol (25.04%), β-bisabolene (8.92%) were identified as a major components for EO1. Also, 1-α-H-himachal-4en-1-β-ol (49.43%), longipinene (13.47%) and β-bisabolene (9.97%) were found as a major components for EO2. Additionally, the anti-cholinesterase activity was determined by using *in vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes inhibition assays. AChE and BuChE inhibitory activity was determined by Ellman *et al.* method using galantamine as the reference compound (Ellman et al., 1961). Essential oils showed potential acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities. EO1 (IC_{50} = 2.125 µM) shows the highest inhibitory activity on AChE. The value was less than that of galantamine (IC_{50} = 2.41 µM). EO2 exhibited the strongest inhibition against BuChE with an IC_{50} value of 11.32 µM, which was 0.6-fold more than that of galanthamine (IC_{50} = 17.38 µM).

Keywords: *Daucus carota* subsp *carota*, essential oil, anti-cholinesterase.

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P-71 Differential response of phytoparasitic nematode species to essential oils

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Abstract

Essential oils (EOs) has been widely reported for their biocidal activity on root-knot nematodes and on the pine wilt nematode *Bursaphelenchus xylophilus*, but only few have been investigated on other nematode species. An *in vitro* study was conducted for a comparative evaluation of effects of EOs from *Artemisia herba-alba*, *Citrus sinensis*, and *Rosmarinus officinalis*, as well as of their main components (thujone, limonene, 1,8-cineole, α-pinene and camphor) on three phytonematode species with a different feeding and reproduction habitus, i.e. *M. incognita*, *P. vulnus* and *X. index*. Infective juveniles of *M. incognita*, mixed stages of *P. vulnus* and adult females of *X. index* were exposed for 24, 48 or 96 hours to 4, 10, 20 and 30 μg mL^{-1} solutions of EOs or their components and then checked for their mortality. Juveniles of *M. incognita* and females of *X. index* resulted highly sensitive to the EOs of *A. herba-alba* and *R. officinalis*, but scarcely or poorly affected by the EO of *C. sinensis*. In contrast, specimens of *P. vulnus* resulted less sensitive than *M. incognita* and *X. index* to EOs of *A. herba-alba* and *R. officinalis*, but showed a higher sensitivity to *C. sinensis* EO. In the assays on major EO components, activity of limonene was almost nil on all three nematode species, as well as a limited mortality occurred also in α-pinene solutions. Activity of thujone and 1,8-cineole was much higher on *M. incognita* than on *P. vulnus* and *X. index*, whereas all the three nematode species were limitedly affected by camphor. The high nematicidal activity of tested EOs and their large availability make them a potential source of new nematicides. As the activity of EOs' components was never as high as that of the whole EOs, a synergistic action of the EO multicomponent mixture may be hypothesized.

Keywords: Essential oils, *Artemisia herba-alba*, *Citrus sinensis*, *Rosmarinus officinalis*, phytoparasitic nematodes

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P-72 Synergistic antibacterial combination of *Lavandula latifolia* Medik. essential oil with camphor

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Abstract

Lavandula sp. (Lamiaceae) are cultivated around the world and are significant for perfume, cosmetic and pharmaceuticals due to their high content and quality of essential oil. In recent years, efforts to increase the antimicrobial efficacy with essential oils due to antibiotic-resistance in infections have gained importance.

In this present study, it was aimed to evaluate the in vitro synergistic antibacterial effect of *L. latifolia* (spike lavender) essential oil with camphor by using checkerboard method against the human pathogens Helicobacter pylori, Staphylococcus aureus and Listeria monocytogenes standard strains to the best of our knowledge for the first time.

L. latifolia essential oil and racemic camphor were obtained from commercial sources in Pharmacopoeia Grade. The components of the essential oil were analysed by GC-FID and GC/MS, simultaneously. In vitro antibacterial activity of essential oil, camphor (MIC: 0.16-20 mg/mL) and standard antimicrobial clarithromycin (MIC: 0.125-16 µg/mL) were carried out by broth microdilution method (CLSI, 2006) against *H. pylori* ATCC 43504, *S. aureus* ATCC 25923, and *L. monocytogenes* ATCC 19111 standard strains, respectively. *L. latifolia* essential oil was combined with camphor using the checkerboard method. Resulting antibacterial effects were evaluated for fractional inhibitory concentrations (FICs) as antagonistic, additive and synergistic effects (de Rapper at all., 2012).

The analytical results showed that the major component was essential oil was linalool and 1,8-cineole in 45.2 and 25.6 %, respectively. Antibacterial effects of essential oil were determined as MIC 0.31 to 5 mg/mL against *H. pylori, S. aureus,* and *L. monocytogenes* standard strains. As a result of the antibacterial activity experiments, essential oil+camphor combinations were identified as "synergistic (FIC≤0.5) and additive (0.5<FIC≤1)" in the respective combinations suggesting a positive aspect in antimicrobial therapy.

Keywords: *Lavandula latifolia,* essential oil, camphor, checkerboard method, antibacterial activity.

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P-73 Essential oil volatile profiles of four *Salvia lavandulifolia* Vahl. subspecies and their antimicrobial activity against opportunistic gut microbiota pathogens

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Abstract

Growing concern among health authorities and consumers about resistance to antibiotics has shifted the focus of research toward naturally occurring antibacterial products. The use of essential oil from aromatic and medicinal plants as possible alternatives to in-feed antibiotic growth promoters in animal nutrition has led to important advances in this respect.

The aerial parts of Spanish sage (*Salvia lavandulifolia*, L.) has been used in traditional Mediterranean medicine for its analgesic, antioxidant, sedative and antiseptic activities since ancient times. In the Iberian Peninsula, 5 different *S. lavandulifolia* subspecies have been defined: subsp. Lavandulifolia; subsp. vellerea; subsp. Oxyodon; subsp mariolensis and subsp. blancoana. While the antibacterial activity of *S. lavanduifolia* Vahl. has previously been described by others researchers, to the best of our knowledge the antimicrobial activity of the essential oils obtained from these five sage subspecies against opportunistic gut microbiota has not be studied. In the present work a survey was made in Murcia and Albacete provinces (south-eastern Spain) in order to define the volatile profiles of the essential oils using Gas chromatography-Mass spectrometry. Their inhibitory growth curves against the following strains were also obtained: *Salmonella enterica* subsp.enterica CECT 443; Staphylococcus *aureus* subsp. aureus CECT 59; Bacillus *cereus* CECT 131; *Listeria monocytogenes* CECT 911; *Escherichia coli* CECT 45 and *Shigella sonnei* CECT Nº 413. A total of 50 plants (10 per subspecies) were prospected, and the analytical results allowed a description of the most common chemotypes: lavandulifolia (1,8-cineole 45% / camphor 11%); vellerea (1,8-cineole 34% / camphor 28%); oxyodon (1,8-cineole 40% / camphor 28%); mariolensis (1,8-cineole 47% / camphor 10%); blancoana (1,8-cineole 19% / camphor 11%; caryophyllene 8%). The inhibition growth curves were monitored for 48h with the essential oil concentrations ranging from 20,000 ppm to 1250 ppm. Among the subspecies studied, subsp. *vellerea* seems to be the most effective against *Salmonella* (48 h/ 2500 ppm), *Staphylococcus* (40 h/ 2500 ppm) *B. cereus* (24 h/10,000 ppm) and *Shigella sonnei* (48h/10,000 ppm) strains. As a major finding, similar relative concentrations (close to 30%) between 1,8-cineol and camphor was seen to improve the antibacterial activity of salvia essential oil.

Keywords: *Salvia lavandulifolia*, subspecies, essential oil, antimicrobial activity.

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P-74 Antibacterial activity of *Mentha pulegium* essential oil components against *Staphylococcus aureus* and *Escherichia coli* in mastitis disease

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Abstract

In recent years, public concern over routine use of antibiotics in livestock for bovine mastitis has increased due to the emergence of antibiotic resistant bacteria that may represent a risk to human health. This study aimed to determine the antibacterial properties of essential oil of pennyroyal (*Mentha pulegium*) on *Staphylococcus aureus and Escherichia coli* by using agar disc diffusion. Essential oils were supplied by Department of Medical Plants of Arak University. Disc-assay was found to be a simple, cheap and reproducible practical method. The inoculum of bacteria (1×10^8 CFU/ml) was spread on sterile Mueller Hinton plate and then sterile discs were placed on the inoculated surface with three replications. Three concentrations (10%, 30% and 50%) of essential oil were prepared by adding dimethyl sulfoxide solvent. Paper discs (6 mm in diameter) were soaked in 10 µl of the essential oil and placed on the inoculated plates and allowed to dry for 15 min, then incubated at 37 ^{0}C for 24h. Antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) surrounding bacterial growth. The model of this study was completely randomized design. In the dose response study, the inhibition zone increased with the increasing concentration of essential oil. Low concentrations (10 µl) of essential oil inhibited weakly the development of bacteria (8-10 mm). However, *E. coli* was more sensitive than *S. aureus* in the medium containing pennyroyal essential oil. At a high concentration (50 µl/ml), the essential oil exhibited inhibition zones ranging from 15–20 mm. There are no significant differences between concentrations of 30% and 50% in *E. coli*. Pennyroyal essential oils have a potential to inhibit and inactivate two microorganisms in agar medium at different concentrations. It is suggested to investigate higher essential oils concentrations than were those used in research.

Keywords: Mastaitis, *Mentha pulegium*, essential oil.

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P-75 Antimicrobial and toxicity activities of *Pimpinella anisum* L. essential oil and its major component

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Abstract

Pimpinella anisum L. (Anise), plant family name Apiaceae, is an annual herb with white flowers and small green to yellow seeds. Anise is endemic to Asia Minor, Egypt, and Greece, and is grown in Europe, South America and many other warm regions of the World.[1,2] Members of this genus are cultivated for their aromatic seeds used in medicine as a condiment, mild expectorant and in treating dyspeptic complaints.[3] In addition, essential oils of some Iraqi *Pimpinella* species have been used to treat some diseases like seizures and epilepsy. The major constituent of the essential oil is trans-anethole.[2]

In the present work, chemical composition of the Pharmacopoeia quality *Pimpinella anisum* essential oil (EO) was investigated. *(E)*-Anethole (93.5%), methyl chavicol (2.5%) were determined as the major components by GC-FID and GC-MS, simultaneously. Moreover, antimicrobial and toxicity activities were also examined EO and its main component.

The antimicrobial activity was evaluated by the microdilution broth susceptibility assay against *Bacillus cereus, Streptococcus sanguinis, Staphylococcus aureus,* and *Corynebacterium striatum.* EO and *(E)*-anethole showed weak antimicrobial effects against *Corynebacterium striatum, Streptococcus sanguinis* and *Staphylococcus aureus*. MIC values was calculated as the EO 1.25 mg/mL and *(E)*-anethole 1.25 mg/mL against *Basillus cereus.*

In vivo animal alternative toxicity tests were carried out for EO and *(E)*-anethole using *Caenorhabditis elegans.* No acute toxicity was observed in the first 4 hours of EO and its main component.

Keywords: *Pimpinella anisum*, *(E)*-anethole, antimicrobial, toxicity.

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P-76 The antifungal effect of three essential oils against *Septoria melissae* Desm. and *Septoria lavandulae* Desm.

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Abstract

In the past 20 years the plant protection of medicinal and aromatic plant (MAP) cultures became more difficult. This is mainly due to the low number of authorized pesticides and the strict requirements of the processing industry on the maximum allowed residue levels. Therefore it is necessary to develop new and environment friendly agents for the protection of MAP cultures. The essential oils are potential candidate materials for this purpose, because they have antimicrobial effects and do not leave toxic compounds in the plants.

The goal of the recent work was to test the *in vitro* effect of the essential oils of *Cinnamomum zeylanicum* Blume, *Thymus vulgaris* L. and *Corinadrum sativum* L. against the most important pathogen of lemon balm, *Septoria melissae* Desm. and a related species *Septoria lavandulae* Desm.

In our experiment we used the agar dilution method to test the inhibitory effect of essential oils in 3 dilutions (0.3%, 0.1% and 0.03%) on the mycelial growth of the test pathogens. Silwet Star wetting agent in 0.02% was used to improve the homogeneity of essential oil-water solutions. The effect of the wetting agent was tested as well.

Dilutions of 0.3% and 0.1% of each essential oil inhibited the growth of the pathogens by 100%. The lowest (0.03%) concentration of essential oils had a moderate effect against the fungal growth. Essential oils of cinnamon, thyme and coriander showed respectively 93%, 69-81% and 47-64% inhibition against both *Septoria* species. The wetting agent had a very weak growth depression effect.

Based on our result, all investigated essential oils can be effective in the concentrations 0.3% and 0.1% against the *S. melissae* and *S. lavandulae* pathogens, although further trials are necessary to test the efficacy among field conditions.

Keywords: *Cinnamomum*, *Coriandrum*, *Thymus*, *Septoria*, antifungal effect, lemon balm, lavender.

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P-77 Factors influencing the antimicrobial efficacy of essential oils in food industrial applications

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Abstract

Essential oils (EOs), owing to their excellent antimicrobial activity, can be used in the food industry for two main purposes: for disinfection of equipment and for preservation of foods. There are some intrinsic and extrinsic factors affecting the antimicrobial efficacy of EOs. Chemical structure of the main and minor components determines the mechanism of antimicrobial action and it is generally accepted that compounds bearing an aromatic ring are the most potent antimicrobials (Bakkali et al. 2008). Extrinsic factors involve pH, temperature, the food matrix, and interaction with other preservatives (Burt, 2004; Hyldgaard, 2012). In our years long research with EOs, we have investigated the modifying effect of food ingredients on the antimicrobial efficacy of the investigated EOs. Milk and meat proteins enhanced the minimal inhibitory concentration of cinnamon, juniper, lemon and marjoram EO while soy protein had no protective effect against the EOs (Tserennadmid et al, 2010, 2012). In non stable, *in vitro* emulsions edible oils decreased the antimicrobial efficacy of juniper and marjoram EOs but in a high fat containing cheese marjoram and cumin EO showed stronger inhibition on mould growth than in a low fat cheese, probably due to the uniform distribution and volatile binding capacity of the fat. Acidic (pH 4.5) and alkaline (pH 9) pH decreased the disinfection time required for cinnamon, marjoram and thyme EO, but had limited effect on juniper and clary sage EO, depending on the chemical structure of the main EO components.

Keywords: Interaction, food matrix, food preservation, disinfection, chemical structure.

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P-78 Chemical and aroma profiles of Northern Limit Yuzu (*Citrus junos*) produced in Rikuzentakata

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Abstract

Citrus fruits are widely cultivated in regions between the tropical and temperate zones, in which they are amongst the most important commercial crops. For example, *Citrus junos* Tanaka (yuzu) was transplanted from the temperate area approximately 70–300 years ago, and is now produced in Rikuzentakata (Iwate prefecture) as what is known as Northern Limit Yuzu (NLY). Yuzu has a strong characteristic aroma and as such, yuzu juice is commonly used in Japanese foods. Indeed, we previously demonstrated that the aroma profile of NLY is significantly different from that of yuzu obtained from other producing districts. In addition, principal component analysis (PCA) based on gas chromatography/mass spectrometry (GC/MS) data suggested that the chemical aroma profile of NLY varies between individual trees grown within the Rikuzentakata area (Kuraya et al. 2016). In this study, we therefore examined the correlation between the clustering classes of the chemical aroma profile of peel oils obtained by PCA and the aroma profile of juice obtained through sensory evaluations to get more information.

All yuzu fruits were provided by producers in Rikuzentakata (3 fruits from each of 8 trees). The juice was extracted using a hand-operated citrus juicer. Eight panelists tested the various juice samples by sniffing each sample and rating the aroma intensities of five attributes using a five-point linear scale from 1 (none) to 5 (very strong). The results were averaged for each attribute and multivariate analysis was conducted. In addition, the volatile compounds present in yuzu peel oils were analyzed by GC/MS, and subsequent PCA was performed using SIMCA (version 13.01; UMETRICS) to examine any differences in the sensory aroma characteristics of individual trees. Five clusters were identified among the various volatile compounds from yuzu peel oils corresponding to individual NLY trees. The aroma profiles of the juices obtained by sensory evaluation also significantly contributed to the observed clustering, with these results confirming that the aroma profile of NLY clearly differed between individual trees. Moreover, the threshold for clustering in PCA was established accordingly.

Keywords: *Citrus junos* (yuzu), peel oils, sensory evaluation, principal component analysis.

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P-79 Aroma profile of *Alpinia zerumbet* flowers

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Abstract

The aromatic perennial plant *Alpinia zerumbet* (Pers.) Burtt & Smith (Zingiberaceae) is widely distributed, occurring in both tropical and sub-tropical regions. Recently, interspecific hybridization of *Alpinia* spp. was reported in Taiwan. In fact, natural hybridization of *Alpinia* is not exclusive; *Alpinia* x *okinawaensis* (hybrid of *A. formosana* and *A. uraiensis*) has been reported in the Ryukyu Islands, Japan. Previously, we demonstrated that *A. zerumbet* aroma profiles were affected by harvest season. We also reported that the fragrance and antioxidant activity of *A. zerumbet* essential oil differed among individuals. On the other hand, gas chromatography–mass spectrometry (GC/MS) and principal component analysis revealed that the chemical composition was not affected by harvest season nor growth area but only by individual plants. To obtain additional information, in this study, we examined the correlation between the essential oils of the chemical aroma profile of leaves and the aroma profile of flowers of *A. zerumbet*.

The leaves of *A. zerumbet* were collected from Okinawa and Ie (8 and 5 samples, respectively) in the Ryukyu Islands from February 2015 to April 2016. They were oven-dried at 40–50 °C to a moisture content of 10%–15% and steam-distilled to obtain essential oils, which were analyzed by GC/MS. Floral volatiles of *A. zerumbet* were collected from three individuals from Okinawa on May 2015 and analyzed using the headspace adsorption technique. The results revealed significant variations in the chromatographic profiles of floral volatiles collected from different individuals. The major volatiles, sabinene, limonene, β-phellandrene, 1,8-cineole, γ-terpinene, camphor, linalool, and borneol, correlated with the essential oil composition in the leaves. Interestingly, in individual plants in which sabinene and γ-terpinene were not present in the essential oils, these compounds were also absent in floral volatiles. The volatiles camphene, β-pinene, myrcene, α-terpinene, *p*-cymene, and terpinen-4-ol did not correlate with the essential oil composition in the leaves.

The flower aroma profile confirmed that floral volatiles of *A. zerumbet* clearly differed between individual plants, similar to leaf essential oils.

Keywords: *Alpinia zerumbet*, floral volatiles, principal component analysis.

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P-80 Antioxidant activity of essential oil of *Artemisia absinthium* L (chemotype: (*Z*)-β-epoxyocimene + (*Z*)-chrysanthemyl acetate)

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Abstract

Essential oils of Artemisia genus show a wide range of biological activities (Abad et al., 2012). Many data related to antioxidant activity of different *Artemisia* species, including *Artemisia absinthium* L, have been reported (Lopes-Lutz et al., 2008). Nevertheless, no data are available concerning the (*Z*)-β-epoxyocimene + (*Z*)-chrysanthemyl acetate chemotype, one of the most extended in Iberian Peninsula (Llorens-Molina & Vacas, 2015). Essential oil belonging to this chemotype was extracted by hydrodistillation and analyzed by GC/MS. Its major components were found: (Z)-β-epoxyocimene (36.0 %); (*Z*)-chrysanthemyl acetate (35,4 %), (*E*)-β-epoxyocimene (10,4 %) and chamazulene (3,7 %). Its radical scavenging activity was evaluated by means of $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ assays. The results (expressed as the mean of triplicate analysis ± standard deviation of IC_{50} -concentration causing 50% inhibition of radical- and TEAC values- trolox equivalent antioxidant capacity-) were the following: IC_{50} = 0.35 ± 0.01; TEAC = 0.100 ± 0.003 □mol/mg, for $DPPH^{\cdot}$ and IC_{50} = 0.123 ± 0.002 mg/mL, TEAC = 0.090 ± 0.002 □mol/mg for $ABTS^{+\cdot}$. This activity comes to be relatively strong with respect other Artemisia absinthium but clearly lower if comparing with origanum or clove oils.

A TLC bioautographic method (Choma et al., 2011) was also applied in order to evaluate the relative activity of their components. The identity of stains showing stronger activity was confirmed by preparative TLC and later GC/MS analysis. They were found to be epoxyocimene isomers and chamazulene.

Keywords: Antioxidant, Artemisia absinthium, DPPH, ABTS, TLC, bioautography.

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P-81 Relevance of *Origanum vulgare* (*hirtum x viridulum*) essential oil chemotype on the antimicrobial activity against opportunistic gut microbiota pathogens

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Abstract

The presence of phenolic compounds as the major components define the chemotype of essential oils and predicts the real effect of oils against opportunistic gut microbiota pathogens. The use of essential oils has been proposed as an alternative to antibiotics in animal feeding. *Origanum vulgare* essential oil exhibits a broad-spectrum of antimicrobial activity against pathogens, and, among the components that confer this biological property, carvacrol, thymol, and γ-terpinene are the most effective. The main goal of the present work was to evaluate the bacterial growth inhibition curves of five *O. vulgare* essential oil chemotypes, containing varying relative concentrations of secondary metabolites, against *Escherichia coli* CECT 45, *Salmonella enterica* subsp. *enteric*a CECT 443, *Staphylococcus aureus* subsp. *aureus* CECT Nº 59, and *Listeria monocytogenes* CECT 911. A total of 15 individual plants (3 per chemotype) were used in the development of this assay. The essential oils were extracted by hydrodistillation and the qualitative and quantitative composition was analysed by a gas chromatograph coupled to a mass spectrometer (GC-MS). The inhibition growth curves were monitored for 48 h and the essential oil concentrations ranged from 1250 ppm to 40 ppm. The essential oil chemotypes were composed of 86% carvacrol; 77% carvacrol/6% thymol; 65% carvacrol/18% thymol; 48% carvacrol/12% γ-terpinene/6% caryophyllenhe; 34% carvacrol/15% γ-terpinene/8% *p*-cymnene /7% caryophyllenhe. The main results confirmed that at a concentration of 625 ppm and exposure time of 48h: all the chemotypes were effective against *E. coli*. In the case of *Salmonella*, concentrations of over 33% carvacrol in the oil are needed in order to inhibit the bacterial growth. Finally, for *Listeria* and *S. aureus* only the chemotypes with carvacrol concentration higher than 60% were effective.

Keywords: *Origanum vulgare*, subspecies, essential oil, antimicrobial activity.

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P-82 Antibacterial activity of essential oils of *Iryanthera polyneura* Ducke (Myristicaceaea)

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Abstract

Brazil is considered the richest country in terrestrial biodiversity, which it is estimated to contain approximately 20% of phanerogamous plants species. From those, a few has been studied in terms of the biological, chemical and pharmacological aspects, transforming this country, particularly the Amazonian Forest, in a source of new discoveries yet to be achieved. The present study evaluated antibacterial activity of essential oils obtained from the leaves of three adult specimens of *Iryanthera polyneura* Ducke (Myristicaceaea), an Amazon rainforest endemic plant, here identified as 22OE, 80OE and 530OE. The aim of this work was to verify the antimicrobial activity of the essential oils from the leaves of *I. polyneura* against pathogenic *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans* and *S. sanguis*, thus contributing to a better understanding of the antimicrobial potential of the Brazilian plant biodiversity. The leaves were periodically collected in a 2-year period and were submitted to Clevenger apparatus extraction in order to obtain the essential oils (EO's). The evaluation of the antibacterial activity was carried out by the microdilution broth assay (MBA) in order to obtain the minimal inhibitory concentrations (MIC) and the minimal bactericidal concentrations (MBC) for each micro-organism. A single-dose assay were firstly made with 42 oils, from which 18 showed to inhibit *E. faecalis*, 41 inhibited *S. aureus* and *S. mutans* and 36 inhibited *S. sanguis*. Then MIC's and MBC's were obtained for the active EO's. *S. aureus* showed to be more susceptible to the EO's activity as the MIC's were 0.75 mg/ml, and both *E. faecalis and S. mutans* were more resistant, once the MIC's were 6 mg/ml. *S. Sanguis* showed susceptibility to some of the EO's and resistance for other ones. EO's obtained from *I. polyneura* showed to be efficient against to Gram positive pathogen micro-organisms.

Keywords: *Iryanthera polyneura*, antibacterial, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococci.*

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P-83 Antioxidant activity of sage essential oil in cooked pork sausages

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Abstract

The effect of sage essential oil (SEO) addition on the lipid oxidation, microbial stability and sensory properties of cooked pork sausages was examined. The SEO was extracted by hydro distillation and analysed by gas chromatography-mass spectrometry (GC-MS). The main compounds were α-thujone (35.44%), camphor (20.43%), eucalyptol (12.23%) and β-thujone (6.57%).

Sausages with different concentrations of SEO (0.1, 0.5 and 1.0 µl/g) and control were prepared. Instrumental parameters of colour (*CIEL*a*b**), thiobarbituric acid-reactive substance (TBARS) values, microbial profile and sensory panel scores have been assessed at the 1st and 30th day of storage.

The addition of SEO in all concentrations resulted in significantly higher (P<0.05) *CIEa** value. TBARS values were lower (P<0.05) in sausages added with 0.1, 0.5 and 1.0 µl/g SEO than in control on the 1st and 30th day of storage. Decrease of total aerobic mesophilic bacteria count was observed in sausages prepared with SEO addition on the 30th day of storage.

This study demonstrates that the sage essential oil could be used in processing of cooked pork sausages in order to improve lipid oxidative and microbial stability as well as to enhance their colour characteristics.

Keywords: Sage essential oil, cooked pork sausage, antioxidant and antimicrobial activity.

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P-84 Composition, antimicrobial and antioxidant activities of essential oils from two *Avicennia schaueriana* Stapf & Leechm. ex Moldenke specimens

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Abstract

A. schaueriana is a native species from the Brazilian mangroves, whose extracts showed antimicrobial activity (Fardin & Young, 2015). This study aimed to compare the composition and the biological activities from the essential oils obtained from two *A. schaueriana* specimens collected at São Paulo Southern seashore, Jureia and Ilha do Cardoso. The essential oils yielded 0.0035% (w/w) for the Jureia oil and 0.0085% for Ilha do Cardoso, after 4 h hydrodistillation. The GC-MS/GC-FID analysis indicated that the Jureia oil was composed mostly by fatty acids, having palmitic (46.5 %) and myristic (11.6 %) acids as major components, while the main components for Ilha do Cardoso were eugenol (19.7%), eugenol acetate (12.9%) and palmitic acid (15,1%). The antioxidant activity by the DPPH method was weak for both oils (EC_{50}> 1.0 mg/mL), if compared with the *A. marina* crude extracts (EC_{50} 80.4 µg/mL) (Patra et al., 2014). The antimicrobial activity was evaluated using the microdilution method in microplates (Moreno et al., 2009) against *Aspergillus niger* (ATCC 6404), *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538), none of the oils presented a considerable activity (MIC > 250 µg/mL), once in screening studies only extracts with MIC < 100 µg/mL can be considered as candidates for developing new antimicrobial agents (Moreno et al., 2013).

Keywords: Antioxidant activity, antimicrobial activity, GC-MS, mangrove plant, *Avicennia.*

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P-85 Antifungal activity of essential oils of flowers and leaves of *Hedyosmum brasiliense* Mart. Ex Miq.

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Abstract

Hedyosmum brasiliense (Chloranthaceae) is a dioecious shrub popularly used in foot fungus treatment [1]. In this work, we searched for the antifungal components present in its essential oils. *H. brasiliense* male and female flowers and leaves were collected at Ilha do Cardoso (São Paulo) and the oils were extracted by hydrodistillation and analyzed by GC/MS [2]. The oil yields were different for the flowers, 0.38% ♀ and 0,24% ♂, but the same for the leaves (0.33%). The major compounds for all oils were sabinene, curzerene and carotol. Despite some differences in the chemical composition, the oils were not discriminated by PCA analysis [3]. The bioautography with *Cladosporium cladosporioides* and *C. sphaerospermum* [4] showed two antifungal bands at R_f's 0.67 and 0.12 in all samples. The bands with R_f 0.67 were isolated from each oil and curzerene was the main compound in these fractions, 97% and 82% flower and leaf oils. The band with R_f 0.12 was still composed by a complex mixture, with predominance of α-terpineol, α-eudesmol and ferula lactone I in different proportions, not allowing to point only one single active compound. These results suggest that curzerene might be the main responsible for the antifungal activity of *H. brasiliense* essential oils, considering it is one of its main components.

Keywords: Antifungal, bioautography, Atlantic Rain Forest, *Hedyosmum brasiliense*, curzerene.

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P-86 Determination of combinatory effect of thymol and carvacrol against *Staphylococcus aureus* using new broth volatilization chequerboard method

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Abstract

The microdilution chequerboard method is a standard assay for the measurement of antibiotic synergy in liquid media [1]. Although the synergistic effect has previously been studied by several authors also in vapour phase [2, 3], there is no standardized method for testing of combinatory effects of volatile compounds. Recently, we developed a new broth volatilization method for simultaneous determination of minimum inhibitory concentrations of volatile compounds in the liquid and the vapour phase using 96-well microtiter plates [4]. With aim to develop effective screening method for determination of synergy of volatile compounds in vapour phase we designed new broth volatilization chequerboard method based on combination of both approaches. Initially, the flanges on the lids are inoculated with bacterial suspension and agar is pipetted into every flange on the lid. Subsequently, classical broth microdilution chequerboard method is performed in the plates, which are also inoculated with bacterial suspension and then fasten together with the lid by the clamps. Combinatory effect is then evaluated according to the means of fractional inhibitory concentration indices (ΣFICI) [1]. In this study, combinatory effect of two volatile compounds, thymol and carvacrol, has been evaluated against S*taphylococcus aureus* by this new method. The results showed additive effects in both, liquid (plate) and vapour (lid) phases. The additive effects were obtained for *Staphylococcus aureus* ATCC 43300 on the lid at combination of 128 µg/mL of carvacrol and 64-128µg/mL of thymol (ΣFICI = 0.5451) and in the plate at combination of 128 µg/mL of carvacrol and 128 µg/mL of thymol (ΣFICI = 0.5611). This method can be potentially applied in development of various medicinal and food applications that are based on antimicrobial combinatory effect of volatile compounds. According to the best of our knowledge, this is the first method enabling determination of FICI values in the vapour phase.

Keywords Antimicrobial activity, volatile compounds, volatilization chequerboard method.

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P-87 Bioactive constituents, antibacterial and antiradical activities of the fruit and leaves essential oil of *Crataegus ambigua* Becker AK.

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Abstract

Crataegus ambigua is a shrub in Eastern Cape Province of South Africa, used in folk medicine for treatment of various diseases including heart complaints, rheumatism, and convulsion. Bioactive constituents of the fruit and leaves volatile oils (FVO and LVO) and their potentials for the management of infectious diseases were studied *in-vitro*. VOs obtained using Clevenger modified apparatus were characterized by GC-MS, while their antiradical and antibacterial properties were tested by spectrophotometric and agar diffusion methods respectively. Limonene (28.16 %), myrcene (16.97 %), caryophyllene (10.99 %) were the dominant compounds found in the FVO, while limonene (25.11%), 1,1,3,3,5,5,7,7,9,9,11,11,13, 13,15,15-hexamethyl octasiloxane (18.30 %), pinene (15. 23 %) and piperidine (11.46 %) were those identified in the LVO. The VOs exhibited strong antibacterial activity against *Escherichia coli*, *Enterococcus faecium* and *Staphylococcus aureus*. The LVO was more active than FVO against test bacteria with MIC ranging between 0.20 mg/ mL – 0.50 mg/ mL. The IC_{50} for LVO (0.43 mg/ mL) showed that the antiradical strength was superior to FVO (0. 62 mg/ mL), BHT 0.83 and vitamin C 1.80 mg/ mL in scavenging $DPPH^{\bullet}$. The oils effectively reduced three other oxidants ($ABTS^{\bullet+}$, LP $^{\bullet}$ and $NO^{\bullet}$) to neutral molecules in dose dependent-manner. To the best of our knowledge this is the first time to report the bioactivity and volatile oil constituents of fruit and leaves of *Crataegus ambigua.* The study indicates that apart from the traditional uses of the plant extracts, the VO contained strong bioactive compounds, with antibacterial and antiradical properties and may be good candidates in the search for lead constiuents for the synthesis of novel potent antibiotics.

Keywords: *Crataegus ambigua,* volatile oil, antibacterial, antiradicals.

P-88 Volatile constituents, radical scavenging and antibacterial properties of the fruit and leaves essential oils of *Syzygium paniculatum* J.Britt.

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Abstract

Syzygium paniculatum (bush cherry) of the family Myrtaceae is a common shrub in Eastern Cape of South Africa. It is used in traditional medicine for management of variety of diseases including heart complaints, rheumatism and mental disorders [1]. The volatile constituents, radical scavenging and antibacterial properties of the fruit, and leaves essential oils and their potentials for the management of communicable and oxidative-stress diseases were studied *in-vitro*. The volatile oils obtained using Clevenger modified apparatus [2] were characterized by GC-MS, while their scavenging and antibacterial properties were tested by spectrophotometric and agar diffusion methods respectively. The predominant constituents in the fruit volatile oil (FVO) were D-limonene (14.26 %), β-ocimene (12.91 %) and 2, 6-dimethyl-7-octen-1-ol (9.18 %) while D-limonene (11.48 %), 2, 6-dimethyl-7-octen-1-ol (9.18 %) and cinnamaldehyde (5.64 %) were some of the major constituents in the leaf volatile oil (LVO). The two volatile oils exhibited strong antibacterial activity against three multi-drug resistant bacterial strains; [*Staphylococcus aureus* (50080), *Escherichia coli* (ATCC700728) and *Enterobacter cloacae* (ATCC 1304)]. The LVO was more active than FVO against test bacteria with minimum inhibition concentration (MIC) ranging between 0.30 - 0.40 mg/ mL and 0.30 - 0.50 mg/ mL respectively. The IC_{50} for the LVO (1.01 mg/ mL) showed that the antiradical it strength was better than FVO (1.74 mg/ mL), β-carotene (2.14 mg/ mL) and rutin (3.01 mg/ mL) in scavenging 2, 2[1]-dipphenyl-1-picrylhydrazyl radicals (DPPH$^{\bullet}$). The oils also effectively reduced (ABTS$^{\cdot+}$, LP $^{\bullet}$ and NO$^{\bullet}$) to neutral molecules in dose dependent-manner. Findings from this study indicates that apart from the traditional uses of the plant extracts, the essential oil has strong bioactive compounds and have strong antibacterial and antiradical properties and may be alternative to synthetic antibiotics in future investigations.

Keywords: *Syzygium paniculatum,* volatile oil, D-limonene, antibacterial, antiradicals.

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P-89 Assessment of the potential of *Cimicifuga dahurica* Huth. oils as inhibitors of α-amylase, lipoxidase, xanthine oxidase and tyrosinase enzymes

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Abstract

The leaf, flower, seed and stem oils of *Cimicifuga dahurica* Huth. (Ranunculaceae) were subjected to investigation for inhibitory activity against α-amylase, lipoxidase, xanthine oxidase and tyrosinase enzymes. The free radical scavenging activity ($DPPH^{\bullet}$), lipid peroxidation inhibition (β-carotene/linoleic acid system) and Trolox equivalent antioxidant capacities ($ABTS^{+\bullet}$) of the oils were also measured (1-3).

The oils demonstrated noteworthy inhibition of α-amylase ranged between 43.5% and 82.6%. The lipoxidase was inhibited with %Inh 16.3-35%, while towards to the xanthine oxidase and tyrosinase enzymes the oils demonstrated weak inhibiton. The leaf and flower oils inhibited lipid peroxidation with %Inh 29-31, while the seed and stem oils did not demonstrate inhibition in this system. TEAC values for the flower, seed and stem oils were found to be as 0.55, 0.33 and 0.24 mM, respectively.

Gas-chromatographic analysis revealed that all the oils contained methyl salicylate (55.6-1.8 %), salicyl aldehyde (20.0-3.9%) and hexadecanoic acid (24.1- 14%) as major constituents. The present work is the first contribution into biological properties of the leaf, flower, seed and stem oils of *C. dahurica* from Mongolia. Results of biological activities tests revealed potential of *C. dahurica* as a source of multifunctional agents for the management of oxidative damage and diabetes mellitus.

Keywords: *Cimicifuga*, oil, α-amylase, lipoxidase, xanthine oxidase, tyrosinase.

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P-90 Antimicrobial and toxicity evaluation of *Matricaria recutita* L. and *Achillea millefolium* L. essential oils and their combinations

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Abstract

Matricaria recutita L. and *Achillea millefolium* L. (Astereacea) essential oils are used also for their antimicrobial activities. In this present study it was aimed to evaluate the *in vitro* antimicrobial activity of *M. recutita* and *A. millefolium* essential oils and their toxicity using an *in vivo* animal alternative experimental model, namely *Caenorhabditis elegans*. Chemical characterizations of Pharmacopoeia grade essential oils were performed by GC-FID and GC-MS systems, simultaneously. *β*-caryophyllene (17.0%), *β*-pinene (13.2%), camphor (10.0%), and sabinene (9.7%) were found as major components for *A. millefolium* essential oil. Bisabolol oxide A (41.6%), *α*-bisabolol (19.4%), (*E*)-*β*-farnesene (17.0%), *α*-bisabolol oxide B (5.2%), *α*-bisabolon oxide A (5.0%), chamazulene (1.6%), and germacrene D (1.2%) were determined as major components for *M. recutita* essential oil. Antimicrobial activities of essential oils were evaluated by microdilution methods against the standard pathogenic strains *Bacillus cereus* NRRL B3711, *Corynebacterium striatum* ATCC BAA-1293, *Streptococcus sanguinis* ATCC 10556, *Staphylococcus aureus* ATCC 700699. Minimal inhibitory concentrations (MIC) were determined. Both essential oils showed inhibitory activity against *B. cereus* (MIC 0.625 mg/mL) and against *C. striatum*, *S. sanguinis*, *S. aureus* (MIC 1.25 mg/mL). Their different combinations showed also varying inhibitory activity. Whereas the lethal doses and acute toxicity tested on *C. elegans* nematodes resulted in non-acute toxicity, indicating relative safe use also in combinations.

Keywords: Matricaria recutita L., Achillea millefolium L., Caenorhabditis elegans, Toxicity, Antimicrobial activities.

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P-91 Chemical composition and antimicrobial activity of the essential oil of *Lippia salamensis* Loes. from Guatemala

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Abstract

As part of the exploration of the economic potential of the aromatic plants of Guatemala, the chemical composition of the essential oils of native Guatemalan species of the genus *Lippia*, is being evaluated. *Lippia salamensis* Loes. (Verbenaceae) is an aromatic shrub found on open slopes or pine-oak forests at heights between 1000 and 1600 m (Standley et al., 1970). No medicinal or edible uses are reported for the species and no information on its essential oil chemical composition was found in the literature. This work aimed to determine the chemical composition and the antimicrobial activity of the essential oil of *L. salamensis*. Plant leaves from a population of *L. salamensis*, at the northern province of Baja Verapaz, were collected in June 2014. The essential oil from dried and ground leaves was obtained by hydrodistillation for 3 h (yield: 1.1 % w/w). The oil components were identified by GC/MS through their mass spectra and retention indices and quantified based on their GC/FID peak areas. The inhibitory activity of the essential oil against two Gram-positive and two Gram-negative bacteria and a yeast strain (*Candida albicans*) was evaluated by the agar diffusion method (Kirby-Bauer method). The major constituents were 1,8-cineol (14.3 %), hedycariol (9.1 %), borneol (6.23 %) and germacrene D (6.0 %). The presence of hedycariol and other sesquiterpenoid alcohols in important amounts differentiate the plant from other Guatemalan *Lippia* species with higher content of monoterpenes. The oil presented activity against Gram-positive bacteria only, with inhibition halos of 15 mm against *S. aureus* and 22 mm against *B. cereus*. The results suggest that the essential oil of *L. Salamensis* has potential as antimicrobial in medicine and in the food industry.

Keywords: Antimicrobial activity, essential oil, *Lippia salamensis*, sesquiterpenoids.

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P-92 Effect of cinnamon oil on the conservation of table grapes

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Abstract

Some aromatic plants produce chemicals as a mechanism of self-protection. These chemicals are potential sources that serve the same function as fungicides, but offer the advantage of being enviro-friendly and having low toxicity. In this work we studied the antifungal activity *"in vitro"* of essential oils Cinnamon (*Cinnamomum verum*) and Bay leaf (*Laurus nobilis*) against *Botryotinia fuckeliana,* and the effect of cinnamon oil on the conservation of table grapes versus *Botryotinia fuckeliana*.

We studied the response of fungus against both essential oils. Antifungal activity was analyzed by an *in vitro* test. Mycelial growth inhibition was calculated on day 7 for each essential oil and fungus, and six replicates dishes were used. Inhibition growth was about 25% after adding laurel oil to culture media. In contrast, addition of cinnamon produced inhibition rates of around 80%. Cinnamon oil displayed effectiveness in the conservation of table grapes against *Botryotinia fuckeliana*. It also lowered the percentage of grapes separated and stained, and improved the turgor and visual appearance of grapes.

Based on the inhibitory potential of cinnamon essential oil, treating crops and stored food with this essential oil is a good alternative to replace the use of agrochemicals as it is more enviro-friendly, is safer for consumers and prolongs the post-harvest shelf-life of table grapes.

Keywords: Antifungal activity, cinnamon, laurel, essential oil, table grapes.

P-93 Evaluation of the antifungal potential of commercial essential oils oregano and clove against *Botryotinia fuckeliana*, *Epicoccum nigrum*, *Curvularia hawaiiensis* and *Aspergillus niger*

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Abstract

Fungal contamination of crops causes both economic and human health problems. Vast economic losses are usually caused by species *Bipolaris*, *Curvularia*, *Fusarium*, *Botryotinia* and *Alternaria*. Infection of seeds is a serious problem because these pathogens can remain viable for 10 years, and are subsequently capable of propagating across other geographical areas and infecting further crops, and thus accomplish global dissemination. In this work we studied the antifungal activity *"in vitro"* of essential oils Oregano (*Origanum compactum*) and Clove (*Syzygium aromaticum*) against *Botryotinia fuckeliana, Epicoccum nigrum, Curvularia hawaiiensis* and *Aspergillus niger*.

We studied the response of fungi against both essential oils. Antifungal activity was analyzed by an *in vitro* test. Mycelial growth inhibition (MGI) was determined on day 7 for each essential oil and fungi, and six replicates dishes were used. After adding oregano oil to culture media, MGI was about 65% in *Botryotinia,* and 100% in *Curvularia* and *Epicoccum*. Addition of clove reached inhibition percentages of between 80-100% in species *Curvularia* and *Epicoccum*.

The results suggest that essential oils are an alternative for practical applications in stored products. Addition of oregano and clove oils can provide an alternative to replace the use of agrochemicals in crops and stored seeds, and could thus extend their shelf life. They can be used as preservatives and additives in foodstuffs, and can be applied to store grain and seeds.

Keywords: Antifungal activity, oregano, clove, essential oil.

P-94 The study on the effect of aroma compounds on airborne microbes for the enhancement of the comfort of vehicle interior

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Abstract

Many Japanese car industries including Toyota Motor Corporation and Fuji Heavy Industries Ltd. have focused on the reduction of airborne microbes, fungi and unpleasant smell in the vehicle interior, because to enhance of the quality of air in the compartment is required by many customers. It seems their effort is also important for the transportation service of elderly day service centre because Japan is expected to face an increasingly aging population and much more people would require this service in near future.

We have reported the use of aroma compounds are pleasant and less harmful than using disinfectants to reduce airborne microbes. In our country car users prefer weaker scents, therefore, we tried to improve our established method to provide for their needs. Then we discovered that even much less amount of aroma compounds are effect to reduce airborne microbes in the compartment. When 100 □l of terpineol (mixture of isomers,□□-terpineol 65%; β-terpineol 10%; γ-terpineol 20%) was spread by using air pump for 1 minute each 5 minutes for 3 times, approximately 85 % of airborne microbes was reduced. The intensity of the smell was quite low, therefore, this method would satisfy car users' needs.

Keywords: Antimicrobial activity, airborne microbe, vehicle interior, aroma compounds, terpineol.

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P-95 Short-term inhalation of lemon balm (*Melissa officinalis* L.) essential oil does not affect human cognitive functions

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Abstract

Different types of *Melissa officinalis* L. (Lamiaceae) extracts were historically and are contemporarily used as mood and cognitive function modulators. Although previous studies pinpointed rosmarinic acid as the main active principle (Scholey et al., 2014), the essential oil, for which lemon balm is renowned for, is poorly investigated in this sense. Thus, this study aimed to evaluate the effects of a short-term inhalation of *M. officinalis* essential oil on cognitive functions of young healthy volunteers. Twenty subjects (mean age 21.4), divided into two equal groups, participated in this study and were tested in laboratory settings on two occasions (firstly - a training session and secondly – an inhalation session). On both occasions the subjects were instructed to follow the PEBL test battery, constructed for this experiment, which evaluated the memory span and reaction time of the subjects. During the second session, the subjects (blinded to the experiment) were instructed to inhale, at a normal respiratory rate, vapors emitted from a vial containing either distilled water or a measured amount of the essential oil for the duration of one minute. Before and after the inhalation the subjects were given a visual analog scale (VAS) in order to evaluate their awakeness, tension and restfulness; and afterwards to estimate odor intensity and its characteristics. All of the obtained data were compared using ANCOVA and ANOVA (SPSS 20.0). No statistically significant effects ($p>0.05$) of the inhaled vapors of the essential oil on the subject memory span or reaction time were ascertained. After the statistical treatment of the data obtained from VAS, the only statistically significant difference between the two groups was found for the inhaled odor intensity ($p=0.001$). The obtained results suggest that short-term inhalation of *M. officinalis* essential oil does not alter the cognitive function of young healthy subjects.

Keywords: *Melissa officinalis*, essential oil, cognitive functions.

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P-96 Synthesis, olfactory evaluation and preliminary environmental impact of citral oxime ethers

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Abstract

Citral is an acyclic monoterpene aldehyde, which can be isolated from natural sources like lemongrass[1] (*Cymbopogon citratus*). It is also prepared in large volumes via synthetic route from isobutylene or isoprene[2]. Accessibility and low price of citral are driving factors for its application as a substrate for preparation of new fragrant compounds. The main goal of our work was synthesis of citral oxime ethers and their olfactory evaluation. Title compounds exhibited various fragrance notes: from fruity (lemon, raspberry, kiwi, fig, quince) to floral (marigold) and herbaceous.

In addition to the synthetic studies preliminary environmental impact of title compounds was assessed on the three trophic levels: bacteria, microalgae, and daphnia. Microtox test was used to evaluate toxicity towards bacteria, Algaltoxfit F towards microalgae, and plate test according to the Reed's Method towards daphnia. All of the tested compounds were toxic to *Vibrio fischerii*, *Pseudokirchneriella subcapitata*, and *Daphnia magna*. It can be concluded that they are toxic to aquatic environment.

Keywords: Citral, oxime ethers, fragrance, olfactory evaluation, aquatic toxicity, environment.

Acknowledgments

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P-97 PCA/CCA correlation of volatile organic compounds and antibacterial activity of essential oils of *Iryanthera ulei* Warb. (Myristicaceaea)

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Abstract

Iryanthera ulei, known in Brazil as *ucuubarana*, is native but not endemic to the Amazon rain forest and is popularly used to clean infected wounds, to treat diarrhoea, oral infections and against fever. The aim of this work was to verify the antimicrobial activity of the essential oils from the leaves of *I. ulei* against *Staphylococcus aureus,* an important micro-organism involved in skin infected wounds*,* and to verify the influence of each volatile organic compound (VOC) over the antibacterial activity (AA). The evaluation of the AA was carried out by the microdilution broth assay (MBA) in order to obtain the minimal inhibitory concentrations (MIC) and the minimal bactericidal concentrations (MBC) for the micro-organism. Essential oils (EO) were obtained from the leaves of two specimens, here named 10OE and 15OE, that were collected for 13 times in a two-year period, by Clevenger apparatus. Oils were evaluated for their chemical composition by CG-MS and 17 volatile organic compounds (VOC's) were identified as terpenes. Principal component analysis (PCA) and canonic correlation analysis (CCA) were applied in the study. Spathulenol, α-cadinol and globulol are considered the major ones according to PCA. It was observed, by means of CCA, that viridiflorol showed a tendency of being related to MBC for specimen 10OE, while α-cadinol, α-muurolene, β-elemene, α-amorphene and viridiflorol showed influence over MIC and β-elemene and α-amorphene influenced MBC in specimen 15OE. None of the major compounds had influenced MIC/MBC, despite α-cadinol for specimen 15OE. Multivariate analyses showed to be an adequate tool to report how VOC's influenced MIC's and MBC's as well as how climate variation can interfere with VOC expression

Keywords: *Iryanthera ulei*, antibacterial, *Staphylococcus aureus*, principal component analysis, canonic correlation analysis.

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P-98 Anti-*Trichophyton mentagrophytes* of essential oils from Rutaceae

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Abstract

The aim of this study was to investigate the anti-*Trichophyton mentagophytes* activity of essential oils from Rutaceae. The essential oil from kaffir lime, lime, and pomelo pericarps were hydrodistillated. The antifungal activity screening was firstly determined by agar disc diffusion assay. Which essential oil(s) exhibited antifungal activity was further determined for its minimum inhibitory concentrations (MICs) by broth macrodilution method. The result showed that kaffir lime, lime and pomelo oils possessed anti-*T. mentagophytes* activity on agar disc diffusion assay. Thus they were investigated for their MICs, all of which expressed equally MICs at 0.5 %v/v.

Keywords: anti-*Trichophyton mentagophytes*, Kaffir lime oil, lime oil, pomelo oil.

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P-99 Herbicidal potencial of *Santolina chamaecyparissus* L. essential oil against *Amaranthus hybridus* L. and *Lolium perenne* L.

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Abstract

Amaranthus hybridus and *Lolium perenne* are to important weeds in many crops. *A. hybridus* have been ranked among the world's 18 worst weeds (Holm *et al*. 1991). *L. perenne* ssp. multiflorum (Lam.) Husnot (Italian ryegrass) is a principal weed problem in *Triticum aestivum* L. ssp. aestivum (wheat), *Gossypium* spp. (cotton) and *Glycine max* L. (soybean) production fields (Salas *et al*., 2012). Both have developed resistance to synthetic herbicides with different mechanisms of action (Heap, 2017). It is necessary to find new solutions to control this weeds. The volatile oil from *Santolina chamaecyparissus* seemed to be a promising alternative to synthetic herbicides as it showed herbicidal activity against *Portulaca oleracea* L. and *Vicia sativa* L. weed species, being less injurious for the crop species *Zea mays* L., *Triticum durum* L., *Pisum sativum* L., and *Lactuca sativa* L. (Grosso et al., 2010). The objective of this work was to test the herbicidal potential of a commercial sample of *S. chamaecyparissus* essential oil to control *A. hybridus* and *L. perenne*. The essential oil was tested *in vitro* conditions at doses of 0.125, 0.25, 0.5, 1 and 2 µl/ml. It was very effective towards *A. hybridus* germination, controlling it completely at all concentrations applied. *L. perenne* was more resistant to this essential oil, reducing its germination only at the three highest concentrations, being the maximum inhibition of 9.33%. Is necessary to test the essential oil *in vivo* conditions and at higher doses to better understand it possibilities for the management of this weeds.

Keywords: Essential oils, natural herbicides, weed control, germination inhibition.

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P-100 Searching for essential oils with antituberculosis activity

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Abstract

Tuberculosis an infectious disease caused by *Mycobacterium tuberculosis* is one of the leading causes of human morbidity and mortality. In 2013 the WHO reported one and half million of deaths and nine millions new cases of active tuberculosis caused by TB (WHO, 2014).The increase in the incidence of clinical tuberculosis is associated with increasing reports of new cases of multi drug resistant (MDR-TB) and extensively multidrug resistant (XDR-TB) strains (WHO, 2010).

Essential oils and their constituents are commonly known for their antibacterial, antifungal and antiparasitic activity, and there are also reports on the antimycobacterial properties.

More than 100 essential oils obtained from different medicinal plant were tested for antituberculosis activity.

Minimal Inhibitory Concentrations (MIC) values for the EO were established with 96-well micro plate method with AlamarBlue (Invitrogen). Reference strain of *Mycobacterium tuberculosis* H37Ra inoculum in Middlebrook 7H9 broth (Difco) was $5x10^5$cfu/ml per well, accordingly to CLSI standards. Serial twofold dilutions of EO ranged from 256 to 8 µg/ml. As the internal control of the method serial twofold dilutions of four first line antibiotics dedicated to the tuberculosis treatment: isoniazid (INH), rifampicin (RMP), ethambutol (EMB) and streptomicin (SM) were used (Palomino et al., 2002; Wayene, P.A., 2011).

Keywords: Tuberculosis, essential oils, *Mycobacterium tuberculosis* H37Ra, MIC.

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P-101 Analysis of essential oil from *Orlaya grandiflora* and evaluation of antibacterial activity

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Abstract

Plants belonging to *Apiaceae* family are well known as coumarins's and essential oils' sources, which are used widely in medicine as well as in traditional phytoterapy.

The aim of our study was the chemical analysis of was the chemical analysis of essential oil obtained from fruits of *Orlaya grandiflora* (*Apiaceae*) (Tutin et al.,1968). Plant material used in all experiments was collected in Botanical Gardem, Maria Curie-Skłodowska University in Lublin, Poland. The air dried and powdered plant material was submitted to hydrodistillation in Deryng apparatus and obtained essential oil has been submitted to chemical analysis.

The GC-MS analysis of the essential oil from mature fruits of *Orlaya grandiflora* showed as the most abundant constituents β-caryophyllene, δ-cadinene, α-amorphene and germacrene-D.

The antimicrobial acitivies of essential oil was also assayed against Gram negative and positive bacterial strains (among them *Mycobacterium tuberculosis*) as well as against human pathogenic fungi (e.g. *C.albicans*, *C. tropicalis*, *C. glabrata*).

Investigated essential oil showed interesting biological profile.

Keywords: *Orlaya grandiflora*, GC-MS, antibacterial activity.

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The authors would like to express their garditude to Director of Botanical Garden of Maria Curie Skłodowska Univeristy in Lublin and specialists from this Garden for plant material

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P-102 Biological activities of *Sideritis taurica* Stephan ex willd essential oil and its infusion

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Abstract

The genus *Sideritis* (Lamiaceae) represented by 46 species and the ratio of endemism is about 80% in Turkey (1-3). Some *Sideritis* species were reported for their anti-inflammatory (4-7), anti-ulcer (4), antioxidant (8), anti-cataract (9), antimicrobial (10), immuno-modulating (11) activities, as well as inhibition of NOS-2 expression in macrophages (12). *Sideritis taurica* was collected from Tokat province, Turkey. Aerial parts of the plant were subjected to hydrodistillation using a Clevenger apparatus. Chemical composition of the oil was investigated with GC-FID and GC-MS techniques. Infusion was prepared from the same parts of the plant. The volatile components of the infusion were determined by HS-SPME method. Both lyophilized material and the essential oil were examined for their antimicrobial and antioxidant activities (free radical scavenging activity by DPPH and ABTS). Main components were found as α-pinene 38.9%, β-pinene 25.6%, β-phellandrene 16.7% and limonene 3.5% of the oil, while α-pinene 20.9%, β-phellandrene 13.5%, β-pinene 11.0% and limonene 5.4% were major components in the infusion. Neither the essential oil nor the lyophilized infusion showed against *Escherichia coli* NRRL B-3008 and *Bacillus cereus* NRRL B-3711 strains.

Keywords: *Sideritis taurica*, essential oil, GC-MS, GC-FID, LC/MS-MS

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P-103 Essential oil composition of *Hypericum perforatum* L. from Istanbul

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Abstract

Previously, essential oil composition of *Hypericum perforatum* from different regions of the world was reported. Essential oil composition of *H. perforatum* from Greece was reported to contain α-copaene (11.3%) and α-longipinene (9.7%) as the main components (Pavlović et al., 2006). Another report from Serbia reports the main component of *H. perforatum* oil with nonane (63.8%) as the major compound (Rančić et al., 2005). In a report from Eskişehir-Turkey, as the major components of the essential oil composition was reported as α-pinene (50.3%), carvacrol (21.9%) and β-selinene (6.6%) (Erken et al., 2001).

In the present study we have identified the essential oil composition of aerial parts of *Hypericum perforatum* naturally growing in Istanbul. The essential oil was obtained by 3h hydrodistillation using the Clevenger apparatus, which afforded a trace amount of essential oil (<0.01 % v/W). Seventy-three compounds were identified in the oil, representing 76.18% of the oil. The major components of the oil were 1-tetradecanol (6.24%), β-selinene (5.34%), α-pinene (5.19%), decanoic acid (4.68%), α- selinene (4.27%), dodecanol (4.05%) and (*E*)-β-farnesene (3.78).

The results of the present study and literature clearly presents that the composition of the essential oil of *H. perforatum* have a high chemical diversity. Additionally, from these results the existence of the chemotypes for this species could be seen clearly. Especially in Turkey a previous report clearly presents chemical variations within this species (Çırak et al., 2010). However, further studies still are needed to determine chemotypes that may have economic importance in Turkey.

Keywords: *Hypericum perforatum*, essential oil, 1-tetradecanol, β-selinene, α-pinene.

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P-104 Essential oils of selected *Thymus* species wild-growing in Croatia: chemical composition and antibacterial activity

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Abstract

The genus *Thymus* (Lamiaceae) is notewory for the numerous plants biosynthesizing remarkable amount of volatile compounds. Present study aimed to evaluate chemical composition and antibacterial activity of essential oils from five selected *Thymus* species originated from Croatia (*T. longicaulis* C. Presl, *T. praecox* Opiz subsp. *polytrichus* (A. Kern. ex Borbás) Jalas, *T. pulegioides* L., *T. serpyllum* L. subsp. *serpyllum* and *T. striatus* Vahl), in comparison with *T. vulgaris* L. The extraction yields (V/m) of essential oils isolated by hydrodestilation from aerial parts of investigated species were in the range 0.2-1.7%. According to GC-MS analysis 95 different components were identified, representing 87.6-99.7% of the total oil content. Thymol (43%), p-cymene (18%) and γ-terpinene (11%) were the most abundant compounds of essential oil of *T. longicaulis*. The main component of *T. pulegioides* essential oil was carvacrol (39%) while 1,8-cineole (37%) was found to be the major compound of the essential oil of *T. serpyllum* subsp. *serpyllum*. The essential oils of mountain species *T. praecox* subsp. *polytrichus* and *T. striatus* were rich in sesquiterpenes, with germacrene-D-4-ol (12% and 12%) and β-caryophyllene (10% and 12%) as predominant compounds. The multivariate analysis of the essential oil constituents from the selected *Thymus* species indicated that the greatest similarity to the thyme oil, thymol type which is officially recognized in European Pharmacopoeia, was presented by *T. longicaulis*. *In vitro* antibacterial activity of the essential oils against Gram-positive and Gram-negative bacterial strains (*S. aureus*, *E. faecalis* and *E. coli*) was determined using the microdilution method. *E. coli* showed the highest susceptibility to all tested essential oils, while *E. faecalis* was the most resistant. MIC values for *E. coli* and *S. aureus* ranged from 0.5 mg/mL to 2 mg/mL, while the MIC value for *E. faecalis* was 4 mg/mL. Our study showed that, in addition to the phenolic compounds (thymol and carvacrol) well-known as strong antimicrobial agents, sesquiterpenes also contribute to the antibacterial activity of the selected *Thymus* species.

Keywords: *Thymus* spp., essential oil, antibacterial effect.

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