47th International Symposium on Essential Oils

47th ISEO - Nice, France

11-14 September 2016

PROGRAM & BOOK OF ABSTRACTS

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47th International Symposium on Essential Oils

(ISEO2016)

PROGRAM, BOOK OF ABSTRACTS AND PARTICIPANTS LIST

11-14 September 2016, Nice, France.

47th International Symposium on Essential Oils (ISEO2016)

Organization :

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Nicolas Baldovini
Institut de Chimie de Nice, UMR 7272
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Organizing Committee Contact:

ISEO2016@unice.fr

President :
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Université de Nice-Sophia Antipolis, CNRS
Parc Valrose
06108 Nice, France
Tél : 33 (0)492076133

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Parc Valrose
06108 Nice, France
Tél : 33 (0)492076577

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CONFERENCE VENUE:

The symposium will be organized at the Parc Phoenix, Nice (http://www.parc-phoenix.org/). The welcome party (Sunday 11 september) will take place in the Novotel Nice Arénas and the Gala dinner in the Hotel Radisson Blu (https://www.radissonblu.com/en).

CONFERENCE WEBSITE:

The Internet homepage of the ISEO is available at http://unice.fr/colloques/iseo

LUNCHES:

Coffee and refreshments will be served in the Poster exhibition room (Salle Emeraude). Lunches (Monday 12 and Tuesday 13) will take place in the Campanile Hotel.

ORGANISATION:

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PUBLICATION :

Authors whose abstracts have been accepted for presentation are invited to submit an article for consideration of publication in a special issue of the Flavour and Fragrance Journal (http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1099-1026) devoted to the ISEO2016, and entitled "ISEO 2016: Essential Oils: From their Production to their Regulation". All contributions to this special issue will be fully refereed according to the usual standards of the journal, therefore acceptance of a paper for presentation at the Conference does not necessarily guarantee acceptance for publication in the Special Issue. The deadline for abstract submission will be November 15th, 2016.

REGISTRATION:

The registration desk will be opened on Sunday 11/09 from 18h in the Novotel Nice Arénas, and in the conference venue during the time of the symposium.

Regular participant and student registration fees include:

Admission to all scientific sessions
Admission to the exhibition
Printed material of the symposium with conference bag
Welcome reception
Coffee breaks and lunches (12th and 13th september)
Wednesday 14th excursion

Accompanying persons’ registration fee includes:

Welcome reception
Lunches (12th and 13th september)
Wednesday 14th excursion

SAFETY RECOMMENDATIONS:

For safety reasons, the access to the conference room and poster exhibition room is **strictly restricted** to participants bearing their Symposium badges. Lunch tickets will be also required at the Campanile restaurant.

SYMPOSIUM LANGUAGE:

The official language of the ISEO2016 is English. There will be no simultaneous translation.

YOUNG SCIENTIST FELLOWSHIPS:

Thanks to the generous support of the International Federation of Essential Oils and Aroma Trade (IFEAT), the ISEO2016 Organizing Committee offered Registration Fellowships to 20 selected young scientists who submit a presentation in ISEO2016. The Registration Fellowship award consists of the registration fee reimbursement. The awardees will also receive a certificate, and their names will be listed in the special issue of the Flavour and Fragrance Journal devoted to the ISEO2016.

The IFEAT Registration Fellowships awardees are:

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-Clémence Vasseur (*Cosmo International Ingredients, Mougins, France*)

SCIENTIFIC PROGRAM

Monday 12 September :

8h45-9h15 :	**Opening ceremony**
9h15-10h :	**PL-1** **Denis Bellenot (Iteipmai, France)** Using Essential Oils in Agriculture: Crop Protection and Animal Farming.
10h-10h20 :	**OP-1** **Nikola M. Stojanovic (University of Niš, Serbia)** The first report on the acute toxicity of the highly valued essential oil of lemon balm (*Melissa officinalis* L., Lamiaceae) in female BALB/c mice.
10h20-10h50 :	**Coffee break**
10h50-11h10 :	**OP-2** **Adam Feyaerts (KU Leuven, Belgium)** Introducing a quantitative assay to assess the volatile antimicrobial activities of essential oils and their components.
11h10-11h30 :	**OP-3** **Katsuya Nagai (Anbas Corp./Osaka University, Japan)** Effects of olfactory stimulation with scents of grapefruit and lavender essential oils on the skeletal muscle sympathetic nerve and muscle blood flow.
11h30-12h30 :	**Young Scientists Session** **YS-1** **Daniel Strub (Wrocław University of Science and Technology, Poland)** Batch and continuous-flow biocatalytic transesterification of chiral fragrant terpenoid alcohols. **YS-2** **Stéphane Andreani (Université de Corse, France)** Chemical investigation of invasive species from Corsica. **YS-3** **Jérémie Topin (Université de Nice Sophia Antipolis, France)** Smell and relax! Assessment of olfactory stimulation protocols in the framework of psychophysiology measurements. **YS-4** **Kaan Polatoglu (İstanbul Kemerburgaz University, Turkey)** Essential oils as potential insecticidal agents for protection of stored products.
12h30-14h :	**Lunch**

14h-14h45 : **PL-2**
Vicente Ferreira (University of Zaragoza, Spain)
A Critical Revision of basic Methodologies for Solving Complex Flavor Systems.

14h45-15h05 : **OP-4**
Yoshinori Asakawa (Tokushima Bunri University, Japan)
Characteristic Scent Components from the Japanese Aromatic Plants, *Alpinia japonica, Elettaria cadamomum, Artemisia annua, Citrus medica, Orixa japonica* and *Litsea cubeba.*

15h05-15h25 : **OP-5**
Esmeralda Cicchetti (Cosmo International Ingredients, France)
Characterization of odour-active compounds in Timur (*Zanthoxylum alatum* Hemsl.) essential oil.

15h25-15h55 : Coffee break

15h55-16h15 : **OP-6**
Toshio Hasegawa (Saitama University, Japan)
Structural Similarities of Odor Compounds for Olfactory Recognition in Odor Materials.

16h15-16h35 : **OP-7**
Sophie Fourmentin (Université du littoral Côte d'Opale, France)
Cyclodextrins: a promising encapsulant material to improve the functionalities of essential oils.

16h35-16h55 : **OP-8**
Valtcho D. Zheljazkov (Oregon State University, U.S.A.)
The Effect of Coal-Bed Methane Water and Gypsum on Lemongrass and Palmarosa.

16h55-18h10 : Coffee break + Poster session (Posters PP1 – PP60)

Tuesday 13 September :

8h45-9h30 : **PL-3**
Peter Q. Tranchida (University of Messina, Italy)
(Low-)flow modulation comprehensive 2D GC-MS: a limited-cost ultrahigh resolution approach for flavour & fragrance analysis.

9h30-9h50 : **OP-9**
Temel Özek (Anadolu University, Turkey)
Preparative gas chromatography for isolation of eudesmane type sesquiterpene ketone from *Prangos heyniae* essential oil.

9h50-10h10 : **OP-10**
Cecilia Cagliero (Università degli Studi di Torino, Italy)
Exploring the Potential of Ionic Liquids as Gas Chromatographic Stationary Phases for The Analysis of Essential Oils and Volatiles from Natural Products.

10h10-10h40 : Coffee break

10h40-11h : **OP-11**
Polina D. Blagojević (University of Niš, Serbia)
Metabolome inspired synthetic libraries facilitate essential-oil analysis.

11h-11h20 : **OP-12**
Céline Roy (European Research Institute on Natural Ingredients, France)
Accurate mass screening using LC-QTOF: opportunities and challenges for the analysis of pesticides in Essential Oil.

11h20-11h40 : **OP-13**
Elvire Messineo (BIPEA, France)
Proficiency-testing scheme for essential oils.

11h40-12h : **OP-14**
Paul Thomas (Kreatis SAS, France)
Towards A New Method for Performing Environmental Risk of Complex Substances (CRANCS).

12h00-13h30 : Lunch

13h30-14h15 : **PL-4**
Michel Schalk (Firmenich SA, Switzerland)
Biotechnology for the Production of Flavor and Fragrance Terpenoids.

14h15-14h35 : **OP-15**
Bernard Blerot (IFF-LMR, France)
Influence of genetic, epigenetic, agricultural and post-harvest factors on the production of Rose and Geranium Essential Oils.

14h35-15h15 : **Focus on Brazilian Essential Oils**
OP-16
Lauro Barata (Federal University of the West Pará, Brazil)
Sustainable Bioproducts from the Amazon rainforest.

OP-17
Humberto Bizzo (Embrapa Food Technology, Brazil)
Brazilian Essential Oils: An Overview.

15h15-15h45 : Coffee break

15h45-17h10 : **Recommended practices in Essential Oil Analysis and Results Presentation**
PL-5
Daniel Joulain (SCBZ Conseil, France)
The Analysis of Essential Oils and Related Natural Products: Avoiding the Pitfalls.

Alain Chaintreau (Flavour and Fragrance Journal),
Recommended practices in Essential Oil Research.

Dominique Davenne (Faculté de pharmacie de Rennes, France)
Biological activities of Essential Oils: Usual weaknesses and real mistakes in posters and papers.

17h10-18h30 : Coffee break + Poster session (Posters PP61 – PP121d)

Wednesday 14 September :

8h45-9h30 : **PL-6**
Matthias Vey (IFRA, Belgium)
Risk Assessment and Management of Fragrance Allergens – ways forward.

9h30-9h50 : **OP-18**
Jane A. Cooper (Waters Corp., UK)
Analysis of Cosmetic Allergens using Ultra Performance Convergence Chromatography (UPC2) with MS Detection.

9h50-10h10 : **OP-19**
Frank David (Research Institute for Chromatography, Belgium)
Advances in instrumental solutions for the determination of the extended list of perfume allergens.

10h10-10h40 : Coffee break

10h40-11h : **OP-20**
Emilie Belhassen (Firmenich SA, Switzerland)
Routine simultaneous quantification of 61 allergens in fragrance raw materials by a GC×GC-qMS method.

11h-11h20 : **OP-23**
Thomas Dutriez (Givaudan, Switzerland)
Implementation of a global program for the quantification of the extended list of suspected allergens and regulated compounds in fragrance materials by GC×GC-TOF-MS.

11h20-12h10 : Round table & Closing session

PLENARY LECTURES ABSTRACTS

(PL)

Using Essential Oils in Agriculture : Crop Protection and Animal Farming

Bellenot Denis[1]

[1]*iteipmai, BP80009 F-49120 CHEMILLE en ANJOU*
Corresponding author: denis.bellenot@iteipmai.fr

Keywords: growth promoters, growth performance, food safety, feed additives, immunostimulation, veterinary products, biopesticides, insecticides, repellents, fungicides, herbicides, sprouting inhibitors.

In the past two decades, researchers have intensively focused their efforts on the development of alternatives to antibiotics as growth promoters in animal farming. Among these alternatives, essential oils were the first substances of natural origin to be tested. Pigs and poultry are the more frequently concerned species in the literature. If antibiotic activity of essential oils was the initial interest of their uses, other properties are now studied: immunomodulation, lipidic metabolism, growth performances and feed conversion ratio or meat oxidative status. If their mechanisms of action are still unclear, some evidences of their influence on animal metabolism are published. The most popular essential oils used in feeds are the ones containing phenols (thymol, carvacrol, eugenol) or cinnamaldehyde.

Beside incorporation of essential oils in feed, their therapeutic use becomes more and more popular. Different essential oils have been recommended for treating dairy cows mastitis or to reduce stress in horses and pets.

The development of organic farming as well as resistance to conventional pesticides have led to the search for natural pesticides. Many essential oil have been tested for their pesticide activities on insects, including larvicidal, antifeedant, repellent properties. Other uses of EO in crop protection are herbicide, fungicide, sprout inhibitors and sometimes as adjuvants.

Examples of these agricultural uses of essential oils will be presented discussed.

Finally, it is pointed out that the very critical point concerning the use of essential oils in agriculture is the regulatory status of these natural products.

A Critical Revision of basic Methodologies for Solving Complex Flavor Systems

Vicente Ferreira, Laura Culleré, María Pilar Sáenz-Navajas

Laboratory for Aroma Analysis and Enology, Facultad of Sciences, University of Zaragoza, Spain.
Corresponding author: vferre@unizar.es

Keywords: Flavor analysis, GC-O, AEDA.

Flavor analytical chemistry has as major goal to be able to understand the chemical bases of the sensory perceptions elicited by a product. For achieving it, this branch of science follows a series of consecutive tasks based sometimes more on experience than on the genuine understanding of the chemical bases of the sensory perception, of the physical chemistry laws related to the isolation and preconcentration of chemicals and of the rules for reliable chemical measurement. Such way of proceeding may imply the consumption of more resources than those genuinely needed for reaching the goal, or worse, the failure in reaching it. This presentation will revisit some of the practices most often reported paying special attention to the more controversial ones.

Maybe the most critical decision is about the type of extract used for beginning the sensory screening process. It will be shown that the most frequently used strategies are extremely ineffective as the extracts are not really representative of the headspaces that the sample can produce. The inevitably biased olfactometric results obtained will have to be further corrected in complicated, effortful, expensive and error-prone steps of determination of OAVs. Different alternatives based on headspace techniques will be discussed.

Another controversial question is related to the strategy used for generating GC-O signals. The most frequent choice of AEDA can be explained just in the attribution of a poor quantitative value to the FD factors. Most effective strategies will be discussed in this context. Following, quantitation and the need for using adequate internal standards will be also discussed. The need to pay attention to the different chemo-physical interactions existing between aroma molecules and matrix components will be highlighted.

Finally, the interest of building models relating well the GC-O signal, well the quantitative information to the sensory properties of the product will be briefly discussed, highlighting some pressing challenges.

Acknowledgements: Work funded by the Spanish MINECO (AGL2014-59840).

(Low-)flow modulation comprehensive 2D GC-MS: a limited-cost ultra high resolution approach for flavour & fragrance analysis

Peter Q. Tranchida[1], Flavio A. Franchina[2], Luigi Mondello[1,2]

[1] *"Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali", University of Messina, Polo Annunziata, viale Annunziata, 98168 – Messina, Italy.*
[2] *Chromaleont s.r.l., c/o University of Messina, Polo Annunziata, viale Annunziata, 98168 – Messina, Italy.*
Corresponding author: ptranchida@unime.it

Keywords: comprehensive two-dimensional gas chromatography; mass spectrometry; flow modulation.

The present contribution will describe on recent evolution of flow modulation (FM) comprehensive two-dimensional gas chromatography (GC×GC), within the context of mass spectrometry (MS) hyphenation. In particular, it will be shown how intensive research on an FM model has enabled a great reduction in gas flows making the combination of GC×GC and MS now much easier. It is noteworthy that the flow-modulation approach enables ultra high-resolution GC separations at a much lower cost compared to cryogenic modulation. Specific examples will be shown involving flavours & fragrances, and their analysis using FM GC×GC combined with single quadrupole, triple quadrupole, and high-resolution time-of-flight mass spectrometry.

Biotechnology for the Production of Flavor and Fragrance Terpenoids.

Michel Schalk

Firmenich SA, Biotechnology, Corporate R&D Division, Geneva, Switzerland.
Corresponding author: Michel.SCHALK@firmenich.com

Keywords: biotechnology, terpenoids, sustainable, Clearwood™.

Synthetic biology opens possibilities of developing cost-effective and sustainable manufacturing processes for molecules not easily available by organic chemistry or by extraction from natural resources. Because of the structural diversity and broad range of application the terpenoid biosynthetic pathway has been extensively studied in recent years and economically viable solution for biochemical production of this family of molecules are emerging.

In the flavor and fragrance industry, terpenoids represent a class of secondary metabolites of great economic importance, with unique olfactory properties, often difficult to replace with synthetic analogs. These molecules are mostly derived from plants and have thus the disadvantage of being subject to fluctuations in price and quality due to climatic or geo-politic factors and are sometimes available only in small concentration in the raw material or only from non-sustainable resources. In addition, given the structural complexity of terpene molecules, cost-effective chemical routes are not available for many terpene compounds important for the industry.

We have therefore investigated the biosynthesis of terpene molecules constituents of key perfume ingredient. The approaches used for the molecular characterization of new terpene biosynthetic pathways will be discussed and examples of successfully elucidated pathways leading to terpene molecules of high value for the perfumery industry will be presented. Finally, recent achievements in cost-effective industrial production of terpene compounds, such as Clearwood™, will be discussed.

The Analysis of Essential Oils and Related Natural Products: Avoiding the Pitfalls

Daniel Joulain

SCBZ Conseil, Les Micocouliers - F3, 99 avenue Sidi Brahim, F-06130 Grasse, France.
dajoulain@wanadoo.fr

Research in natural product chemistry often starts with an identification task. Until the early sixties, this was frequently achieved by isolating a quantity of pure substance, which was then converted by a variety of degradation and derivatization reactions into more readily analyzable molecules. These, or crystallized derivatives, could in turn be identified by comparison with known ones, e.g. with the help of elemental analysis and melting points. Today, a battery of advanced nondestructive spectroscopic methods exists for the expeditious assignment of structures to highly complex molecules isolated from nature in milligram or sub-milligram quantities. In the domain of flavour and fragrance related substances, this is mostly achieved by GC-MS, even though more sophisticated hyphenated techniques are becoming more popular. However, GC-MS in isolation practically always restricts identification to previously known substances. Misidentifications and gross errors are observed frequently when precautions are not taken to avoid biases or pitfalls generated by an excessive confidence in the data generated by this powerful instrumentation. Such drawbacks apply to both qualitative and quantitative analysis. Examples and strategies to avoid their occurrence will be presented.

As in the old days, the identification of new components in a natural mixture (either essential oil or solvent extract) still requires today the isolation of a sufficient amount of pure substance, and then to submit it to standard spectroscopic analysis in order to determine the molecular structure, ideally including absolute configuration. Among the many preparative separations which can be used, some techniques have sadly been abandoned or neglected over the years; such methods include high efficiency fractional distillation and preparative GC. The benefits of rejuvenating such techniques will be discussed, with examples.

Risk Assessment and Management of Fragrance Allergens – a way forward

Matthias Vey

IFRA, Arnulfstrasse 24, 40545 Düsseldorf, Germany.
mvey@ifraorg.org

Keywords: Fragrance allergens, induction, elicitation, risk assessment and risk management, IDEA.

Contact allergy to fragrance ingredients observed in clinics remains a topic of high attention by stakeholders and regulators. The fragrance industry has developed its own risk assessment system for fragrance allergens, the so called quantitative risk assessment (QRA). The QRA allows to derive use levels that should prevent the majority of the population to become induced (develop a contact allergy). This risk assessment system is in place for several years now and recently in the context of the International Dialogue on Fragrance Allergens (IDEA; www.ideaproject.info) has undergone a critical revision. Major elements of the revision have been the underlying safety assessment factors and the incorporation of aggregate exposure. Details on this activity will be provided as well as a deeper insight in all the aspects covered under IDEA. Further activities in the context of the EU Cosmetics Regulation linked to fragrance allergens will be presented.

ORAL PRESENTATIONS ABSTRACTS

(OP)

The first report on the acute toxicity of the highly valued essential oil of lemon balm (*Melissa officinalis* L., Lamiaceae) in female BALB/c mice

Nikola M. Stojanović[1,] *, Pavle J Randjelović[2], Ivan R. Ilić[3],
Marko Z. Mladenović[4], Niko S. Radulović[4,] *

[1] *Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000 Niš, Serbia.*
[2] *Department of Physiology, Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000 Niš, Serbia.*
[3] *Department of Pathology, Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000 Niš, Serbia.*
[3] *Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia.*
Corresponding author : nikola.st90@yahoo.com, nikoradulovic@yahoo.com

Keywords: *Melissa officinalis*, Lamiaceae, citronellal, acute toxicity, BALB/c mice

The perennial herb *Melissa officinalis* L. (Lamiaceae) is commonly known as lemon balm, owing to its citrus aroma. The essential oil of *M. officinalis* leaves is held in high esteem for its use in aromatherapy; however, due to the low yield of the essential oil, its production cost is very high. A myriad of beneficial biological activities of this essential oil was reported and its composition has been extensively studied. Strangely, up to date, no studies exist on the acute toxicity of this essential oil. Prompted by this, in this work, the toxicity of parenterally administered *M. officinalis* essential oil was assessed. The hydrodistilled essential oil used in the current study was obtained from fresh plant material (leaves; yield 0.087%, w/w) collected from wild-growing populations in the vicinity of the city of Niš (SE Serbia). Detailed analyses (GC and GC/MS) showed that the tested essential oil contained high amounts of geranial (22.1%), neral (17.6%), citronellal (4.2%), nerol (1.3%) and geraniol (1.2%), as expected for *M. officinalis*. The acute toxicity was evaluated in female BALB/c mice that were injected intraperitoneally with the essential oil (the used doses were in the range 0.5-2 g/kg). Near-lethal doses (over 1 g/kg) of the essential oil caused a decrease in motor activity (hypoactivity), abnormal movement patterns, tremors, ataxia and paralysis of the hind legs with consequential hypothermia, also myoclonic jerks and the loss of righting reflex were observed. Twenty-four hours following the essential-oil injections, the animals that survived were sacrificed, their blood was withdrawn, liver and kidneys were removed. Serum levels of liver (ALT, AST and ALP) and kidney (urea and creatinine) damage-related parameters were significantly increased in animals injected with the doses of essential oil higher than 1 g/kg. A microscopic analysis of liver and kidney sections, routinely stained with H&E, revealed a different degree of blood stasis and perivascular mononuclear cell infiltration. Moderate degeneration of kidney tubule cells and hepatocytes were also found. In conclusion, by causing a wide panel of both behavioral alterations and parenchymatous organ changes in female mice, *M. officinalis* essential oil can be deemed as moderately toxic.

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

Introducing a quantitative assay to assess the volatile antimicrobial activities of essential oils and their components

Adam Feyaerts[1,2], Lotte Mathé[1,2], Walter Luyten[3], Patrick Van Dijck[1,2]

[1] VIB Department of Molecular Microbiology, KU Leuven, 3001, Leuven, BELGIUM
[2] Laboratory of Molecular Cell Biology, KU Leuven, 3001, Leuven, BELGIUM
[3] Department of Biology, KU Leuven, 3000, Leuven, BELGIUM
**Corresponding author: Email: adam.feyaerts@mmbio.vib-kuleuven.be*

Keywords: antimicrobial activity, distance-related effects (DRE) assay, volatile MIC

Many studies are devoted to the antimicrobial activities (AMAs) of essential oils (EOs). Typically, their minimal inhibitory concentrations (MICs) are reported; indicating the lowest concentration necessary to inhibit microbial growth in a given assay. This has been the gold standard defining the antimicrobial potential of agents against a specific microorganism under specific conditions[1]. For single-compound antimicrobials it is a fixed value. However, antimicrobials such as EOs are highly complex natural mixtures of diverse EO components (EOCs), and hence their MIC value may vary between similar EOs or different batches of the same EO, although the differences generally are within a fairly narrow range. Various MIC-derived parameters have been introduced, for instance, to report the cidal activities of antimicrobials such as the minimal fungicidal activity, or to report the MIC for specific growth conditions of the microorganism such as like sessile MIC (commonly abbreviated as sMIC). Assays to measure a (derived) MIC of an antimicrobial against a specific microorganism have in common that they are all water-based assays and that the component is dissolved or emulsified in the liquid medium. For relatively hydrophobic antimicrobials such as EO(C)s this can be a challenge although different (co-)solvents are available to facilitate dissolution. However, EO(C)s are also volatile and can have biological effects e.g. antimicrobial activity, at a distance. To the best of our knowledge, there is not yet a gold standard assay in place for such effects.

In an attempt to close this knowledge gap, we developed the Distance-Related Effect (DRE) assay. This is the generic name of a novel, relatively inexpensive, easy to handle, quantitative (semi-)high throughput assay to measure the DREs of EO(C)s which, by extension, can be used with any volatile component. When the DRE assay is used to quantitatively measure the volatile MIC (vMIC) of components against planktonic microorganisms, then there is an objective way to compare the volatile AMAs between different antimicrobials. For specific growth conditions like sessile growth e.g. to assess anti- biofilm activity, we also introduce vsMIC.

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Effects of olfactory stimulation with scents of grapefruit and lavender essential oils on the skeletal muscle sympathetic nerve and muscle blood flow

Katsuya Nagai[1,2], Yuko Horii[1], Yoshiyuki Fujisaki[1],
Risa Fuyuki[1], Yoshiko Misonou[1]

[1]ANBAS Corporation, 4-12-10 Toyosaki, Kita-Ku, Osaka, Osaka 531-0072, Japan
[2]Osaka University, 1-1 Yamadaoka Suita, Osaka, 565-0871, Japan
Corresponding author : knagai@anbas.co.jp

Keywords: *Citrus paradisii, Lavandula angustifolia*, olfactory stimulation, skeletal muscle, sympathetic nerve, blood flow, rat

Previously, it was observed that olfactory stimulation with scents of grapefruit and lavender oils reciprocally affected the autonomic nerves innervating the white adipose tissue, brown adipose tissue, adrenal glands, kidney and stomach in rats [1]. That is, scent stimulation with grapefruit oil facilitated sympathetic nerves innervating the white adipose tissue, brown adipose tissue, adrenal glands and kidney, and inhibited the vagal (parasympathetic) nerve innervating the stomach. White, scent stimulation with lavender oil inhibited sympathetic nerves innervating the white adipose tissue, brown adipose tissue, adrenal glands and kidney, and facilitated the vagal (parasympathetic) nerve innervating the stomach. Furthermore, we observed evidence that scent stimulation with grapefruit oil increases lipolysis, thermogenesis, the blood pressure and blood glucose, and decreases appetite in rats [1,2]. On the other hand, we observed evidence that scent stimulation with lavender oil decreases lipolysis, thermogenesis, the blood pressure and blood glucose, and elevated appetite in rats [1,2].

Since the suppression and facilitation of the skeletal muscle sympathetic nerve increases and decreases the blood flow (BF) of the skeletal muscle, respectively [3], effects of olfactory stimulation with scents of a grapefruit oil (*Citrus paradisii*, Laboratoire Rosier Davenne) and a lavender oil (*Lavandula angustifolia*, Laboratoire Rosier Davenne) on the sympathetic nerve innervating the skeletal muscle and skeletal muscle blood flow (BF) were examined. Consequently, it was found that the former stimulation suppressed and the latter facilitated the sympathetic nerve and the BF, respectively. These facts suggest that the scent stimulation with the grapefruit oil inhibits and that with the lavender oil enhances the muscle BF via these autonomic changes. These findings suggest possibilities that scent stimulation with the grapefruit oil may suppress and that with the lavender oil may enhance the muscle recovery from fatigue and muscle growth, respectively. These must be examined in future.

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Characteristic Scent Components from the Japanese Aromatic Plants, *Alpinia japonica*, *Elettaria cadamomum*, *Artemisia annua*, *Citrus medica*, *Orixa japonica* and *Litsea cubeba*

Yoshinori Asakawa[1], Kazutoshi Sakurai[2], Kenichi Tomiyama[2], Yukihiro Kawakami,[2] and Yoshihiro Yaguchi[2]

[1]Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan; [2]Corporate Research and Development Division, Takasago International Corporation, Hiratsuka, Kanagawa 254-0073, Japan
Corresponding author: asakawa@ph.bunri-u.ac.jp

Keywords: *Japanese aromatic plants,* fenchone, artemisia ketone, *cis*-jasmone, neral, geranial

1. Introduction

Alpinia japonica, *Elettaria cardamomum* (Zingiberaceae), *Artemisia annua* (Asteraceae), *Citrus medica* var. *sarcodactylis*, *Orixa japonica* (Rutaceae) and *Litsea cubeba* (Lauraceae) emit characteristic odor. The present paper deals with the identification of characteristic aroma from each Japanese aromatic plant.

2. Materials and methods

HS-SPME-GC/MS analysis: Each adsorbed material was analyzed by GC/MS: BC-WAX (50 m x 0.25 mm I.D.x 0.15μm d_f), 70-220°C at 4°C/min temp. program, inject. temp. 250°C, split ratio 10:1, He (1ml/min). γ-DeX 225 was used as a chiral column. 70-120°C at 1.0°C/min. temp. program.

3. Results and Discussion

The rhizomes, roots, leaves and flowers of *A. japonica* are rich sources of the fenchane-type monoterpenoids, fenchene, (S)-(+)-fenchone (%*ee*), fenchyl alcohol and fenchyl acetate. However, the major volatile component of the fresh fruits was 1,8-cineol (69.4%). Surprisingly the roots contained bornyl acetate (15.1%) as the major component. Fenchone found both in the dried and fresh rhizomes of *E. cardamomum*, also showed the same chirality, 99% *ee*. The strong sweet odor of both plants might be due to fenchane-type monoterpenoids.

The major volatile components of the leaves of *A. annua* were β-artemisyl acetate (23.6.%), artemisia ketone (24.0%) and 1.8-cineol (7.2%). The second major group was *ci*s-crysantenyl acetate, (6*E*)-farnesene, β-caryophyllene, germacrene D, and benzyl 2-methyl butylate.

The major components of the immature and mature fruits of *C. medica* var. *sarcodactylus* were limonene and γ-terpinene, together with a small amount of myrcene, α-pinene, β-pinene and geranial whose composition was similar to that of lemon oil. On contrary, the leaves contained geranial and neral (73.9% as citral) and limonene as the predominant components, along with geraniol, nerol and citronellal and (2*E*)-hexenal as the minor components. The presence of citral and (2*E*)-hexenal is responsible for the green-lemon like aroma of the leaves of this species. GC/O analysis indicated that the powerful lemon and lemongrass fragrance of the leaves were due to a large amount of citral.

The major volatile component in both the flowers and the leaves of *O. japonica* was linalyl aceate (33.9 and 33.2%). Both organs also contain linalool (5.0-6.4%). In the flowers, five monoterpene acetates, neryl acetate, geranyl acetate, *cis*-linalool-3,6-oxide acetate, *trans*-linalool-3,6-oxide acetate, and α-terpinyl acetate were identified. The most significant difference between the two volatiles was that a relatively high amount of *cis*-jasmone (10.7%) was identified in the flowers, but the leaves did not. The presence of linalyl acetate and *cis*-jasmone along with the other acyclic monoterpene acetates may play an important role in the fragrant characteristics of the flowers of Japanese *O. japonica*.

The major components of the ether extract of *Litsea cubeba* was measured by BC-WAX column. In this case, (8*Z*)-heptadecene and geranial were detected as the major components. When the same extract was measured by OD column, gernial and neral were identified as the major monoterpenoids, together with *trans*-β-farnesene. On the other hand, the volatile component profile which was analyzed by SPME method was totally different from the above methods, except for the presence of a large amount of (8*Z*)-heptadecene. A predominant content of neral and geranial plays an important role in sweet-lemon fragrance of the flowers. The role of a high content of (8*Z*)-heptadecene might play a significant role in the dispersion of the volatile monoterpene hydrocarbons and aldehydes.

Characterization of odour-active compounds in Timur (*Zanthoxylum alatum Hemsl.*) essential oil

Esméralda Cicchetti[1], Marion Perez[1], Laura Sizaire[1], Clémence Vasseur[1]

[1] *Cosmo International Ingredients, R&D department, Analytical laboratory, 855 av. Maurice Donat, 06250 Mougins*
Corresponding author: *esmeralda.gonzales@cosmo-ingredients.com*

Keywords: *Zanthoxylum alatum Hemsl., Zanthoxylum armatum DC.,* volatile compounds, sulfur compounds, impact odorant, GC-MS, RRF, GC-olfactometry, Time Intensity Method, Detection Frequency, derivatization, SPE, SPME, Head Space, DOE

The smell of Timur (*Zanthoxylum alatum Hemsl*. Syn. *Zanthoxylum armatum DC*) fruit, an asian pepper, is a combination of spicy, peppery, citrus notes together with grapefruit, blackcurrant and tropical facets. The essential oils of the seeds and the pericarp are already known to be rich in linalool, methyl cinnamate, limonene and beta phellandrene [1-4] explaining the spicy/citrus aspect but to our knowledge none of the published data reported volatile compounds that could participate to the interesting sulfury odour of Timur.

The present contribution will describe the characterization of the main volatile constituents of the essential oil of Timur seeds, from different regions and crops, with their identification by GC-MS and their quantification by GC-FID with the use of predicted relative response factors [5]. The volatile constituents in the head space of the seeds will also be studied using static head space and SPME with the use of design of experiments to improve the extraction conditions. The work will also focus on the determination of the key odorant-active compounds of Timur by GC-olfactometry using a hybrid methodology between detection frequency and time intensity methods. Additional experiments to identify the most potent aroma chemicals of the oil will also be presented like derivatization, sample fractionation and solid phase extraction.

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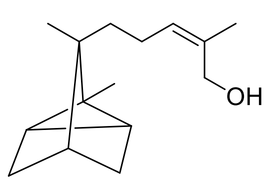
Structural Similarities of Odor Compounds for Olfactory Recognition in Odor Materials

Toshio Hasegawa[1], Takashi Fujihara[1], Hideo Yamada[2]

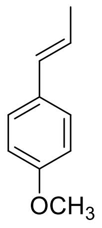
[1]*Department of Chemistry, Graduate School of Science and Engineering, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama 338-8570, Japan*
[2] *Yamada-matsu Co., Ltd., Kamigyo-ku, Kyoto 602-8014, Japan*
Corresponding author: toshihas@mail.saitama-u.ac.jp

Keywords: structural similarity, odor compound, odor material, olfactory recognition

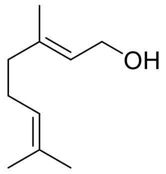
The aromas of natural odor materials, such as sandalwood, consist of many odor compounds. In some cases, we have found that the odors of the materials arise from a group of constituents with similar structures [1]. Recent studies on the olfactory mechanism show that several olfactory receptors interact with one odorant molecule with different intensities and one olfactory receptor responds to different odorant molecules with similar structures [2]. This mechanism means that the odor of the material is not the sum of the odors of each constituent. The interactions of several constituents with similar structures are important for the aroma profile. We observed a change in the response pattern of olfactory receptors that indicated a change in odor when the structure of an odorant molecule was changed. Therefore, we sought to understand the mechanism underlying the structural similarity of olfactory receptors by collecting systematic data on structure-odor relationships of odorants. A series of derivatives of α-santalol, anethole, and geraniol were synthesized to obtain information about their structural similarities for olfactory recognition. We investigated the structure-odor relationships and found the following important structural factors for odor. (1) Molecular skeleton; (2) number of double bonds; (3) double bond configuration (*E* vs *Z*); (4) groups containing oxygen; and (5) the isoprene moiety.



α-Santalol



Anethole



Geraniol

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Cyclodextrins: a promising encapsulant material to improve the functionalities of essential oils.

Sophie Fourmentin[1], Miriana Kfoury[1,2], Lizette Auezova[2], Hélène Greige-Gerges[2]

[1] *Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV, EA 4492), SFR Condorcet FR CNRS 3417, ULCO, F-59140 Dunkerque, France*
[2] *Bioactive Molecules Research Group, Faculty of Sciences-2, Lebanese University, Fanar, Lebanon*
Corresponding author: lamotte@univ-littoral.fr

Keywords: cyclodextrins, encapsulation, solubility enhancement, photostability.

There is a strengthening and increasing concern in the use of essential oils (EOs) in different industrial formulations. In addition to their main function as flavoring and aromatic substances, EOs could be used as natural preservatives, antioxidants and antimicrobial agents. However, there are still many challenges ahead for the food, cosmetics, and pharmaceutical industries. EOs suffer from very poor aqueous solubility and stability as well as high volatility and susceptibility for degradation. Encapsulation in cyclodextrins (CDs), natural cage molecules obtained from starch, could enhance EOs functionalities and maintain their biological activities [1,2]. In this study, we investigated the ability of different CDs to encapsulate twelve EOs. Static-headspace-gas chromatography (SH-GC) was used to determine the affinity between the EOs components and CDs. A new Total Organic Carbon (TOC) method was applied to evaluate the effect of CDs on the aqueous solubility of EOs by carrying out phase solubility studies [3]. NMR spectroscopy and molecular modeling were also used to characterize inclusion complexes of EOs components. Then, the ability of CDs to generate controlled release systems was examined by multiple headspace extraction (MHE) and the effect of the encapsulation on the photostability of EOs was studied under UVC irradiation. Results proved the ability of CDs to encapsulate EOs. They also underlined that the binding strength strongly depends on the space filling of the CD cavity and the geometric fit between the latter and the EO component. Moreover, CDs considerably enhanced the aqueous solubility of EOs. A relationship between the intrinsic solubility of EOs and the solubilizing potential of CD was proposed. CDs also provided a photoprotective effect to EOs and allowed the generation of controlled release systems.

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The Effect of Coal-Bed Methane Water and Gypsum on Lemongrass and Palmarosa

Valtcho D. Zheljazkov[1], Charles L. Cantrell[2], Ekaterina A. Jeliazkova[3], Tess Astatkie[4], Vicki Schlegel[5], Urszula Norton[6]

[1]Oregon State University, Crop and Soil Science Department, 109 Crop Science Building, 3050 SW Campus Way, Corvallis, OR 97331 U.S.A.
USDA, Agricultural Research Service, NPURU, University, MS 38677, U.S.A
[3]Oregon State University, Columbia Basin Agricultural Research Station, 48037 Tubbs Ranch Road P.O. Box 370, Pendleton, Oregon 97801 U.S.A.
[4]Dalhousie University Faculty of Agriculture, Agricultural Campus, Department of Engineering, PO Box 550, Truro, NS, B2N 5E3 Canada
[5]University of Nebraska – Lincoln, Department of Food Science and Technology, 327 Food Technology Complex, Lincoln, NE 68583, U.S.A.
6Universtiy of Wyoming, Department of Plant Sciences, Ag Bldg. 4014, 1000 E. University Ave. Laramie, WY 82071 U.S.A.
Corresponding author: Valtcho.Jeliazkov@oregonstate.edu, Valtcho.pubs@gmail.com

Keywords: antioxidant, coal-bed methane, produced water, *Cymbopogon flexuosus Steud., Cymbopogon martini Roxb.*

Coal bed methane has become important energy source for many countries. The coal bed methane production coproduces a vast amount of coal bed methane water (CBMW), saline-sodic waste water with high concentrations of S, Na, and salts, and is considered a waste material from the U.S. Environmental Protection Agency. The disposal of CBMW is associated with environmental and economic issues. The hypothesis of this study was the CBMW may be used for irrigation of high-value non-food crops if amended with gypsum. Therefore, the objective of this study was to assess the effect of CBMW on the growth, essential oil content, composition, and antioxidant activity of two industrial crops, lemongrass (*Cymbopogon flexuosus* (Nees ex Steud.) Will.Watson), and palmarosa (*Cymbopogon martinii* (Roxb.) Wats). The treatments were: (1) water treatment at two level, CBMW and a tap (drinking) water, and (2) gypsum at four levels (0, 500, 2500, 7500 kg/ha). This was a container study, all treatments were in 4 replicates. The CBMW and the tap water used in this study had very different properties such as pH, EC, and in some cases very different concentration of various elements. Growth medium pH was higher in the CBMW treatments than in the tap water treatments. Gypsum application reduced growth medium pH in all CBMW treatments, but did not have an effect on growth medium pH in the Tap water treatments. Overall, biomass and essential oil yields were reduced by the application of CBMW. CBMW application increased significantly growth medium Na and tissue Na and affected the concentration of several other nutrients in growth medium and plants. CBMW also had an effect on the concentration of some of the essential oil constituents of lemongrass and palmarosa oils, as well as on their antioxidant capacity. These results along with our previous studies indicate CBMW needs to be diluted with good quality water before it is used for irrigation of agricultural crops.

Preparative gas chromatography for isolation of eudesmane type sesquiterpene ketone from *Prangos heyniae* essential oil

T. Özek[1], G. Özek[1], E. Bedir[2], N. Tabanca[3,4,5], A. Ali[3], A. Duran[6], I.A. Khan[3],
K.H.C.Başer[7]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskisehir, Turkey.*
[2]*Department of Bioengineering, Izmir Institute of Technology, Urla, 35430, Izmir, Turkey.*
[3]*National Center for Natural Products Research, The University of Mississippi, University, MS 38677, USA.*
[4]*Department of Entomology and Nematology, Emerging Pathogens Institute, University of Florida, Gainesville, FL 32610, USA.*
[5]*USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL, 32608, USA.*
[6]*Department of Biology, Faculty of Science, Selçuk University, 42075, Selçuklu/Konya, Turkey.*
[7]*Department of Pharmacognosy, Faculty of Pharmacy, Near East University, Lefkoşa (Nicosia) N. Cyprus.*
Corresponding author: tozek@anadolu.edu.tr

Keywords: *Prangos heyniae*, eudesmane sesquiterpene, preparative gas chromatography, larvicidal.

Prangos Lindl. (Apiaceae) is represented in the world by 40 species and altogether 43 taxa. In the Flora of Turkey 17 taxa belonging to 16 species are recorded. Herbal parts of *Prangos* species called caksir are used as fodder and the roots are used as aphrodisiac like members of some other related genera such as *Ferula* L. and *Ferulago* W. Koch [1].

In the present work, an endemic species *Prangos heyniae* H.Duman & M.F.Watson was collected from Konya: Korualan area and subjected to hydrodistillation in a Clevenger type apparatus to yield the essential oil (EO). GC/MS and GC-FID analyses revealed that the EO was rich in elemol (27.3%) and unidentified constituent (15.5%) with [M^+218]. Using an HP Innowax preparative capillary column connected to a preparative fraction collector, an unknown eudesmane type constituent was isolated in a rapid one-step manner with >95.0% purity. Structure determination accomplished from 1D- and 2D-NMR spectroscopic data resulted with 3,7(11)-eudesmadien-2-one.

The essential oil was subjected to investigation for larvicidal activity against 1st instar *Aedes aegypti* L. The oil demonstrated significant activity and resulted in 100% mortality at concentration 125 and 62.5 ppm and the mortality dropped to 90% at 31.25 ppm, while no mortality was recorded at 15.625 ppm.

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Exploring the Potential of Ionic Liquids as Gas Chromatographic Stationary Phases for The Analysis of Essential Oils and Volatiles from Natural Products

Cecilia Cagliero[1], Barbara Sgorbini[1], Patrizia Rubiolo[1], Carlo Bicchi[1]

[1] *Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, I-10125 Torino, Italy. Corresponding author: cecilia.cagliero@unito.it*

Keywords: ionic liquids stationary phases, peculiar selectivity, improved inertness towards polar analytes, analysis of aqueous samples.

Analyses of essential oils and volatiles from natural products have to cover a large range of topics, from quality control to marker identification and quantitation, thus requiring a combination of efficiency and selectivity of the chromatographic system suitable to produce a baseline separation of all markers in a single run. Samples in these fields are, in general, complex mixtures often consisting of isomeric components with similar structural and physical characteristics (e.g., mono- and sesquiterpenoids). Isomeric compounds are sometimes difficult to identify because of their similar mass spectra thus making chromatographic data indispensable for their correct identification, and, in some cases, challenging to separate with conventional GC stationary phases. A continual search for new stationary phases with different selectivity and, at the same time, with good chromatographic properties, is therefore necessary to obtain separation patterns different from those of the currently used based on polysiloxane and polyethylene glycol. The introduction of the ionic liquids (ILs) as stationary phases for GC have opened new perspectives because of their uncommon selectivity completely different from those mentioned above in particular after having overcome some problems of inertness with polar analytes of the first generation IL columns [1, 2].

This communication will explore the potential of commercially available IL stationary phases when applied to the analysis of essential oils and volatiles from natural products. In particular, it will report some examples on how IL peculiar selectivity can be exploited in challenging separations and component identification(s), also in view of the high inertness towards polar analytes reached from the last generation of IL columns. Furthermore, this contribution will discuss possible applications in this field of a new line of water compatible IL columns especially dedicated to the analysis of aqueous samples.

Acknowledgements: The authors are indebted to Len Sidisky (Supelco, Bellefonte, PA, USA) for helpful discussion and advice.

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Metabolome inspired synthetic libraries facilitate essential-oil analysis

Polina D. Blagojević, Niko S. Radulović

Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, Niš, Serbia
Corresponding author: blagojevicpolina@gmail.com

Keywords: libraries of synthetic compounds, plant metabolome, volatilome.

Despite evident advancement in the field of modern analytical techniques, the analysis of essential oils (EOs) is still a challenging task [1]. Complexity of the matrix and structural likeness (*i.e.* similar chemical/physical properties) of EO constituents often render preparative (chromatographic) separation, as the necessary step in structural elucidation, inapplicable. Thus, a combination of GC and GC/MS still remains the first choice in the analysis of EOs: identification of EO constituents relies on the comparison of their mass spectra (MS) and retention indices (RI) with those from the literature and, where possible, on the co-injection of EO samples with appropriate pure standards. But what to do in the cases of possibly new (minor) compounds, for which no MS/RI data and standards are available? The possible solution for this problem could be found in the insufficiently exploited "synthetic library" (SL) approach, which enables simultaneous identification not only of a single new compound, but of a series of its homologs/analogs. This includes the following steps: 1) preliminary GC and GC/MS analysis of the EO sample (metabolic profiling of a selected plant species), 2) recognition of a possible "parent structure" structurally/spectrally related to the unknown EO constituent (a "template" for the synthetic library), 3) a library design, retrosynthetic analysis and direct synthesis of the library compounds, 4) characterization of the library compounds (e.g. MS, RI data mining) and 5) parallel (re-)analysis of the synthetic library and the EOs containing unknown components: confirmation or rejection of the potential hit structures. Synthesis of a library of structurally related compounds also opens up the possibility to assess their safety, potential biological activity and gain insights into SAR/SPR relationships. Up to know we have successfully applied this methodology for the identification and pharmacological evaluation of a number of minor new EO constituents (e.g. allylmetoxyphenyl, *trans*-sabinyl, bornyl and fragranyl esters or long-chain 3-methyl-2-alkanones) [1,2]. The advantages of SL-approach will be illustrated in the cases of these (series of) compounds.

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Accurate mass screening using LC-QTOF: opportunities and challenges for the analysis of pesticides in Essential Oil

Céline ROY[1], Yoann FILLATRE[1], François-Xavier GRAY[1]

[1] *ERINI, Espace J.L. Lions, 4 Traverse Dupont, 06130 Grasse France.*
Corresponding author: celine.roy@erini-institute.com

Keywords: pesticides analysis, ring test results, Quadrupole-Time of Flight High Resolution Mass Spectrometer (QTOF-HRMS), target and non-target analysis, screening, collaborative action.

In a context where the competitive, environmental and societal pressures encourage strongly to produce perfectly safe and ecofriendly natural extracts, companies of the cosmetic and aromatic sector are increasingly concerned by the presence of pesticide residues in EOs. The ability to determine precisely the presence or absence of pesticide residues in their products becomes thus a key factor. Nevertheless, the physico-chemical properties of EOs (high hydrophobicity and multi components product), the numerous processing methods and the exposure to a wide range of agricultural practices according to geographical origins makes very challenging the determination of pesticide residues in EOs.

The classical methods are traditionally based on a targeted multiresidue analysis approach using a very sensitive and specific MS/MS detection (Selected Reaction Monitoring mode) to detect a large panel of pesticides covering most of the traditional cultivations. It classically represents 200 to 500 active substances among around 1150 selected according to national considerations mainly (regulation and monitoring plans of environmental contaminants).

This approach leads often to disconcerted results for the contracting companies which do not understand the relevance of pesticides lists as well as some differences in analytical performance from one lab to another.

In this context, new analytical strategies based on untargeted screenings of compounds, which exploit the resolution capacities of Quadrupole-Time of Flight High Resolution Mass Spectrometer (QTOF-HRMS), appear as promising alternative methods to classic targeted ones using triple quadrupole mass spectrometer. The opportunities they offer in the case of pesticide residues analysis in EOs will be exposed and illustrated by results obtained from a collaborative project orchestrated by the PASS cluster, and performed by ERINI with 11 industrial partners. Issues concerning method performance and validation will be also evoked as challenges to overcome before to adopt definitively this kind of approach.

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Proficiency-testing scheme for essential oils

Elvire Messineo[1], Athina VARFI[1], Jonathan MINZIERE[1], Abdelkader BOUBETRA[1]

[1] *BIPEA, 189 rue d'Aubervilliers, 75018 PARIS - FRANCE.*
Corresponding author: emessineo@bipea.org

Keywords: Proficiency-testing schemes, quality control, GC-FID profile, laboratory performance

BIPEA (http://www.bipea.org) organizes regular proficiency-testing schemes (PTS) in many analytical domains, including the analysis of essential oils. As an example, in September and October 2015, two tests were conducted using pine essential oil as a matrix, with respectively 27 and 32 participating laboratories. The first test was intended for routine quality-control analyses (density, refractive index optical rotation, flash-point, peroxide value, and acid value), while the second was intended for the analysis of the composition of this essential oil, by determination of its profile by gas chromatography with flame-ionization detection (for 22 compounds of this particular essential oil).

Participating laboratories were required to return their results on a dedicated website after a period of one month, and a statistical treatment of the data was as usual performed by BIPEA according to ISO 13528 [1]. Assigned (consensus) values were calculated from the participants' results and the performances of the laboratories could then be evaluated individually and collectively according to ISO 17043 [2].

These tests allow participating laboratories to draw up a general inventory of their analytical skills, and are a very useful tool to detect bias or non-compliant results; they act as a warning signal for the implementation of corrective and/or curative actions in the laboratory.

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Towards A New Method for Performing Environmental Risk of Complex Substances (CRANCS)

Paul THOMAS[1] Pauline Remuzat[2], Sylvie Betat[3] Nicolas Delpit[3] Benjamin Madru[3] Pascal Bicherel[1]

[1] *KREATiS SAS, 23 rue du Creuzat 38090 L'ISLE D'ABEAU, FRANCE*
[2] *CEHTRA SAS, 23 rue du Creuzat 38090 L'ISLE D'ABEAU, FRANCE*
[3] *LPL, rue des Ecoles 64150 LAGOR, FRANCE*

contact@kreatis.eu

Keywords: mixtures, aquatic toxicity, sediments, environmental risk assessment, modeling, calculation, partitioning

Complex mixtures pose a significant problem for environmental risk assessors in Europe as current guidance is unclear how to deal with numerous parameters required as input data. Notably, while ecotoxicity data can be generated using the Water Accommodated Fraction (WAF) method, there is some concern that such data cannot be easily validated and how it should be used to derive a PNEC in other compartments than the aquatic compartment. In fact it is likely that a complex mixture which has been deposited into receiving water will differ in the way it partitions between the aquatic compartment and the sediment, potentially leading to a significantly different constituent profile from the original mixture.

The objective of the Compartmentalised Risk Assessment for Natural Complex Substances (CRANCS) project is to develop a predictive method for environmental risk assessment in aquatic medium for Complex Substances with applied examples of Natural Complex Substances (NCS), notably essential oils. The main characteristic of CRANCS is to provide a more realistic picture of the adsorption profile of mixture constituents within the aquatic environment (water/sediment) and then to relate the exposure profile to the corresponding aquatic toxicity.

Initially, concentrations of the constituents of known mixtures in characterised sediment water systems will be measured and standard methods (e.g. EP method) will be compared to the measured concentrations of each constituent to determine their accuracy. If insufficient alternative partitioning calculations will be used to quantifying the partitioning of constituents between the two phases. This information will be used to define the $PEC_{aquatic}$ and $PEC_{sediment}$.

The spiked compartments will then be separated and tested as sediment and water WAFs then used in order to evaluate risk in both aquatic compartments: pelagic (water) *versus* benthic (sediments). In order to validate the use of the method for risk assessment both acute and chronic toxicity to organisms in both phases will be measured.

Finally the experimental results obtained from two complex mixtures (one prepared specifically for the purposes of this study, containing not readily biodegradable, stable substances and a second one on a known NCS) will be used to develop and validate several calculation methods to predict experimental results.

Influence of genetic, epigenetic, agricultural and post-harvest factors on the production of Rose and Geranium Essential Oils.

Bernard Blerot[1], Sylvie Baudino[2], Bernard Toulemonde[1]

[1]International Flavors & Fragrances - Laboratoire Monique Rémy (IFF-LMR) P.I des Bois de Grasse, Av. Louison Bobet – 06130 Grasse - France (bernard.blerot@iff.com)
[2]1Université de Lyon, F-42023, Saint-Etienne, France; Université de Saint-Etienne, F-42000, Saint-Etienne, France; Laboratoire BVpam, EA3061, 23 rue du Dr Michelon, F-42000, Saint-Etienne, France
Corresponding author: Bernard.blerot@iff.com

Keywords: Geraniol, ‘Pelargonium rosat’, Rose

Hybrids of Pelargonium with a rose scent, originated from South Africa, were created during the 18th century and are cultivating around the world for the production of essential oil (EO) [1]. Rosa damascena is originated from Syria and cultivated for its EO in Asia Minor since the 15th century. Both vegetal are producing a rose smell EO containing similar molecules (geraniol, citronellol) but synthesized through a different way. When the Pelargonium is using a classic terpene synthase to produce geraniol (unpublished result), the rose is using a completely different and unique way to do that [2]. The storage and emission are also different. Pelargonium stores its EO in glandular trichomes located at the leave surface [3] which has the consequence to repellent the predator insects. On the contrary, Rose fragrance molecules are produced and emit continuously from in conical, papillate or flat shape epidermal flower cells which have the effect to attract the pollinators [4]. These fundamental differences influence deeply the EO producer to adapt his cultivation practices. Indeed, the influence of the environment parameters (soil, temperature…) is crucial as the cultivation, harvest practices and the logistic around the crop. All these parameters are forming a chain that it’s necessary to manage and understand deeply each link in the chain to develop the best product.

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Sustainable Bioproducts from the Amazon rainforest

Lauro Barata

Federal University of the West Pará UFOPA-PROPPIT (www.ufopa.edu.br)
Email: lauroesbarata@gmail.com

The economy of the Amazon region is primarily focused on producing commodities such as meat, soy, energy and ore from mining operations[1]. The main export item that comes from the largest tropical forest in the world is timber, with 14 million m^3 being exported in in 2010[2], with non-timber forest products occupying a negligible share (0.1% of total exports) in this economic context. In order to be preserved, the Amazon basin must go through a Research & Development (R & D) process that would transform Biodiversity into Bioproducts for society. The R & D of Amazonian natural products should be the basis of sustainable development, integrating technology with socioeconomic aspects.

Our laboratory, The P&DBIO - Research & Development Laboratory on Natural Bioactive Products in Amazon, in the heart of the forest, uses Biotechnology to standardize raw materials for the cosmetics and perfumery markets. The Brazilian cosmetic market is one of the most important in the world with US$ 11 billion in sales in 2014 (ex-factory), and it was also the most important perfume market with sales of US$ 6 billion in 2012. Nevertheless, almost all raw materials are imported and the Amazon practically does not participate in this market. An important item in the Cosmetic industry is essential oils. More than a thousand aromatic plants have been recorded in the Amazon, however only the essential oil of rosewood (*Aniba rosaeodora*) – an endangered species - is exported to the main perfumery companies. Barata et al. (2012)[3] cultivated cassava intercropped with rosewood in the Amazon, and the sustainable procedure of trimming trees (4 years of age) produces a fragrant essential oil in use now for fine perfumes and aromatherapy. This know-how has been adapted by a small enterprise in the Amazon, which is now exporting rosewood oil at the price of US$ 250/kg. In another agro-industrial project, the P&DBIO developed a Priprioca (*Cyperus articulatus*) plantation on two family farms in the western Amazon leading to the harvest of 1,000 kg of rhizomes in 2014, and from that harvest the authors obtained an essential oil that is already being used to create fine fragrances. The technology from the agro-industrial project with P&DBIO partnership is about to be transferred to small local producers who will be able to access the perfume market in the near future, bringing economic opportunities to families in Amazon communities, and thereby offering an alternative to the devastation of the Amazon forest.

Brazilian Essential Oils: An Overview

Humberto R. Bizzo

Embrapa Food Technology – Avenida das Américas, 29501 Rio de Janeiro 23020-470 Brazil.
Corresponding author: humberto.bizzo@embrapa.br

Keywords: *Citrus*, *Eucalyptus citriodora*, *Varronia curassavica*, essential oil trade, Brazil.

Brazil is the sixth larger essential oil exporter in value, but the first in quantity of exported oil. This market share is due to citrus oils, orange oil mainly, a byproduct of the world largest citrus industry. In 2015, more than 26 tons were exported, representing US$ 155,000,000. Brazilian commercial production begun in the 1920's with rosewood oil (*Aniba roseaodora* var. *amazonica*) and grew along the last century. For a while, Brazil stood as the largest world supplier of sassafras (*Ocotea pretiosa*) and Japanese mint (*Mentha arvensis*) oils, but not anymore. Besides citrus, *Eucalyptus* oils are also distilled and exported. *E. citriodora* accounts for 85 to 90 % of the total, with small quantities of *E. globulus* and *E. staigeriana*. Several Poaceae, like palmarosa, vetiver, citronella and lemongrass were introduced during the 1950's intending oil production, some with quite reasonable success. In Northeast Brazil, a large vetiver cultivation was established during the last decade. Its oil production rise up to 4.7 tons in 2010, but suddenly drop to only 30 kg in 2013. It is quite peculiar, however, that despites Brazilian extremely rich biodiversity, nearly all commercial oils come from exotic species. More recently, some oils from native plants have being produced and consumed internally, representing successful commercial cases. "Priprioca" (*Cyperus articulatus*, Cyperaceae) and "erva baleeira" (*Varronia curassavica*, syn *Cordia verbenaceae*, Boraginaceae) are good examples. A 0.5% dispersion of *V. curassavica* essential oil, with anti-inflammatory activity, is commercialized by a pharmaceutical company as an ointment or aerosol. The success was so pronounced that it caused a drop in the sales of the regular prescription anti-inflammatory chemotherapic. On the other hand, some biomes, like the Cerrado in Central Brazil, were poorly studied so far, and could account for new fragrance, flavouring and medicinal raw materials. The Cerrado and the Atlantic Rainforest are biodiversity hotspots, and the continuous pressure from the agricultural frontier is restricting the occurrence of endogenous species to a few parks and protected areas, as well as increasing the official list of endangered species. The main difficulty, however, in bringing new native materials to commercial use is the development of sustainable production systems. Simple extractivism has long proven to be deleterious to the survival of the species collected.

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Analysis of Cosmetic Allergens using Ultra Performance Convergence Chromatography (UPC^2) with MS Detection

Jane A. Cooper[1], Michael Jones[1], and Falk-Thilo Ferse[2]

[1] *Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, SK9 4AX, UK*
[2] *Waters GmbH, Helfmann-Park 10, 65760 Eschborn, Germany*
Corresponding author : jane_cooper@waters.com

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

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

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 moisturiser), 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.

There are many limitations / consideration / challenges that need to be addressed for any method used for allergen analysis. Specificity needs to be considered, the resolution achieved between analytes, between isomers and with matrices components needs to be optimized, and the sensitivity of the method needs to be at least 1 ppm (greater preferred).

Convergence Chromatography (CC) is a separation technique that uses carbon dioxide as the primary mobile phase, with a co-solvent such as acetonitrile or methanol to give similar selectivity as normal phase LC.

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.

ADVANCES IN INSTRUMENTAL SOLUTIONS FOR THE DETERMINATION OF THE EXTENDED LIST OF PERFUME ALLERGENS

Frank DAVID[1], Christophe DEVOS[2], Bart TIENPONT[3] and Pat SANDRA[4]

[1]*Frank DAVID, Research Institute for Chromatography, Pres. Kennedypark 26, B-8500 Kortrijk, Belgium*
[2]*Christophe DEVOS, Research Institute for Chromatography, Pres. Kennedypark 26, B-8500 Kortrijk, Belgium*
[3]*Bart TIENPONT, Research Institute for Chromatography, Pres. Kennedypark 26, B-8500 Kortrijk, Belgium*
[4]*Pat SANDRA, Research Institute for Chromatography, Pres. Kennedypark 26, B-8500 Kortrijk, Belgium*
Corresponding author : frank.david@richrom.com

KEYWORDS: perfume allergens, extended list, GC-MS, multidimensional techniques

Recently the list of fragrance allergens that should be measured in perfumes and cosmetic products has extended to more than 60 target solutes [1]. Taking into account the complexity of perfumes and cosmetic samples in terms of number of solutes and their large concentration range, the analysis of this extended list of target compounds is a challenge for control laboratories.

In this presentation, analytical solutions based on two-dimensional GC and GC-MS approaches will be presented and discussed. These solutions combine heart-cut two-dimensional configurations applying flexible capillary flow technology, low thermal mass column heating and intelligent software [2, 3]. The presented configurations are compatible with single quadrupole, triple quadrupole and time-of-flight mass spectrometers. The advantages and limitations of each of these MS systems will be highlighted.

The presented configurations were validated using a wide range of samples and the obtained results will be compared to classical analysis and data analysis [4].

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Routine simultaneous quantification of 61 allergens in fragrance raw materials by a GC×GC-qMS method

Emilie Belhassen[1], Philippe Merle[1], Chiara Cordero [2], Carlo Bicchi [2], Alain Chaintreau[1]

[1] *1 Route des Jeunes, 1217 Genève, Suisse.*

.

[2] *Dipartimento di Scienza e Tecnologica del Farmaco, Università degli Studi di Torino, via Pietro Giuria 9 – 10125 Torino, Italy.*

Corresponding author : emilie.belhassen@firmenich.com

Keywords: Comprehensive GC×GC, quadrupole-MS, Allergens, Quantification.

In the context of the forthcoming regulation on the extended list of 61 allergens[1], Flavours & Fragrance companies are requested to provide their clients with quantitative data regarding the content of allergens in their formula. In order to submit the results to clients at least two years before the application of the new regulation and considering the few thousands of raw materials to be analyzed, a specific analytical strategy is required. The first step has been to extend the existing method for the 24 [2] to the 61, which was carried out through the Analytical Working Group at IFRA. Considering the number of analytes to be quantified and raw materials to be scrutinized, there is a clear need to shorten the analytical time of the current official European norm, while maintaining the requirements on the quality of results.

One of our important constraints has been to develop an analytical method which can be used routinely in quality control/assurance (QC/QA) laboratories. To reach this objective, it has been decided to develop a dedicated one-run GC×GC-qMS method for the whole set of 61 analytes.

This presentation also aims at giving some key points related to the evaluation of the performance of this GC×GC method. Nowadays, the most efficient way to assess the performance of a quantitative method, is obtained through the use of accuracy profiles [3]. Based on this approach, preliminary validation results will be given. Additionally we will briefly discuss the industrial challenges in a broader perspective from samples sourcing to final labelling of consumer products.

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Implementation of a global program for the quantification of the extended list of suspected allergens and regulated compounds in fragrance materials by GC×GC-TOF-MS

Thomas Dutriez[1], Laetitia Duffour[1], Diane Pétermann[1], Neil Owen[2], Greg Adamson[3]

[1] *Givaudan, RAPS Analytical Laboratory, Vernier, Switzerland*
[2] *Givaudan, RAPS Analytical, Ashford, United Kingdom*
[3] *Givaudan, Global Regulatory Affairs, Product Safety & Sustainability, East Hanover, United States.*
Corresponding author : thomas.dutriez@givaudan.com

Keywords: Comprehensive two-dimensional chromatography, Regulated substances.

Proposed European Cosmetics legislation is to extend the current list of 24 allergens related to cosmetic product labeling to 57 [1]. As a consequence fragrance organizations must adapt for their analytical approach. Current methodology is based on two parallel GC-qMS separations with SIM acquisitions [2]. This approach usually overcomes coelutions with most matrix solutes. However, this compromises identification confidence with complex naturals, and especially when considering the new list of allergens including diastereoisomers, allergen precursors and additional regulated substances, making about 100 targets.

By adopting comprehensive two-dimensional gas chromatography (GC×GC-TOFMS), an enhanced resolution and higher peak capacity (>4000) have be achieved, after a deep investigation of operating parameters, such as the selection of stationary phases (normal, reverse or hybrid combinations). An ultimate degree of identification confidence is therefore achieved when implementing data processing filters: ^{1}tr and ^{2}tr deviations, MS match and qualifier ions ratios. A rigorous reviewing step finally provides a high degree of confidence for quantitative data from ppm to % range.

Demonstrating this as more than just an R&D device, GC×GC-TOFMS is fully integrated into an industrial environment in a “QC-type” infrastructure in terms of liquid nitrogen facility, IT server, sample delivery from local QCs and automatic uploading of results to a global Regulatory Management system (SAP). Therefore, a global program for monitoring new regulated substances in fragrance ingredients is now in place.

Analytical results will be presented focusing of essential oils, e.g. Ylang-Ylang, Patchouli, Vetiver…, whereas further analytical perspectives will be finally discussed, such as online GC×GC-MS / FID for high % range and low eV fragmentation for a further identification.

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YOUNG SCIENTISTS PRESENTATIONS ABSTRACTS

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Batch and continuous-flow biocatalytic transesterification of chiral fragrant terpenoid alcohols.

Daniel Strub[1], Zofia Hrydziuszko[1], Jolanta Bryjak[1], Katarzyna Szymańska[2], Andrzej Jarzębski[2,3], Stanisław Lochyński[1,4]

[1]*Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology, 27 Wyspiańskiego Shore, 50-370 Wrocław, Poland*
[2]*Department of Chemical Engineering and Process Design, Faculty of Chemistry, Silesian University of Technology, 7 ks. M. Strzody street, 44-100 Gliwice, Poland*
[3]*Institute of Chemical Engineering, Polish Academy of Sciences, 5 Bałtycka street, 44-100 Gliwice, Poland*
[4] *Institute of Cosmetology, Wrocław University College of Physiotherapy, 4 Tadeusza Kościuszki street, 50-038 Wrocław, Poland*
Corresponding authors: jolanta.bryjak@pwr.edu.pl, daniel.strub@pwr.edu.pl

Keywords: terpenoid alcohols, transesterification, lipases, enzyme immobilization

Lipase from *Pseudomonas cepacia* was immobilized on three different granular supports (acrylic, silica, cellulose). Batch processes were carried out in shake flasks (100 rpm) with vinyl acetate as a transesterification reagent, and hexane as a solvent for 24h. For the means of this study we chose (+)-(*SR*)-1-[(1*S*,5*R*)-6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)]ethanol [1,2] as a model fragrant chiral terpenoid alcohol (*S:R* 85:15; fruity with peppermint note). The best results were obtained for the lipase immobilized (covalently and adsorptively) on the acrylic support. Operational stability studies have shown that during first five batch processes the support had been being saturated with (*S*)-acetate (product sorption), and since the 6th process only (*S*)-acetate and (*R*)-alcohol were present in the reaction mixture. Desorption of the product haven't been observed. Operational stability studies were carried out in fifteen - 24 h batch processes. The best results were obtained for the adsorbtively immobilized lipase (operational stability on the level of 100% after 15 processes).

Biocatalytic transesterification was also carried out under continuous-flow conditions using monolithic microreactor. The same lipase from *P. cepacia* was immobilized adsorptively on the monolith's pores. A mixture of the model substrate, and vinyl acetate in hexane was passed through the monolithic microreactor at 37°C. For the flow 0.2 ml/min (residence time ~7 min.) only (*S*)-acetate and (*R*)-alcohol were present in the effluent.

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Chemical investigation of invasive species from Corsica

Stéphane Andreani[1], Julien Paolini[2], Jean Costa[2], Alain Muselli[2]

[1]*Institut de Chimie de Nice (ICN), UMR CNRS 7272,* 28 avenue Valrose, 06108 Nice Cedex 2
[2]*Université de Corse, UMR CNRS 6134 SPE, Laboratoire Chimie des Produits Naturels, BP 52, 20250 Corte, France.*
Corresponding author: Stephane.ANDREANI@unice.fr

Keywords*:* invasive species, essential oils, antioxidant activities, anticorrosive activities

The proliferation of invasive species is a global problem. The introduction of exogenous species is after pollution and habitat destruction major cause of the disappearance of the worldwide plant and animal biodiversity [1]. Corsica is not spare to the phenomenon and every year there are numerous dangerous species which are introduced to the island ecosystem. In 2008, we already reported more than 60 taxa implanted on the island territory [2]. Principal causes are the introduction of plant for ornamental way, but also the trade from the mainland [3]. The control of the proliferation of this species consist essentially to manual or mechanic uproot or using the chemical or biological agents: the vegetal is systematically destroyed without possible recovery way.

As these invasive plants are potential M.A.P.P. with interesting secondary metabolites, we imagine a possible valuation pathway by the production of essential oils. Indeed, in addition to generating an economic opportunity, our project deals with the spread-control of these weed species. In the present work, four Asteraceae: *Xanthium italicum*, *Xanthium spinosum*, *Senecio angulatus* and *Senecio inaequidens,* living wild in Corsica and listed as invasive species were investigated.

Phytochemical studies have performed and the antioxidant and the anticorrosive activities of their essential oils were assessed. GC and GC/MS analysis allowed to identify 107 and 74 components which accounted 98.7 and 93.5% of the *X. italicum* and *X. spinosum* essential oils and 62 and 61 compounds representing 94.4 and 98.8 % were identified in the *S. angulatus* and *S. inaequidens* essential oils.

Results affected an effective antioxidant activity of both the *Xanthium* essential oils and the higher anticorrosive activity to *S. inaequidens* essential oil which exhibited cacalohastine, a naphtofuranic compound.

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Smell and relax!
Assessment of olfactory stimulation protocols in the framework of psychophysiology measurements.

Jérémie Topin[1], Julie Brêteau[1], Claire Alice de March[2], Jérôme Golebiowski[1,3]

[1]*Institut de Chimie de Nice, Université Nice Sophia Antipolis, Nice France*
[2]*Duke University Medical Center, Durham USA*
[3]*Department of Brain and Cognitive Science, DGIST, Daegu Republic of Korea*
Corresponding author: jerome.golebiowski@unice.fr

Keywords: odor stimulation, physiological parameters, emotion

Essential oils are used in traditional medicine from different civilizations for the treatment of a broad range of diseases. Their antifungal or antibacterial activities have been rigorously demonstrated through well-established protocol. Essential oils are also frequently cited as efficacious and well-tolerated alternatives as a pharmacological treatment for patients suffering from anxiety. For example, it has been shown that the use of orange or lavender scent leads to a reduction of anxiety level of dental patients. However, the assessment of physiological measurements upon odor stimulations through a standardized protocol is still to be done. Methodological issues include gender choice, time of stimulation or evaluation of anxiety in animal models.

In the present study, the effect of odor stimulation on the response driven by the autonomous nervous system was monitored. A group of 8 people was stimulated with different essential oils and breathing frequency, heartbeat, and skin conductance were recorded. In addition, a survey was conducted to assess the edibility, relaxing and/or stimulating effect, as well as the familiarity of odorants. In particular, the influence of the stimulation on the results was evaluated by varying the mode of delivery and the length of exposure. The final protocol optimizes the consistency between physiological measurements and surveys. It will allow comparing the effect of different essential oils on human emotion.

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Essential oils as potential insecticidal agents for protection of stored products

Kaan Polatoğlu[1], Hüseyin Servi[2], Ömer Cem Karakoç[3],Yasemin Yücel Yücel[4], Salih Gücel[5], Betül Demirci[6], Kemal Hüsnü Can Başer[6,7]

[1] İstanbul Kemerburgaz University, Faculty of Pharmacy, Department of Analytical Chemistry.
[2] Yıldız Technical University, Graduate School of Natural & Applied Sciences.
[3] Çankırı Karatekin University, Yapraklı Vocational School, Department of Crop & Animal Protection.
[4]İstanbul Kemerburgaz University, Faculty of Pharmacy, Department of Biochemistry.
[5]Near East University, Institute of Environmental Sciences.
[6]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy.
[7]Near East University, Faculty of Pharmacy, Department of Pharmacognosy.
Corresponding author: kaan.polatoglu@kemerburgaz.edu.tr

Keywords: Asteraceae, Apiaceae, Lamiaceae, Rutaceae, Coleopteran insects.

Stored product insects cause the most important damage to the product, among many other factors (humidity, temperature, microorganisms, rodents, avians etc.). The primary (*S. granarius, S. oryzae, R. dominica* etc.) and secondary (*T. confusum, T. castaneum, O. surinamensis* etc.) stored product insect species cause serious damage to the product during storage [1]. Especially if modern storage methods are not employed the product losses could reach from 40% to 100% [2]. Currently, in large scale pest management of stored products against insects, synthetic chemical agents (organophosphorus compounds) that have contact and fumigant activity are used. There is growing interest on natural pest management agents because of environmental and health concerns related the use of these synthetic insecticides. New regulations have been put in action due to these concerns such as European Pesticide Regulation (EC) No. 1107/2009 [3]. Through our screening studies on the insecticidal activity of the Turkish and Cypriot plants we have evaluated essential oils of many species. *Achillea biebersteinii, A. teretifolia, Chrysanthemum coronarium, Inula viscosa*, *Matricaria chamomilla* var. *recutita, Tanacetum macrophyllum, T. abrotanifolium, T. parthenium* of Asteraceae family, *Ferulago cypria, Crithmum maritimum* of Apiaceae family, *Salvia veneris* of Lamiaceae family and *Ruta chalepensis* of Rutaceae family afforded considerable contact and fumigant insecticidal toxicity against Coleopteran insects.

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POSTER PRESENTATIONS ABSTRACTS

(PP)

Essential oil vapors as potential agents against *Haemophilus* species

Kamilla Ács[1], Béla Kocsis[2], Andrea Böszörményi[3], Györgyi Horváth[1]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs, 7624 Pécs, Rókus str. 2., Hungary*
[2]*Department of Medical Microbiology and Immunology, Medical School, University of Pécs, 7624 Pécs, Szigeti str. 12., Hungary*
[3]*Semmelweis University, Faculty of Pharmacy, Institute of Pharmacognosy, H-1085, Budapest, Üllői str. 26., Hungary*
Corresponding author: kamilla.acs@aok.pte.hu

Keywords: vapor phase, respiratory tract, *Haemophilus influenzae, H. parainfluenzae*

Haemophilus species could be one of the major causative pathogens in respiratory tract infections (e.g. sinusitis, tracheobronchitis, pneumonia), especially, in the case of patients with suppressed immune system or other debilitating diseases. The growing antibiotic resistance of these bacteria reveals a therapeutic challenge and necessitates the discovery of new agents which could effectively support the medication. Investigation of essential oil (EO) vapors could be reasonable considering that these substances can directly reach the respiratory system via inhalation. Therefore, the aim of our study was the antibacterial screening of essential oil (cinnamon bark, clove, thyme, scots pine, eucalyptus, citronella, and peppermint) vapors against two *Haemophilus* species by *in vitro* optimized vapor-phase (VP) technique.

EOs were obtained from a Hungarian distributor (Aromax Ltd.). Their quality was verified by GC-MS and GC-FID analyses. The antibacterial activity was tested against *H. influenzae* (DSM 4690) and *H. parainfluenzae* (DSM 8978) by a modified and optimized VP method based on a previously published study by Kloucek et al. [1]. We used four-section Petri dishes (PDs) poured with chocolate agar. The minimum inhibitory concentrations (MICs) were determined after 48 h incubation at 37°C. The MIC values were expressed in µL/L referred to the free space in the PD. All tests were carried out in triplicate.

EO of cinnamon bark produced similar MIC value (15.62 µL/L) against both bacterial strains. We found that EOs of clove, peppermint, citronella, eucalyptus, and thyme showed better inhibition in the case of *H. influenzae* (MIC: 25-125 µL/L) in comparison with *H. parainfluenzae* (MIC: 31.25-200 µL/L). Scots pine was ineffective against *H. parainfluenzae* in our test system.

In our future study, we would also like to focus on the anti-inflammatory effect and mode of actions of these EOs in *in vivo* mouse models.

Acknowledgements: Our work was supported by NKFI 104660 grant (previously OTKA PD 104660).

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Chemical investigation of *Xanthium spinosum* L., essential oil an invasive species of Corsica

Stéphane Andreani[1], Julien Paolini[2], Jean Costa[2], Alain Muselli[2]

[1]*Institut de Chimie de Nice (ICN), UMR CNRS 7272,* 28 avenue Valrose, 06108 Nice Cedex 2
[2]*Université de Corse, UMR CNRS 6134 SPE, Laboratoire Chimie des Produits Naturels, BP 52, 20250 Corte, France.*
Corresponding author: Stephane.ANDREANI@unice.fr

Keywords: *Xanthium spinosum*, Invasive species, Essential oil, Chemical variability, Eudesma-4(15),7-dien-1-*β*-ol

Xanthium spinosum L. (spiny cocklebur) is a highly invasive plant widespread throughout the world [1] as well as in Corsica island, where is considered as a worrying species because it's good adaptation to Mediterranean environment [2-3]. The present work deals to determine the contribution of the volatile secondary metabolites to the plant-ecology of this weed taxa of agriculture crops. For this purpose, the chemical composition of *X. spinosum* essential oils from 25 Corsican locations was investigated using gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Seventy-four components, which accounted for 96.2% of the total amount, were reported for the first time in the essential oil from aerial parts. The main compounds were eudesma-4(15),7-dien-1-*β*-ol (21.3%), germacrene-D (8.8%), cadalene (8.7%) and *β*-elemol (6.3%).

The chemical composition of the essential oils obtained during one vegetative cycle was also studied to gain more knowledge about of the plant-ecology. The production of oxygenated sesquiterpenes seems implicated in the plant-flowering process and indirectly to dispersal of this invasive species.

A study of the chemical variability was performed using PCA and CA analysis. Two clusters were discriminated by the contents of oxygenated compounds which were higher in the oil samples of *Cluster I* (49.9-60.9%) than in the oil samples of *Cluster II* (31.7-44.0%). Results focused on direct correlation between the chemical composition of individual oil samples and the morphological characters of the plant. *Cluster I* included the sample oils from narrow leaves specimens (20 mm long, 10 mm large) while *Cluster II* the essential oils from specimens with larger leaves (55 mm long, 25 mm large).

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The essential oil components of *Cymbopogon citratus* (Lemon grass) from South-West Nigeria: A study of the effect of drying methods

Olayinka Taiwo Asekun, Isaac Saint Njoku, Oluwole Babafemi Familoni

Department of Chemistry, University of Lagos, Nigeria
Corresponding author : oasekun@unilag.edu.ng

Keywords: *Cymbopogon citratus,* drying methods, sesquiterpenoids, citral

The methods of moisture removal from plant materials affect the chemical composition of some essential oils [1]. The chemical content of the essential oils determine their quality [2]. The leaves of lemongrass (*Cymbopogon citratus*) were dried using three different drying methods (sun, air and oven drying). The powdered, dried plant material from each of the drying methods and fresh plant were hydrodistilled to obtain the essential oils using a Clevenger-type apparatus. GC-MS analysis was carried out on the essential oils obtained from the fresh and dried plants to identify the chemical components. Comparison of the yield and composition of the essential oils showed differences in the chemical contents of leaves dried by the different methods; air-dried leaves yielded the most essential oil (0.30 %) compared to other drying methods which gave approximately the same essential oil percentage yields; sun-dried (0.16%), oven-dried (0.16%) and fresh plant (0.16 %). Sesquitepenoids dominate the oils (81.25%-90.30%). Citral, the major component in the fresh plant oil (33.68%), was also identified as the most prominent compound in the air and oven dried oil but occurred in low content, 8.25% and 8.82%), it was observed that only 5 out of the 21 compounds identified were present in all the four oils. Some compounds such as farnesene, farnesol, farnesal and caryophllene oxide absent or in trace amounts in the fresh oil emerged in the dried leaf oils. The drying methods show significant effect on the chemical content of lemongrass oil and this may be ascribed to chemical transformation of the various components.

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Thyme essential oil inhalation decreases endotoxin-induced acute airway inflammation and hyperreactivity in a mouse model

Amir Reza Ashraf[1], Eszter Csikós[1], Kamilla Ács[1], Andrea Böszörményi[2], László Kereskai[3], Béla Kocsis[4], Ágnes Kemény[5], Kata Csekő[5], Zsuzsanna Helyes[5,6], Györgyi Horváth[1]

[1]*Department of Pharmacognosy, University of Pécs, 7624 Pécs, Rókus str. 2., Hungary*
[2]*Department of Pharmacognosy, Semmelweis University, 1085 Budapest, Üllői str. 26., Hungary*
[3]*Department of Pathology, University of Pécs, 7624 Pécs, Szigeti str. 12., Hungary*
[4]*Department of Microbiology, University of Pécs, 7624 Pécs, Szigeti str. 12., Hungary*
[5]*Department of Pharmacology and Pharmacotherapy, University of Pécs, 7624 Pécs, Szigeti str. 12., Hungary*
[6]*Janos Szentagothai Research Centre & MTA-MAP B Chronic Pain Research Group, 7624 Pécs, Ifjúság str. 20., Hungary*
Corresponding author: gyorgyi.horvath@aok.pte.hu

Keywords: *Thymus vulgaris* L., acute airway inflammation, LPS, myeloperoxidase activity

Inflammatory lung diseases affect a large population at every age worldwide. Essential oils (EOs) can easily reach the respiratory tract via inhalation due to their volatility. The anti-inflammatory effect of EOs is weakly studied and there are only few *in vivo* data. Therefore, we examined the chemical composition and effects of thyme EO in the endotoxin-induced acute airway inflammation model of the mouse. Thyme EO was selected on the basis of its potent antibacterial activity in our preliminary experiments.

The chemical composition of the EO was determined by GC-FID and GC-MS. Lung inflammation was evoked by 60 µL intratracheal endotoxin administration (*E. coli* 083 lipopolysaccharide: LPS) in female C57BL/6 mice (n=7-10/group). Thyme EO was inhaled 3 times for 30 min during the 24-h period. Airway responsiveness in unrestrained animals was measured by whole body plethysmography. Lung myeloperoxidase (MPO) activity was determined by spectrophotometry and semiquantitative histopathological scores on hematoxylin-eosin- and Periodic Acid-Schiff-stained sections were performed.

Thymol (46.3%) was the main component in thyme EO. Its inhalation significantly reduced LPS-induced airway hyperresponsiveness compared to paraffin oil-treated controls, but had no effect on other respiratory parameters, such as relaxation time, inspiration and expiration times. Thyme EO significantly reduced neutrophil and macrophage MPO activity and also the extent of perivascular edema and perivascular/peribronchial inflammation. However, it had no effect on goblet cell hyperplasia.

Thyme EO inhalation decreases acute pneumonitis and hyperresponsiveness. Therefore, it can be considered as a potential treatment in such conditions. Further experiments are planned to measure the inflammatory cytokine profile and mechanism of action.

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Chemical Composition And Antibacterial Activity Of *Mentha Pulegium* Essential Oil From West Algeria

ATTOU Amina[1*], DAVENNE Dominique [2], BENMANSOUR Abdelhafid[1], and LAZOUNI Hamadi Abderahmene[1]

[1] Department of Biology, faculty of sciences, Laboratory of natural products: biological activities and synthesis, University Abou Bekr Belkaid, P.B.119 Tlemcen, 13000, Algeria.
[2] University of Rennes, Faculty of Pharmacy, Departement of Pharmacognosy, 35043, France
Laboratory Rosier Davenne, Company Specialized in Essential Oils, Avignon, 84140, France
**Corresponding author: email: heyfraise21@yahoo.fr*

Keywords: *Mentha pulegium*, clinical strains, antibacterial activity

For many years, a variety of different chemical and synthetic compounds has been used as antimicrobial agents, but the widespread of their use causes ecological and medical problems.

That's why; there is a renewal interest in the use of herbs and spices that have been used since ancient times for their perfume, flavor and preservative properties.

Among natural antimicrobials are the essential oils extracted from many plants, and have been reported to possess a wide spectrum of antibacterial activity.

Mentha pulegium (pulegium means repulse fleas) is an aromatic plant belonging to the family Lamiaceae and widespread in northern Europe, the Mediterranean region and Asia, called by locals Fliou, it is a great emmenagogue, digestive, tonic and sudorific, leaves and flowers are commonly used to repel insects, but also against colds and flu, reduce asthma attacks, combat fever and regulate menstruation, used also in some culinary preparations to flavor sauces, desserts and drinks.

Our sample of *Mentha pulegium* from west Algeria has been subjected to hydrodistillation for 3 hours and then analysis using a CPG/SM clarus model.

The antibacterial activity was evaluated by the method of discs on agar, which is a qualitative technique based on measuring the diameters of inhibition in mm, against a sample of 130 hospital strains.

Our essential oil has a good yield of essential oil (1.36% w/w) with the main component is pulegone (45,17%) and menthenone (33,98%).

Mentha pulegium essential oil is generally moderately active against 83.84% of the strains with ++ (2 crosses) index:

- For Enterobacteriaceae: 84.31% are sensitive;
- Staphylococcus aureus: 81.25% are sensitive;
- 66.67% of Streptococci are sensitive.

Chemical composition and biological activities of Essential oil and hydrosol extract of *Calendula arvensis* (vaill.) from Algeria

Rania Belabbes[1], Imane Mami[1], Fatima Zahra Benomari[1], Mohamed El Amine Dib[1], Nassim Djabou[1], Jean Costa[2], Alain Muselli[2]

[1]Université de Tlemcen, Laboratoire COSNA, BP 119, Tlemcen 13000, Algeria.
[2]Université de Corse, UMR CNRS 6134, Laboratoire CPN, BP 52, 20250 Corte, France.
**Corresponding author: muselli@univ-corse.fr*

Keywords: *Calendula arvensis*, *Penicillium expansum*, hydrosol extract, biological activities.

The volatile secondary metabolites of Algerian *Calendula arvensis* (vaill.) have been investigated using GC/RI and GC-MS. For this, we investigated the essential oil obtained by hydroditillation and the extract obtained by LLE from the hydrosol. The antioxidant activity was established by DPPH, FRAP and the bleaching test β-carotene methods. The antifungal activity was established against *Penicillium expansum* and *Aspergillus niger*, two phytopathogenic fungi causing rot on apple fruits.

C. arvensis essential oil exhibited zingiberenol 1 (28.5 %), (E,Z)-farnesol (18.5 %), zingiberenol 2 (17.1 %) and τ-cadinol (8.5 %) as main components and zingiberenol 1 (33.1%), (E,Z)-farnesol (23.7 %), zingiberenol 2 (21.2 %) and eremoligenol (11.1 %) were identified as main compounds in the hydrosol extract.

The antioxidant tests showed that the LLE extract obtained from *C. arvensis* hydrosol exhibited highest activity than the essential oil. In addition, the essential oil showed strong *in-vitro* antifungal activity against *P. expansum* and *A. niger* pathogens and the tests realized on infested fruits showed that very low concentrations of essential oil and hydrosol in air contribute to limit the apple infection.

This activity can be correlated with the oxygenated components of both extract tested. Essential oil and hydrosol of *C. arvensis* could be used for management of pathogens as alternative source of ecological fungicides or an ideal alternative to control *P. expansum* infection during apples storage.

Chemical composition, Insecticidal and Larvicidal activities of Essential oil of *Mentha piperita* from Western Algeria

Fatima Zahra Benomari[1], Hanane Senouci[2], Nassim Djabou[1], Kenza Medjdoub[2], Mohamed El Amine Dib[1], Nassira Gaouar Benyellels[2], Alain Muselli[4], Jean Costa[4]

[1]*Université de Tlemcen, Département de Chimie, BP 119, Tlemcen 13000, Algérie.*
[2]*Université de Tlemcen, Laboratoire LECGEN, BP 119, Tlemcen 13000, Algérie*
[3]*Université de Corse, UMR CNRS 6134, Laboratoire CPN, BP 52, 20250 Corte, France.*
**Corresponding author: chimiste-tema@hotmail.fr*

Keywords: *Mentha piperita*, *Bactrocera oleae*, insecticidal and larvicidal activities, *olea europaea* fruits.

Chemical composition of essential oils of *Mentha piperita* of 17 different localities from Western Algeria has been investigated; using GC/RI and GC-MS. *In-vitro* antifungal and i*n-vivo* insecticidal and larvicidal activities has been established, from collective essential oil, against *Bactrocera oleae* in fly and larvae shape. Essential oils from different localities were qualitatively similar but differ quantitatively by percentage of major components. The main components of essential oils from different localities were: linalool (34.3-45.3%), linalyl acetate (8.9-32.6%), α-terpineol (6.4-10.4%) and geraniol (2.2-7.5%). On the other hand, collective essential oil of *M. piperita* demonstrates moderate *in-vitro* antifungal activity against *Alternaria alternata, Cladosporium herbarum* and *Aspergillus niger*, fungi caused by *Bactrocera oleae* after injection, with the percentages of inhibition of 100%, 23 % and 14 % at 10 µl/ml, respectively. In the other hand, collective oil showed spectacular insecticidal and moderate larvicidal activities against *Bactrocera oleae* (olive fly). Very low quantity (0.2 µl) of collective oil caused 100% of mortality of *Bactrocera oleae* fly after only 10 seconds and 70% of mortality of *Bactrocera oleae* larvae after 4 hours. LD_{50} was established and value was 21.124 g/kg from flies and 84.500 g/kg from larvae. This result was very promising compared to chemical insecticides uses against *Bactrocera oleae* (Spinosad® LD_{50} 3.738 g/kg and Thiaclopride® LD_{50} 2.500 g/kg) [1].

Those results demonstrated that *Mentha piperita* essential oils from Western Algeria can be used as bio-pesticide against *Bactrocera oleae* flies, to substituted chemical pesticide treatment.

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CHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITIES OF *Plectranthus amboinicus* ESSENTIAL OIL IN TWO CROP SYSTEMS

Suzan Kelly V. Bertolucci[1], Simony C. Mendonça[2], Smail Aazza[3], Krisnanda Kelly C. Souza[4], José Eduardo Brasil P. Pinto[5]

[1,2,4,5] Federal University of Lavras (UFLA), Agriculture Department, Mailbox: 3037, 37.200-000, Lavras - MG, Brazil.
[3]University of Algarve, IBB- Center for Plant Biotechnology, Faculty of Science and Technology, Gambelas fields, 8005-139 Faro - Portugal.
Corresponding author : suzan@dag.ufla.br

Keywords: *Plectranthus amboinicus*, carvacrol, free radical.

Essential oils from *Plectranthus amboinicus* (Lour) Spreng. (Lamiaceae) leaves have been reported to possess antioxidant, antibacterial, anti-dyspeptic and fungitoxic activities [1]. However, due to the geographical region, variety and agricultural management, the activity and chemical composition of essential oils may be vary. This study aimed to determine the chemical composition and antioxidant activities of essential oil from *P. amboinicus* cultivated in two different crop systems at southeastern Brazil. Fresh leaves from field and protected crops, harvesting in the morning and in the afternoon, were subjected to hydrodistillation in Clevenger apparatus for 120 minutes. The contents of oils were 0.049% in field crop and 0.102% in the protected crop. The chromatographic analysis (GC-FID and GC-MS) identified sixteen chemical constituents, being carvacrol the major compound with 68-72% in the field crop and 74-75% in the protected crop of total chemical composition. Positive correlation was observed between carvacrol content and the total antioxidant capacity (1,549 to 1,905 mg of ascorbic acid equivalents per g of fresh leaf). In the same way, the correlations between IC_{50} (mg/mL) and carvacrol content were observed in free radicals scavenging assays (DPPH – 0.36 to 0.51 and ABTS – 0.058 to 0.078). The best results were obtained in protected crop with harvesting time during in the morning. Anyway, *P. amboinicus* essential oil, rich in carvacrol, showed high antioxidant capacity by different mechanism of free radicals scavenging.

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A thermodynamically-based step by step calculation method to assess aquatic toxicity of mixtures

Pascal BICHEREL[1], Paul C. THOMAS[1]

[1] *KREATiS SAS, 23 rue du Creuzat 38080 L'ISLE D'ABEAU, FRANCE*
Corresponding author: pascal.bicherel@kreatis.eu

Keywords: mixture; ecotoxicity; essential oils; activity; WAF

More recently, research has been conducted in order to determine if an additivity approach can be used to predict aquatic toxicity of mixtures of chemicals or whether independent action predominates. Nowadays the favoured hypothesis is the Concentration Addition (CA) where toxic units of mixture constituents with same toxic mode of action can be summed [1],[2]. In this work, the chemical activity concept has been used to normalise the relative part of toxicity of each constituent [3],[4]. Schmidt *et al.* (2013) [5] and Smith *et al.* (2013) [6] have both shown that aquatic and terrestrial ecotoxicity can be determined by summing constituent activities of sub-lethal levels of constituents in a mixture comprised of solid PAHs. However unlike solids compounds [7], liquids partition into each other thus reducing the overall toxicity of the mixture. Therefore the initial rule of CA modified according to the chemical activity has also to be completed by an additional step where the non-bioavailability phase is remove from the calculation. Based on thermodynamic liquid-liquid equilibrium, aquatic toxicity can finally be accurately calculated for mixtures of liquid hydrophobic compounds. This method can provide results within a factor of 2 of experimental results while the CLP additivity method results are at least a factor of 10 lower.

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Antimicrobial activity of essential oils from some Verbenaceae and Asteraceae from Brazilian Cerrado

Humberto R. Bizzo[1], Marcelly C. S. Santos[1], Roberto F. Vieira[2], Fátima R. V. Goulart [3], Celuta S. Alviano, Daniela S. Alviano[3]

[1]Embrapa Food Technology - Avenida das Américas, 29501 Rio de Janeiro 23020-470 RJ Brazil.
[2]Embrapa Genetic Resources - Brasilia DF Brazil.
[3]Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-590 RJ Brazil.
Corresponding author: humberto.bizzo@embrapa.br

Keywords: *Lippia lacunosa, Lippia origanoides, Baccharis reticularia, Hoehnephytum trixoides.*

Cerrado (savannah-like vegetation) is the second largest Brazilian biome, covering almost 2 million km^2 (the same size as Western Europe). It concentrates a huge amount of endemic species, many of which have great potential for producing essential oils. Only a small part of these species has been studied so far. Six samples of essential oils, two from *Lippia lacunosa*, and one from *L. origanoides* (Verbenaceae), two from *Baccharis reticularia* (Asteraceae) and one from *Hoehnephytum trixoides* (Asteraceae) were obtained by hydrodistillation of the aerial parts of the plants. Voucher specimens were deposited at Embrapa Genetic Resources herbarium. The oils were analyzed by gas chromatography and mass spectrometry for components identification (unpublished data). They were then tested against human pathogens *Escherichia coli* ATCC 11229, methicillin-resistant *Staphylococcus aureus* (MRSA) BMB 9393, *Candida albicans* ATCC 10231 and *Cryptococcus neoformans* T 444 according to CLSI/NCCLS standard procedure [1]. All the oils tested were active against the *C. albicans*, *C. neoformans* and MRSA, with minimum inhibitory concentration (MIC) ranging from 78 to 5000 µg/mL. Best MIC was observed for *L. origanoides* (78 µg/mL) for MRSA and *C. albicans*. This oil is rich in carvacrol (50%) and thymol (21.4%). Very poor or no activity was verified against *E. coli*, except for *L. origanoides* oil (MIC 156 µg/mL).

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The most frequently encountered volatile contaminants of essential oils introduced during the isolation procedure: fast and easy profiling

Polina D. Blagojević, Niko S. Radulović

Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, Niš, Serbia
Corresponding author: blagojevicpolina@gmail.com

Keywords: volatile contaminants, average mass scan of the total ion chromatogram.

Well-known volatile contaminants (VC) of synthetic/artificial origin (e.g. GC and GC-MS analyzable plasticizers, chemicals used for solvent stabilization, butylated hydroxytoluene, etc.) are sometimes identified as (major) constituents of essential oils (EO) or plant extracts (PE), and considered to be native plant metabolites. In a few cases, these compounds were even isolated in pure state from the (contaminated) "biologically active" EO/PE and designated as potential carriers of such activity, or subjected to various biological/pharmacological studies without the recognition of their true origin [1,2]. Organic solvents, which play a critical role in natural product chemistry, and packaging materials (used in the transport of plant material or isolated EOs) are among of the most probable sources of VC. Thus, to explore the possibility of an early recognition and to create a list of some of the most common VCs of EO/PE, we have performed detailed GC and GC-MS analyses (a standard methodology commonly applied for EO/PE investigation) of the evaporation residues of six commercially available diethyl ether samples (a routinely used solvent for the isolation of EO) and of a plastic bag hydrodistillate. Almost 200 different compounds, subdivided into two groups, were identified in the analyzed samples: (i) compounds that could only be of a synthetic/artificial origin, such as butylated hydroxytoluene and *o*-phthalic acid esters, i.e. requiring exclusion from the list of identified plant constituents (type 1); (ii) compounds possibly of synthetic and/or natural plant origin, i.e. compounds derived from the fatty acid metabolism or products of anaerobic intracellular/microbial fermentation (type 2). To address the serious problem of type 2-contaminats, we have developed a new methodology, based on the AMS (average mass scan of the total ion chromatogram) profiles of the solvent/packaging samples, that enables fast, reliable and easy recognition of EO/PE VCs introduced during the isolation procedure and provides information on the likelihood of rejection/acceptance of contaminants as possible plant constituents. AMS profiling, in combination with the herein generated database of the most common VCs, allows even inexperienced researchers to validate the results of EO/PE analysis.

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Chemical compositions of the essential oils of both *Glebionis* species from Corsica

Marion Brunel[1,2], Stéphane Andreani[1], Xavier Vitrac[2],
Jean Costa[1], Alain Muselli[1]

[1] *Université de Corse, UMR-CNRS 6134 SPE, Laboratoire Chimie des Produits Naturels, Campus Grimaldi, BP 52, 20250 Corte, France.*
[2] *Laboratoire PHENOBIO SAS, Centre Montesquieu, 1 Allée Jean Rostand, 33650 Martillac, France.*
Corresponding author: muselli@univ-corse.fr

Keywords: *Glebionis coronaria*, *Glebionis segetum*, Irregular terpenes, Acetylenic compounds.

The genus *Glebionis* belongs to the Asteraceae family and consists of two Mediterranean annual weeds: *G. coronaria* (L.) and *G. segetum* (L.). They are previously included to the genus *chrysanthemum* and now are classified among the *Glebionis* on account of achenes forms [1]. In Corsica Island, both species are widespread and are quite distinct according to the forms of the leaves [2]. To our knowledge, the chemical composition of *G. segetum* essential oil was never studied, while a few studies reported these of *G. coronaria*, so we investigated the chemical compositions of 20 sample oils of *G. coronaria* and 20 sample oils of *G. segetum* from Corsica using GC/RI and GC-MS.

G. coronaria essential oils were characterized by myrcene (16-31%), (*Z*)-*β*-ocimene (7-31%), lyratyl acetate (2-12%), camphor (2-15%) and both cis- and trans-chrysanthenyl acetates (0,1-8% / 0,1-0,3-%, respectively). In addition, we reported the occurrence of the lyratyl 2-methylbutyrate (0.2-4.6%) and the trans-chrysanthemyl acetate (0,2-7%), which were never reported in the *G. coronaria* essential oils. *G. segetum* exhibited essential oils dominated by acetylene compounds: capillene and 5-phenyl-1,3-pentadiyne were identified as main constituents (41-60 % and 7-22 %, respectively).

Our results suggest that volatile secondary metabolites could be used as taxonomical markers in the systematics of the Asteraceae family. Indeed, the *G. coronaria* essential oils approach to these of *Chrysanthemum* genus [3-4] while *G. segetum* essential oils contain compounds reported in *Artemisia* and *Santolina* genus [5].

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Designing Odorants using a Computational Nose

Bushdid C. [1], Topin J. [1], Briand L. [2] & Golebiowski J.* [1,3]

[1]*Institut de Chimie de Nice, Université Nice Sophia-Antipolis, UMR CNRS 7272, 06108 Nice cedex 2, France,*

[2]*Centre des Sciences du Goût et de l'Alimentation, INRA UMR1324, CNRS UMR6265, Université de Bourgogne,2100 Dijon, France,*

[3]*Department of Brain & Cognitive Sciences, DGIST, 333, Techno JungAng Deaero, HyenongPoong Myen, Daegu, Republic of Korea*

Corresponding author: jerome.golebiowski@unice.fr

Keywords: odorant receptor, computational tool, rational design

Understanding how chemicals code for a certain type of odor is a fundamental step for the development of a rational method to create new odorants. It is all the more urgent since restrictive regulations are regularly updated regarding toxicologic properties of these fragrant chemicals: the 48th amendment to the IFRA regulation dates from summer 2015 and the latest removal from a flavoring substance was in October 2015 (CE no. 1334/2008 *source:EFFA*). Therefore, the demand for new molecules that have similar smells but dissimilar structures and physicochemical properties is increasing.

By the means of our research project, we establish the basis for developing a *virtual nose*. This computer program would be capable to rationally design new odorant molecules or odorous analogs of existing molecules. The actual consensus states that the code to olfactory perception is a combinatorial one. According to the combinatorial code, each one of our 396 odorant receptors (ORs) is differentially activated when interaction with an odorant molecule occurs. [1]

ORs are the cornerstone of olfactory perception: they transform the molecular message into a series of action potential spikes. Understanding how each of them recognizes a certain set of molecules would allow to build a bioinspired computational nose. [2]

As a proof of principle, OR5P3, a human odorant receptor, was investigated through a joint approach combining *in vitro* data and computational modeling. We showed how the responsiveness of this receptor is encoded within its structure. More specifically 5 residues within the cavity control its specificity.

By computationally screening the cases in which ORs get activated by certain ligands, we are one step closer to establishing the activational pattern associated with any odorant. The application of this knowledge will result in a computational biomimetic nose that predicts odor from stucture.

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Preparation and characterization of inclusion complex of β-cyclodextrin and two phenolic compounds aiming agriculture applications

Campos E.V.R[1,2], Oliveira, J.L[1]., Proença, P.L.F[1]., Fraceto, L.F[1,2].

[1]*Departament of Environmental Engineering, São Paulo State University, Sorocaba, SP, Brazil*
[2]*Departament of Biochemistry, Institute of Biology, Campinas State University,Campinas, SP, Brazil*
Corresponding author: estefaniavan@gmail.com

Keywords: *Origanum vulgare, Aniba rosaeodora, inclusion complex, β-cyclodextrin, carvacrol and linalool*

Carvacrol and linalool are phenolic compounds extracted from *Origanum vulgare* and *Aniba rosaeodora* respectively. The first compound is known for its insecticide activity while linalool only promotes repellency to insects [1]. These compounds present problems such as high volatility and photosensitivity, which leads to decreased of insecticide/repellent activity as function of time and consequently failure in pest control. The encapsulation of volatile compounds has been showed a potential tool to overcome these problems [2]. β-cyclodextrin are cyclic oligassarides composed of seven units of glucopyranose linked by α-(1,4). Due to the conical structure of cyclodextrin as well the orientation of hydroxyl to outside, cyclodextrin are soluble in aqueous medium and can carrying hydrophobic compounds in their cavity. The main goal of this work was to prepare and characterize inclusion complex of between β-CD and carvacrol and β-CD and linalool. Both inclusion complex were prepared by kneading methodology. Differential scanning calorimetry, X-Ray diffraction and infrared spectroscopy were employed to verify the inclusion complex formation. The apparent stability constant were determined in different temperatures (20-40 °C) by solubility isotherm studies. Thermodynamic relationship were calculated by van't Hoff plot [3]. The X-ray analysis showed a reduction in the crystallinity of the inclusion complex when compared with β-CD. Thermal analysis of the inclusion complex showed that the characteristic peaks of carvacrol and linalool were not observed indicating the inclusion complex formation. These results indicate the formation of inclusion complexes between carvacrol or linalool and β-CD. Isotherm experiments showed linear relationship for all tested temperatures indicating that the solubility of the active compounds increase as function of the increasing in cyclodextrin concentration. Also, these results showed that the complexation between cyclodextrin and linalool (or carvacrol) is 1:1. The values of enthalpy and entropy determined for the carvacrol complexation with β-CD were 7.14×10^{-3} kJ/mol and 1.83 kJ/mol/K respectively and for linalool complexation it was -0.105 kJ/mol and -32.10 kJ/mol/K respectively. The value of Gibbs free energy calculated from these values was -0,31 and -4,71 kJ/mol for carvacrol and linalool respectively, indicating favorable complexation. These initial results will help to better understand the interactions between cyclodextrin with linalool and carvacrol in order to use these systems in crop protection.

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Developing Standard Reference Materials for Essential Oils: Investigation of Various Species of Cinnamon Bark using GC-MS and GC-IRMS

Richard E. Carlson, Brett J. Murphy, Ariel Poulson, Tyler M. Wilson, Brett L. Smith, R. Michael Buch, and D. Gary Young

Department of Research and Development, Young Living Essential Oils, 3125 W. Executive Pkwy, Lehi, UT, USA

Richard Carlson: richcarlson@youngliving.com

Keywords: *Cinnamomum verum, syn. Cinnamomum zeylanicum, Cinnamomum burmanii, Cinnamomum cassia, and Cinnamomum loureiroi,* GC-IRMS, GC-MS

Understanding, obtaining, and developing standard reference materials for natural products in general, and especially, essential oils is a challenging process. As a result, Young Living has implemented a process to generate and catalog essential oil reference materials as part of our Research and Development and Quality Assurance processes. This paper will describe the investigation of various cinnamon bark species based on Gas Chromatography/Mass Spectrometry and Gas Chromatography Pyrolysis Isotope Ratio Mass Spectrometry results. True cinnamon is defined by the species, *Cinnamomum verum syn. Cinnamomum zeylanicum*. On the other hand, several different species are referred to as Cassia (*e.g. Cinnamomum burmanii, Cinnamomum cassia,* and *Cinnamomum loureiroi).* True cinnamon and cassia are often used interchangeably in the food and flavor industry; however true cinnamon seems to be preferred in the essential oil market. In this preliminary study, our goal was to examine essential oils from various cinnamon and cassia species as well as their geographical variation. True cinnamon and cassia bark was sourced directly from the following countries: Sri Lanka, Zanzibar, Madagascar, Indonesia, China, and Vietnam. Both synthetic and natural trans-cinnamaldehyde standards were also purchased. The essential oil was extracted from the bark using a stove-top steam distiller and then analyzed using GC-MS and GC-IRMS. Using GC-MS analysis, trans-cinnamaldehyde was determined to be the main essential oil constituent ranging from 46%-89% (n=7). The concentration of eugenol, (not detected to 4.2%) and linalool (not detected to 2.7%) also varied within the samples tested. GC-IRMS analysis was performed on cinnamaldehyde in the samples against natural and synthetic standards. Synthetic and natural cinnamaldehyde standard results ranged from +370 to + 292 $\delta^2H_{V\text{-}SMOW}$ for the synthetic, and -170 to -180 $\delta^2H_{V\text{-}SMOW}$ for the natural standard. The isotope results obtained from various species of cinnamon samples (t-cinnamaldehyde) ranged from -134 to -200 $\delta^2H_{V\text{-}SMOW}$.

Volatile fingerprints from plant food supplements and veterinary botanical preparations

Remigius Chizzola[1], Ana Bozic[1], Chlodwig Franz[1]

[1]*Institute of Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine Vienna, Veterinaerplatz 1, 1210 Vienna, Austria.*
Corresponding author : Remigius.Chizzola@vetmeduni.ac.at

Keywords: plant food supplements, veterinary botanical preparations, *Matricaria recutita,* microdistillation, SPME.

Various botanical preparations containing parts or extracts from essential oil bearing plants are available as veterinary pharmaceutical preparations, feed additive or plant food supplements. Most of the products are complex mixtures containing more than one herbal component and pharmaceutical excipients. For the present study products containing chamomile (*Matricaria recutita*) according to their instruction leaflet were chosen. These products were present in different forms (liquid, lotion, capsule, pellet, powder, solid).

The volatiles were isolated by microdistillation and/or SPME and analyzed by GC and GC/MS. The SPME method proved to be more sensitive than the microdistillation. Each product had his individual composition. Some products were very low in volatiles or showed no volatiles at all in their microdistillates. Compounds typical for chamomile as α-bisabolol, the bisobololoxides and *E*-β-farnesene could be detected in most products. Components from other herbals, such as thyme, fennel, peppermint, sage or cinnamon were detectable when they were part of the product concerned. Some preparations had the methyl and/or ethyl esters of medium chain fatty acids as major volatile compounds.

To give some examples:

Two plant food supplements intended to support the gastro-intestinal tract contained presumably a deodorized chamomile extract so that no or only traces of the bisaboloids could be detected.

A lotion for animal skin care made with thymol, chamomile flowers, marigold, sage and hamamelis had thymol as main compound and camphor, thujone and 1,8-cineol and low amounts of bisabololoxide A in the volatile fraction.

Pellets for horses containing 27% peppermint leaves had menthone, menthol and pulegone as main volatiles by SPME.

A liquid peat bog preparation with herbs for calves displayed geraniol, linalool and the bisabolol oxides in the headspace when assessed by SPME.

In conclusion, in some cases, good results could be achieved with the simple sample preparation described here while further analyses are necessary for a final evaluation of other samples.

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Volatile composition of the essential oil of Timur (*Zanthoxylum alatum Hemsl*) seeds from Nepal.

Esméralda Cicchetti[1], Leslie Duroure[1], Marion Perez[1], Laura Sizaire[1], Clémence Vasseur[1]

[1]Cosmo International Ingredients, 855 avenue du Dr Maurice Donat, 06250 Mougins, France.
Corresponding author: esmeralda.gonzales@cosmo-ingredients.com

Keywords: *Zanthoxylum alatum Hemsl., Zanthoxylum armatum DC.,* volatile composition, DOE, factorial analysis, gas chromatography, liquid chromatography, derivatization, HRMS, isotopic dilution

Timur (*Zanthoxylum alatum Hemsl*), is a spice belonging to the Rutaceae family sometimes misnamed pepper in the culinary world. Both seeds and corresponding essential oils exhibit a combination of grapefruit, zesty, coriander leaf odour. The volatile composition of the seed oil from India, China, Pakistan or Vietnam has already been reported mainly in the aim of finding a natural source of linalool, one of the main constituents, or in the context of antifungal or antimicrobial activity studies. Most of the times, only major constituents were identified in the oil and they were not quantified properly, with the reporting of FID or MS percentages.

This poster will report the volatile composition of Timur seed essential oil from Nepal determined by GC/MS and GC/FID with the use of predicted Relative Response Factors (RRF [1]). Essential oils from different crops and regions in Nepal were studied and the results were submitted to factorial analysis. The head space of the corresponding seeds was analyzed after static-HS extraction and SPME with optimized conditions as determined by DOE. Finally attempts to identify thiols in trace amount in the oils, using complementary techniques of derivatization, isotopic dilution and HRMS liquid chromatography, will be presented in this poster.

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Two-dimensional gas chromatography analysis for an easier identification of *d*-limonene and linalool hydroperoxides in complex fragrance matrices after triphenylphosphine reduction

Elise CORBI[1], Naïlée EBIBI[1], Christophe PERES[1], Nathalie DAVID[1]

[1] *Chanel, Laboratoire de Création et de Développement des Parfums,*
Laboratoire Recherche et Analyses, 20 rue Victor Noir, 92521 Neuilly-sur-Seine Cedex.
Corresponding author: elise.corbi@chanel-corp.com

Keywords: linalool hydroperoxides, limonene hydroperoxides, triphenylphosphine reduction.

Oxidation of natural terpenes to air exposure (autoxidation) has largely been investigated :
the result is the generation of primary volatile species which move towards secondary compounds such as alcohols, ketones, aldehydes and polymers. Despite low shelf life, those compounds have been identified at very low levels in essential oils and fragrance finished products [1]. Due to the important use of terpenes in fragrance creation a robust and sensitive quantitative analytical method for complex matrices is needed.

Different analytical approaches have been published to quantify such compounds, mainly based on LC-MS or GC/MS with derivatization steps. A GC/MS method based on the quantification of hydroperoxides by-products after triphenylphosphine (PPh_3) reduction of the more stable linalool and *d*-limonene hydroperoxides (linalool-6-OOH, linalool-7-OOH *d*-limonene-2-OOH, and *d*-limonene-1-OOH) [2] has been tested in finished fragrance goods. In some cases coelutions make data treatment difficult, leading to overestimated concentrations. Heart cutting and TOF detection can tackle this analytical issue and improve existing methods. The principle of the presented work is based on the separation of the compounds on a first dimension (apolar column phase) connected to a Deans switch to guide samples to the second dimension (polar column) with specific cut programs. This methodology has previously been successfully applied for the analysis of allergens in fragrances [3]. Illustrative examples will demonstrate how one single 2D-GC run coupled with high resolution mass detector can simplify complex matrices data treatment.

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Effect of cinnamon and citronella essential oils in endotoxin-evoked acute airway inflammation mouse model

Eszter Csikós[1], Amir Reza Ashraf[1], Kamilla Ács[1], Andrea Böszörményi[2], László Kereskai[3], Béla Kocsis[4], Ágnes Kemény[5], Kata Csekő[5], Zsuzsanna Helyes[5,6], Györgyi Horváth[1]

[1]*Department of Pharmacognosy, University of Pécs, 7624 Pécs, Rókus str. 2., Hungary*
[2]*Department of Pharmacognosy, Semmelweis University, 1085 Budapest, Üllői str. 26., Hungary*
[3]*Department of Pathology, University of Pécs, 7624 Pécs, Szigeti str. 12., Hungary*
[4]*Department of Microbiology, University of Pécs, 7624 Pécs, Szigeti str. 12., Hungary*
[5]*Department of Pharmacology and Pharmacotherapy, University of Pécs, 7624 Pécs, Szigeti str. 12., Hungary,*
[6]*Janos Szentagothai Research Centre & MTA-MAP B Chronic Pain Research Group, 7624 Pécs, Ifjúság str. 20., Hungary*
Corresponding author: gyorgyi.horvath@aok.pte.hu

Keywords: cinnamon, citronella, LPS, acute airway inflammation, whole body plethysmography, myeloperoxidase activity

Respiratory tract diseases (RTDs) associated with inflammation affect a large number of patients worldwide. Essential oils (EOs) are traditionally used for treating RTDs via inhalation due to their volatile property. The *in vivo* anti-inflammatory effect and mode of action of EOs are weakly studied. Therefore, the aim of our experiments was the investigation of the effect of cinnamon bark and citronella EOs in the endotoxin-induced acute airway inflammation model of the mouse. These EOs showed potent antibacterial activity in our preliminary tests.

GC-FID and GC-MS were used to determine the chemical composition of cinnamon bark and citronella EOs. In female C57BL/6 mice (n= 7-10/group), lung inflammation was evoked by 60 µL intratracheal endotoxin administration (*E. coli* 083 lipopolysaccharide: LPS). Mice inhaled cinnamon bark or citronella EO 3 times for 30 min during the 24-h period. Airway responsiveness in unrestrained animals was measured by whole body plethysmography. Myeloperoxidase (MPO) activity from homogenized lung tissue samples was determined by spectrophotometry.

Trans-cinnamaldehyde (74.0%) and citronellal (36.2%) were the main components in the EO of cinnamon bark and citronella, respectively. Inhalation of cinnamon bark oil significantly reduced LPS-induced airway hyperresponsiveness, inspiration and expiration times compared to paraffin oil-treated controls, but it had no effect on neutrophil and macrophage MPO activity. Contrary, citronella EO significantly impaired the most of the respiratory parameters, and the MPO activity was also increased.

Inhalation of cinnamon bark EO decreases acute pneumonitis and hyperresponsiveness. Therefore, it can be considered as a potential treatment in such conditions. Further experiments are planned to measure the inflammatory cytokine profile.

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Essential Oil and Volatile Glucosinolate Breakdown Products of *Descurainia sophia* (L.) Webb ex Prantl (Brassicaceae)

Milan S. Dekić[1], Niko S. Radulović[2], Jelena B. Danilović-Luković[3] and Dalibor Z. Stojanović[3]

[1]*Department of Chemical and Technological Sciences, State University of Novi Pazar, Vuka Karadžića bb, 36300 Novi Pazar, Serbia*
[2]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia*
[3]*Department for Biomedical Sciences, State University of Novi Pazar, Vuka Karadžića bb, 36300 Novi Pazar, Serbia*
Corresponding author: mdekic@np.ac.rs

Keywords: *Descurainia sophia*, glucosinolates, isothiocyanates, arctigenin

Descurainia sophia (L.) Webb ex Prantl 1891 (syn. *Sisymbrium sophia* L. 1753; *Discurea sophia* (L.) Schur 1866), commonly called flixweed or *strižica* in Serbian, is an annual weed plant commonly found in whole Europe, in Asia to India and the Himalayas, in Siberia, China, Japan, North Africa, in North and South America and New Zealand. Flixweed has been widely used in folk medicine for reducing jaundice and in the case of stomach disorders.

The scarce literature data on the volatile chemistry of this plant species has prompted us to investigate the volatile profile of this taxon originating from Serbia. Furthermore, as this species belongs to the Cruciferae, a plant family characterized by the presence of glucosinolates, another goal was set to detect and identify the volatile autolysis products of these secondary metabolites.

Volatile constituents obtained by autolysis of the aerial and underground parts of *D. sophia* and the essential oil obtained by hydrodistillation of whole plant samples were analyzed in detail by GC and GC-MS. In total, 71 constituents were identified, accounting for more than 90% of the detected total peak areas. Both aerial and underground autolysates contained considerable amounts of lignan arctigenin and cuticular wax compounds. The essential oil was dominated by the glucosinolate breakdown product 4-pentenenitrile. Glucosinolate degradation products identified in the essential oil and autolysates, 3-butenyl isothiocyanate, 4-pentenenitrile and allyl isothiocyanate, suggested the presence of gluconapin and sinigrin in this species as the most likely “mustard oil” precursors. The relatively high contribution of arctigenin in *D. sophia* aerial parts autolysate, a lignan with an important biological activity such as antioxidative, anti-HIV-1 and anti-inflammatory, as well as selective cytotoxicity, imply that this species could serve as good source of this compound.

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Volatiles of *Xeranthemum cylindraceum* and *X. annum* (Asteraceae): Composition and Antimicrobial activity

Milan S. Dekić[1], Niko S. Radulović[2], Vladimir N. Ranđelović[3],
Zorica Z. Stojanović-Radić[3] and Bojana P. Veljković[4]

[1]*Department of Chemical and Technological Sciences, State University of Novi Pazar, Vuka Karadžića bb, Novi Pazar, Serbia*
[2]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, Niš, Serbia*
[3]*Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, Niš, Serbia*
[4]*Department of Biomedical Sciences, State University of Novi Pazar, Vuka Karadžića bb, Novi Pazar, Serbia*
Corresponding author: mdekic@np.ac.rs

Keywords: *Xeranthemum cylindraceum*, *Xeranthemum annum*, sesquiterpene lactones, antimicrobial activity, multivariate statistics

Detailed GC and GC-MS analyses of the essential oils and Et_2O extracts of two *Xeranthemum* species – *X. cylindraceum* and *X. annum* – resulted in the identification of 254 components, in total. Terpenoids constituted the major part of both *X. cylindraceum* and *X. annum* essential oils and extracts. Among the sesquiterpenoids, the extracts of both investigated taxa contained the guaianolide xerantholide, its 11,13-dihydro derivatives, and two additional sesquiterpene lactones (STLs): an eudesmanolide, 11,13-dihydroisoalantolactone, and a pseudoguaianolide, confertin. The isolated extracts of *X. cylindraceum* and *X. annum* were tested in a broth microdilution assay against a panel of microorganisms. The tested extracts demonstrated significant antimicrobial inhibitory activity, ranging from 30 to 260 µg/ml, being most active against *Bacillus cereus* and *Staphylococcus aureus*, an important human pathogen, with MIC close in value to those of chloramphenicol. A comprehensive literature survey focusing on the reported STLs for the species of the genera up to the level of the tribe Cardueae was conducted (55 taxa in total, divided into five taxonomical groups: Arctium, Saussurea, Carduus, Onopordum, and Xeranthemum). As a result of this survey, dozens of compounds were used to test the statistical significance of the variation in the STL profiles among these species at intergeneric level using multivariate statistical analyses. The Xeranthemum group was found to be the most distant of all other related ones and this indicates a significant degree of congruence of the chemical and molecular systematics markers.

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Essential oil composition of hop accessions cultivated at Southern Brazil.

Cicero Deschamps[1], Beatriz Helena L.N. Sales Maia[2], Aurea P. Ferriani[1], Roger Cipriano[1], Erik Gomes[1], Felipe Francisco[1], Rubens Mattos[3], Andre Luiz Tagliaferro[3]

[1] *Plant Science Department– Federal University of Parana – UFPR, P.O. Box 19032, CEP80035-050, Curitiba – PR, Brazil.*
[2] *Chemistry Department – Federal University of Parana – UFPR, P.O. Box 19032, CEP81531-980, Curitiba – PR, Brazil.*
[3]*Brasil Kirin Brewery Company, Rod. Marechal Rondon, Km 111, Itu – SP, Brazil*
Corresponding author: cicero@ufpr.br

Keywords: *Humulus lupulus* L., *chemical diversity, aromatic profile, myrcene, humulene, caryophyllene, breeding programs.*

Hop is one of the three species of the *Humulus* genus and has special economic importance mainly because of the use by the brewery companies. The hop production in Brazil is still incipient and the cultivation occurs only in small farms and with low agronomic technology. The main objective of this work was to evaluate the cone aromatic profiles of hops grown under different climatic conditions in Southern Brazil. Mature cones of seven accessions cultivated in Parana and São Paulo state were collected and the essential oil was extracted by hydrodistillation during two hours and analyzed by gas chromatography-mass spectrometry. The results of the volatile fraction of all accessions showed myrcene (36.57-86.96%) and humulene (7.8-21.45%) as the main constituents. Caryophyllene (2.97-6.59%) was identified in six of the evaluated accessions. Other minor constituents as selinene, isoamyl butyrate, cadinene, ocimene, chamigrene and aristolochene were also identified in accessions 3, 6 and 7. The phytochemical variability among the accessions suggests its potential use in breeding programs in Brazil as an initial strategy to introduce the commercial cultivation in the country. The improvement of cultivated varieties adapted to different regions could result on increase of cultivated areas to attend the national industry demand.

Chemical composition, Seasonal variations, Antifungal and Larvicidal activities of essential oils of *Marrubium vulgare*, growing wild of West-Algeria

Amina Tabet Zatla[1], Imane Mami[1], Samira Bouayad Alam[2], Ali Medbouhi[1], Mohamed El Amine Dib[1], Nassim Djabou[1], Alain Muselli[3], Jean Costa[3]

[1] Université de Tlemcen, Département de Chimie, BP 119, Tlemcen 13000, Algérie.
[2]Université de Tlemcen, Laboratoire LECGEN, BP 119, Tlemcen 13000, Algérie
[3]Université de Corse, UMR CNRS 6134, Laboratoire CPN, BP 52, 20250 Corte, France.
**Corresponding author: nassim_djabou@yahoo.fr*

Keywords: *Marrubium vulgare*, seasonal variation, fungal infections, larvicidal activity, tomato fruits.

Essential oil of *Marrubium vulgare*, collected at the same locality throughout the three seasons (winter, spring and summer), was compared in terms of essential oil composition and yields. Mostly quantitative, rather than qualitative variation was observed in the oil composition. Considerable changes were noted for E-β-caryophyllene (4.5-23.5 %), E-β-farnesene (3.4-22.9 %) and α-humulene (tr-14.8 %). On the other hand, collective essential oil of *M. vulgare* collected during month of April and June **(ColEO)** was evaluated for its insecticidal and antifungal activity. ColEO showed good in-vitro antifungal activity against *Penicillium expansum* and *Alternaria alternata* with the percentages of inhibition of 94 % and 100 % at 10 µL/L, respectively. Due to the promising results of in-vitro tests, essential oil of *M. vulgare* was chosen to determine protective effects in tomato fruits against infection caused by *Alternaria alternata*. Observations showed that the treatment of tomato fruits with ColEO resulted remarkable reduction of disease severity compared with the controls. ColEO used with the concentration of 50 µL/L air showed a very interesting protective activity against black rot of tomatoes caused by *Alternaria alternata*, up to 15th day of storage, compared with the control. However, essential oil collected during month of February **(FEO)** exhibited no effect of antifungal activity against the phytopathogens tested. On the other hand, the ColEO of *M. vulgare* exhibited also a good insecticidal activity. At the concentration of 20 µL/L air, the oil caused mortality over 100% for all larval stages and adults of *Tuta absoluta* after 24 h of exposure. Thus, that ColEO can be used as a potential source to develop control agents to manage some of the main pests and fungal diseases of tomato.

Chemical variability and biological activities of essential oils and hydrosol of *Mentha rotundifolia* from Western Algeria

Fatima Zahra Benomari[1], Nassim Djabou[1], Ali Medbouhi[1], Nadjiya Merad[1], Mohamed El Amine Dib[1], Alain Muselli[2], Mourad Bendahou[3], Jean Costa[2]

[1]*Université de Tlemcen, Département de Chimie, BP 119, Tlemcen 13000, Algérie.*
[2]*Université de Corse, UMR CNRS 6134, Laboratoire CPN, BP 52, 20250 Corte, France.*
[3]*Université de Tlemcen, Laboratoire LAMAABE, Faculté SNV-STU, BP 119, Tlemcen 13000, Algérie.*
**Corresponding author: nassim_djabou@yahoo.fr*

Keywords: *Mentha rotundifolia*, chemical variability, biological activities.

Chemical composition of the aerial part of hydrosol extract and essential oils of North Algerian *Mentha rotundifolia* has been investigated, using GC/RI and GC-MS. Biological activities of the hydrosol, hydrosol extract and essential oil, were assayed for their antioxidant and *in-vivo* and *in-vitro* antifungal activities. GC and GC/MS analysis of *M. rotundifolia* hydrosol extract and essential oil from 79 stations allowed the identification of 32 and 80 compounds accounting for 93.3 and 99.2 % respectively for hydrosol extract and collective essential oil, after fractionation by column chromatography using an Automatized Combi Flash apparatus. The main components of hydrosol extract obtained by liquid-liquid extraction with diethyl ether were: piperitenone (22.9-30.2%), menthone (6.2-29.7%), pulegone (10.9-25.1 %) and piperitenone oxide (5.8-10.7%). The main components of collective essential oil were Menthone (19.5%), Pulegone (19.1%), iso-menthone (13.1%), piperitenone oxide (10.3%), Z and E piperitone oxide (7.8%) and neo-menthol (7.8%). Intra-species variations of the chemical compositions of essential oils from 79 Algerian sample locations were also investigated using statistical analysis. Three different groups were illustrated: major group **G1** with menthone, pulegone, iso-menthone and neo-menthol as major components, and two localized groups **G2** and **G3** with respectively E and Z piperitone oxide from **G2** and piperitenone oxide from **G3**.

Antioxidant activity of collective essential oil and hydrosol extract reducing DPPH radical formation and the bleaching test β-carotene with IC_{50} 40.90µg/mL and 2.95µg/mL respectively from DPPH and IC_{50} of 40.87µg/mL and 0.51µg/mL respectively from β-carotene. Collective essential oil and hydrosol extract showed strong *in-vitro* and *in-vivo* antifungal activity against *Aspergillus niger* and *Penicillium italicum*. The data indicate that hydrosol extract at 5 µg/mL produced total inhibition against *Penicillium italicum* (100 %) and a potent inhibitory effect on the growth of *Aspergillus niger* with a percentage of 88 %. *Citrus sinensis* fruits infected by *Penicillium italicum* pathogen were treated *in-vivo* with hydrosol water. Very low concentrations (5 mL of hydrosol water/L air) were needed for the absence of orange infection and very low disease incidence. Those results demonstrated that hydrosol of *Mentha rotundifolia* can be used as conservative solution to protect orange in storage.

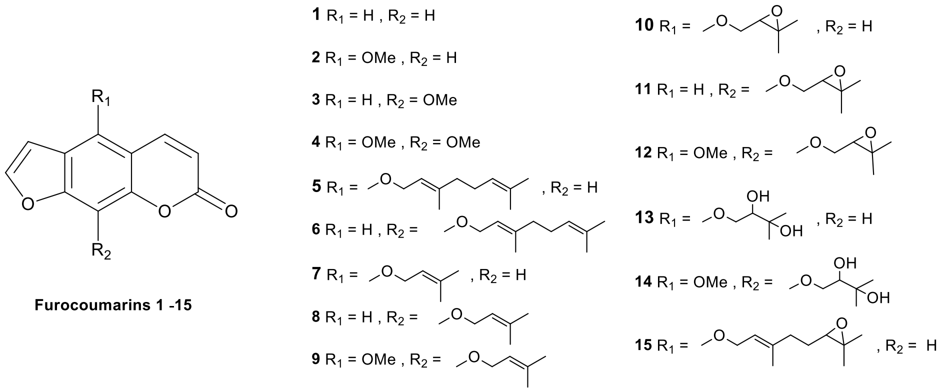
Validated UHPLC-MS QqQ method for the quantification of selected furocoumarins

Duroure Leslie[1], Bertrand Thomas[1], Sizaire Laura[1], Cicchetti Esmeralda[1]
[1]*Cosmo International Ingredients, 855 avenue du Docteur Maurice Donat, 06250 Mougins, France*
Corresponding author : esmeralda.gonzales@cosmo-ingredients.com

Keywords: *Furocoumarins, UHPLC-MS-QqQ, validation, accuracy profiles*

Due to their phototoxicity, furocoumarins are limited in cosmetics and perfumery [1,2]. The current IFRA method allows the quantification of the 15 most common furocoumarins (**1** to **15)** in citrus oils with a LOQ of 10 ppm by reverse phase HPLC-UV at 310nm in simple matrices [3]. Mass spectrometry detection in SRM or MRM mode [4-6] improved specificity and limits of UV detection. To our knowledge, none of the published methods refers to the quantification of **1-15** together while some of them exhibit the same mass spectrum and could lead to lack of specificity in case of coelution and/or only partial validation data are available.

This poster will present the development and the validation of a UHPLC method with MS QqQ detection in MRM mode according to ICH Q2 (R1) Guideline [7] and the performance of the method will be displayed as individual and global accuracy profiles for compounds **1** to **15**. The development of the chromatographic method will be discussed and especially the simplification of the IFRA method eluents, the choice of sub 2 micron C18 modified column to solve isomers coelution while saving time. The impact on the validation results of the solvent of calibration and the regression type will also be underlined in the poster.



Furocoumarins 1 -15

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Separation performances of GCxGC versus 1DGC for the speciation the extended list of suspected allergens and regulated compounds in fragrance materials

Thomas Dutriez[1], Laetitia Duffour[1], Diane Pétermann[1], Neil Owen[2], Greg Adamson[3]

[1] *Givaudan, RAPS Analytical Laboratory, Vernier, Switzerland*
[2] *Givaudan, RAPS Analytical, Ashford, United Kingdom*
[3] *Givaudan, Global Regulatory Affairs, Product Safety & Sustainability, East Hanover Ridgedale, United States.*
Corresponding author: thomas.dutriez@givaudan.com

Keywords: Comprehensive two-dimensional chromatography, Fragrances, Allergens.

In 2011, the Scientific Committee on Consumer Safety (SCCS) proposed an extension of the current list of 24 suspected volatile allergens to 57 compounds, including isomeric forms [1]. As a consequence, a new European legislation, related to the labelling of cosmetic products, is expected in the coming months regarding the information to consumers, to which fragrance industry organizations must adapt their analytical approaches to meet.

Typically, the analytical methods currently applied to the 24 volatile allergens are based on two parallel GC-qMS separations (apolar and mid-polar stationary phases) with SIM acquisitions. This approach usually overcomes coelutions of the current "24" suspected allergens with matrix solutes, where specific m/z ions help for assigning target. However, this approach has clear limitations in terms of identification confidence with complex naturals, and even more so when considering the new list of allergens, especially with diastereoisomers, allergen precursors and additional regulated substances, in total about 100 analytical targets.

Comprehensive two-dimensional gas chromatography (GCxGC) involves two GC columns connected in serie and an "on the fly" sampling device, i.e. modulator. By adopting GCxGC with adapted stationnary phases and operating parameters, enhanced resolution and higher peak capacity (>4000) can be achieved, resulting in analytical targets eluting to the MS with no or limited coelution, even in complex essential oils. Together with TOF-MS acquisition, where full range m/z ions are recorded up to 200 Hz, using deconvolution software can provide a "pure" mass spectrum for each regulated compound. GCxGC approach is then providing an ultimate degree of identification confidence for regulated substances on fragrance materials for a futher quantification step.

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Industrial integration of GCxGC-TOFMS for the quantification of the extended list of suspected allergens and regulated compounds in fragrance materials

Thomas Dutriez[1], Laetitia Duffour[1], Diane Pétermann[1], Neil Owen[2], Greg Adamson[3]

[1] *Givaudan, RAPS Analytical Laboratory, Vernier, Switzerland*
[2] *Givaudan, RAPS Analytical, Ashford, United Kingdom*
[3] *Givaudan, Global Regulatory Affairs, Product Safety & Sustainability, East Hanover Ridgedale, United States.*
Corresponding author: thomas.dutriez@givaudan.com

Keywords: Comprehensive two-dimensional chromatography, Allergens.

A new European legislation, related to the information to consumers of cosmetic products, is expected in the coming months, based an extension of the current list of 24 suspected volatile allergens to 57 compounds [1]. Comprehensive two-dimensional gas chromatography (GCxGC-TOFMS) can overcome the limitations of the typical analytical method applied to the 24 volatile allergens in complex naturals, i.e. GC-qMS with 2 parrallel runs [2], in terms of peak capacity (>4000), chromatographic resolution between analytical targets and available separation space for major diastereoisomers and even additional regulated compounds.

An ultimate degree of identification confidence is therefore achieved by GCxGC-TOFMS and by demonstrating the data processing filters which have been implemented: retention time in both dimensions with accepted deviations, MS match with dedicated library and qualifier ions ratios. A quantification procedure has been setting-up including, analytical standards, automated sample preparation, dilution ranges, control samples and a full reviewing procedure, such as a high degree of confidence is obtained for a wide target dynamic range (ppm to high % level).

Demonstrating this as more than just an R&D device, GCxGC-TOFMS is fully integrated into an industrial environment in a “QC-type” infrastructure in terms of liquid nitrogen facility, IT server, sample delivery from local QCs and automatic uploading of results to a global Regulatory Management system (SAP). Therefore, a global program for monitoring new regulated substances in fragrance ingredients is now in place, including essential oils, e.g. Ylang-Ylang, Patchouli, Vetiver…,

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Low eV fragmentation as a complementary tool for the extended list of suspected allergens by GC×GC-TOFMS – Application to essential oils

Thomas Dutriez[1], Laetitia Duffour[1], Diane Pétermann[1], Laura McGregor[2], Nick Bukowski[2], Neil Owen[3], Greg Adamson[4]

[1] Givaudan, RAPS Analytical Laboratory, Vernier, Switzerland
[2] Markes International Ltd, Llantrisant RCT, CF72 8XL, United Kingdom
[3] Givaudan, RAPS Analytical, Ashford, United Kingdom
[4] Givaudan, Global Regulatory Affairs, Product Safety & Sustainability, East Hanover Ridgedale, United States.
Corresponding author: thomas.dutriez@givaudan.com

Keywords: Low eV, Comprehensive two-dimensional chromatography, Allergens.

In the context of the new forecast European legislation, related to suspected allergenic compounds in cosmetic products (57 compounds including isomeric forms [1]), and the clear limitations of the classical GC-MS separation with apolar and mid-polar stationary phases, comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC–TOF MS) applied to fragrance materials has been recently introduced. This approach clearly provides a much enhanced separation efficiency and therefore a higher confidence of quantitative data for regulatory purpose. Identification filters are already numerous with a GC×GC-TOFMS approach, e.g. Library MS match, 1D and 2D deviation, qualitative MS ion ratios; therefore adequate confirmation tools are provided for the majority of samples with trained operators. However, for some residual cases, e.g. very complex matrices and/or compounds which are spectrally similar, an additional identification dimension would be beneficial in delivering a further enhanced confirmation.

Recently, Markes International introduced a new electron impact technology (Select-eV) which allows the tuning of ionization voltages (related to ionization energies) from 10 to 70 eV without compromising sensitivity and with no source-switching. With low eV fragmentation, typically 12-14 eV, mass spectra containing initial structurally-significant fragment ions are greatly enhanced. This therefore induces an additional confirmation tool for compound assignment. Very recently, Markes International has released a fast-switching of electron energies to collect both hard (70 eV) and soft (10-20 eV) electron ionisation spectra within a single run, i.e. "Multiplexing".

The Select-eV approach has been successfully applied to the forecast extended list of suspected volatile allergens and other regulated compounds. It has provided a powerful and useful identification filter, especially for isomers such as Farnesols, Damascones, ISO E Super and Terpinenes, for which 70 eV mass spectrum are normally very similar. An application to two essential oils is finally presented with the above approach.

PTV-GCMS Quantitation of benzene in benzaldehyde

Nailee EBIBI[1], Elise CORBI[1], Christophe PERES[1], Nathalie DAVID[1]

*[1]Chanel, Laboratoire de Création et de Développement des Parfums,
Laboratoire Recherche et Analyses, 20 rue Victor Noir, 92521 Neuilly-sur-Seine Cedex.*

Corresponding author: elise.corbi@chanel-corp.com

Keywords: benzene, benzaldehyde, Programmable Temperature Vaporizer.

Quantification of benzene in fragrance raw materials is a critical analysis since this compound is regulated and should not exceed 1 ppm in the fragrance compound.
This analysis is usually performed by HeadSpace GC/MS [1] with isotope labeled internal standard. Calibration is performed in diethyl phtalate, an appropriate and almost universal dilution solvent of raw materials.
Difficulties in quantitative work has been reported when analysing benzaldehyde raw material especially when dilution was required (ie when calibration range upper level is exceeded). It was observed that the more the raw material was diluted in diethyl phtalate, the more the quantity of benzene increased, leading to highly erroneous results.
Benzene generation has been largely described in food domain [2,3]. Simultaneous presence of ascorbic acid and sodium benzoate under certain conditions of temperature, pH and presence of traces of metals are often presented as an explanation of this phenomenon.

This poster describes a technical solution to accurately measure benzene in benzaldehyde.
First, experiments with fluorobenzaldehyde are presented. They demonstrate that the benzene comes from benzaldehyde degradation at hot temperature under high pressure (split/splitless injection conditions).
Second a HS-GC/MS method with PTV injector has been developed and prevents from benzene overestimation. Calibration is linear from 1 to 10 mg/L with standard deviation lower than 10 %.

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Antibacterial Activity of *Salvia* Species from Turkey

Emir Tan[1], Merve Bilgin[1], Nur Tan[2], Seçil Yazıcı-Tütüniş[2], Emine Akalin[3]

[1]*Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Yeni Yuzyıl University, 34010 Istanbul, Turkey*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Istanbul University, 34116 Istanbul, Turkey*
[3]*Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, 34116 Istanbul, Turkey,*

Corresponding author: etan57@gmail.com

Keywords: *Salvia*, antibacterial activity

The aim of this study is to determine and to compare the antimicrobial activities of the different essential oils of endemic *Salvia* species from Turkey. The genus *Salvia* L. (Lamiaceae) comprises about 900 species world-wide, while it is presented with 89 species and 93 taxons in Turkey, approximately half of which is endemic. *Salvia* species are used in folk medicine for the treatment of a variety of diseases, including infectious diseases [1-3]. The antimicrobial activity of essential oils of seventeen *Salvia* species was determined against common Gram (+) and Gram (-) reference bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis,* methicillin resistant *Staphylococcus aureus* (MRSA)*, Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Proteus mirabilis*) using the standard microbroth dilution method modified with rezasurin [4,5]. *Salvia recognita*, *S. syriaca* and *S. potentilifolia* showed better activity compared to the others, especially against *S. aureus* and *B. subtilis*, followed by *S. aramiensis, S. cassia, S. nigdeggerii, S. euphratica, S. microstegia, S. heidreichiana, S. virgata, S. chionantha* and *S. glutinosa* which showed good antibacterial activity MIC values of 0, 16 to 0, 63 mg/mL against *S. aureus*, MRSA, *E. faecalis* and *B. subtilis*. This study shows that these essential oils may be effective as bakteriostatic agents Gram (+) and Gram (-) microorganisms.

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Chemical composition and cytotoxic mechanism of *Baccharis milleflora* essential oil

Paulo Vitor Farago[1], Camila Bugnotto Pereira[2], Carla Cristine Kanunfre[1], Débora Maria Borsato[1], Beatriz Helena Noronha Sales Maia[2], Marilis Dallarmi Miguel[2]

[1]*State University of Ponta Grossa, 4748, Carlos Cavalcanti Ave., Zip Code 84030-900, Ponta Grossa, Paraná, Brazil*
[2]*Federal University of Paraná, 632, Prefeito Lothário Meissner Ave., Zip Code 80210-170, Curitiba, Paraná, Brazil*
Corresponding author: pvfarago@gmail.com

Keywords: Apoptosis, cancer, necrosis, tumor treatment.

Baccharis L. is traditionally used for many medicinal purposes. In particular, some *Baccharis* species have currently shown cytotoxic properties [1]. Moreover, to the best of our knowledge, this is the first report devoted to investigate the cytotoxicity of *B. milleflora*. The aim of this work was to investigate the chemical composition of the essential oil from cladodes of *B. milleflora* and its cytotoxic activity against Jurkat, Raji and HL-60 cells as well as to explore cell mechanisms in order to elucidate how the cytotoxic process occurs. The presence of volatile compounds: bicyclogermacrene (12.16%), D-germacrene (11.18%), (*E*)-caryophyllene (9.28%), and *α*-humulene (8.05%) was detected by GC-MS method. In general, IC_{50} values lower than 50 µg/mL were obtained for all tumor cells at 24, 48 and 72 h by MTT assay. The decrease of cell DNA content was demonstrated due to inhibition of Jurkat, Raji and HL-60 cells proliferation by *B. milleflora* essential oil as a result of cytotoxicity. In particular, Raji cells presented the greatest inhibition of cell proliferation using propidium iodide and it was then used for investigation of cell death mechanisms. *B. milleflora* essential oil promotes G0/G1 arrest and also induces cell fragmentation which was represented by the increase of sub G0 population indicating cell death induced by apoptosis. The selectivity index was 3.97. Necrotic cell death coupled with low level apoptotic cell death was observed by conventional EB/AO and Hoechst 33342 staining assays, demonstrating that this essential oil acts via both necrotic and apoptotic mechanisms. The current research proves that *B. milleflora* essential oil have a cytotoxic effect against the investigated tumor cell lines and shows an antitumor mechanism by apoptosis and necrosis processes.

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Inoculation of *Pinus halepensis*, *P. pinaster*, *P. pinea* and P. *sylvestris* with the pinewood nematode *Bursaphelenchus xylophilus*: Essential oils and monoterpene hydrocarbon enantiomers responses

Ana M. Rodrigues[1], Marta D. Mendes[1,#], Ana S. Lima[1,2], Pedro M. Barbosa[3], Lia Ascensão[1], José G. Barroso[1], Luis G. Pedro[1], Manuel M. Mota[3,4], A. Cristina Figueiredo[1]

[1]Centro de Estudos do Ambiente e do Mar (CESAM), Faculdade de Ciências da Universidade de Lisboa, Centro de Biotecnologia Vegetal (CBV), Edifício C2, Campo Grande, 1749-016 Lisboa, Portugal
[2]Instituto Politécnico de Bragança, Escola Superior Agrária, Centro de Investigação Montanha, Campus Santa Apolónia, Apartado 1172, 5031-855 Bragança, Portugal
[3]NemaLab, Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade de Évora, Núcleo da Mitra, Apartado 94, 7002-554 Évora, Portugal.
[4]Departamento Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, EPCV, Campo Grande 376, 1749-024 Lisboa, Portugal
[#]Currently at Centre for Novel Agricultural Products, Department of Biology, University of York, York YO10 5DD, United Kingdom.
Corresponding author: acsf@fc.ul.pt

Keywords: pine wilt disease, chemotypes, enantiomeric composition

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is the causal agent of pine wilt disease (PWD), a serious threat to global forest populations of conifers, especially *Pinus* spp. A time-course study of the essential oils (EOs) of 2-year-old *Pinus halepensis, Pinus pinaster*, *Pinus pinea* and *Pinus sylvestris* following inoculation with the PWN was performed. The constitutive and nematode inoculation induced EOs components were analyzed at both the wounding or inoculation areas and at the whole plant level. The enantiomeric ratio of optically active main EOs components was also evaluated. External symptoms of infection (yellowing of the needles and stem necrosis and phenolization) were observed only in *P. pinaster* and *P. sylvestris* 21 and 15 days after inoculation, respectively. The EO composition analysis of uninoculated and unwounded plants revealed the occurrence of chemotypes for *P. pinaster*, *P. halepensis* and *P. sylvestris*, whereas *P. pinea* showed a homogenous EO composition. When whole plants were evaluated for EO and monoterpene hydrocarbon enantiomeric chemical composition, no relevant qualitative and quantitative differences were found. Instead, EO analysis of inoculated and uninoculated wounded areas revealed an increase of sesquiterpenes and diterpenic compounds, especially in *P. pinea* and *P. halepensis*, comparatively to uninoculated whole plants EOs. These may play a role in the plant defence response and should be further investigated.

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The "new old" α-furanopinguisanol and its oxidation product from the essential oil of the liverwort *Porella cordaeana* (Hub.) Moore (Porellaceae)

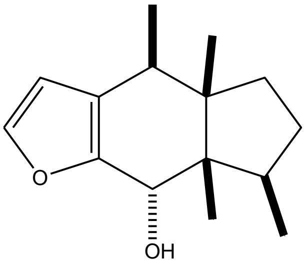
Sonja I. Filipović [1,*], Niko S. Radulović[1,*], Dragan B. Zlatković[1],
Miljana R. Đorđević[1], Vladimir N. Ranđelović[2]

[1]Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, RS-18000 Niš, Serbia
[2] Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, RS-18000 Niš, Serbia

Corresponding author : SF: sonjafilipovic86@yahoo.com NR: nikoradulovic@yahoo.com

Keywords: *Porella cordaeana* (Porellaceae), α-furanopinguisanol, furanopinguisanone, structural elucidation

Porella cordaeana (Hub.) Moore (Porellaceae, Jungermanniales), cliff scalewort, is a leafy marchantiophyta, classified in the non-pungent group of *Porella* L. species that are known as a prolific source of biologically active sesquiterpenoids among which many belong to the rare pinguisane-type, exclusively found in liverworts. Previous phytochemical investigations of *P. cordaeana* revealed a great variation of its secondary metabolism which was found to be highly dependent on geographical origin of plant material. In this work, we wish to report on the identification of two new natural products, sesquiterpenes with a very rare carbon-skeleton, α-furanopinguisanol (**1**) and furanopinguisanone (**2**), identified in the essential oil of *P. cordaeana* collected from the slopes of Suva planina mountain (southeastern Serbia). Although α-furanopinguisanol was previously reported in the literature, herein, we present arguments that its structure was actually erroneously assigned to a different, related compound. Structure elucidation was accomplished by spectral means (various 1D and 2D NMR experiments in $CDCl_3$ and C_6D_6, IR, UV and MS) and the structures corroborated by chemical transformations. The relative configuration of the compounds was additionally verified by an analysis of shift changes produced by a lanthanide shift reagent. A complete assignation of all ^1H and ^{13}C NMR signals was performed.



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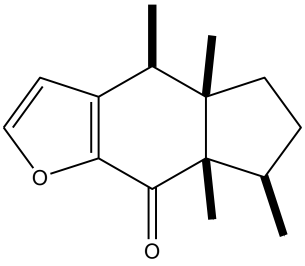

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What to do with old, auto-oxidized tetrahydrofuran? Simple, make a perfume out of it.

Milan Nešić, Milica Stevanović, Sonja Filipović, Niko S. Radulović

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

Corresponding author: NR nikoradulovic@yahoo.com SF sonjafilipovic86@yahoo.com

Keywords: Tetrahydrofuran acetals, auto-oxidation, 2-hydroperoxytetrahydrofuran, acetal of citronellol, sensory evaluation

Recently, we developed a diversity-oriented synthetic methodology for the preparation of acetals employing PPh_3-CCl_4 reagent combination. The methodology utilizes stoichiometric amounts of an aldehyde and an alcohol and can be successfully applied to a wide scope of both carbonyls and alcohols. Herein, we wish to report on a related new synthesis of acetals derived from tetrahydrofuran-2-ol and various alcohols, starting from, presumably, 2-hydroperoxytetrahydrofuran present in auto-oxidized tetrahydrofuran (THF). In this particular case, PPh_3 acts both as an *in situ* reducing (2-THF-OOH to 2-THF-OH) and, in combination with CCl_4, a dehydrating agent.
The procedure was applied to a series of aliphatic and monoterpene alcohols and the corresponding acetals (several new compounds) were obtained in medium-to-good yields (up to 70%) under very mild conditions (room temperature, no acid added, simple reaction work-up). All of the synthesized compounds were fully spectrally characterized (1D- and 2D-NMR, MS, IR) and all NMR data assigned. Additionally, purified acetals were evaluated by a trained sensory panel (from local vinery). A number of acetals derived from monoterpenols were shown to bare a pleasant flowery or fruity smell that was different when compared to the original alcohols. For example, a mixture of diastereomers of 2-((3,7-dimethyloct-6-en-1-yl)oxy)tetrahydrofuran, derived from racemic citronellol, was found to have an odor reminiscent of roses.
The source of 2-THF-OOH used in this work was an old bottle of THF, in which the amount of peroxides was determined by iodometric titration. If, one would want, the commercially available THF, stabilized with an antioxidant (such as butylated hydroxytoluene, BHT), a straightforward distillation at atmospheric pressure is sufficient to obtain a BHT-free THF which auto-oxidizes by a much faster rate (days instead of years). The results of the peroxide determination were used to assess the amount of PPh_3 needed to completely reduce 2-THF-OOH, thus, enabling the addition of the exactly required (1 equivalent for the reduction of the organic hydroperoxide + 1 equivalent for acetal formation).

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Potential use of *Lippia origanoides* Kunth essential oil for the control of the nematode *Meloidogyne javanica*

Franceli da Silva[1], Fábio Nascimento de Jesus[1], Simone Teles[1] Ana Cristina Fermino Soares[1]

[1] *Centre for Agricultural, Environmental and Biological Sciences, Federal University of Recôncavo da Bahia/UFRB, University Campus, Cruz das Almas/BA,44380-000 Bahia, Brazil.*
Corresponding author: franceli.silva@gmail.com

Keywords: *Meloidogyne javanica*, carvacrol, *Lippia origanoides*.

Essential oils (EO's) composition is comprised by a significant number of components capable to act as natural control agents against pests and diseases. In order to explore these properties, in this work, the nematicidal activity of *Lippia origanoides* Kunth EO was tested against the nematode *Meloidogyne javanica* in *in vitro* assays. EO's from *L. origanoides* were extracted by hidrodistillation and were characterized by by gas chromatography coupled with flame ionization detector (GC/FID) and by gas chromatography coupled to a mass spectrometry detector (GC/MS). After that the EO's nematicidal activity was tested *in vitro* at different concentrations (0, 10, 20, 30 and 40 µL mL^{-1}) for 24 h at 28 ºC. The number of dead and alive nematodes was counted. Nematicidal activity of EO's increased with concentration of EO, with mortality rates over 85% with concentrations ≥ 20 µL mL^{-1}. Through a regression analysis, the estimated concentration of 31.52 µL mL^{-1} retrieved a mortality rate of approximately 96% of the nematodes tested. The results obtained could be related to carvacrol, thymol and linalool, main components of *L. origanoides* EO. It was concluded that *L. origanoides* EO's could be an alternative and natural resource to control the nematode *M. javanica*.

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Aroma Profile Analysis of Incense from a New Perspective

Toshio Hasegawa[1], Natsumi Sato[1], Asako Yamazaki[1], Yuka Sekiguchi[1], Takashi Fujihara[1], Hideo Yamada[2]

[1]Department of Chemistry, Graduate School of Science and Engineering, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama 338-8570, Japan
[2] Yamada-matsu Co., Ltd., Kamigyo-ku, Kyoto 602-8014, Japan
Corresponding author: toshihas@mail.saitama-u.ac.jp

Keywords: aroma profile analysis, incense, odor evaluation, olfactory mechanism

Traditional Japanese incenses have characteristic odors, which are produced by the complex interactions of their odor constituents. Recently, the response mechanism between olfactory receptors and odorants has been elucidated, in which one odorant molecule is recognized by several olfactory receptors, and one olfactory receptor responds to different odorant molecules with similar structures. This mechanism indicates that the combination of several constituents with similar structures creates an odor that is not the sum of the odors of each constituent.

We have studied the aroma profiles of several kinds of incense materials, including sandalwood, vetiver, patchouli, star anise, turmeric, frankincense, myrrh, and benzoin gum. However, it is difficult to clarify the aroma of these incense materials by conventional gas chromatography (GC) analytical methods (GC-olfactometry and GC-mass spectrometry). The odor of the aroma constituents is not necessarily involved in their aroma profile owing to the complex olfactory mechanism. To solve this problem, we developed a new analytical method, aroma profile analysis, in which the odor constituents of materials are divided into several groups containing constituents with similar molecular structures. We used this approach to identify a key group of compounds of the characteristic odor of sandalwood.

Next, we used our method to investigate the odor profile of Korean mint and *Lysimachia foenum-graecum*. GC analyses showed that these incense materials had complicated odor component structures. We performed a detailed analysis of the odor by our proposed method and found that the aroma profile of Korean mint arose from the combination of two groups of compounds. However, we were not able to clarify the aroma profile of *L. foenum-graecum*. The material contained a large number of constituents with diverse structures, and it was difficult to divide the constituents into groups with similar structures. Analyzing the relationship between the components and the odor of the fractions showed that the lack of some constituents caused the loss of the odor similarity to the fragrance material. This indicates that all of the aroma components in the material are necessary for the characteristic odor. We revealed that the aroma profile of *L. foenum-graecum* by our proposed evaluation method. Thus, our method is useful for elucidating the complex aroma profiles of odor materials, which are difficult to analyze by conventional methods.

Antimicrobial and anti-inflammatory activities of *Thymus vulgaris* L. essential oils of Western Algeria

Martina Höferl[1], Jürgen Wanner[2], W. Abdelli[3], Fouad Bahri[3], Abderrahmane Romane4, Erich Schmidt[1], Leopold Jirovetz[1]

[1]Department of Pharmaceutical Chemistry, University of Vienna, Center of Pharmacy, Althanstrasse 14, A-1090 Wien, Austria.
[2]Kurt Kitzing GmbH, Hinterm Alten Schloß 21, 86757 Wallerstein, Germany.
[3]Laboratoire de Microbiologie et Biologie Végétale, Université de Abdelhamid Ibn Badis, Faculté des Sciences de la nature et de la vie, Mostaganem, Algérie.
[4]Laboratoire de Chimie Organique Appliquée, Equipe Analyse et Contrôle de Qualité, Faculté des Sciences Semlalia, Université Cadi Ayyad, Marrakech, Morocco
Corresponding author : martina.hoeferl@univie.ac.at

Keywords: *Thymus vulgaris* L., Algeria, antimicrobial activity, acute toxicity, anti- inflammatory activity.

For the present investigation, leaves and stems of *Thymus vulgaris* were collected in the regions of Tlemcen and Mostaganem in the west of Algeria. Hydrodistillation yielded 4.2% (Tlemcen) and 2.2 % (Mostagenem) of essential oil, respectively. The essential oil samples were analyzed by GC-FID and GC-MS. The main compounds of the Tlemcen sample were thymol (67.3 %), γ-terpinene (10.1 %) and *p*-cymene (6.0 %). The sample from Mostagenem was mainly comprised of thymol (59.5 %), γ-terpinene (8.7 %), α-pinene (5.7 %), *p*-cymene (5.6 %) and linalool (5.1 %).

The antimicrobial activity was assessed by antibiotic aromatogram and broth dilution methods. The essential oils had a considerable effect on the seven reference strains tested, particularly on *Bacillus cereus* ATCC 10876 and *Candida albicans* ATCC 10231. MIC, MBC and CMF values were obtained ranging respectively from 0.312 to 5 µl /ml, 0.312 to 10 µl/ml and at 1.25 µl/ml for EO of T. vulgaris of Tlemcen and from 0.312 to 1.25 µl/ml, 0.312 to 10 µl/ml and from 0.625 to 2.5 µl/ml, respectively for that of Mostaganem. Testing for acute toxicity test demonstrated that the oil from Tlemcen was toxic from a dose of 4500 mg/kg, whereas the oil from Mostagenem did not show signs of toxicity even at the highest dose tested (5000 mg/Kg). The paw edema test induced by carrageenan injection in mice, resulted at respective doses of 100, 200 and 400 mg/kg essential oil, respectively, to inhibit inflammation by 31.2%, 48% and 58.4% using thyme oil of Tlemcen and by 29.6%, 43.2% and 50.4% for thyme oil of Mostaganem, compared to diclofenac as reference drug, which gave an inhibition percentage of 85.6% at a dose of 10 mg/kg.

β-Amyrin biosynthesis: catalytic function of the highly conserved aromatic amino acid residues

Tsutomu Hoshino[1], Chika Nakada[1], Ikki Kaneko[1]

[1]*Graduate School of Science and Technology, and Department of Applied Biological Chemistry. Faculty of Agriculture, Niigata University, Ikarashi 2-8050, Nishi-ku, Niigata, Japan 950-2181*

Corresponding author: hoshitsu@agr.niigata-u.ac.jp

Keywords: β-amyrin, triterpene, 2,3-oxidosqualene, *Euphorbia tirucalli*, biosynthesis.

β-Amyrin, a triterpene, is widely distributed in plants and its glycosides confer important biological activities (a sweetener, licorice). β-Amyrin synthase is one of oxidosqualene cyclases (OSCs). Mutagenesis studies on β-amyrin synthase are very limited. This study was conducted to elucidate the function of the highly conserved Y259 and W257 residues in *Euphorbia tirucalli* β-amyrin cyclase. Few reports describing the expression levels of OSC mutants are available. In order to assess the *in vivo* enzymatic activities, the quantities of the OSC protein expressed (Western blot analyses) and the triterpene products (GC quantification) accumulated in the host lanosterol-deficient yeast must be estimated. To address the function of these aromatic residues, the side-directed mutants were constructed. We have reported the functional analyses of F728 and F413 residues [1-3]. Here, we report the functions of Y259 and W257 residues. Y259 residues were site-specifically mutated into some aliphatic amino acids, resulting in the significantly decreased activity for the formation of β-amyrin, but the Phe variant afforded the equivalent activity to the wild type, this result having also given the definitive evidence for the cation/π interaction as the catalytic mechanism. The aliphatic mutants conferred significantly increased amounts of tetracyclic products, derived from dammanrenyl cation, thus Y259 residues acts to stabilize the baccharenyl cation via cation/π-interaction. The mutation of W257 into aliphatic amino acids such as Ala, Valine and Ileu decreased the enzyme activity and conferred significantly large amounts of lupeol. However, the Phe and Tyr variants showed relatively higher activity than the aliphatic amino acid mutants. Thus, it is likely that W257 residue stabilizes the oleanyl cation via cation/π-interaction. Our recent studies using the substrate analogs revealed that this enzyme tightly binds 23*Z*-methyl group (Me-30) of 2,3-oxidosqualene to form the normal folding of a chair-chair-chair-boat-boat conformation [4]. To seek for the binding site to hold Me-30, many residues (except Y259, W257 and F728 residues) surrounding Me-30 were mutated, but any mutant did not afford tetracyclic product, but only β-amyrin. In contract, incubation of the analog with CH_2OH substituent at the 23Z-position afforded tetracyclic dammanrenyl cation-trapped products, indicating that hydrophobic interaction between M-30 and the binding site is involved. Thus, three residues Y259, W257 and F728 possibly would have roles for not only cation/π but also CH/π interactions to form β-amyrin scaffold.

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PP39

Effects of two volatile esters of *N*-methylanthranilic acid in CCl_4-induced acute nephrotoxicity rat model

Ivan R. Ilić[1], Nikola M. Stojanović[2], Pavle J. Randjelović[3], Ana B. Miltojević[4], Mirjana Ilić[5], Vesna V. Živković[1], Ratko S. Ilić[1], Niko S. Radulović[4]

[1]*Institute of Pathology, Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000 Niš, Serbia.*
[2]*Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000 Niš, Serbia.*
[3]*Department of Physiology, Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000 Niš, Serbia.*
[4]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia.*
[5]*Pediatric Clinic, Laboratory of Biochemistry, Clinical Center and Medical Faculty of Niš, University of Niš, 18000 Niš, Serbia.*
Corresponding author: ilicko81@gmail.com

Keywords: *N*-methylanthranilic acid esters, Rutaceae, kidney damage, histochemical staining.

Methyl *N*-methylanthranilate (MA) and isopropyl *N*-methylanthranilate (IA) are two naturally occurring plant constituents reported from a number of essential oils of Rutaceae taxa (e.g. *Citrus reticulata* Blanco, *Choisya ternata* Kunth. etc.). Herein, we investigated the potential protective effects of the two esters in a rat model of acute intoxication with carbon tetrachloride (CCl_4) by tracking changes in kidney tissue morphology and function. Wistar rats were treated daily with MA and IA for seven days in a dose of 200 mg/kg. Twenty-four hours after a CCl_4 injection, rats were sacrificed and blood samples were used for the evaluation of urea and creatinine. Kidney tissue specimens were stained with hematoxylin and eosin, periodic acid-Schiff (PAS) and Jones stain and evaluated for morphological changes. Quantification of kidney glomeruli structural changes was assessed using ImageJ software. The administration of CCl_4 caused significant abnormalities in renal morphology: proximal tubules necrosis, parenchymatous degeneration (cloudy swelling), increase in glomerular cellularity and infiltration of mononuclear cells mostly localized in subcapsular and juxtamedullary regions of the kidney cortex. Moreover, several foci with loss of basement membrane in proximal tubules and increase of PAS positive tubular casts were noted. IA and MA applied in high doses did not cause any significant damage to kidney tissue. A pretreatment with MA prior to the administration of CCl_4 significantly prevented the increase of serum levels of decreased kidney function markers, while that of IA did not. Histopathological evaluation of the kidneys also revealed that MA reduced the incidence of kidney lesions. Our experiments showed that MA, and not IA, possesses a protective potential against CCl_4-induced kidney damage in rats. The results are of interest due to the presence of natural or synthetic MA in the human diet and their known potent analgesic properties.

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Prep-MDGC: cheap, robust and reliable tool to collect volatile components present at trace level in complex matrices for NMR characterization

Alain Jaquier[1], Alessandro Casilli[1,2], Estelle Delort[1]

[1] *Analytical Innovation, Corporate R&D Division, Firmenich SA, Geneva, Switzerland*
[2] *Universidade Federal do Rio de Janeiro, Instituto de Química, LBCD-LADETEC, Ilha do Fundão, Rio de Janeiro, RJ 21941-909, Brazil.*
Corresponding author: alain.jaquier@firmenich.com

Keywords: Preparative MDGC, Trace components analysis, Deans Switch, Low Thermal Mass, *Citrus australasica.*

In GC/MS analysis, the elucidation of unknown peaks present at trace level remains a tedious and time-consuming task. One approach is based on the interpretation of the mass spectrum fragmentation followed by synthesis of the hypothesis. This requires strong expertise and if the hypothesis is wrong, the process has to be reiterated. Another approach is based on the direct isolation of the unknown peak of interest followed by NMR characterization. In this case, a consistent amount of high purity standard is necessary for unambiguous identification of the isolated compound. With the higher sensitivity of spectroscopic techniques and last-generation analytical tools (MDGC, Deans Switch, Low Thermal Mass), the second approach is now more readily accessible.

In this investigation a preparative MDGC (prep-MDGC) system based on Capillary Flow Technology (CFT) and Low Thermal Mass (LTM) has been designed, assembled and optimized. The instrument is a conventional GC, equipped with one mega-bore column, one injector and two FID detectors, and two Deans Switch systems in the oven. A second column is located in an LTM module. The configuration was successfully applied in combination with enrichment by flash-chromatography for the discovery of two new volatile constituents found in a citrus extract.

First, the two unknown compounds present at low concentration (<0.4 TIC%) in an extract of Australian finger lime (*Citrus australasica*) peel [1] were enriched respectively to 12.2 and 8.4 TIC% by flash-chromatography. Secondly, repetitive collections by prep-MDGC allowed to obtain a pure sample of each unknown compound with a high purity (92 and 95 TIC%) allowing their NMR characterization.

The full analytical procedure is opening the door to the discovery of trace volatile constituents from various complex matrices [2].

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VARIATION OF LEDOL CONTENT IN ESSENTIAL OIL FROM IN VIVO AND IN VITRO BIOMASSES OF RHODODENDRON TOMENTOSUM (LEDUM PALUSTRE)

Jesionek A[1], Wlodarska P[1], Kokotkiewicz A[1], Zabiegala B[2], Luczkiewicz M[1]

[1]*Department of Pharmacognosy, Medical University of Gdansk, al. Gen. J. Hallera 107, 80-416 Gdansk, Poland.*
[2]*Department of Analytical Chemistry, Gdansk University of Technology, ul. Gabriela Narutowicza 11/12, 80-233 Gdansk, Poland.*
Corresponding author : annajesionek@gumed.edu.pl

Keywords: *Rhododendron tomentosum, Ledum palustre,* ledol, *in vitro* cultures, HPTLC, GC

Rhododendron tomentosum (formerly *Ledum palustre*) is an aromatic shrub which grows in bogs in northern Europe, Asia and North America [1]. It has been traditionally used to treat rheumatic diseases due to analgesic and anti-inflammatory properties. Its scientifically proven biological activity is related mainly to the volatile fraction content [2]. However, one of its main consituents, ledol, is regarded as the potentially toxic compound which could affect the possibility of the essential oil use in rheumatoid arthritis prevention or therapy [1].

The ledol content in volatile fractions, obtained from the plant material from different natural habitats and from *in vitro* cultures which were established as the alternative source of the essential oil of the endangered discussed species was determined by gas chromatography (GC/MS). Additionally, the effect of various drying and extraction methods on the chemical composition of *R. tomentosum* volatile fractions was specified. Simultaneously, the optimized HPTLC analysis of the *R. tomentosum* essential oils was conducted not only for a quick recognition of the qualitative and quantitative chemical profiles, but mainly for the plant material screening for the terpenoids biosynthesis. Afterwards, the validation of the developed method was performed for the determination of the ledol content in the studied biomasses. Finally, the results of HPTLC and GC separation were compared and combined with literature data. The proposed HPTLC screening test of plant matrices allows to determine the quality and toxicity of the ground plant material as well as to control the correctness of the various stages of the biotechnological process quickly and effectively.

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Volatile components as chemical markers of botanical origins of honey bee from Guiana

Jiang Weiwen[1,2], Robinson Jean-Charles[1], Bereau Didier[1], Battesti Marie-José[2], Jean Costa[2], Bercion Sylvie[3], Paolini Julien[2]

[1] UMR Qualisud. Université de Guyane (Campus de Troubiran), Cayenne Guyane française .
[2] UMR CNRS 6134 Laboratoire de chimie des produits naturels. Université de Corse. Corse.
[3] UMR Qualisud. Université des Antilles (Campus de Fouillole), Guadeloupe.
Corresponding author:paolini@univ-corse.fr ; jiangweiwen973@gmail.com

Keywords: Honey, french Guianea, SPME/GC

French Guiana has a rich flora, including approximately 6000 species and more than 160 plants are considered as endemic. Three biotopes had been recognized having a beekeeping interest : the mangrove (*Avicennia sp.* and *Rhisophora sp.*), the savanna (various herbaceous, shrubs and palms) and the forest (essentially trees) [1]. Despite the presence of *Apis mellifera,* the beekeeping did not start until 1990s [1]. Currently the honey production was around 5 tons per year. For the development of this agricultural sector, honeys from Guiana must be certified by quality labels according to botanical and geographical origins. Many authors suggested that honey aroma might be affect by several factors like botanical origin and nectar composition [2]. Therefore, volatile constituents of honeys can be used as chemical markers of melliferous plant contribution, especially for monofloral honeys [3].

The aim of the present work was to establish for the first time, the botanical origins of Guianese honeys using melissopalynological studies and volatile composition analysis. For this purpose, 17 honey samples were collected from three distinct biotopes (mentioned above) during the honeyflow (August to January). The volatile fraction of each sample was characterized by HeadSpace-Solid Phase Micro-Extraction (HS-SPME), Gas Chromatography (GC) and GC coupled with mass spectrometry (GC-MS). Using this analytical approach, 60 components were identified including aliphatic, phenolic and terpenic compounds (oxygenated and hydrocarbons). Chemical variability of volatile composition was observed according to samples locations and botanical origins of honeys. For instance, hotrienol was reported for the first time as major compound of honey volatile fraction from tropical area. In order to establish correlations between this chemical diversity of Guianese honeys and their botanical origins, further studies were actually conducted by melissopalynological analysis associated with statistical analysis.

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Biological activities of volatile and non-volatile components from Australian *Pittosporum angustifolium* are consistent with its use in traditional Aboriginal medicine

Jones GL[1] and Sadgrove NJ[2]

[1]*Pharmaceuticals and Nutraceuticals Group, School of Science and Technology, University of New England, Armidale, New South Wales 2350, Australia*
[2]*Department of Botany and Plant Biotechnology, University of Johannesburg, South Africa*
Corresponding author Email: gjones2@une.edu.au

Keywords: *Pittosporum angustifolium,* saponins, essential oils, solvent extracts, eczema, gumbi, Australian Aboriginal.

Pittosporum angustifolium (Pittosporaceae) often called ‘Gumbi’ by Aboriginal people was and to an extent still is involved in a significant number of traditional medicinal applications. The most common of these are related to the treatment of coughs and colds, for lactagogue activity and in the treatment of eczema and psoriasis. More recently, a number of anecdotal reports have surfaced related to, *ia,* cancer inhibition, autoimmune conditions in the intestines and antimicrobial activity. Previous studies have supported potential anticancer activity, as well as possible antiviral (Ross River Fever) activity. Previous characterisation of volatiles from *P. angustifolium*, demonstrated compounds with structural similarities to previously described chemosemiotic compounds identified in mother-infant bonding, including acetic acid decyl ester or 1-dodecanol (Sadgrove & Jones, 2013). Perhaps these compounds are involved in the traditional use as a lactagogue, particularly because the usage modality involved heating a compress of leaves to produce such volatiles to fumigate the breasts of the nursing woman. Another study has identified several novel triterpenoid glycosides, with pittangretoside A as the most abundant (Bäcker *et al.*, 2013).

Here we compare the antibacterial and antifungal activity of various solvent extracts with that of the essential oil hydrodistilled from both leaves and fruit of *Pittosporum angustifolium* and the related species *Pittosporum undulatum* and consider aspects of intra and inter specific markers of phytochemical variation of possible importance in commercial cultivar selection.

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GC-MS detection of dimethyl trisulfide as a chemical marker of shallot extract and oil in Thailand

Narisa Kamkaen[1], Nirun Vipunngeun[2], Thanapat Songsak[3]

[1,2,3]*Faculty of Pharmacy, Rangsit University, 52/347 Muang Ake, Pathumthani, Thailand.*
Corresponding author: narisa.k@rsu.ac.th

Keywords: *Allium ascalonicum*; Dimethyl trisulfide

Shallot (*Allium ascalonicum)* has been characterized by their rich content in sulfur compounds that are responsible for the organoleptic parameters and contribute to the antioxidant and antimicrobial activities [1]. The volatile flavor components of shallot oils can be classified in the following categories: thiols, monosulfides, disulfides, trisulfides, thiophenes and oxygen compounds that can be detected by gas chromatography-mass spectrometry (GC-MS) [2]. In the present study, dimethyl trisulfide has been suggested as a chemical marker of shallot extract and oil in differentiation from four regions of Thailand. It is noticed that the highest amount of dimethyl trisulfide obtained from raw shallot grown in the north-eastern part of Thailand that should be considered as a good source of raw material for cosmeceutical product development.

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Cytotoxicity of *Eucalyptus alba* essential oil on human tumor cell lines

Carla Cristine Kanunfre[1], Thais de Oliveira Borsato[1], Beatriz Helena Noronha Sales Maia[2], Jane Manfron Budel[1], Paulo Vitor Farago[1]

[1]*State University of Ponta Grossa, 4748, Carlos Cavalcanti Ave., Zip Code 84030-900, Ponta Grossa, Paraná, Brazil.*

[2]*Federal University of Paraná, 632, Prefeito Lothário Meissner Ave., Zip Code 80210-170, Curitiba, Paraná, Brazil*

Corresponding author: cckanunfre@gmail.com

Keywords: Volatile oil; cancer, tumor treatment, IC50.

Cancer is a disease with high incidence rates. However, its treatments present many clinical limitations as high intensive collateral effects. New therapies have been investigated in order to provide effective and safe treatment for cancer. In that sense, many *in vitro* and *in vivo* studies demonstrated the use of essential oils (EO) for treating cancer. The use of *Eucalyptus* EO is widespread in folk medicine, particularly for treating respiratory diseases. Considering previous reports of its potential antitumor effect, the aim of this work was to evaluate the cytotoxicity of *Eucalyptus alba* Reinw. ex Blum EO on human tumor lines HeLa, HRT-18, Jurkat and Calu-3. The aerial parts of *E. alba* were collected in Governador Manoel Ribas Complex in Ponta Grossa (latitude: 24° 4'45.29"S and longitude: 54°11'17.10"W), Paraná, Brazil. The *Eucalyptus* EO was extracted through hydrodistillation using Clevenger apparatus for 6 hours. This EO was characterized by GC-MS comparing to volatile standards. The main volatile components found in *E. alba* EO were 1,8-cineole (55.24%), α-terpinyl acetate (21.64%); α-pinene (7.94%) and furfuryl acetone (6.45%). The determination of cytotoxicity of *E. alba* EO was performed by cell viability assay using MTT reduction. IC50 values were higher than 1000 mg/mL for HeLa and HRT-18 cell lines. For Jurkat and Calu-3 cells, IC50 values of 391.43 ± 34.57 mg/mL and 689.79 ± 29.34 mg/mL were obtained, respectively. Considering that a higher cytotoxic effect was verified for Jurkat cells, cell cycle and DNA fragmentation analyses were performed for *E. alba* EO against this tumor cell line in order to investigate the cell death mechanism. The cytometric flow assay showed a decrease in G2/M phases at 500 µg/mL. In addition, DNA fragmentation was recorded for Jurkat cells when treated with *E. alba* EO at 500 µg/mL. These results are involved in the possible mechanism of cytotoxicity of *E. alba* EO against Jurkat cells. The current work shows that *E. alba* EO has a weak cytotoxicity against Jurkat and Calu-3 cancer cell lines. However, *E. alba* EO cannot be used as a potential antitumor drug due to its high IC50 value.

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Selection of optimal operating conditions for extraction of *Myrtus communis L.* essential oil

Durmus Alpaslan Kaya[1], Elena Danila[2], Mihaela Violeta Ghica[3],
Madalina Georgiana Albu Kaya[2], Musa Turkmen[1]

[1] *Department of Field Crops, Faculty of Agriculture, Mustafa Kemal University, 31034, Antakya-Hatay, Turkey.*
[2] *Collagen Department, Division Leather and Footwear Research Institute, The National Research & Development Institute for Textiles and Leather, 031215, Bucharest, Romania.*
[3]*Department of Physical and Colloidal Chemistry, Faculty of Pharmacy, Carol Davila University of Medicine, 020956, Bucharest, Romania.*
Corresponding author : elena.danila23@yahoo.com

Keywords: *Myrtus communis L.*, yield, Taguchi approach

Myrtus communis L. is one of the important aromatic and medicinal species to the Mediterranean area [1]. It is used in various fields such as culinary, cosmetic, pharmaceutical, therapeutical and industrial traits [2]. Essential oils of Myrtus species consisting in phenolic acids, flavonoids, tannins, anthocyanin, pigments and fatty acids [3].

In order to establish essential oils best yield, quality and cost, and also saving environment, reducing cost energy, a Box-Wilson factorial design was applied to evaluate the effect of major process variables on the essential oil extraction from *Myrthus communis L.* herba. The input variables considered as significant operating conditions were: X1 – boiler occupancy rate (boilers were filled to 50%, 75% and 100%), X2 – the distillation duration (distillations was continued 60, 75 and 90 minutes), and respectively X3 – particle size (herba were cut in size 10, 20 and 30 mm via guillotine). The dependent variables selected were: Y1 – the essential oil volum obtained (mL) and Y2 – the extraction process yield (%).

The steps of the classical statistical experimental design technique were complemented with Taguchi method to improve the extraction process of essential oil from *Myrtus communis L.*, and the optimum parameter conditions were selected. This suggested approach could be extended for the optimization of a wide variety of essential oils from vegetable products.

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Chemical and aroma profiles of *Citrus junos* (yuzu) peel oils produced in Rikuzentakata

Eisuke Kuraya[1,*], Akiko Touyama[1], Shina Nakada[1],Toshio Hasegawa[2] and Shigeru Itoh[1]

[1] *National Institute of Technology, Okinawa College, 905 Henoko Nago City, Okinawa, JAPAN*
[2] *Graduate School of Science and Engineering, Saitama University, 255 Shimo-Ohkubo, Sakura-ku Saitama, Saitama, JAPAN*
[*] Corresponding author:kuraya@okinawa-ct.ac.jp

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

Citrus fruits, some of the most important commercial crops, are widely cultivated in the regions between the tropical and temperate zones. *Citrus junos* Tanaka (yuzu) has a strong characteristic aroma and yuzu juice is used in Japanese foods. Yuzu produced in Rikuzentakata (Iwate prefecture) is called “Northern Limit Yuzu (NLY)” and was transplanted from the temperate area approximately 70–300 years ago. We have previously demonstrated that the aroma profile of NLY is significantly different from that of yuzu from other producing districts [1]. In addition to revealing the differences between NLY and other yuzu samples, principal component analysis (PCA) has also suggested that the NLY aroma profile varies between individual trees grown within the Rikuzentakata area. In the present study, we have evaluated the chemical and aroma profiles of essential oils extracted from yuzu peel to understand how these characteristics differ between individual plants using multivariate analysis of gas chromatography/mass spectrometry (GC/MS) data.

All yuzu fruits were provided by producers in Rikuzentakata (NLY, 15 samples), Kochi (yuzu cultivars were ‘Kumon’ and ‘Nagano’), and Saitama (2 samples, yuzu cultivar was unnamed), respectively. The yuzu peel oils were extracted using hexane and the volatile compounds were analyzed by GC/MS. Prior to statistical data analysis, an ion matrix generated using the Profiling Solution software (version 1.1; Shimadzu Corp.) was extracted and the intensity of all the significant ions plotted against *m/z* values at the particular retention time. PCA was performed using the SIMCA software (version 13.01; UMETRICS) to determine whether the differences in the chemical composition of peel oils could be correlated to the district of their production and to individual plants. The NLY profiles were different from those of other districts, and some clusters were identified among the approximately 3,000 ions corresponding to NLY individual trees. These results confirm that the aroma profile of NLY is clearly different from that of yuzu grown in other districts and that it significantly varies between individual trees.

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Essential oil yield and antioxidant activity from *Alpinia zerumbet* grown in the Ryukyu Islands

Eisuke Kuraya*, Rui Yamashiro, Akiko Touyama, Shina Nakada, Kenta Watanabe, Akira Iguchi, Shigeru Itoh

National Institute of Technology, Okinawa College, 905 Henoko Nago City, Okinawa, Japan
**Corresponding author e-mail: kuraya@okinawa-ct.ac.jp*

Keywords: *Alpinia zerumbet*, volatiles yield, antioxidant activity

The aromatic perennial plant *Alpinia zerumbet* (Pers.) Burtt & Smith (Zingiberaceae) is widely distributed, occurring in both tropical and sub-tropical regions; in Japan, it grows from southern Kyushu to the Ryukyu Islands. The essential oil of *A. zerumbet* has a distinct aroma and exhibits antioxidant activity. Recently, interspecific hybridization of *Alpinia* spp. has been reported in Taiwan [1], and it may also occur in Japan; this type of hybridization may produce detectable differences in essential oil characteristics. Previously, we demonstrated the contribution of *A. zerumbet* essential oil to differences in fragrance and antioxidant activity among individuals [2]. Here, we evaluated the yields, antioxidant activity, and volatile compound composition of essential oils extracted from the leaves of *A. zerumbet* to better understand how these characteristics may differ between growth areas and among individual plants.

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. The leaves were oven-dried at 40–50 °C to a moisture content of 10%–15%, and steam-distilled to obtain their essential oils, which were analyzed by gas chromatography/mass spectrometry (GC/MS). Antioxidant activities were measured by free radical scavenging assays using 1,1-diphenyl-2-picrylhydrazyl.

The Trolox equivalent (TE) antioxidant capacities ranged from 1.5 to 8.8 μmol TE/g oil. The yields of essential oil ranged from 0.32 to 3.64 g/kg leaf dry weight and decreased with increasing antioxidant activity ($r^2 = 0.831$). Although the antioxidant activity and yield of these essential oils differed significantly between individuals and collecting seasons, there was an inverse correlation between the yields of essential oils and their antioxidant activity.

The GC/MS and antioxidant activity assays revealed that highly polar compounds were responsible for the antioxidant effects detected in the essential oils. The GC/MS analysis also revealed significant variation in the chromatographic profiles of volatile oils extracted from different individuals. These results suggest that the essential oils from different individuals possess different antioxidants, and that the yield of essential oil may depend on the amount of terpenes produced in the leaves. Moreover, these results are in close agreement with those of our previous study [2]. Lastly, the chemical composition within individual plants was not influenced by the season or the growth area.

To summarize, the essential oil of *A. zerumbet* differed significantly among individuals with respect to its yield, volatile compounds, and odor. This pronounced variation among individuals in the yield, composition, and antioxidant activities of *A. zerumbet* essential oil may be related to interspecific hybridization of *Alpinia* spp. in the Ryukyu Islands.

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Qualitative analysis of selected furocoumarins by HPTLC in essential oils

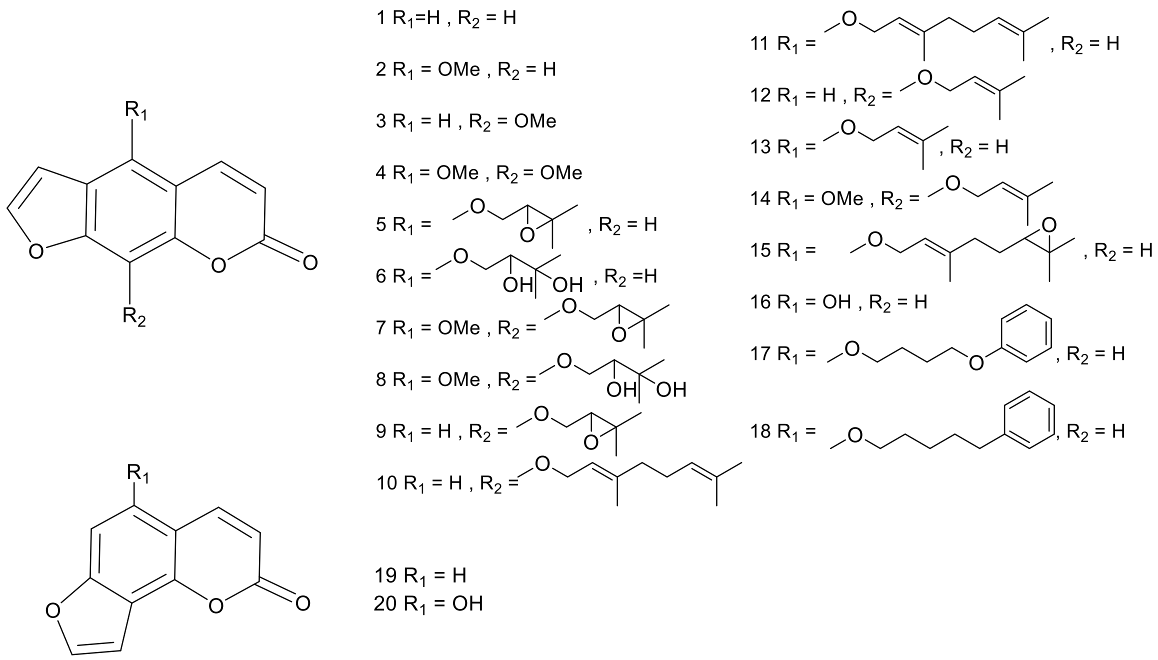
Erell Le Borgne[1], Thomas Bertrand[1], Esméralda Cicchetti[1]

[1]*Cosmo International Ingredients, 855 avenue du Dr Maurice Donat, 06250 Mougins, France.*
Corresponding author: esmeralda.gonzales@cosmo-ingredients.com

Keywords: Furocoumarins, HPTLC

Because of their phototoxic properties, furocoumarins are now regulated in cosmetics and perfumery. The quantification of the furocoumarins (**1-15)** most commonly found in essential oils can be carried out by HPLC/UV [1]. But this method is time and solvent consuming for routine analysis. High Performance Thin Layer Chromatography (HPTLC) is a simple, fast and cheap technique and a method of choice in quality control to evaluate the presence of analytes down to a target level or to monitor a process development.

In this context, two HPTLC methods (1D and 2D) were developed to analyze the 15 compounds cited above along with 5 additional furocoumarins (**16-20**). These compounds were detected at 366 nm and their identification was based on the Rf and the color of the spots. The influence on the color of the position of the substituent(s) on the furocoumarinic moety will be discussed in this poster together with the order of elution on the plates. The specificity of these methods will be determined by analyzing the most common coumarins and analogs found in essential oils. In only 35 minutes, the 1D method leads to a first estimation of the furocoumarin composition of several samples simultaneously. The 2D method allows discriminating more furocoumarins compare to the 1D analysis but only one sample can be analyzed at a time. These methods were found to be easy to use for routine analysis and to save time compare to HPLC methods. To our knowledge, this is the first time a HPTLC method is provided to analyze **1-20** simultaneously in essential oils.



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THE QUANTITATIVE MEASUREMENT OF WATER BY GC USING IONIC LIQUID CAPILLARY COLUMNS

Leonard M. Sidisky[1], Gustavo Serrano[1], James L. Desorcie[1], Greg A. Baney[1], Katherine K. Stenerson[1], Michael Halpenny[1], Michael D. Buchanan[1], Klaus Bucklendahl[2]

[1]MilliporeSigma, Bellefonte, PA, United States of America
[2] Merck, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany.
Corresponding author: klaus.buckendahl@sial.com

Keywords: ionic liquid GC capillary columns, determination of water content, small sample size, quantitative GC method.

The determination of water content in solvents, alcoholic beverages and various consumer products such as foods, pharmaceuticals, petroleum, and essential oils products is one of the most common types of chemical testing. Many techniques such as gravimetric analysis, Karl Fischer Titration, near infra-red spectroscopy, gas chromatography (GC) and others have been used for water quantification with good results. [1] However, the limitations of these approaches can include high limits of detection, large sample sizes required for trace analysis, side reactions, the use of expensive consumables, and the production of chemical waste. Previous work by Prof. Daniel Armstrong and co-workers has described the use of ionic liquid GC capillary columns for the trace analysis of the water content in a wide variety of solvents. [2] These columns were capable of providing a rapid and quantitative determination of water contents using very small sample amounts. This paper further examines the characteristics of ionic liquid capillary columns for the GC analysis of water.

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Development and characterization of microemulsions containing essential oils of rosemary (*Rosmarinus officinalis*) and basil *(Ocimum basilicum)*.

Viviane S. Lobo[1], Claudia C. Machado[1], Karina G. F. Colombo[1], Maurício Rosa[2]

[1]Technological Federal University of Paraná – Campus Toledo.
[2]State University of Western Paraná – Campus Toledo.
Corresponding author :lobovivíane@gmail.com

Keywords: Phase diagrams; Microemulsion, *Rosmarinusofficinalis*, *Ocimumbasilicum*

Due to the antioxidant and antimicrobial properties attributed to the essential oils [1] the use of such compounds in water-based products becomes an interesting strategy. Therefore, microemulsions have become an alternative solution to enhance the solubilization capacity of these oils in water. Aiming to produce microemulsions of essential oils we carried out experiments using essential oils of rosemary (*Rosmarinusofficinalis*) and basil (*Ocimumbasilicum*), distilled water and polysorbate 80 as surfactant. From the mixture of these components, varying the concentration every 5%, the two phase diagrams were developed (Fig. **1**). Both, for the basil oil and for the rosemary oil, 19 microemulsion formulations were obtained. For each oil, two microemulsion formulations with a smaller amount of surfactant were chosen and specific tests were carried out, as follows: rosemary oil (50; 5; 45) (oil, water, surfactant) and (45; 5; 50), formulations 1 and 2, respectively; basil oil (45; 10; 45) and (40; 10; 50), formulations 3 and 4, respectively. The pH measurement was in triplicate, and the following results were found: 6.49, 6.93, 6.55 and 6.57 for the formulations 1, 2, 3 and 4, respectively. These four formulations were kept in rest for 48 hours, having a satisfactory macroscopic performance. The same formulations were subjected to centrifugation for one hour, and no phase separation had been observed. For thermal stress analysis, the solutions were immersed in water bath, and the temperature was gradually increased from 20 to 50° C and they remained homogeneous. The formulations were submitted to three freezing and thawing cycles, being each cycle of 12 hours in a refrigerator and 12 hours in an oven at 40 °C, and remained stable. Therefore, all microemulsion formulations analyzed showed to be adequate.

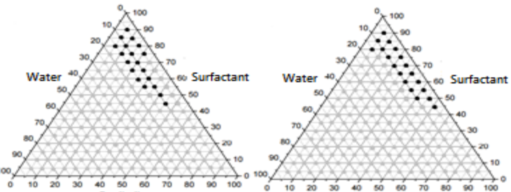

Fig. 1–Phases diagram of microemulsion containing essential oil of basil and rosemary.

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Essential oils form *Cymbopogon* species – chemical composition, antioxidant and antimicrobial activity

Agnieszka Ludwiczuk[1], Krystyna Skalicka-Woźniak[1], Tomasz Baj[1],
Anna Kiełtyka-Dadasiewicz[2], Agnieszka Grzegorczyk[3], Anna Malm[3]

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

[2] *Department of Plant Production Technology and Commodities, University of Life Sciences,*
15 Akademicka Str., 20-954 Lublin, Poland.

[3] *Chair and Department of Pharmaceutical Microbiology with Laboratory for Microbiological Diagnostics,*
Medical University of Lublin, 1 Chodzki Str., 20-093 Lublin, Poland.

Corresponding author : aludwiczuk@pharmacognosy.org

Keywords: *Cymbopogon* species, lemongrass oil, citronella oil, palmarosa oil, chemotypes, antioxidant and antimicrobial activity

The genus *Cymbopogon* Spreng. (Poaceae) is characterized by its species possessing great variability in morphology and chemotypes [1]. Most species of the genus are aromatic and yield volatile oils of important commercial values. The most common is lemongrass oil obtained from *C. citratus* or *C. flexuosus*, and citronella oil from *C. nardus* or *C. winterianus* [2]. The aim of this work was the chemical analysis of 8 commercially available essential oils (EO) obtained from different *Cymbopogon* species (*C. nardus*, *C. winterianus*, *C. flexuosus*, *C. schoenanthus*, *C. martini*). Additionally, for the first time, the EOs were hydrodistilled from leaves, stems and roots of *C. citratus* cultivated in Poland. The yield of EO from the aerial parts of the Polish material was about 1.5 % (v/w), which is comparable to plants growing in tropical and subtropical regions.

GC/MS analysis of *Cymbopogon* essential oils showed the differences in the chemical composition. The analyzed EOs can be divided into three chemotypes. Chemotype I (*C. nardus*, *C. winterianus*) contains mainly citronallal, while neral and geranial are the most important compounds found in chemotype II (*C. citratus*, *C. flexuosus*, *C. schoenanthus*). *C. martini* known as palmarosa, belong to the chemotype III. This EO instead of the monoterpene aldehydes produces alcohols: nerol and gearniol. The chemical composition of EO obtained from the leaves from *C. citratus* (chemotype II) was very similar to that of the EO hydrodistilled form stems, with the exception of the presence of sesquiterpene alcohol, elemol in stems. This compound was the major constituent of the EO obtained from roots. The findings on the composition of EO from *Cymbopogon* species were similar to those previously reported except for elemol which was found in *C. citratus* from Poland.

EOs classified in chemotypes I and II showed good antioxidant activity (EC_{50} 0.43 – 1.96 mg/ml). *C. martini* (chemotype III), showed no antioxidant activity. All EOs were tested for their antimicrobial activity against 16 reference strains of Gram-positive, Gram-negative bacteria and yeasts. EOs belonging to chemotype II have the strongest activity, especially against Gram-positive bacteria like *B. subtilis* (MIC = 7 µg/ml, MBC = 30 µg/ml) and *M. luteus* (MIC = 30-60 µg/ml, MBC = 60-125 µg/ml). These EOs also showed strong activity against *Candida* species with MIC values ranging from 60-125 µg/ml. EOs of lemongrass also cultivated in Poland can be a good source of antimicrobial agents.

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Influence of seasonality on the chemical composition of the essential oil of three *Baccharis* species.

Beatriz Helena L.N. Sales Maia[1], Tania F. Dlugoviet[1], Aurea P. Ferriani[2], Noemi Nagata[1], Francisco A. Marques[1]

[1]*Chemistry Department – Federal University of Paraná – UFPR, P.O. Box 19032, CEP81531-980, Curitiba – PR, Brazil.*
[2]*Plant Science Department– Federal University of Paraná – UFPR, P.O. Box 19032, CEP81531-980, Curitiba – PR, Brazil.*
Corresponding author : noronha@ufpr.br; bhsalesmaia@gmail.com

Keywords: *Baccharis calvescens, Baccharis retusa, Baccharis uncinella*, stastistical analysis, seasonality.

Baccharis belongs to Asteraceae family and is well represented in southern and southeastern Brazil with many of them used in popular medicine. The aim of this study to investigate the chemical composition of the essential oils obtained from leaves of three *Baccharis* species (*B. calvescens* DC., *B. retusa* DC. and *B. uncinella* DC.), using multivariate analysis (PCA and HCA), to find similarities and differences in the oils' chemical constitution, trying to relate them with the seasonality. There are not studies with *B.calvescens* and the others are little investigated. The leaves, collected in Piraquara – PR, were submitted to hydrodistillation and in triplicate in a modified Clevenger-type apparatus for 4 hours each. The yields were between 0.05 – 0.29%, and in winter there were the highest yields and in autumn, the minors. The oils were analyzed by GC-MS in Shimadzu GC-2010 systems coupled with a mass spectrometer detector Shimadzu GCMS-QP2010 Plus. The GC-MS measurements were performed using a nonpolar capillary column Rtx-5MS and the carrier gas was helium. Oil components were identified by comparison of both arithmetical index and mass spectra with literature and spectral library. After identification of the constituents of essential oils, the data obtained were subjected to multivariate statistical procedures: Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). The PCA and HCA were performed using PLS Toolbox 3.0 and Matlab 7.0. The major compound of the essential oil from *B. uncinella* in all seasons was spathulenol (> 10%), and this specie showed minor variations due to seasonality. For the essential oil of *B. retusa*, there were large variations during the seasons, and α-pinene was the main constituent in the spring, limonene in the summer, spathulenol in the winter and D germacrene in the autumn. The essential oils of *B. calvescens* had as major constituent D germacrene in the autumn and spathulenol in other seasons. The PCA demonstrated clearly which compounds differentiated the three species in function of the seasonality. It could be useful to future investigations about biological activities.

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Multivariate analysis of the antibacterial activity of essential oils from the leaves of *Iryanthera polyneura* related to climatic changes.

Martins, E.R.[1]; Silva, N.H. [1] & Suffredini, I.B.[2]

[1] *Graduate Program in Environmental and Experimental Pathology., University Paulista, São Paulo, Brazil.*
[2] *Center for Research in Biodiversity, University Paulista, São Paulo, Brazil.*

Corresponding author: eramosmartins@gmail.com

Keywords: Myristicaceae, *Iryanthera polyneura*, antibacterial activity, multivariate analysis, climatic changes

Fourty-five essential oils (EO's) obtained from leaves of three adult individuals of *Iryanthera polyneura* Ducke (Myristicaceae), identified as 22OE, 80OE and 530OE, were collected during a 2-year period. Correlations between minimal inhibitory concentrations (MIC's) and minimal bactericidal concentrations (MBC's) of each oil and the presence of El Niño and La Niña, climate variables as maximum temperature, relative humidity, total daily irradiation and total daily precipitation were assessed by discriminant analysis (DA) and canonic correlation analysis (CCA), as a contribution to understand how climate changes in a tropical forest may influence antibacterial activity in a 2-year period. EO's were obtained from fresh leaves by hydrodistillation, and their MIC's and MBC's were obtained by microdilution broth assay, using dilutions of 10%, 5%, 2.5% and 1.25% in dimethylsulfoxide (DMSO). Results were obtained by the analysis of turbidity, with a posterior subculture in agar medium. DA performed with 22OE, 80OE and 530OE showed separation of two distinctive groups 'summer' and 'winter', indicating a possible correlation of MIC's and MBC's and climate changes and El Niño-La Niña phenomena with both typical tropical seasons. Such results were confirmed by CCA findings, which suggest that there was a variation in MIC values related to the occurrence of El Niño, in individuals 22OE and 530OE, as a tendency. Multivariate analyses DA and CCA showed to be important tools to seek for correlations in the variation of MIC's/MBC's and climate variables related to EO's that was collected from three individuals from *I. polyneura*, in a 2-year period.

Oxygenated heterocyclic compounds used for differentiating *Citrus spp.* essential oils through metabolomic strategies

Jérôme Masson[1,2*], Erica Liberto[2], Jean-Claude Beolor[1], Hugues Brevard[1],
Carlo Bicchi[2], Patrizia Rubiolo[2]
[1] *Robertet SA, Research Division, 37 Avenue Sidi Brahim, F-06130 Grasse, France,*
[2] *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via P. Giuria 9, I-10125 Torino, Italy,*
**Corresponding author: jerome.masson@robertet.com*

Keywords: *Citrus spp.,* metabolomic strategy, oxygenated heterocyclic compounds, UHPLC/TOF-MS, multivariate data analysis, discriminant markers

Citrus Essential oils, mainly obtained by cold pressing, are important natural flavouring materials. The authentication of the species of Citrus EOs was well known and was generally based on the volatiles compounds [1, 2]. In 1971 Stanley [3] introduce the hypothesis that a method based on the Oxygenated Heterocylcic Compounds (OHC) distribution allows to authenticate and detect adulteration.

In this context this study aimed to characterize and discriminate 44 authenticated commercial samples of citrus EOs from seven species (bergamot, lemon, bigarade, orange, mandarin, grapefruit, lime) by analyzing the non-volatile OHCs by UHPLC/TOF-HRMS, multivariate data analysis (PCA, PLS-DA) and metabolomic strategies. The OHC fraction includes coumarins, furocoumarins, and polymethoxylated flavonoids. Two different approaches were adopted:

-(i) targeted profiling based on the quantification of 18 furocoumarins and coumarins,

-(ii) targeted fingerprinting based on 140 OHCs reported in citrus EOs, from which 38 discriminant markers were defined.

This study showed that metabolomic strategies applied to C*itrus spp.* is an efficient tool for discriminating samples. In house mixtures were easily discriminated. Additionally, this methodology was able to provide quantitative information on FCs and Cs, limited or not, by current and future regulations.

This methodology is already extended to numerous natural extracts and its development will be supported by further technological and software implementations.

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A metabolomic approach to quality determination and authentication of raw plant materials. Iris rhizomes: a case study

Jérôme Masson[1,2*], Erica Liberto[2], Jean-Claude Beolor[1], Hugues Brevard[1],
Carlo Bicchi[2], Patrizia Rubiolo[2]
[1] Robertet SA, Research Division, 37 Avenue Sidi Brahim, F-06130 Grasse, France,
[2] Dipartimento di Scienza e Tecnologia del Farmaco, Universitàdi Torino, Via P. Giuria 9, I-10125 Torino, Italy,
**Corresponding author: jerome.masson@robertet.com*

Keywords: Iris species and origins, metabolomic strategy, phytochemical analysis, UHPLC/TOF-MS, statistical data treatment, discriminant markers

Raw plant materials used in the flavor and fragrance field often differ in terms of species and origins and, as a consequence, they present variable chemical compositions, resulting in different characteristic organoleptic properties.

Iris rhizome essential oil, commonly known as “Orris butter”, is one of the most important raw materials used in the fragrance field. Over the last 30 years, the determination of the geographical and botanical origins of orris butter was mentioned in several studies, mostly based on the isomeric abundances of irones and iridals [1], and more recently also on the flavonoid fractions, whose composition was used to discriminate *I. germanica* from *I. pallida* from Morocco [2].

This study aimed to discriminate 22 samples of commercial Iris rhizomes (orris root) by species and origin, applying the strategy derived from those adopted in metabolomics. Three *Iris spp*. (albicans, gemanica and pallida), originated from three locations (China, Italy and Morocco) were studied.

A strategy combining UHPLC/TOF-HRMS, in positive and negative modes, with multivariate statistical methods was applied. Exact Mass/Retention Time pairs (EMRT) were submitted to statistical processing by principal component analysis (PCA), partial least square discriminant analysis (PLS-DA), and then orthogonal partial least square-discriminant analysis (OPLS-DA), for extracting the discriminating EMRT pairs. Discriminant markers were selected on the basis of their trend views. Some of them were tentatively identified from the reference database created on the basis of Iris genus components reported in the literature. The reliability of this strategy was confirmed by identifying species and origin of unknown samples submitted to the same analytical procedure.

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Inter-Laboratory validation of a LC/MS method for atranol and chloratranol quantification in moss extracts: preliminary results

CST PRODAROM
Jean-Claude Bayle[1], Fabienne Bettini[1], Hugues Brevard[2], Esméralda Cicchetti[3],
Nathalie David[4], Yoann Filatre[5], Jean-François Goursot[6], Odile Janinet[7],
Jérôme Masson[2*], Céline Roy[5], Christine Schippa[7]

[1] IFF France, SAS LABORATOIRE MONIQUE REMY Av. Louison Bobet 06130 GRASSE, France,
[2] Robertet SA, Research Division, 37 Avenue Sidi Brahim, F-06130 Grasse, France,
[3] Cosmo International Ingredients 855 AV du Dr. M. Donat 06250 Mougins, France,
[4] Chanel, 135 Av Charles de Gaulle 92521 Neuilly-sur-Seine CEDEX, France
[5] ERINI, Espace Jacques-Louis Lions - 4 Traverse Dupont 06130 Grasse, France
[6] RODAROM, 48 avenue Riou Blanquet BP 2101706131 GRASSE CEDEX, France
[7] V. MANE Fils, 06620 Le Bar-sur-Loup, France
**Corresponding author: jerome.masson@robertet.com*

Keywords: moss, *Evernia prunastri, Pseudoevernia furfuracea*, atranol, chloratranol, LC-MS, method validation, inter-laboratory Ring test.

Moss extracts from the lichen *Evernia prunastri (L.) Arch (oak moss) and Pseudoevernia furfuracea,* are important ingredients used in fragrance industry [1]. These materials have been found to initiate a skin sensitization. Consequently atranol and chloroatranol were two substances limited at 100 ppm in moss extracts by IFRA (International Fragrance Association) [2]. Different methodologies using DAD-UV detection were developed and tested in preliminary inter-laboratory studied but did not allow obtaining satisfactory sensitivity.

The quantification of these compounds at low levels is particularly difficult due to the complexity of the natural extracts. In this context, PRODAROM (French national Association of Fragrance Manufacturers) decided to carry out an inter-laboratory ring-test in order to evaluate and validate a HPLC-MS method in SIM mode for their quantification in commercialized extracts. The preliminary collaborative validation was performed with six different laboratories. The performance of the method was evaluated in methanolic solution and method linearity and limits will be displayed in the poster together with accuracy profiles [3]. Spiked mosses were then submitted to quantitation. LC-MS method brings useful qualitative information in SCAN mode. It was also demonstrated that it was an accurate method when used in SIM mode for quantifying atranol and chloratranol in natural extracts at ppm levels. Additionally, during this ring test, it has been shown that the target compounds were not stable in solution over the time, leading to underestimated values. As a consequence, quantification required to be performed on fresh prepared samples.

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Chemical composition and biological activities of essential oil of *Eryngium triquetrum* from Western Algeria

Ali Medbouhi[1,2], Nadjiya Merad[1], Nassim Djabou[1], Mourad Bendahou[3], Jean Costa[2], Alain Muselli[2]

[1]*University of Tlemcen Laboratory COSNA, BP 119, 13000 Tlemcen, Algeria.*

[2]*University of Corsica, CNRS UMR 6134, Laboratory CNP, BP 52, 20250 Corte, France.*

[3]*University of Tlemcen Laboratory LAMAABE, Faculty SNV-STU, BP 119, Tlemcen 13000, Algeria*

**Corresponding author: muselli@univ-corse.fr*

Keywords: *Eryngium triquetrum*, falcarinol, biological activities

The chemical compositions of essential oils from the aerial parts and the separated organs (leaves, flowers, stems, roots) of Algerian *Eryngium triquetrum* were investigated, using GC/RI and GC-MS. Antibacterial activity was established against eight species of micro-organisms using paper disc diffusion and dilution agar assays and the antioxidant activity was established using DPPH and FRAP methods. To our knowledge, the present study is the first report on the chemical composition of *E. triquetrum* essential oil and their biological activities.

GC and GC-MS analysis of the essential oils allowed the identification of 20 components accounting for 94.4 % of the total composition. Falcarinol is highly dominant in the essential oils from total aerial parts and roots (90.6 - 95.45%), while it is present in moderate amounts in the stems and the flowers (36.5% and 39.6%, respectively) with octanol (32.7% and 28.5%, respectively).

The Algerian *E. triquetrum* essential oil from the aerial parts has a interesting antibacterial activity against *S. aureus* and *P. aeruginosa* according to disc diffusion assays and the MICs of were measured at 0.125 mg/mL for *L. monocytogenes*, *S. aureus, P. aeruginosa* and *K. pneumoniae*. Essential oil shows strong antioxidant activity with IC_{50}= 28.68 µg/mL, close to the reference BHT (IC_{50}=17.90 µg/ml). These activities could be attributed at falcarinol, the ultra-dominant component of the essential oil tested. This C-17 bisacetylenic alcohol is considered as a natural pesticide that contribute to protect naturally the plant such as carrot in which is present [1].

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Effect of drying on the volatile profile of *Morus alba* L. and *M. nigra* L. leaves

Vojkan M. Miljković[1], Marko M. Mladenović[2], Goran Nikolić[1], Niko S. Radulović[2]

[1]*Faculty of Technology, University of Niš, Bulevar Oslobodjenja 124, Leskovac, Serbia.*
[2]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia.*
Corresponding authors: VM vojkanmm_serbia@yahoo.com, NR nikoradulovic@yahoo.com

Keywords: *Morus alba*, *Morus nigra*, storage, bovolide, metabolomics.

The genus *Morus* L. (Moraceae), commonly known as mulberries, comprises about 16 species of deciduous, wild-growing/cultivated trees that are spread across many temperate world regions, especially in Northern hemisphere. *Morus alba* L. (white mulberry or 'beli dud' in Serbian) is a short-living, fast-growing, small to medium-sized tree. Today, white mulberry, native to China, is a worldwide naturalized species cultivated to feed the silkworms in the commercial production of silk. *Morus nigra* L., known as black mulberry or 'crni dud' in Serbian, is a flowering, native to Asia (Iran, Afghanistan) deciduous tree with richly flavored, dark purple fruits. To the best of our knowledge, there are limited data of the composition of *M. alba* leaves essential oil and no previous studies of essential-oil constituents of *M. nigra* leaves.

Detailed GC and GC/MS analyses of the essential-oil samples obtained by hydrodistillation of *M. alba* L. and *M. nigra* L. leaves (four samples) allowed the identification of 131 constituents of essential oils that represented 95.1 – 96.4% of the total GC peak areas. The most abundant compounds classes were alkanes, diterpenoids, carotenoid derivatives and fatty acid-related constituents with *trans*-phytol (7.9 – 71.2%), (*E*,*E*)-geranyl linalool (0.2 – 8.0%), bovolide (8.1%) and *n*-chain alkanes (tricosane (2.0 – 2.8%), pentacosane (2.4 – 10.9%), heptacosane (1.9 – 7.9%), nonacosane (3.4 – 12.4%) and hentriacontane (2.4 – 12.4%)) as the major constituents of the analyzed essential-oil samples.

In general, there were only quantitative differences noted between *M. nigra* essential oils from fresh and dry leaves. The most discernable changes included a variation of the content of the major constituents (e.g. the relative amount of *trans*-phytol decreased for 5.8%, while that of (*E*,*E*)-geranyl linalool and the total alkanes increased for 7.8% and ≈ 1%, respectively). On the other hand, the composition of *M. alba* essential oil was much significantly affected by the drying process. The highest quantitative differences were noted for *trans*-phytol, geranyl acetone, and all isomers of 4,6,8-megastigmatrien-3-one. Also, a rare plant metabolite bovolide, characteristic of leaf senescence, was only identified (8.1%) in the essential oil of *M. alba* dry leaves.

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Metabolites of two essential-oil constituents, methyl and isopropyl *N*-methylanthranilates, in rat urine

Ana B. Miltojević[1,2], Niko S. Radulović[2], Nikola M. Stojanović[3],
Pavle J. Ranđelović[3], Marina T. Stojanović[1]

[1] *Faculty of Occupational Safety, University of Niš, Čarnojevićeva 10a, 18000 Niš, Serbia.*
[2] *Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš.*
[3] *Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000, Niš, Serbia.*
[3] *Department of Physiology, Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000, Niš, Serbia.*
Corresponding authors: AM: anamiltojevic@yahoo.com, NR: nikoradulovic@yahoo.com

Keywords: *Choisya ternata* Kunth, methyl and isopropyl *N*-methylanthranilates, metabolism, Sephadex LH-20 column chromatography.

A new alkaloid, isopropyl *N*-methylanthranilate (IMA), and a related one, methyl *N*-methylanthranilate (MMA), have been recently identified from the essential oil of *Choisya ternata* Kunth (Rutaceae) [1]. These volatile *N*-methylanthranilic acid esters have been proven to possess a spectrum of pharmacological activities, including antinociceptive, gastro-, hepato- and nephroprotective activities, anxiolytic and antidepressant properties, as well as a prolongation of the diazepam-induced sleep [2]. Herein we report on the identification of the metabolites of IMA and MMA in the urine of rats pretreated with these two substances (200 mg/kg, *i.p*, 7 days). Pure urine metabolites of the two esters were isolated after Sephadex LH-20 column chromatography of a diethyl-ether extract of the urine of the treated rats. The obtained fractions were analyzed by NMR, GC-MS, FTIR and UV methods. Products of hydroxylation, isopropyl 5-hydroxy-*N*-methylanthranilate, isopropyl 5-hydroxy-anthranilate and isopropyl 3-hydroxy-anthranilate, were identified as the principal urine metabolites of IMA. On the other hand, two major metabolites detected in the urine of rats pretreated with MMA were 2-(methylamino)benzamide, a product of amonolysis, and *N*-methylanthranilic acid, a product of hydrolysis of the ester group. It appears that in the case of the isopropyl ester of *N*-methylanthranilic acid, the predominant metabolic pathway was hydroxylation, probably due to a steric hindrance imposed on the ester carbonyl by the isopropyl group, while in the case of the methyl ester the principal pathway was the hydrolysis of the ester functional group.

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Quantitative structure–property relationship modeling of GC retention indices of organosulfur compounds

Ana B. Miltojević[1,2], Niko S. Radulović[2], Polina D. Blagojević[2],
Tatjana D. Golubović[1]

[1]*Faculty of Occupational Safety, University of Niš, Čarnojevića 10A, 18000 Niš, Serbia.*
[2]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia.*
Corresponding authors: AM: anamiltojevic@yahoo.com, NR: nikoradulovic@yahoo.com

Keywords: *Allium ursinum* L., QSPR modeling of GC retention indices, organo(poly)sulfides.

Retention indices (RI) serve as one of the important criteria for the identification of constituents of essential oils analyzed by GC and GC/MS. However, the commercially available databases of RI values are naturally limited to a set of several hundred entries. Researchers are often faced with a situation that there is no RI data reported in the literature for the compound of interest. Organosulfur compounds represent an especially problematic class of volatiles in this sense. In this situation, QSPR (quantitative structure–property relationship) modelling of RI values could be the solution. 0D/1D descriptors are directly available from the molecular structure without the usage of any specialized software. In this work, we used these simple structural descriptors for QSPR modelling of RI values of alk(en)yl (poly)sulfides ($R1(S)_nR2$). For this purpose, several series of alk(en)yl (poly)sulfides were prepared and their experimental RIs determined on a DB-1 column. QSPR modeling of RIs was performed using the following descriptors: the total number of C-atoms (C), number of C-atoms in each of the alk(en)yl chains (R1 and R2), the total number of C-C double bonds (pi), the total number of (*E*)- and (*Z*)-C-C double bonds (E and Z), the total number of S atoms (S), the total number of C-S single bonds (C-S), the total number of branches and/or the total number of –SR' substituents of R1/R2 at positions 1, 2 or 3 from the C-chain terminus (Branch-1 to Branch-3). Regression analysis (correspondence of experimental and calculated RIs) showed that the QSPR equation $RI = 24.2 + 86.9[R1] + 102.2[R2] + 221.0[S] + 27.9[C\text{-}S] - 15.9[pi] + 21.8[E] + 19.4[Z] - 48.8[Branch\text{-}2] - 69.1[Branch\text{-}3]$ has excellent predictive power ($RMSE<6$ and $r^2=0.999$). This model was applied for the analysis of 12 different essential-oil samples hydrodistilled from fresh/dried aerial parts and fresh inflorescence of 5 wild-growing populations of *Allium ursinum* L. (Liliaceae) in which we have detected a large number of homologous/isomeric organosulfur compounds. This approach (a combination of GC/MS, QSPR and synthesis) allowed us to successfully identify around 80 different *A. ursinum* sulfur-containing volatiles (more than in any other previous study).

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Vapour phase antimicrobial activity from *Pimenta pseudocaryophyllus* (Gomes) Landrum essential oils

Paulo R. H. Moreno[1], Fábio Fiorucci-Filho[2], Marcos A. A. Pereira[1], Telma M. Kaneko[1], Marcos E. L. Lima[3], Maria Cláudia M. Young[3], Inês Cordeiro[3].

[1]Prog. Pós Grad. Fármacos e Medicamentos, Fac. Ciências Farmacêuticas – USP. Av. professor Lineu Prestes, 580 - CEP 05434-070, São Paulo (SP), Brasil.
[2]Universidade Paulista, Campus Vergueiro, Rua Apeninos 267 – CEP 01533-000, São Paulo (SP), Brasil.
[3]Instituto de Botânica SMA/SP, Av. Miguel Estéfano, 3496 – CEP 04301-902, São Paulo (SP), Brasil.
Corresponding author: prmoreno@iq.usp.br

Keywords: *Pimenta pseudocaryophyllus*, Myrtaceae, antimicrobial, vapour phase, fumigant

Essential oil fumes have been used for their sanitizing properties since ancient times. However, antimicrobial activity is commonly tested with the microorganisms dispersed in a liquid medium. Lately, essential oil vapours gained some interest because they can be an interesting alternative treatment of hospital environment due to their ability in preventing biofilm formation [1]. *Pimenta pseudocaryophyllus* (Gomes) Landrum (Myrtaceae) is a native species that is used for culinary and medicinal purposes whose essential oils have already demonstrated antimicrobial activity [2]. Thus, the aim of the present work was to analyse the *P. pseudocaryophyllus* essential oil composition and the antimicrobial activity of its vapours from plants collected in Cajati (São Paulo, Brazil). The oil was obtained by hydrodistillation for 4 h, and the component identification was performed by GC/MS [3]. The antimicrobial activity of the EO vapours was evaluated by the inverted plate method [4] against *Staphylococcus aureus* (ATCC 25923), using formaldehyde as positive control. The average essential oil yield was 1.2% (w/w), presenting as major components *p*-cymene (19%), eugenol (18%), terpinolene (14%), α-terpinene (14%) and myrcene (9%). The oil vapours were able to inhibit the *S. aureus* growth with Minimum Inhibitory Concentration (MIC) of 285 mg/L while for formaldehyde it was 22.4 µg/L. These results indicated that the oil vapours were active against *S. aureus*. Although they were less active than formaldehyde, the oil vapours have the advantage as sanitizers because they do not require direct application on surfaces, which is suitable for the use as room disinfectants and air decontaminants even in inhabited areas due to their lower toxicity than formaldehyde.

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Acetylcholinesterase inhibition of *Hedyosmum brasiliense* flowers essential oils

Cynthia Murakami[1], Marcos E. L. Lima[1], Rodrigo Sant'Ana Cabral[2], Paulo R. H. Moreno[3], Maria C. M. Young[4]

[1] Pós Graduação em Biodiversidade Vegetal e Meio Ambiente, Instituto de Botânica, São Paulo, Brasil.
[2] Depto de Química, Universidade Federal de São Carlos, São Paulo, Brasil.
[3] Depto de Química Fundamental, Instituto de Química, Universidade de São Paulo, São Paulo, Brasil.
[4] Núcleo de Pesquisa em Fisiologia e Bioquímica, Instituto de Botânica, São Paulo, Brasil.
Corresponding author: cynthia.murakami@uol.com.br

Keywords: *Hedyosmum brasiliense*, acetylcholinesterase inhibition, Brazilian Atlantic Forest.

Hedyosmum brasiliense (Chloranthaceae) is a dioecious shrub commonly used in the Brazilian folk medicine [1]. The aim of this work was to compare the chemical composition and acetylcholinesterase inhibition from the essential oils from *H. brasiliense* flowers collected at Paranapiacaba and Cardoso's Island, two Atlantic Forest reservation areas. The essential oils of fresh flowers of male and female plants were extracted by hydrodistillation and analyzed by GC/MS [2]. Acetylcholinesterase inhibition was tested in microplates by Ellman *et al.* [3] and Rhee *et al.* [4] methods, using preliminary concentration of each essential oil (300 µg/mL) and fisostigmine (1.56 µg/mL) as positive control. Statistical analysis was performed by one-way analysis of variance (ANOVA, $P<0.05$). Cardoso's Island flowers presented the highest essential oil yields (0.38% ♀ and 0.24% ♂) than those from Paranapiacaba (0.20% ♀ and 0.07% ♂). However, Paranapiacaba flower oils presented the highest antiacetylcholinesterase activities, 85% (♀) and 67% (♂), while those from Cardoso's Island presented 42% (♀) and 35% (♂). The essential oil composition was also different for both localities, as Paranapiacaba oils showed predominance of sesquiterpene and monoterpene hydrocarbons, with major compounds (Z)-α-bisabolene (18% ♀ and ♂) and sabinene (11% ♀), and the Cardoso's Island oils contained more monoterpene hydrocarbons and oxygenated sesquiterpenes, having as main components curzerene (12%♀ and 11%♂), carotol (9%♀ and 10%♂) and sabinene (8%♀ and 10%♂). These results suggest that the hydrocarbon terpenes are more efficient in inhibiting the Cholinesterase activity than their oxygenated counterparts.

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Physiological and psychological effects of rose aroma and appearance on human

Nakamura M, Tomi K, Tsuda M, Hayashi T

Faculty of Agriculture, Kindai University, 3327-204, Nakamachi, Nara, Japan.
Corresponding author : tomi@nara.kindai.ac.jp

Keywords: *Rosa damascena*, autonomic nervous system, POMS, physiological effect

Rose essential oil is commonly used for aromatherapy. Several studies have reported physiological effects of rose essential oil: anti-stress [1] and anti-anxiety [2]. In our previous study, it is indicated that the recognition of smelling fresh rose flower itself affects the physiological and psychological effects of rose aroma inhalation on human [3]. In this study, we evaluated the physiological and psychological effects of not only rose aroma inhalation but also rose appearance on human, especially in focusing the synergistic effect of olfactory and visual stimulation.

We used the preserved flower of rose in order to unify the volatile components and flower color and flower form. Fifteen healthy students who do not smoke attended the physiological and psychological measurements. Four samples were prepared as follows: rose essential oil (E), preserved flower of rose (F), combination of rose essential oil and preserved flower of rose (E-F), and pure water (control). The measurements were carried out by recording ECG (electrocardiogram) for 30 min: 10 min for rest time and 20 min for sample presentment. Power spectral analysis on R-R interval of heartbeat was applied for evaluating the physiological effects of the samples on human autonomic nervous activity. POMS（profile of mood state）test was used to reveal the psychological effects of samples on human. Each of the subjects attended 4 times to evaluate all the samples, by randomized order.

Effects of the E-F group's samples on autonomic nervous activity resulted in the significant increase of sympathetic nerve activity ($p<0.05$, in comparison with control, 2-way ANOVA). E and F groups tended to increase sympathetic nervous activity during the measurement time. The parasympathetic nervous activity in the E-F group was significantly lower than in the control group ($p<0.05$, 2-way ANOVA). The significant differences were not observed in the POMS scores. These results imply that the aroma and appearance of rose give physiological effects, but not psychological effects.

In conclusion, the strongest physiological effects were obtained in the presence of both olfactory and visual stimulation. These findings indicate the possibility of utilizing fresh rose flowers for aromatherapy and phytotherapy.

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Chemical composition and *in vitro* antibacterial activity of the essential oils from leaves, resin and stem-bark of *Dacryodes edulis* (G. Don) H. J. Lam

Florentine M-C NDOYE FOE[1], *Sara Honorine RIWOM*[2], *Maximilienne Ascension NYEGUE* [1,2,3], *Reskingjoe AMBE WANKI*[2], *Steve VOUNDI Olugu*[2] *Chantal MENUT and François-Xavier ETOA*[2]
[1]*University of Yaoundé I, Department of Biochemistry, Laboratory of Phytobiochemistry and Medicinal Plant Study, PO Box 812 Yaoundé- Cameroon,*
[2]*University of Yaoundé I, Department of Microbiology, Laboratory of Microbiology, PO Box 812 Yaoundé –Cameroon,*
[3]*Equipe de Recherche "Glyco et Nanovecteurs pour le criblage Thérapeutique"-IBMM, BP 14491 34093 Montpellier-France.*
Florentine M-C. NDOYE FOE: chantfoe@yahoo.com

Keywords: *Dacryodes edulis*, chemical composition, antibacterial activity.

Dacryodes edulis (G. Don) H. J. Lam (*Burseraceae*) is a medicinal plant used traditionally in Africa (Cameroon) in the form of its decoction and steam bath [1]. The essential oils obtained by hydrodistillation of leaves (0,01%), resin (11,47%) and stem-bark (0,07%) harvested in 2013 at Etoug-Ebe (Yaounde-Cameroun), were analyzed by Gas Chromatography (GC) and GC coupled to Mass Spectrometry (GC-MS) and their antimicrobial activity were assessed on *Bacillus cereus*, *Escherichia coli, Staphylococcus aureus*, *Salmonella thyphi,* and *Shigella* spp by Agar well diffusion and microdilution [2]. The resin essential oil contained *p*-cymene (30.32%), *α*-thujene (28.58%), *α*-phellandrene (27.14%) and *β*-phellandrene (10.16%) as the main components; the stem-bark essential oil was characterized by its high percentages of *p*-cymene (35.14%), *trans*-carveol (22.60%), *α*-thujene (14.86%), *β*-phellandrene (8.65%) and *β*-elemene (5.22%). The leaves essential oil differs by its chemical composition, dominated by sesquiterpenic compounds (elemol, 29.22%, caryophyllene oxide, 15.26% and spathulenol, 6.28%) accompanied by *trans*-carveol (11.80%). The leaves essential oil was the most active with MIC and MBC value of 18.75 mg/mL on *Bacillus cereus*, the most susceptible strain. The stem-barks essential oil had a MIC of 50 mg/mL and MBC of 100 mg/mL on *Escherichia coli, Staphylococcus aureus*, *Salmonella thyphi,* and *Shigella* spp meanwhile the resin essential oil had a bacteriostatic effect at 200 mg/mL all the strains. As far as this species is widespread in Africa and considering the large use of its fruits in food, the exploitation of its leaves could reasonably be considered, despite the low yield in essential oil, for their bactericidal activity.

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To dry or not to dry? Significant changes in the chemical composition of the essential oil of *Tripleurospermum inodorum* (L.) Sch.Bip. occur during plant material storage

Milica Nikolić, Niko Radulović, Polina Blagojević

Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, RS-18000 Niš, Serbia
Corresponding author: milica.nikolic.1990@gmail.com

Keywords: *Tripleurospermum inodorum*, matricaria esters, matricaria lactones, (*E*)-β-farnesene, storage, drying of plant material

Although significant changes in essential-oil yield accompany plant material storage, due to, primarily, water evaporation, the changes in the chemical composition are generally not considered to be so extensive. Storage-induced changes of plant volatiles could have an important impact on the quality of the essential oil and, consequentially, on all of the conclusions drawn from the compositional studies (e.g. in chemotaxonomy). Comparative analyses of chemical compositions of essential oils isolated from fresh and dried plant material are rarely performed within a single study. Herein, we report on an extreme instance of such storage-induced changes noted in the case of the essential oils hydrodistilled from fresh and dried aboveground parts of *Tripleurospermum inodorum* (L.) Sch.Bip. (Asteraceae), scentless mayweed, collected from the urban settings of the city of Niš (SE Serbia). Unexpectedly, the yield of the essential oil appeared to decrease during storage (10 days, room temperature, without exposure to direct sunlight), from 0.57% to 0.40% (w/w) from fresh and dried plant material, respectively. GC and GC-MS analyses (including true quantitations using calibration curves for the major constituents) revealed a profound difference in the composition of these essential oils. The major constituent detected in the essential oil from fresh plant material was (*Z,Z*)-matricaria ester (60.6%), whereas (*E*)-β-farnesene (65.8%) was the major component of the essential oil from the dried sample. The relative amount of all other acetylenic volatiles (matricaria and lachnophyllum lactones and esters, and other related compounds) was also significantly lower (*ca.* 10 times) in the dried plant material. Since these changes are not expected to be the consequence of evaporation of either (*Z,Z*)-matricaria ester or (*E*)-β-farnesene (low vapor pressures at room temperature), the acetylenic ester must have undergone a chemical transformation during storage resulting in non-volatile products. This conduct/instability of matricaria esters/lactones was not noted previously.

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Identification of matricaria esters and lactones based on a correlation between GC retention indices and their molecular-level properties

Milica Nikolić, Niko Radulović, Polina Blagojević

Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, RS-18000 Niš, Serbia
Corresponding author: milica.nikolic.1990@gmail.com

Keywords: *Tripleurospermum inodorum*, matricaria esters, matricaria lactones, QSPR

We encountered an essential-oil sample (*Tripleurospermum inodorum* (L.) Sch.Bip.) that contained all possible diastereoisomers of matricaria esters and matricaria lactones. Faced with a general lack of GC retention data for these acetylenic compounds (there is no single study reporting all of them) and an overall inconsistency between the literature data coming from different research groups, we decided to attempt to isolate the pure diastereomers by column chromatography on SiO_2 from the leaves essential oil of the mentioned taxon; however, this was successful in only two cases, (*Z,Z*)-matricaria ester and (*Z,E*)-matricaria lactone. Having their stereochemistry inferred from their NMR spectra, we were still confronted with the problem of the identification of the remaining stereoisomers. For this purpose, a possible correlation between a number of structural descriptors and their retention indices, on a DB-5 column, was considered. The conformation of the 4 ester + 4 lactone isomers was optimized at MM+ level in ChemBio3D Ultra 12.0 (CambridgeSoft). After a number of trail-and-error attempts, where the used descriptors were varied and the peak-to-stereochemistry assignation swapped within a series of isomers, the following descriptors (molecular-level properties) were found to give the best correlation coefficients (R^2=0.9999, for both esters and lactones) during the setup of the structure-retention data model: Connolly accessible area, molecular area and solvent excluded volume, and the distance between the carbonyl oxygen and the farthest carbon atom. When a joined model, that included both esters and lactones, was developed, based on the mentioned four structural descriptors and the corresponding molecular masses, as the fifth, the correlation somewhat deteriorated but remained satisfying (R^2=0.897). Based on the developed QSPR equations, matricaria esters and lactones were found to elute from a GC column in the following order: (*Z,Z*)-matricaria lactone (1463), (*E,Z*)-matricaria lactone (1490), (*E,Z*)-matricaria ester (1513), (*Z,Z*)-matricaria ester (1519), (*Z,E*)-matricaria lactone (1529), (*Z,E*)-matricaria ester (1551), (*E,E*)-matricaria ester (1565) and (*E,E*)-matricaria lactone (1578).

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Chemical composition and in vitro antibacterial activity of the essential oils of *Psidium guajava* (L.) and *Lantana camara* (L.) leaves

Maximilienne Ascension NYEGUE[1,2,3], Gislaine Aurelie KEMEGNE[1], Ghislain SEUYIM KOUOGUEU[1], Sylvain Leroy SADO[1], Florentine M-C NDOYE FOE[3], Jean Justin ESSIA NGANG[1], Chantal MENUT[1] and François-Xavier ETOA[1]

[1] *University of Yaounde I, Department of Microbiology, Laboratory of Microbiology, PO Box 812 Yaounde-Cameroon*

[2] *Equipe de recherche "Glyco et nanovecteurs pour le criblage thérapeutique" Institut des Biomolécules Max Mousseron (IBMM), BP 14491 34093 Montpellier-France*

[3] *University of Yaounde I, Department of Biochemistry, Laboratory of Phytochemistry and Medicinal Plant Study, PO Box 812 Yaounde-Cameroon*

Maximilienne Ascension NYEGUE: maxy_nyegue@yahoo.fr

Keywords: *Psidium guajava* (*Myrtaceae*), *Lantana camara* (*Verbenaceae*), chemical composition, antibacterial activity

Infectious diarrhea is a major world concern and a crucial problem in Africa. In general, for adults we observe 5 to 7 days of sickness while among infants under 5 years, 0.2% of patients death is observed [1]. Among all the plants used to treat diarrhea, leaves of *Psidium* guajava L. and *Lantana camara* L. where selected for extraction of their essential oils by hydrodistillation, determination of their chemical composition (GC-FID and GC-MS) and evaluation of their antibacterial activity (agar disc diffusion and microdilution [2]) on three enteric strains: *Staphylococcus aureus*, *Shigella* and *Escherichia coli*. The major compounds identified (>5%) in *L. camara* essential oil (0.0007% of yield) where (*E*)-nerolidol (26.4%), (*E*)-β-caryophyllene (12.7%), sabinene (8.4%), α-zingiberene (7.4%) and 1,8-cineole (6.9%). (*E*)-β-caryophyllene (26.5%), β-bisabolol (8.9%), benzaldehyde (7.8%), (*E*)-nerolidol (7.2%) and 1,8-cineole (5.8%) were identified in *P. guajava* essential oil (0.09% of yield). *P. guajava* essential oil presented the best bacteriostatic effect on *S. aureus* (MIC=18.75 ppm); for the same strain, MIC= 150 ppm where in the case of *L. camara* essential oil. The two essential oils presented the same activity on *Shigella* and *E.coli*: MIC=150 ppm for *P. guajava* and MIC=600 ppm for *L. camara*. The MBC measurement showed bactericidal effect of *P. guajava* essential oil on *Shigella* at 150 ppm (MBC/MIC=1) as well as *L. camara* essential oil on *E. coli* at 1200 ppm (MBC/MIC=2) [3]. This work demonstrated that these essential oils represent a potential source of antibacterial substances for combating bacteria implicated in diarrheal infection.

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Bioactive constituents, bactericidal and antiradical properties of the leaves and stem essential oil of *Peperomia pellucida* (L.) Kunth

Sunday O Okoh[1, 2*], Benson C Iweriebor [2,] Omobola O Okoh[3], Anthony I Okoh[1, 2]

[1] *SAMRC Microbial Water Quality Monitoring Center, University of Fort Hare, Private mail Bag X1314, Alice 5700, Eastern Cape Province, South Africa.*

[2] *Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, South Africa.*

[3] *Department of Pure and Applied Chemistry, University of Fort Hare, Alice 5700, South Africa.*

[*] *SAMRC Microbial Water Quality Monitoring Center, University of Fort Hare.*

Corresponding author, E-mail: sokoh@ufh.ac.za; alternative E-mail: sunnyokoh2003@yahoo.com

Keywords: *Peperomia pellucida,* bactericidal, antiradical, limonene, cinnamaldehyde, β-caryophyllene

Bioactive properties of the leaf and stem essential oils (EOs) of *Peperomia pellucida* and their potential for the management of oxidative-stress related diseases were studied *in-vitro*. EOs obtained using modified Clevenger apparatus were characterized by GC-MS, while the antiradicals and antibacterial properties investigated by spectrophotometric and agar diffusion techniques respectively. The EOs exhibited strong antibacterial activity against *Escherichia coli*, *Enterococcus faecium* and *Staphylococcus aureus.* The leaves essential oil (LEO) was more active than the stem essential oil (SEO) against test bacteria with minimum inhibitory concentration (MIC) ranging between 0.150 – 0.30 mg/mL and 0.20 - 0.40 mg/mL for SEO. The LEO was bactericidal at 0.25 mg/mL against *E. faecium,* while SEO was bacteriostatic at 0.40 mg/ mL after 24 h. LEO IC_{50} value (2.40 mg/ mL) showed that its antiradical capacity is superior to SEO (3.01 mg/mL) and vitamin C (3.4 mg/ mL) in scavenging $DPPH^{\bullet}$. The EOs reduced other radicals ($LP^{\bullet}$, $ABTS^{\cdot+}$, and $NO^{\bullet}$) in dose dependent-manner. Limonene 14.25, cinnamaldehyde 3.77, cumarin 3.46 %, citronellol 3.40 %, and β-caryophyllene 3.30 %, were the prominent bioactive monoterpene and sesquiterpeniods in both EOs. Findings from this study suggest that apart from the traditional uses of the plant extracts, the EO has strong bioactive compounds, noteworthy antibacterial and antiradical properties and may be good candidates in the search for lead compounds for the synthesis of novel potent antibiotics.

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Geraniol encapsulated in chitosan/gum arabic nanoparticles: preparation and characterization

Oliveira, J. L.[1], Marquardt, L. C.[1], Campos, E. V. R.[1,2], Fraceto, L. F.[1,2]
[1]Departament of Environmental Engineering, São Paulo State University, Sorocaba, SP, Brazil
[2]Departament of Biochemistry, Institute of Biology, Campinas State University,Campinas, SP, Brazil
Corresponding author : jholuisoliveira@hotmail.com

Keywords: Geraniol, Nanoparticles, Chitosan, Gum Arabic

Geraniol (trans 3,7-dimethylocta-2,6-dien-1-ol) is an acyclic monoterpene alcohol. It is a common constituent of many essential oils such as citronella, palmarosa, rose and others. One of its properties is its action as repellent for pest control [1]. The use of this compound in agriculture may be limited, due its high sensitivity to: i) light, ii) humidity, iii) temperature and also by the microorganism degradation. In this way, nanotechnology has shown potential for developing formulations to increase the stability and efficacy of natural products [2]. Many matrices have been used to development of nanostructured system, such as natural polymers. One example is chitosan, which is derived from chitin and is present in the crustacean exoskeleton [3]. The main objective of this study was to prepare and characterize chitosan/gum arabic nanoparticles loaded with geraniol. This is a new method of encapsulation, because it was not found in the literature works that encapsulated geraniol in nanostructured systems based on chitosan and gum arabic. The nanoparticles were prepared based on the method described by [4] with slight modifications. The experimental conditions to nanoparticles preparation was performed using a 2^3 factorial design. It was investigated the influence of chitosan, gum arabic and Tween 80 in the nanoparticles preparation. To analyze the results of the factorial design were investigated the mean size distribution, polydispersity index, zeta potential and encapsulation efficiency of the geranial into the nanoparticles. The physicochemical stability of the formulations was investigated over 120 day. The nanoparticles were characterized by dynamic light scattering and nanoparticles tracking analysis. Geraniol was quantified by high-performance liquid chromatography. The results of the factorial design for chitosan/gum arabic nanoparticles showed that changes in the composition of the nanoparticles can influence significantly the zeta potential and polydispersity index. Based on the factorial design, the best formulation was selected (with higher zeta potential) and the colloidal properties were performed over 120 days. This formulation showed a good physicochemical stability with encapsulation efficiency over 80%, a mean diameter of 250 nm (with a concentration of 3.79×10^{12} particles/mL), polydispersity index of 0.3 and zeta potential of 28 mV. These results showed that this system presented good characteristics to be an alternative for pest control.

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Chemical diversity of *Aster tataricus* volatiles from Mongolia

G. Özek[1], S. Yur[1], S. Shatar[2], A. Altantsetseg[2], K.H.C.Başer[3], T. Özek[1]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskisehir, Turkey.*
[2]*Institute of Chemistry and Chemical Technology of the Mongolian Academy of Science (MAS), 211051, Ulaanbaatar, Mongolia.*
[3]*Department of Pharmacognosy, Faculty of Pharmacy, Near East University, Lefkoşa (Nicosia) N. Cyprus.*
Corresponding author: gozek@anadolu.edu.tr

Keywords: *Aster tataricus*, volatiles, diversity, MSD-SPME.

Aster tataricus Turcz. (Compositae) is native to Southeast Siberia, Northern China, Mongolia, Korea and Japan, where it grows in meadows and wetlands [1].

This study investigates the chemical diversity of *A. tataricus* essential oils and the quantitative changes of main constituents according to different plant organs. The plant materials (leaves, flower, stem and seeds) were collected in Mongolia and subjected separately to hydrodistillation in Clevenger type apparatus to yield essential oils (EOs). GC/MS and GC-FID analyses revealed significant difference in chemical compositions of the EOs of different organs. Hexadecanoic (13.4-58.0 %) acid was detected in all the EOs. Besides, the leaf oil was characterized with phytol (12.6%), hexahydro-farnesylacetone (8.0%), eudesma-4(15),7-dien-1-β-ol (7.4%), neophytadiene isomer I (6.6%) and torilenol (6.0%). The flower oil contained myrcene (7.6%), geranyl acetate (5.5%), and γ-amorphene (5.1%). Tetradecanoic acid (5.4%), geraniol (4.8%), β-pinene (2.6%) and α-pinene (2.5%) were detected in the stem oil, while (Z)-9-hexadecenoic acid (30.5%) was found in the stem oil. In addition, microsteam distillation - solid phase microextraction (MSD-SPME) technique applied to the leaves resulted with rose furan epoxide (20.5%), methyl salicylate (11.1%), isocaryophyllene (8.2%), caryophyllene (8.0%), neophytadiene isomer I (7.5%) and spathulenol (5.4%). MSD-SPME technique enabled of an isolation of the volatiles with PDMS-DVB fiber (blue type) from 0.3 g plant material for short time (3 min) [2].

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Chemical Composition and Antioxidant Activity of *Sium sisarum* L. Essential Oil

Gozde OZTURK[1], Betul DEMIRCI[2], Ahmet DURAN[3], Mustafa ÇELIK[4], Fahim ALTINORDU[5], K. Husnu Can BASER[6]

[1]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskisehir, 26470, Turkey
[2]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskisehir, 26470, Turkey
[3]Selçuk University, Faculty of Science, Department of Biology, Konya, 42075, Turkey
[4]Selçuk University, Faculty of Science, Department of Biology, Konya, 42075, Turkey
[5]Selçuk University, Faculty of Science, Department of Biology, Konya, 42075, Turkey
[6] Near East University, Faculty of Pharmacy, Department of Pharmacognosy, Nicosia, 99138, Cyprus
Corresponding author : g.ozturkau@gmail.com

Keywords: *Sium sisarum* L., gas chromatography, gas chromatography and mass spectrometry, DPPH antioxidant activity.

The genus Sium (Apiaceae) is presented by perennials with fibrous roots, inflorescence paniculate-corymbose, white petals, minute sepals, slightly lateral compressed fruits in Flora of Turkey [1]. The essential oils of fruits and aerial parts of *Sium sisarum* L. collected from two different localities Kavak and Ladik in Samsun, Turkey, were obtained by hydrodistillation. The essential oils were coded as EO1 (fruit, Kavak), EO2 (aerial parts, Kavak), EO3 (fruit, Ladik), EO4 (aerial parts, Ladik). All samples were analyzed by gas chromatography (GC) and gas chromatography and mass spectrometry (GC/MS), simultaneously. *γ*-terpinene (71.8, 64.2, 42.3, 38.5 %) and *p*-cymene (16.4, 23.4, 33.8, 33 %) were found as major constituents of all samples. *β*-pinene (7.7 %) and limonene (8.3 %) were identified as a major components for EO3. Also, *β*-pinene (4.0 %), limonene (10.0 %) and ar-curcumene (2.0 %) were found as a major components for EO4. Additionally, the quantitive study of DPPH was conducted to determine the antioxidant activity. UV absorbance was read at 517 nm at room temperature using a microplate spectrophotometer. 50% inhibitory concentration values (IC_{50}) were calculated. According to the results, antioxidant activity of essential oils were determined as weak. ($IC_{50} > 30$ mg/ml).

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Volatile Constituents of the Corsican liverwort *Frullania tamarisci*

Anaïs Pannequin[1], Aura Tintaru[2], Jean-Marie Desjobert[1], Jean Costa[1] and Alain Muselli[1]

[1]*Université de Corse, UMR CNRS 6134, Laboratoire Chimie des Produits Naturels, BP 52, 20250 Corte, France*
[2]*Université Aix-Marseille, UMR CNRS 7272, Equipe SACS, Campus Saint Jérôme, 13013 Marseille, France*

Keywords: *Frullania tamarisci*, Hydrosol, SPME, Sesquiterpene lactones.

F. tamarisci is a liverwort living wild on the rocks of deciduous broadleaved forests of Corsica [1]. To our knowledge, only one study deals with the chemical compositions of *F. tamarisci* essential oils from USA and from Sweden [2]. Tamariscol and some hydrocarbon sesquiterpenes, as pacifigorgiane derivatives, were identified. Except for tamariscol (56%), no quantitative data were reported for the other constituents.

The volatile components of the Corsican *F. tamarisci* essential oil (EO) and hydrosol (HY) obtained by hydrodistillation (HD) and the volatile fraction (FV) concentred by HS-SPME were investigated using an analytical procedure including LC, LLE, GC-FID, GC-MS and Carbon-13 NMR spectroscopy.

The main components of *F. tamarisci* essential oil were tamariscol (30.4 %), γ-cyclocostunolide (20.1%) and (4S,7R)-germacra-(1(10)E,5E)-dien-11-ol (3.6%). The main components of the extract obtained by LLE from the *F. tamarisci* hydrosol were γ-cyclocostunolide (36.6%) and tamariscol (11.0 %). The main compounds identified in the volatile fraction obtained by HS-SPME were tamariscol (31.7%), pacifigorgia-1,10-diene (12.6%), pacifigorgia-2,10-diene (11.6%) and 1-octen-3-yl propionate (5.1%).

As tamariscol is a taxonomical marker for the systematics of *Frullania* genus, Corsican liverwort *F. tamarisci* is original with the occurrence of (4S,7R)-germacra-(1(10)E,5E)-dien-11-ol and 1-octen-3-yl propionate which were, to the best of our knowledge, never reported in the genus *Frullania*.

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Zein nanoparticles loaded Citronellal for future agriculture applications

Pascoli, M.[1,2], Oliveira, J. L.[1], Lima, R.[2], Fraceto, L. F.[1]

[1]Departament of Environmental Engineering, São Paulo State University, Sorocaba, SP, Brazil
[2]Departament of Biothecnology, Sorocaba University, Sorocaba, SP, Brazil
Corresponding author : mo.pascoli@msn.com

Keywords: *Cymbopogon nardus*, Citronellal, Zein Nanoparticles.

World food production has suffered the great loss caused by the insects and pests action. In order to avoid losses, the producers has been used a large amount of synthetic pesticides. But excessive use these compounds have caused health problems for farmers and environmental contamination [1]. In this context, the use of natural compounds in agriculture has gained prominence due their low harmful effects. Citronellal or rhodinal (3,7-dimetiloct-6-en-1-al) is a monoterpene and is the main constituent of Citronella essential oil (*Cymbopogon nardus*) [2]. Nanotechnology has demonstrated potential for the development of formulations capable of increasing the stability and efficiency of natural products. Proteins have been used to development of nanostructured system, zein is the major storage protein of maize and can develop different systems [3]. The main objective of this study was to prepare and characterize zein nanoparticles loaded with citronellal in chiral form (R)(+) citronellal and (S)(+) citronellal. The zein nanoparticles were prepared according to the method of antisolvent precipitation, described by Hu & McClements [4] with slight modifications. Characterization and stability (120 days) of formulations were analyzed by different techniques: Dynamic light scattering (average size and polydispersity); Nanoparticles tracking analysis (concentration and size distribution); Microelectrophoresis (zeta potential); Ultrafiltration/centrifugation (encapsulation efficiency). The formulation loaded R-Citronelal and S-Citronelal showed a mean diameter of 170 nm and 210 nm, respectively, and increase in their size were observed along 120 days. Both formulations presented polydispersity index of around 0.3 with increased over the stability period (120 days). The zeta potential of the formulations was negative (about -37 mV). The nanoparticle tracking analysis showed a smaller mean diameter from the nanoparticles (around 156 nm) for both formulations and a mean concentration of 1.1 x10^{12} particles/mL. Essential oils (R and S citronellal) showed good encapsulation efficiency in zein nanoparticles with values higher than 97%. These results open perspectives for the use of nanotechnology formulations containing citronellal in agriculture.

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Chemical composition and antinociceptive activity of *Stevia serrata* Cav. from a Guatemalan population

Francisco Pérez-Sabino[1], Max Mérida-Reyes[1], Edwin A. Taracena[1], Manuel A. Muñoz[1], Bessie Oliva[1], José Vicente Martínez[1], Millena S. Cordeiro[2], Thais B. S. Giorno[2], Patricia D. Fernandes[2], Daniel L.R. Simas, Antonio J.R. da Silva[3]

[1]Facultad de Ciencias Químicas y Farmacia, Universidad de San Carlos de Guatemala, EdificioT-12, zona 12, Guatemala City, Guatemala. 01012.
[2]Facultad de Agronomía, USAC, Edificio T-9, zona 12, Guatemala City, Guatemala.01012.
[3]Instituto de Ciências Biomédicas, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Bloco J, Ilha do Fundão, Rio de Janeiro, RJ Brazil, CEP 21941-902
[4]Instituto de Pesquisas de Produtos Naturais, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Bloco H, Ilha do Fundão, Rio de Janeiro, RJ Brazil, CEP 21941-590
Corresponding author: fpsabino@usac.edu.gt

Keywords: Antinociceptive activity, chamazulene, *Stevia serrata.*

Stevia serrata Cav. (Asteraceae) grows in various habitats from Yucca-Opuntia scrub to pine forests usually over 1500 m, from southern Arizona to Ecuador. In Guatemala it is found at the western highlands as perennial herbs to 8 dm tall [1]. The aerial parts of *S. serrata* were collected in September 2014, from a population at the province of Sololá at 2341 m, 146 km west from Guatemala City. A deep blue oil was obtained by hydrodistillation (yield: 0.2% w/w). The oil, analyzed by GC-FID and GC/MS, showed a high content of sesquiterpenes, with chamazulene (60.1%), a deep blue substance and already found in *S. serrata* from Mexico [2], as the major component. The oil was administered as gavage to female Swiss Webster mice at doses of 10, 30, 100 mg/kg in a final volume 0.1 mL. The control group was composed by vehicle (oil). In the formalin test, the mice received an injection of 20 µL of formalin (2.5% v/v) into the left hind paw, and the time that the animal spent licking the injected paw was recorded. The nociceptive and inflammatory response consists of two phases: the first phase lasts until 5 min after the formalin injection (neurogenic pain response), and the second phase occurs 15–30 min after the formalin injection (inflammatory pain response). The animals were pre-treated with oral doses of essential oil 60 min before the administration of formalin. The oral administration of *S. serrata* Cav. essential oil produced a marked antinociceptive activity. The results enabled us to ascertain that the tested *S. serrata* Cav. essential oil has a significant impact on both characteristic phases of its response.

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Esters of (irregular) monoterpenols, a hallmark of *Artemisia absinthium* L. root essential oil

Marko S. Pešić, Polina D. Blagojević, Niko S. Radulović

Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, Niš, Serbia
Corresponding author: marko.pesic89@yahoo.com

Keywords: *Artemisia absinthium* L., monoterpenol esters.

Artemisia absinthium L. (Asteraceae; wormwood) is an economically important, highly aromatic plant species, utilized in foodstuff (e.g. preparation of absinth and related beverages). Antihelmintic, stomachic, antibacterial, antifeedant, antifertility, antipyretic, cytostatic, antitumor and antimalarial properties of its (volatile) constituents are known/utilized from the ancient times. Thus, it is not surprising that this species was and still is extensively studied. For example, almost 100 different reports on *A. absinthium* essential oil (EO) (aboveground parts; SciFinder search of the CAS database) were published in the last 5 years, confirming the relatively high variability of wormwood volatile profile (existence of different chemotypes). Surprisingly, the corresponding root essential oil was investigated on only few occasions [1,2]. Therefore, the aim of this study was to reinvestigate the EO obtained from the roots of *A. absinthium*, to probe its variability and to try to detect and identify not only the major root volatiles, but also those present in trace amounts. In order to do this, GC and GC/MS analyses of *A. absinthium* (population from SE Serbia) root EO was combined with "dry flash" chromatography (DFC), which enabled a successful detection and identification of c.a. 150 different constituents (significantly more than in any of the previous studies) divided in 8 DFC fractions of different polarity; many of these were present in low relative amount (less than 0.05% of the total EO). The dominant EO constituents were α-fenchene (13.9%), β-myrcene (9.0%) and bornyl acetate (9.0%). Nonetheless, lavandulol and its esters (acetate, propanoate, butanoate, isobutanoate, valerate, isovalerate and 2-methylbutanoate) comprised 23.0% of the EO. Citronellol, geraniol, nerol, fragranol and their esters made up for an additional 12.0% of the sample. As some of these seem to be new compounds (no MS/RI data available in the literature), to confirm the identity of the alcohol moieties of the esters (pure standards available for co-injection), both the EO and its esters-containing DFC fractions were subjected to transesterification using sodium methoxide; the resulting mixtures were re-analyzed using GC and GC/MS. Previous studies also reported monoterpenol esters as the hallmark of *A. absinthium* root oil. However, to the best of our knowledge, this is the very first time (esters of) irregular lavandulol and fragranol were found in *A. absinthium* root EO.

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Conformational analysis and molecular docking-based prediction of olfactory properties of homologues of *endo*-fenchyl acetate

Marko S. Pešić, Polina D. Blagojević, Niko S. Radulović

Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, Niš, Serbia
Corresponding author: marko.pesic89@yahoo.com

Keywords: *endo*-fenchyl acetate, olfactory properties, molecular docking, conformational analysis.

Monoterpenol *endo*-fenchol (F) and its acetate (FA) can be found in the essential oils of a number of plant species belonging to different plant genera/families (*Alpinia* Roxb. (Zingiberaceae), *Lavandula* L., *Sideritis* L. (Lamiaceae), *Chaerophyllum* L. *Foeniculum* Mill. (Apiaceae), *Helichrysum* Mill. (Asteraceae), *Eucalyptus* L'Her (Myrtaceae) etc.). Both F and FA are used as flavoring agents and are included in the FEMA (Flavor & Extract Manufacturers Association) list of GRAS (generally recognized as safe) substances. Despite their high structural resemblance, their flavor characteristics are different: while *endo*-fenchol has camphor-like odor with citrus notes and a bitter, lime-like flavor, its acetate has a mild, sweet odor reminiscent of fir-needle oil [1]. In order to rationalize these differences, and try to predict olfactory properties of several other FA homologues (formate, propanoate, butanoate, isobutanoate, pentanoate, isopentanoate, 2-methylbutanoate and tiglate), we decided to compare the outcomes of the molecular docking of these (potential) odorants into several homology models of mammalian olfactory receptors, based on the six three-dimensional structures of G protein-coupled receptors [2]. To do this, we first performed a search of the conformational space of the selected (new) *endo*-fenchyl esters (these were synthesized by employing the Steglich procedure), including FA. This was done by using a combination of experimental and theoretical tools (1D and 2D NMR analysis, ^{1}H NMR iterative full spin analysisand DFT molecular modeling). The minimum geometries (those with the C=O moiety oriented towards bridging methylene group) of the studied esters were docked into the selected homology models using *AutoDock Vina* program (blind, flexible docking) [3]. The differences in the most favorable orientations of the studied ligands and in the values of the predicted binding energies suggested that by changing the length or available degrees of freedom of the acid part of the molecule, one could finely tune olfactory properties of *endo*-fenchyl esters and thus create new molecules with desired characteristics.

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Effect of Colored Shade Nets and intensity of light in the essential oil content and composition in leaves and roots of *Piper aduncum* L.

José Eduardo Brasil P. Pinto, Fernanda V. Pacheco, Rafaella de P. Avelar, Ivan Caldeira A. Alvarenga, Suzan Kelly V. Bertolucci, Amauri A. de Alvarenga

Federal University of Lavras (UFLA), Agriculture Department, Mailbox: 3037, 37.200-000, Lavras - MG, Brazil.
Corresponding author :jeduardo@dag.ufla.br

Keywords: irradiances, medicinal plant, apiole, *E*- nerolidol, environment

Light is both the source of energy and a major regulatory factor in plant life. And the responses depend on both the quantity and quality of light. Thus, the purpose of the study was to evaluate the influence of different colored shade nets and intensities in the essential oil content and composition in leaves and roots of *Piper aduncum* L. Plants were cultivated for 150 days under ChromatiNet 50% red or blue netting and under full sunlight (100%), 30% (70% shade) and 50% (50% shade). The essential oils was obtained by hydrodistillation with Clevenger-type apparatus for 90 min from leaves and roots and analyzed by GC / MS. Roots showed higher amount of essential oil (0.11% w/w), which had no influence on light conditions. On leaves the highest oil content was observed in 50% of irradiance (0.085% w/w). The essential oil of leaves was characterized by a high content of sesquiterpenes and roots of phenylpropanoids. The leaves is rich in *E*-nerolidol, linalol (14.28-16.65%); α-humulene (8.45-10.62%); cis-cadin-4-en-7-ol (7.48-12.24%) and caryophyllene (7.93-12.24%). While the root is rich in apiol (16.27-29.51%). The compositions of the oils varied according to the quality and intensity of light. Most major compounds increased in the environment where the plants grown under blue ChromatiNet. And the production of *P. aduncum* L. essential oil depends on the light conditions and can be related to phenotypic and genetic factors. Therefore control of the light environment in growing conditions makes it necessary for obtaining raw material quality.

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Insecticidal activity and essential oil composition of *Ruta chalepensis* L.

Kaan Polatoğlu[1], Hüseyin Servi[2], Ömer Cem Karakoç[3], Yasemin Yücel Yücel[4], Salih Gücel[5], Ayşe Nalbantsoy[6]

[1]Istanbul Kemerburgaz University, Faculty of Pharmacy, Department of Analytical Chemistry.
[2]Yıldız Technical University, Graduate School of Natural & Applied Sciences.
[3]Çankırı Karatekin University, Yapraklı Vocational School, Department of Crop and Animal Protection.
[4]Istanbul Kemerburgaz University, Faculty of Pharmacy, Department of Biochemistry.
[5]Near East University, Institute of Environmental Sciences.
[6]Ege University, Faculty of Engineering, Department of Bioengineering.
Corresponding author: kaan.polatoglu@kemerburgaz.edu.tr

Keywords: Rutaceae, *Ruta chalepensis,* postharvest insects, insecticidal activity, AChE & BChE inhibition.

Previously essential oil composition of *R. chalepensis* was reported to contain 2-undecanone, 2-nonanone, 2-nonyl acetate and similar ketones [1,2]. Insecticidal activity of *R. chalepensis* oils were also reported for different insects [3]. However there is no comprehensive report on the insecticidal activity of *R. chalepensis* against post-harvest pest. Here we present the compositon and insecticidal activity of essential oil of aerial parts of *R. chalepensis* from Cyprus. The essential oil yield was 1.27% (v/w). The essential oil composition was investigated by GC/MS analysis which is replicated three times. The main components of the oil were 2-undecanone (21.52 ± 0.21%), 2-nonanone (18.31 ± 0.27%), 2-nonyl acetate (13.22 ± 0.19%) and pregeijerene (3.60 ± 0.09%). The fumigant and contact toxicity of the essential oil was tested against post-harvest insects including *Sitophilus granarius, Sitophilus oryzae, Tribolium castaneum, Tribolium confusum, Rhyzopertha dominica* and *Oryzaephilus surinamensis*. Highest fumigant activity (insect mortality after 48h, 1µL oil/10 mL) was observed against *S. granarius* (100.00 ± 0.00%) and *T. castaneum* (80,69 ± 1,66%). Highest contact toxicity was (24 h, LD_{50} µL/insect) observed against *R. dominica* (0.018 µL/insect). The insecticidal activity usually manifest itself on the cholinergic system; because of this, acetylcholinesterase and butyrylcholinesterase inhibitory effects of the oil were also evaluated. The AChE and BChE inhibitory effect (at 10 mg/mL concentration) of the oil were 5.29 ± 1.20% and 42.55 ± 0.71% respectively.

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Essential oil composition of *Salvia candidissima* VAHL., *S. tomentosa* MILLER. and *Salvia heldreichiana* BOISS. EX BENTHAM from Turkey.

Kaan Polatoğlu[1], Hüseyin Servi[2], Hilal Bardakçı[3]

[1]*İstanbul Kemerburgaz University, Faculty of Pharmacy, Department of Analytical Chemistry.*
[2]*Yıldız Technical University, Graduate School of Natural & Applied Sciences.*
[3]*Acıbadem University, Faculty of Pharmacy, Department of Pharmaceutical Botany.*
Corresponding author: kaan.polatoglu@kemerburgaz.edu.tr

Keywords: Lamiaceae, *Salvia candidissima, Salvia tomentosa*, *Salvia heldreichiana*, Sclareol, Borneol, α-pinene.

Previously *S. candidissima* from Greece was investigated and main components were reported as α-pinene and 1,8-cineole [1]. Essential oil (EO) of *S. tomentosa* and *S. heldreichiana* from Turkey reported to have 1,8-cineole, cyclofenchene, δ-cadinene and linalool, α-pinene, 1,8-cineol, borneol main components respectively [2,3]. EO compositions of *S. candidissima* Vahl. ssp. *candidissima* Vahl., *S. tomentosa* Miller., S. *heldreichianna* Boiss. Ex Bentham. from Turkey were investigated. The EO yields were 0.05%, 0.26% and 0.06% (v/w) respectively. The EO compositions were determined with GC/MS analysis. The relative percentage of the components were obtained by the integration of the GC/MS peaks. Identification of EO components were done by comparison of their retention times with authentic samples or by comparison of their RRI to a series of *n*-alkanes. Computer matching against commercial MS databases (Wiley 8th Ed./NIST 05, Adams Essential Oil, Pallisade 600K Complete Mass Spectra libraries). Main components of *S. candidissima* ssp. *candidissima* EO were sclareol 15.9 %; hexadecanoic acid 4.3%; *m*-cymene 3.5%; 1,3-*epi*-manoyl oxide 3.4%; sclareoloxide 3.2%; caryophyllene oxide 3.1%; α-selinene 3.0%; manool 2.7%; manool oxide 2.6%; linalool 2.3%; bornylacetate 2.2%; β-caryophyllene 2.1%. Main components of *S. tomentosa* EO were α-pinene 15.3%; borneol 7.8%; δ-cadinene 4.0%; β-myrcene 3.2%; camphor 2.9%; 1,8-cineol 2.7%; calamenene 2.5%; spathulenol 2.4%; α-copaene 2.2%; γ-cadinene 2.1%; α-calacorene 2.1%. Main components of *S. heldreichianna* EO were α-pinene 9.0%; borneol 8.5%; caryophyllene oxide 6.9%; terpinen-4-ol 4.7%; α-cadinol 3.6%; α-terpineol 3.3%; camphor 2.4%; spathulenol 2.3%;1,8-cineol 2.2%; β-pinene 2.1%; hexadecanoic acid 2.0%. The main components of the investigated *Salvia* essential oils differed from the previously reported oils considerably.

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Essential oil composition of *Arabis* species from Cyprus

Kaan Polatoğlu[1], Hüseyin Servi[2], Özge Özçınar[3], Salih Gücel[5], Ayşe Nalbantsoy[6]

[1]İstanbul Kemerburgaz University, Faculty of Pharmacy, Department of Analytical Chemistry.
[2]Yıldız Technical University, Graduate School of Natural & Applied Sciences.
[3]Ege University, Faculty of Pharmacy, Department of Pharmacognosy.
[5]Near East University, Institute of Environmental Sciences.
[6]Ege University, Faculty of Engineering, Department of Bioengineering.
Corresponding author: kaan.polatoglu@kemerburgaz.edu.tr

Keywords: Brassicaceae, *Arabis purpurea, Arabis cypria*, nonacosane, hexahydrofarnesyl acetone.

There are very few reports on the essential oil composition of the *Arabis* L. (Brassicaceae) species in the literature. In our phytochemical screening study of Cypriot plants here we present essential oil composition of two endemic *Arabis* species from Cyprus. The essential oils of aerial parts of *Arabis purpurea* Sm. and *Arabia cypria* Holmboe. afforded very low oil yields (0.01> yield v/w). The essential oil compositions of these plants were analyzed by GC/MS with three replications. Results are given as mean ± standard deviation. The relative percentage of the components were obtained by the integration of the GC/MS peaks. Identification of EO components were done by comparison of their retention times with authentic samples or by comparison of their RRI to a series of *n*-alkanes. Computer matching against commercial MS databases (Wiley 8th Ed./NIST 05, Adams Essential Oil, Pallisade 600K Complete Mass Spectra libraries). Sixty six compounds were identified in the essential oil of *A. purpurea* that represent 82.75 ± 0.21 % ($n = 3$) of the oil. The major components of the oil were nonacosane 16.18 ± 0.13 %, heptacosane 14.91 ± 0.17 %, hexahydrofarnesyl acetone 12.44 ± 0.10 % and phytol 7.36 ± 0.10 % ($n = 3$). Forty three compounds were identified in the essential oil of *A. cypria* which represent 81.28 ± 1.55 % ($n = 3$) of the oil. The major components of the oil were nonacosane 20.25 ± 0.47 %, heptacosane 9.13 ± 1.88 %, hexahydrofarnesyl acetone 9.03 ± 0.44 % and 1-tetradecanol 4.38 ± 2.60 % ($n = 3$). To the best of our knowledge this is the first report on the essential oil compositions of these species.

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Inhibition of *Chromobacterium violaceum* Quorum Sensing by Corsican essential oils

Jean-Pierre Poli[1], Elodie Guinoiseau[1], Anne Lucciani[1], Yann Quilichini[2], Liliane Berti[1], Vannina Lorenzi[1]

[1]CNRS, UMR 6134 SPE, laboratoire de Biochimie et de Biologie Moléculaire du végétal, 20250 Corte, France.
[2] CNRS, UMR 6134 SPE, laboratoire « Parasites et Ecosystèmes Méditerranéens », 20250, Corte, France
Corresponding author : poli_jp@univ-corse.fr

Keywords: Quorum sensing, *Chromobacterium violaceum*, Biofilms, acyl homoserine lactone.

Nowadays antibiotics resistance causes major public health issues, that's why research is focusing on new ways to suppress bacterial virulence. To grow and survive bacteria can form biofilms, which are heap structured with bacterial cells coated by a polymeric matrix and attached to a biological (intestinal mucous, teeth) or inert surface (medical implants, rocks). Biofilms formation is subjected to a regulation mechanism called Quorum Sensing (QS). It allows a real "communication" between bacterial cells *via* the secretion of specific molecules known as "autoinducers". At a given bacterial concentration these molecules are going to lead to the expression of some genetic factors (biofilms, virulence factors, bioluminescence...).

The aim of this work was to identify in essential oils some molecules able to inhibit the mechanism of QS. The strain used in this study, *Chromobacterium violaceum* (CIP 103350), is a Gram negative bacteria able to synthetize a purple pigment (violacein) and also to form biofilm under the control of the QS.

A screening of 10 essential oils was made to select the most active ones. For that purpose we determine the minimal QS inhibiting concentration (MQSIC) corresponding to the inhibition of 50% of the violacein production without killing bacteria. Essential oils of *Inula graveolens* and *Eucalyptus polybractea* respectively showed MQSIC of $2{,}5.10^{-3}$ % and 5.10^{-3} %, they are the most active among all essential oils tested. Those results were confirmed by scanning electron microscopy observations. Indeed we can see strong morphological changes of the biofilms (matrix deterioration). Although used at sublethal concentrations, essential oils seem to also exert an action on planktonic bacteria altering their surface.

Taken together, those results highlight the interest of essential oils in QS inhibition. For this reason, an in-depth study is required to investigate the activity of other Corsican essential oils and to identify the active molecules.

Synthesis of (8S)-(–)-p-Mentha-1,3-dien-9-ol, a new odorous monoterpene alcohol

Georges Radoias, Alin Bosilcov

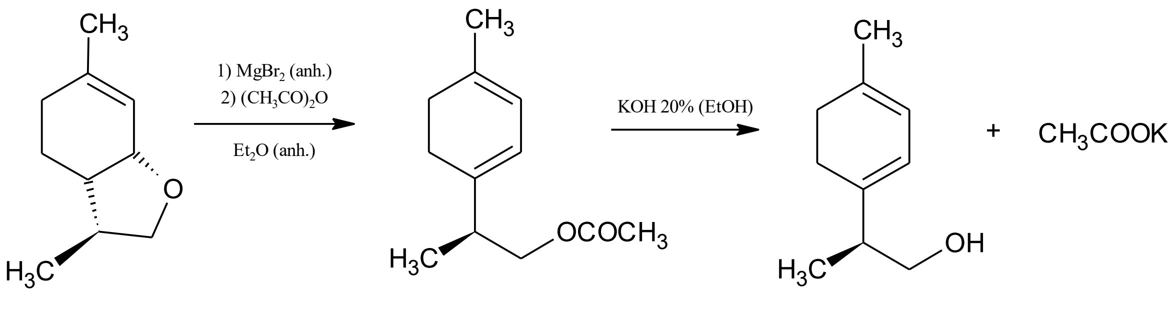
Brüder Unterweger GmbH, Thal-Aue 13, A-9911 Thal-Assling, Austria
Corresponding author : alin.bosilcov@unterweger-oils.com

Keywords: p-mentha-1,3-dien-9-ol, p-mentha-1,3-dien-9-yl acetate, dill ether, dill weed oil, *Anethum graveolens*

Surprisingly, the monoterpenoid p-mentha-1,3-dien-9-ol **1** was never mentioned as a component of any essential oil, being reported only once as a volatile constituent from the nectar of linden blossoms (*Tilia cordata* Mill.) [1].

In the present work, enantiomerically pure (8S)-(–)- p-mentha-1,3-dien-9-ol **1** was synthesized for the first time, via its acetate **3** using dill ether **2** as the starting material. Compound **2** is a common constituent (up to 30%) of dill weed oil (*Anethum graveolens* L.) from which it has been isolated with more than 98% purity by means of fractional distillation. It is known that in dill weed oil only the (3S,3aS,7aR) stereoisomer of dill ether **2** is present, having a significant contribution to the organoleptic properties of the dill plant [2,3].

In the first step, dill ether **2** was transformed into the acetate **3** by treatment with anhydrous magnesium bromide, followed by the addition of acetic anhydride. The reaction mixture was obtained with 99% yield and had a 90% content of compound **3**, with p-cymen-9-yl acetate as the principal by-product. Hydrolysis of this reaction mixture with potassium hydroxide (yield 97%) led to the formation of compound **1** in 89% purity, together with p-cymen-9-ol as the main by-product. The chemical identity of compound **1** was confirmed by GC/MS and ^{1}H-NMR. Chiral analysis with cyclodextrin capillary columns has shown that compound **1** consists of enantiomerically pure (8S)-(–)- p-mentha-1,3-dien-9-ol.



2 **3** **1**

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Methyl 2-methylalkanoates from the essential oil of *Humulus lupulus* L.

Milena Z. Živković, Niko S. Radulović, Marija S. Genčić

Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, RS-18000 Niš, Serbia
Corresponding author: nikoradulovic@yahoo.com

Keywords: *Humulus lupulus*, minor volatile constituents, methyl esters, methyl 2-methylalkanoates

For hundreds of years strobiles from *Humulus lupulus* L. (Cannabaceae) have been used to flavor beer and in the enthnopharmacology of many nations. The essential oil is produced by the female inflorescences (strobuli lupuli) which contain the oil bearing glands. Up to now, more than 200 essential-oil constituents have been identified; however, a large number still remains unknown, either due to their low relative abundance in the essential oil or due to a natural variation of the composition. These minor components are known to significantly contribute to the overall sensory impression, and should not be neglected. Prompted by this, we subjected a sample of hydrodistilled essential oil of fresh hop cones (yield 0.6%, w/w), collected from a wild-growing population of *H. lupulus* near the city of Niš (SE Serbia), to gradient "dry flash" SiO_2 chromatography. A fraction that eluded with 2% (v/v) diethyl ether in pentane had an odor that was reminiscent of the original essential oil. Initial GC and GC-MS analyses revealed that this fraction consisted of a myriad of methyl esters of normal and branched aliphatic carboxylic acids. A series of 2-methylalkanoates (C_8-C_{11}) was tentatively identified based on their mass spectra and retention data. Since, according to a literature survey, methyl 2-methylnonanoate and methyl 2-methyldecanoate were never previously reported for *H. lupulus*, we decided to prepare the two compounds by synthesis and confirm their presence in *H. lupulus* by GC co-injection experiments. The corresponding lithium enolates, obtained in the reaction of methyl nonanoate and methyl decanoate with lithium bis(trimethylsilyl)amide at -78 °C, were alkylated with methyl iodide, and, in this way, following a chromatographic purification, we acquired pure samples of the two esters. The subsequent co-injection experiments corroborated the initial tentative identifications, i.e. this is the first report on the occurrence of methyl 2-methylnonanoate and methyl 2-methyldecanoate in *H. lupulus* essential oil.

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PP85

Cytotoxicity of octyl esters from the essential oil of *Tordylium maximum* L. (Apiaceae) towards rat peritoneal macrophages

Niko S. Radulović[1], Milena Lj. Krstić[1], Nikola M. Stojanović[2],
Pavle J. Ranđelović[2]

[1]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia*
[2]*Department of Physiology, Faculty of Medicine, University of Niš, Zorana Đinđića 81, 18000, Niš, Serbia*
Corresponding author: nikoradulovic@yahoo.com

Keywords: *Tordylium maximum*, octyl esters, synthetic library, octyl 2-methylbutanoate.

Hartwort (*Tordylium* spp.), a genus of umbelliferous plants, was used in Greece in drugs and medicinal wines and also as potherbs and culinary herbs. According to Aristotle, a deer will find and eat hartwort immediately after giving birth to young [1]. Such rich ethnopharmacological and culinary usage of taxa belonging to this genus prompted us investigate the essential oil composition and possible biological activity of the only representative of this genus in Serbia, *Tordylium maximum* L. The essential oil of the aerial parts of this species was studied only once and no reports on the volatiles from its schizocarps exist to date. Herein, we wish to report on an analysis of the volatiles obtained by hydrodistillation from the dried fruits of *T. maximum* that included a synthetic effort to prepare a library of the detected constituents. Initial GC and GC-MS analyses of the essential oil revealed a series of esters of 1-octanol and acetic, propanoic, acrylic, methacrylic, butanoic, isobutanoic, pentanoic, isovaleric, 2-methylbutanoic, senecioic, tiglic and angelic acids. 1-Octyl tiglate, isovalerate and isobutanoate were the major constituents of the analyzed essential oil. Chemotaxonomically speaking, this is in agreement with the compositions of essential oils of other Tordylieae tribe taxa. All of the twelve mentioned esters were prepared by a Steglich procedure starting from 1-octanol and the corresponding acids. Besides allowing us to perform quantitations using calibration curves, this approach permitted us to test the effect of these esters on rat macrophage viability. An 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT)-assay, based on the mitochondria-dependent reduction of the dye by viable cells, showed that three (tiglate, methacrylate and isobutanoate) out of six tested octyl esters were devoid of any effect towards rat peritoneal macrophages, while octyl 2-methylbutanoate, isovalerate and senecioate demonstrated prominent cytotoxicity with LD_{50} values (in $moldm^{-3}$): $1.2x10^{-4}$, $8.9x10^{-3}$, $5x10^{-4}$, respectively (cf. the positive control, 5-fluorouracil, $LD_{50} = 1.5x10^{-5}$ $moldm^{-3}$). Interestingly, the Michael acceptor (α,β-unsaturated ester moiety), present in two inactive esters, appears not to play an important role in the observed activity.

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A High Accuracy QSAR based on rabbit data to predict the human skin irritation potential of individual constituents and mixtures

Faizan SAHIGARA[1], Carole CHARMEAU-GENEVOIS[2], Pascal BICHEREL[1], Mélissa PEREA[2], Satinder SARANG[3], Charles EADSFORTH[3], Tom AUSTIN[3], Paul THOMAS[1,2]

[1]1KREATiS, 23 rue du Creuzat, 38080 L'Isle d'Abeau, France
[2]CEHTRA, 23 rue du Creuzat, 38080 L'Isle d'Abeau, France
[3]Shell Services International Limited, Brabazon House, Threapwood Road, Concord Business Park, Manchester M22 9PS, United Kingdom
Corresponding author: faizan.sahigara@kreatis.eu

Keywords: skin irritation, corrosion, alternative, *in silico*, QSPR, QSAR, model, mixtures

To evaluate the potential for skin irritation of a substance, normally experimental tests (*in vitro/in vivo*) must be performed. Currently, this means an *in vitro* test first, but if inconclusive it is followed by an OECD 404 *in vivo* study, where observations of erythema and oedema are recorded as Draize scores, to indicate the potential for skin irritation/corrosion of the substance. Since 2004, with the EU ban on animal testing of finished cosmetic products and with REACH in 2007, the validation of alternative approaches such as *in vitro* methods and the use of Read-Across approaches has progressed. Consequently, testing in animals for some endpoints has been reduced, but not totally avoided.

To date QSARs have not been completely successful for the determination of skin irritancy. The QSARs Mix Challenge sponsored by Shell [1], was initiated in 2015 under the NC3Rs CRACK-IT programme with the aim of using QSARs to address the 3Rs issues associated with assessing skin and eye irritation endpoints in animal studies, so that these can ultimately be replaced *by in silico* models, particularly to examine the effects of mixtures.

The Challenge was completed in March 2016 with the development and validation of the Skin Irritation module of "iSafeRabbit" – the High Accuracy QSAR (HA-QSAR). The modelling strategy will be briefly outlined and the poster will focus on the validation of the models for individual substances and for mixtures. The predictive power of the iSafeRabbit model will be compared to those derived from other existing in silico models for a validation set comprising of chemicals across various chemical groups.

References

[1] www.crackit.org.uk/challenge-19-qsars-mix

A High Accuracy QSAR based on rabbit data to predict the human eye irritation potential of individual constituents and mixtures

Faizan SAHIGARA[1], Carole CHARMEAU-GENEVOIS[2], Pascal BICHEREL[1], Mélissa PEREA[2], Satinder SARANG[3], Charles EADSFORTH[3], Tom AUSTIN[3], Paul THOMAS[1,2]

[1] *1KREATiS, 23 rue du Creuzat, 38080 L'Isle d'Abeau, France*
[2] *CEHTRA, 23 rue du Creuzat, 38080 L'Isle d'Abeau, France*
[3] *Shell Services International Limited, Brabazon House, Threapwood Road, Concord Business Park, Manchester M22 9PS, United Kingdom*
Corresponding author: faizan.sahigara@kreatis.eu

Keywords: eye irritation, corrosion, alternative, *in silico*, QSPR, QSAR, model, mixtures

An experimental test must be performed following OECD Test Guideline 405 for the assessment of potential eye irritation/corrosion of a substance. In the past this was performed using an *in vivo* study test where a chemical was instilled into the conjunctival sac of albino rabbits and then injuries of cornea, iris, and conjunctivae were daily evaluated according to the Draize scores. Depending on the scale of different effects, the test substance was classified or not as irritant/corrosive to the eyes. Since 2004, with the EU banning animal testing of finished cosmetic products, and with REACH coming into force in 2007, along with the validation of alternative approaches such as *in vitro* and *in silico* methods, animal testing has been reduced. However, this has not totally avoided animal testing. There has been growing efforts made by the regulatory authorities to minimise the use of animal testing by switching to alternative approaches such as QSARs and Read-Across. The QSARs Mix Challenge, sponsored by Shell [1], was initiated in 2015 under the NC3Rs CRACK-IT programme to address the 3Rs issues associated with assessing skin and eye irritation endpoints in animal studies. This Challenge was aimed at the development of a reliable QSAR model to predict the skin and eye irritation potential of the individual constituents as well as mixtures to replace the experimental studies.

The Challenge was completed in March 2016 with the development and validation of the eye Irritation module of "iSafeRabbit" – the High Accuracy QSAR (HA-QSAR). The modelling strategy will be briefly outlined and the poster will focus on the validation of the models for individual substances and for mixtures. The predictive power of the iSafeRabbit model will be compared to those derived from other existing *in silico* models for a validation set comprising of chemicals across various chemical groups.

References

[1] www.crackit.org.uk/challenge-19-qsars-mix

Release and permeation kinetics of *Melaleuca alternifolia* (tea tree) essential oil bioactive compounds from cosmetic formulations

Barbara Sgorbini[1], Cecilia Cagliero[1], Monica Argenziano[1], Roberta Cavalli[1], Carlo Bicchi[1], Patrizia Rubiolo[1]

[1]Dipartimento di Scienza e Tecnologia del Farmaco – Università degli Studi di Torino,
Via Pietro Giuria 9, 10125 Torino (Italy)
Corresponding author: barbara.sgorbini@unito.it

Keywords: *Melaleuca alternifolia*, cosmetic formulations

Melaleuca alternifolia essential oil (tea tree oil, TTO) is well known because of its antimicrobial, antifungal and anti-inflammatory properties [1]. Thanks to these properties, TTO is employed as active ingredient in several skin cares.

This study aims to investigate and evaluate permeation and release kinetics of TTO bioactive marker compounds from several semisolid formulations (creams, ointments, gels) containing different percentages of TTO (from 5 to 30% p/p).

In vitro permeation and release tests were carried out using Franz diffusion cells with synthetic and pig skin membranes at different times (from 10 minutes to 72 hours). Each test was repeated three times. The recovered receptor phases were analyzed by Headspace-Solid Phase Micro-Extraction (HS-SPME) in combination with GC-MS. Permeation and release kinetics were evaluated on some selected markers known to be responsible of the TTO biological activities, i.e. 1,8-cineole, 4-terpineol and α-terpineol.

The results of permeation and release tests demonstrated that all compounds show the same kinetics, although lag time and amount of substances released are meaningfully different. Finally, the released markers were quantified by both standard addition approach and multiple headspace solid phase microextraction. Some preliminary experiments were carried out to determine the amount of the above markers accumulated into skin.

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Chemical constituents and biological activities of essential oils from Tajikistan plants

Farukh Sharopov[1,2], Michael Wink[1]

[1]*Institute of Pharmacy and Molecular Biotechnology, Heidelberg University,*
Im Neuenheimer Feld 364, Heidelberg, 69120, Germany.
[2]*University of Central Asia, Toktogul Str. 138, Bishkek, 720001, Kyrgyzstan.*
Corresponding author: sharopov@uni-heidelberg.de; shfarukh@mail.ru

Keywords: *Polychrysum tadshikorum, Ferula clematidifolia, Galagania fragrantissima, Tajikistan.*

Well-known analytical, chemical and biological methods were used in order to investigate the chemical constituents and biological activities of essential oils from 25 medicinal plants from the flora of Tajikistan. Many of these plants are endemic plants of Tajikistan and Central Asia; they are widely used in traditional medicine.

For the first time, the chemical composition and bioactivity of the essential oils of *Ferula clematidifolia, Galagania fragrantissima* and *Polychrysum tadshikorum* were investigated. The main components of the essential oils of *Ferula clematidifolia, Galagania fragrantissima* and *Polychrysum tadshikorum* were analyzed by Gas Chromatography Mass Spectroscopy (GLC-MS) and identified as pinene, 2E-dodecenal, and terpinen-4-ol, respectively.

The antioxidant activity of essential oils are strongly dependent to the presence of phenolic metabolites. Essential oil of *Origanum tyttanthum* which contain phenols (carvacrol and thymol) exhibited a high antioxidant activity with an IC_{50} value of 0.1-0.3 mg/ml. The essential oil of *Galagania fragrantissima* was very active against gram-positive bacteria (methicillin resistant *Staphylococcus aureus*). MIC and MBC values were 0.04 and 0.08 mg/ml respectively. *Galagania* oil shows high anti-inflammatory activity, it inhibited 5-LOX enzyme with an IC_{50} value of 7.3 µg/ml.

Essential oils from *Origanum tyttanthum*, *Galagania fragrantissima* and *Mentha longifolia* exhibited a high cytotoxic effect against five human tumour cell lines (HeLa, CaCo-2, MCF-7, CCRF-CEM and CEM/ADR 5000). Their IC_{50} values ranged between 7.5-78 µg/ml. Combinations of doxorubicin with essential oils of *Mentha longifolia, Anethum graveolens, Origanum tyttanthum, Galagania fragrantissima* and *Artemisia absinthium* exhibited synergistic activity. The IC_{50} values of doxorubicin could be enhanced in dual combinations with essential oils 3-15 fold. Synergistic effects of essential oils seem to be a promising area for future research.

In conclusion, essential oils from *Galagania fragrantissima* and *Origanum tyttanthum* are interesting candidates for a use in phytotherapy.

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The influence of natural terpenoids on the activity of tuberculostatic antibiotics.

Elwira Sieniawska[1], Marta Swatko-Ossor[2], Rafał Sawicki[2], Grażyna Ginalska[2]

[1] *Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Poland*
[2] *Department of Biochemistry and Biotechnology, Medical University of Lublin, Poland*
Corresponding author : elwira.sieniawska@gmail.com

Keywords: *Mycobacterium tuberculosis*, terpenoids, tuberculostatic antibiotics, MIC

The natural terpenoids are known for their antimicrobial properties and the deleterious effect on structure and function of microbial membrane and cell wall, has been generally used to explain the antimicrobial action of the essential oils and their components [1]. Antimycobacterial activity of some terpenoids was described against *Mycobacterium tuberculosis* and *M. bovis* [1] and the synergistic *in vitro* interactions between oleanolic acid and isoniazid, rifampicin or ethambutanol against *M. tuberculosis* were reported [2].

In this study we aimed to describe the influence of (R)limonene, (S)limonene, myrcene, sabinene, α-pinene and β-elemene on the antimycobacterial activity of first line tuberculostatic drugs against *Mycobacterium tuberculosis* H37Ra.

The values of the minimal inhibitory concentration (MIC) for tested terpenoids, as well as for combinations of terpenoids with tuberculostatic antibiotics, were determined by log2 dilution method in the range from 125 to 0.059 µg/ml. The concentration of terpenoids in the sample was equal to obtained MICvalues.

Compounds	**MIC(µg/ml)**	**Compounds**	**MIC(µg/ml)**	**Compounds**	**MIC(µg/ml)**
Ethambutol	16	Isoniazid	32	Rifampicin	16
S-limonene	64	R-limonene	64	β-elemene	2
S-limonene + ethambutol	0.475	R-limonene + ethambutol	0.95	β-elemene + ethambutol	0.475
S-limonene + isoniazid	0.475	R-limonene + isoniazid	15	β-elemene + isoniazid	0.475
S-limonene + rifampicin	0.237	R-limonene + rifampicin	0.475	β-elemene + rifampicin	0.237
α-pinene	16	sabinene	32	myrcene	32
α-pinene + ethambutol	125	sabinene + ethambutol	3.9	myrcene + ethambutol	3.9
α-pinene + isoniazid	125	sabinene + isoniazid	1.95	myrcene + isoniazid	0.95
α-pinene + rifampicin	0.475	sabinene + rifampicin	0.475	myrcene + rifampicin	0.475

β-elemene and S-limonene gave strong synergistic effect with every antibiotic. Combinations of myrcene, R-limonene and sabinene with antibiotics decreased MIC for both, terpenoid and antiobiotic, while combinations of α-pinene with ethambutol and isoniazid resulted in increased MIC values. Rifampicin gave synergistic increase in activity with every compound tested.

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Being the main constituent, carotol is not responsible for cytotoxic activity of carrot seeds essential oils

Elwira Sieniawska[1*], Łukasz Świątek[2], Barbara Rajtar[2], Małgorzata Polz-Dacewicz[2] and Krystyna Skalicka-Woźniak[1]

[1] Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland
[2] Department of Virology, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland
**Corresponding author. Email: elwira.sieniawska@gmail.com*

Keywords: Carrot seed essential oil, *Daucus carota*, carotol, cytotoxicity

Carrot seed essential oil (CSEO) is a common fragrance component in cosmetics and perfumes, as well as a flavour ingredient in different categories of food products, mainly desserts, meat products and soups, usually in rather low use levels (<0.003%). Because the main source of commercially available CSEO is *Daucus carota* L. ssp. *carota*, which contains carotol as a main constituent, it is very important to evaluate the cytotoxicity of carotol-rich essential oils. What is more, no information about cytotoxicity of pure carotol is available.

In this work we aimed to compare the chemical composition of commercially available (Moroccan and French) and hydrodistilled (Polish) wild carrot seed essential oils (*Daucus carota* L. ssp. *carota*), to evaluate cytotoxicity of these essential oils, as well as of isolated carotol, on VERO (green monkey kidney) and FaDu (human pharynx squamous cell carcinoma) cell lines.

The gas chromatography-mass spectrometry analysis of three carrot seed essential oils (CSEOs) of different origin (Polish, French, Moroccan) revealed that carotol is the main constituent amounting 19-33% of the sum of compounds. α-Pinene, sabinene, myrcene, limonene, geranyl acetate, bisabolene, cayophyllene oxide and daucol were identified as other main compounds. The comparison of cytotoxicity of tested essential oils on VERO and FaDu cell lines indicated that Moroccan and French EOs have similar cytotoxicity (35.3-46.1 µg/mL), while Polish EO showed lower cytotoxic effect (70.9-96.3 µg/mL). Carotol being the main constituent of all studied EOs showed moderate cytotoxicity on both tested cell lines without any selectivity (39.7 and 38.3 µg/ml on VERO and FaDu cell lines, respectively). The results suggest that carotol is not responsible for cytotoxic properties of CSEOs, because its cytotoxicity is equal to cytotoxicity of Maroccan EO in which it constitute 33%. Geranyl acetate having very detrimental cytotoxic activity may be main cytotoxic compound. This could explain why French and Moroccan EO having higher geranyl acetate content than Polish EO were also more cytotoxic.

In vitro antibacterial activity of *Eucalyptus globulus* essential oils against *Enterococcus faecalis* and *Lactobacillus rhamnosus*

Carmen M. Sinche Ambrosio[1], Severino M. de Alencar[1], Eduardo Micotti da Gloria[1]

[1] *Department of Agroindustry, Food and Nutrition-College of Agriculture "Luiz de Queiroz" University of São Paulo, Piracicaba-São Paulo, BRAZIL..*
Corresponding author: carmen.milagros8@usp.br and emgloria@usp.br

Keywords: *Eucalyptus globulus*, Essential oil, Antibacterial activity, *Enteroccoccus faecalis*, *Lactobacillus rhamnosus*.

In livestock, antibiotics were used worldwide as antimicrobial growth promoters (AGP) and as therapeutics to bacterial diseases. However, the European Community, since 2006, based on the concern with bacterial resistance, has prohibited the use of antibiotics as AGP, hence, the need to find alternatives to replace them has risen. Essential oils, for their recognized antimicrobial properties, have appeared as a potential alternative. On the other hand, it has been shown that beneficial bacteria are important to maintain an ideal gut microbiota composition. Therefore, it is desirable that essential oils have little or no effect on beneficial bacteria. The aim of this study was to evaluate the antibacterial activity of the essential oil from *E. globulus* against a model of pathogenic bacterium, *E. faecalis* ATCC 25912 and, a model of beneficial bacteria, *L. rhamnosus* ATCC 7469. The oil composition was analyzed by CG-MS. The antibacterial activity of the *E. globulus* oil was evaluated by time-kill assay and Bacterial Tolerance to Sequential Doses (BTSD). The BTSD assay was proposed in our study in order to simulate the sequential exposure of the animal gut bacteria population to the oil, through the sequential intake of feed containing it. BTSD was performed by exposing bacteria to sequential doses of the oil (0.411%) every 3h during 9h of treatment. Besides, in order to contrast the BTSD results, a concomitant treatment was performed by exposing bacteria to the oil only once (single dose) at the start of the 9-hour treatment. The GC-MS analysis detect 1.8-cineole (83.65%) as the major compound of this oil. The results of the time-kill assay showed that the *E. globulus* oil (1.645%), at 30s of contact, killed the total population of *E. faecalis*, and caused a reduction of only ~3Log in the *L. rhamnosus* population. The BTSD results showed that the first dose (0-3h) of the *E. globulus* oil caused the reduction of ~2Log, and the second dose (3-6h) killed the total population of *E. faecalis*, whereas the exposure to a single dose (0-9h) reduced ~2Log of the *E. faecalis* population during the first 3h, and no additional reductions were later observed. For *L. rhamnosus*, the exposure to sequential doses or to a single dose caused a similar effect, with a total reduction of ~2Log (0-9h) in both cases. The two assays showed that the *E. globulus* oil had a selective antibacterial effect between pathogenic and beneficial bacteria.

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Influence of olfactory adaptation on the effect of sandalwood oil under the aspect of smoking behavior

Iris Stappen[1], Emine Kader[1], Erich Schmidt[1], Jürgen Wanner[2]

[1]*Department of Pharmaceutical Chemistry, University of Vienna, Althanstr. 14, Vienna, Austria.*
[2]*Kurt Kitzing GesmbH., Hinterm Alten Schloss 21, Wallerstein, Germany.*
Corresponding author: iris.stappen@univie.ac.at

Keywords: Adaptation, smoking behavior, sandalwood oil, psychophysiology.

Adaptation can be defined as reversible decrease in sensitivity to an odor during prolonged exposure. It is the ability of the olfactory system to adjust its sensitivity at different odorant intensities. The effect of smoking behavior on the olfactory function has been investigated in recent years, showing controversial outcome: some authors found significant results between smokers and nonsmokers [1], others failed to show an influence on subjects' olfactory ability [2,3].

To address the aspect of smoking behavior on the effect of essential sandalwood oil (*Santalum austrocaledonicum* Vieillard, SO) on psychophysiology under adaptation conditions, 45 smokers and 45 nonsmokers aged between 18 and 35 years were tested in 90 sessions, each lasting for 30 minutes. Subjects were randomly assigned to three groups representing three conditions: an "adaptation-condition" (subjects were continuously exposed to SO), an "intermitted-condition" (SO was presented for one minute followed by four minutes of no odor, repeatedly for six times over 30 minutes), and a control condition (no odor). Each group consisted of 15 smokers and 15 nonsmokers, respectively. Fragrance stripes with odors were placed directly under participants' noses. An intensity rating was performed every five minutes during each session to access adaptation. 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 (SO or control) were rated at the end of each session.

A main effect was found for the psychological parameter calmness/restlessness. In comparison to the other groups nonsmokers in the "intermitted-condition" became calmer in time than smokers. Disregarding odor conditions, systolic blood pressure decreased significantly more in smokers. They also became significantly more alert and bad tempered in time than nonsmokers. All participants rated SO as calming as control.

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Analysis of tropical flower secondary metabolites as natural ingredients for cosmetic products

Elena Stashenko, Lady Sierra, Jesica Mejía, Fausto Prada, Yuri Córdoba, Corina Bernal, Jairo René Martínez

Research Center for Biomolecules, CIBIMOL, Research Center of Excellence CENIVAM, Universidad Industrial de Santander, Carrera 27, Calle 9, Bucaramanga, Colombia.
Corresponding author : elenastashenko@gmail.com

Keywords: Tropical flower; GC-MS; LC-MS; HRTOF-MS; Antioxidant; UV-irradiation.

Flowers are very sophisticated and important plant parts. Without them there are no fruits and there is no life continuation. Flowers and their metabolites have been studied to lesser extents than other plant parts. Their secondary metabolites include non-volatile, semi-volatile, and very volatile compounds, whose isolation and analysis cannot be accomplished with a single methodology. Their study is an analytical challenge for both sample preparation (high recovery, non-invasive solventless methods, no artefact formation) and for instrumental analysis (high sensitivity, peak resolution, confirmatory identification). This work presents results of the chromatographic and mass spectrometric examination of several aspects of tropical flower secondary metabolism. Over 20 different tropical flowers were investigated, obtained from the CENIVAM experimental garden (Bucaramanga, Colombia). The taxonomic identification of the vegetal material was done at the Colombian National Herbarium (Bogotá). Volatiles analyses were performed by GC-MS and by GCxGC-MS/TOF (Leco Pegasus HRT-MS) with data systems which included the ADAMS, NIST and WILEY spectral libraries. Flower extracts were also analyzed by LC-MS with electrospray ionization in positive and negative modes, and high-resolution mass spectrometry detection (r-TOF as well as Orbitrap) with MassHunter and Excalibur software, respectively, for data analysis. Sampling *Cananga odorata* flower volatiles with solid-phase microextraction and ionic polymer coatings showed a larger relative amount of polar compounds, when compared with those obtained with a CAR/PDMS coating. GC-MS analysis permitted the identification and quantification of typically, over 90% of the flower fragrance constituents. ORAC antioxidant capacity values for flower extracts were higher than those observed for reference antioxidants BHT and α-tocopherol. Matrix solid-phase dispersion, used to isolate alkaloids from tropical flowers, resulted more efficient and quantitatively reproducible compared with the traditional Soxhlet, or sonication-assisted solvent extraction. LC-MS analysis was used to examine changes in flower secondary metabolite composition resulting from exposure to UV-A radiation (350 nm). Several flower extracts were tested for their antigenotoxic and photoprotective activities. These attractive properties (photoprotective, antioxidant) make them excellent candidates as natural ingredients for cosmetic products.

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Immunomodulatory activity of pinguisane-type sesquiterpenes from the essential oil of the liverwort *Porella cordaeana* (Hub.) Moore (Porellaceae)

Nikola M. Stojanović[1,*], Niko S. Radulović[2,*], Sonja I. Filipović[2], Dragan B. Zlatković[2], Miljana R. Đorđević[2], Pavle J. Randjelović[3], Katarina V. Mitić[2], Tatjana M. Jevtović-Stoimenov[4], Vladimir N. Ranđelović[5]

[1] *Faculty of Medicine, University of Niš, Bulevar dr Zorana Đinđića 81, RS-18000 Niš, Serbia.*
[2] *Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, RS-18000 Niš, Serbia.*
[3] *Department of Physiology, Faculty of Medicine, University of Niš, Bulevar dr Zorana Đinđića 81, RS-18000 Niš, Serbia.*
[4] *Institute of Biochemistry, Faculty of Medicine, University of Niš, Bulevar dr Zorana Đinđića 81, RS-18000 Niš, Serbia.*
[3] *Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, RS-18000 Niš, Serbia.*
Corresponding author : NS: nikola.st90@yahoo.com; NR: nikoradulovic@yahoo.com

Keywords: *Porella cordaeana* (Porellaceae), α-furanopinguisanol, furanopinguisanone, rat splenocytes, blast like cells

Two sesquiterpenes, α-furanopinguisanol (1) and furanopinguisanone (2), identified in the essential oil of the liverwort *Porella cordaeana* (Hub.) Moore (Porellaceae, Jungermanniales), were assessed for their immunomodulatory properties. Their influence on rat splenocytes (SPCs) was monitored through MTT, trypan blue and neutral red assays, microscopic investigation of cells, comet assay, and protein, RNA and DNA contents. The alcohol (1) and its ketone (2) caused mutually different alterations of SPC functioning, as inferred from the results of the three viability assays. Bigger, activated lymphocytes (over 15 μm) were observed in average in 49±7% among the cells treated with compound 1, while compound 2 significantly reduced the number of viable SPCs. In the case of compound 1, an increased DNA and RNA content verified the existence of lymphocytes undergoing cell division. Interestingly, in higher concentration (10^{-4} M), α-furanopinguisanol induced a blast-like transformation of SPCs, while in lower ones (10^{-8}-10^{-6} M) it acted as a cytotoxic agent. On the other hand, furanopinguisanone exerted prominent cytotoxicity in all concentrations. The lowering of levels of DNA in the SPCs treated with compound 2 strongly suggested that cell death caused by this compound occurs via apoptosis. The compounds did not demonstrate *in vitro* genotoxicity after a 24 h-treatment based on the results of the comet assay.

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PCA and CCA multivariate analyses and the correlation between VOC's percentage and climate variation

Ivana B. Suffredini[1,2], Natalina H. da Silva[2], Mateus L.B. Paciencia[2] …

[1]*Center for Research in Biodiversity, Paulista University, Av. Paulista, 900, 1 andar, São Paulo, SP, Brazil, 01310-100.*
[2]*Graduate Program in Environmental and Experimental Pathology, Paulista University, R. Dr. Bacelar, 1212, São Paulo, SP, Brazil, 04026-002.*
Corresponding author : ibsuffredini@yahoo.com.br; extractlab@unip.br

Keywords: *Iryanthera ulei*, Myristicaceae, climate changes, multivariate analysis.

Iryanthera ulei is popularly known as *ucuubarana*, in Brazil, and it is used to treat oral infections, diarrhoea, malaria and anaemia. Essential oils (EO) were obtained from the leaves of two specimens that were collected for 13 times in a two-year period. EO's were tested against 11 microorganisms of human and veterinary pathological interest. Oils were evaluated for their chemical composition by CG-MS and 17 volatile organic compounds (VOC's) were identified as terpenes, of which spathulenol, α-cadinol, globulol and cubenol-1,10-di-epi are the major ones. Climate parameters as maximum temperature, relative humidity, total daily irradiation and daily precipitation were compared to terpene percentage using multivariate analyses such principal component analysis (PCA) and canonic correspondence analysis (CCA), in order to verify any alterations in terpene concentrations in relation to climate variations in each of the individuals, then in both together, so as to prospect for a pattern in terpene occurrence. PCA showed that there are three compounds that can be assigned as major VOCs. CCA analyses showed that the expression of α-muurolene, β-elemene and ledol in specimen 10OE was influenced by relative humidity, but was not influenced by daily precipitation, while α-muurolene, α-amorphene and ledol were also influenced by total daily irradiation. Only α-muurolene was influenced by higher temperatures. The expression of trans-10-calamenenol in specimen 15OE was influenced by total daily precipitation, while β-elemene was influenced by relative humidity and 1,10-di-epi cubenol was influenced by higher temperatures. EO's showed expressive antibacterial activity against *Staphylococcus aureus, Enterococcus faecalis* and *Streptococcus mutans*, but no activity against *Escherichia coli, Pseudomonas aeruginosa* or *Candida albicans*. In relation to the anti-Staphylococcal activity and its relationship with the VOC's expression, it was observed that viridiflorol showed a tendency of being related to minimal bactericidal concentration (MBC) for specimen 10OE, while α-cadinol, α-muurolene, β-elemene, α-amorphene and viridiflorol showed influence over minimal inhibitory concentration (MIC) and β-elemene and α-amorphene influenced MBC in specimen 15OE. None of the major compounds were influenced by climatic changes or 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.

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Essential oils and antioxidants from ten *Salvia* spp. isolated by high pressure extraction methods

Vaida Šulniūtė, Petras Rimantas Venskutonis, Renata Baranauskienė

Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų rd. 19, Kaunas, LT-50254, Lithuania
Corresponding author : vaida91@yahoo.com

Keywords: *Salvia* spp., high pressure extraction, distillation/solvent extraction, antioxidant capacity.

The genus *Salvia* (sage) is one of the largest and most important Lamiaceae family genera of medicinal and aromatic plants, which is widespread throughout the world. Essential oils of various Lamiaceae plants have been widely used in food industry, for medical purposes or in cosmetic preparations as aromatic and bioactive ingredients. *S. officinalis* and *S. sclarea* are the most thoroughly studied *Salvia* species, whereas the information about phytochemicals in many other *Salvia* species is rather scarce. To fill this gap, our study was aimed at comprehensively evaluation of the composition of essential oils and antioxidant potential of ten *Salvia* spp., including the above mentioned well-known species and remarkably less investigated such as *S. forsskaolii*, *S. stepposa* and *S. verticillata*.

Supercritical carbon dioxide extraction (SFE-CO_2), pressurized liquid extraction (PLE) with various polarity solvents and simultaneous distillation/solvent extraction methods were used for separating sage material into several soluble fractions. Volatile compounds were analysed by GC×GC/MS, antioxidant properties of different fractions were evaluated by using several *in vitro* antioxidant capacity assays, the composition of other constituents was screened by UPLC-Q/TOF.

Biorefining process of different *Salvia* spp. by SFE-CO_2 (45 MPa, 60 °C, 60 min) yielded from 1.84 ± 0.03 % (*S. stepposa*) to 5.22±0.2 % (*S. sclarea*) of lipophilic fraction. Further PLE (10.3 MPa, 140 °C, 30 min) of SFE-CO_2 residues was performed consecutively using ethanol (96 %) and water. The highest yields were obtained with water (30.2±0.18 – 43.7±0.14 %), while ethanol gave approximately 2-fold lower yields from different *Salvia* spp. The antioxidant potential of various fractions obtained at different steps of biorefining process was evaluated measuring overall antioxidant potential of *Salvia* spp. and expressed as a sum of values of all extracts obtained with different solvents. Total phenolic content, which in gallic acid equivalents (GAE) was in the range of 20.9–48.6 mg GAE/g DWP, ABTS cation radical scavenging properties, which in Trolox equivalents (TE) was in the range of 236–543 µmol TE/g DWP and oxygen radical scavenging capacity, which was in the range of 597–1872 µmol TE/g DWP). The antioxidant potential of such species as *S. forsskaolii* and *S. verticillata* was the highest and comparable with that of *S. officinalis*. GC×GC/MS resulted in identification and quantification of numerous valuable constituents in *Salvia* spp. extracts. The results obtained may serve as valuable information for the valorization of *Salvia* spp. as raw materials for the isolation of functional ingredients for human nutrition and other purposes.

Chemical composition and antioxidant activity of *Ocimum basilicum* L. in single and intercropped culture with *Lactuca sativa* L.

Ediclan Soares Machado[1], Simone Teles[1]*; Franceli da Silva[1]

[1]*Centre for Agricultural, Environmental and Biological Sciences, Federal University of Recôncavo da Bahia/UFRB, University Campus, Cruz das Almas/BA,44380-000 Bahia, Brazil*
**Corresponding author:* telessimone@gmail.com

Keywords: *Ocimum basilicum*; composition; properties; crop system

Basil is highly valorized due to their chemical components with bioactive properties, which enhanced research and interest in this species. This work studied the chemical composition and anti-oxidant activity of the essential oil (EO) from *Ocimum basilicum* L. grown in single and intercropped with *Lactuca sativa* L. with and without commercial fertilizer. EO's were extracted by hydrodistillation and were characterized by gas chromatography coupled with flame ionization detector (GC/FID) and by gas chromatography coupled to a mass spectrometry detector (GC/MS). The anti-oxidant activity, specifically antiradical activity, was measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) identified 24 volatiles, with contents of linalool above 50% in all EO's and with a marked influence of the cropping system in eugenol contents, increasing 60% comparing intercropped system with fertilization and single crop system without fertilization. The differences verified in chemical composition affected the anti-oxidant activity of EO's. The antiradical activity was higher in the fertilized plants in both cropping systems. With the present results, it can be concluded that fertilization improves the properties of basil EO's, by the enhancement of their chemical composition. Such findings could be explored in a wide variety of sectors, from the agriculture and food industrial to the pharmaceutical sector.

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Chemical diversity and antimicrobial activity of volatile compounds from *Zanthoxylum zanthoxyloides* fruits

Yoro Tine[1,2], Abdoulaye Diop[3], Franck Renucci[1], Cheikh Saad Bouh Boye[3], Jean Costa[1], Alassane Wélé[2], Julien Paolini[1]

[1] *Université de Corse, UMR CNRS 6134 SPE, Laboratoire de Chimie des Produits Naturels, Campus Grimaldi, BP 52, F-20250 Corte, France.*
[2] *Laboratoire de Chimie Organique et Thérapeutique, Faculté de Médecine, Pharmacie et Odontologie, Université Cheikh Anta Diop, BP: 5005 Dakar-Fann, Sénégal.*
[3] *Laboratoire de Bactériologie-virologie, Hopital Aristide Le Dantec, BP : 3001, Dakar, Sénégal.*
Corresponding author: paolini@univ-corse.fr

Keywords: *Zanthoxylum zanthoxyloides*, fruits, coumarin, antimicrobial activity, GC-MS, LC-MS/MS.

Zanthoxylum zanthoxyloides, belonging to Rutaceae family, is a medicinal and aromatic plant widely used in ethnopharmacology and also as spice throughout central and West Africa [1]. Like other plants of Rutaceae family such as *Citrus* sp., *Z. zanthoxyloides* oils could be used in cosmetic products for their biological activities and/or flavouring properties. For this purpose, the diversity of chemical composition of the essential oils from *Z. zanthoxyloides* growing wild in Senegal were studied in term of terpenic and aliphatic compounds as well as coumarin components, because they have phototoxic effects on human skin [2]. The oil composition was investigated using an original targeted approach based on the combination of Gas Chromatography (GC) and Liquid Chromatography (LC) coupled with mass spectrometry (MS). The volatile composition of *Z. zanthoxyloides* fruits exhibited relative high amounts of hydrocarbon monoterpenes (24.3-55.8%) and non-terpenic oxygenated compounds (34.5-63.1%). The main components were (*E*)-*β*-ocimene (12.1-39%), octyl acetate (11.6-21.8%) and decanol (9.7-15.4%). The GC and GC-MS profiling of fruit essential oils showed a chemical variability according to geographical locations of plant material. The LC-MS/MS analysis of fruit oils allowed the detection and quantification of seven coumarins: 6,7-dimethylesculetin (1.69 mg/l), herniarin (8.67 mg/l), psoralene (344.64mg/l), xanthotoxin (490.00 mg/l), bergaptene (258.21 mg/l), iopimpinellin (52.14 mg/l) and imperatorin (330.71 mg/l). The fruit essential oil exhibited interesting microbial activities against *S. aureus* and *C. albicans*, particularly the alcohol fraction of the oil.

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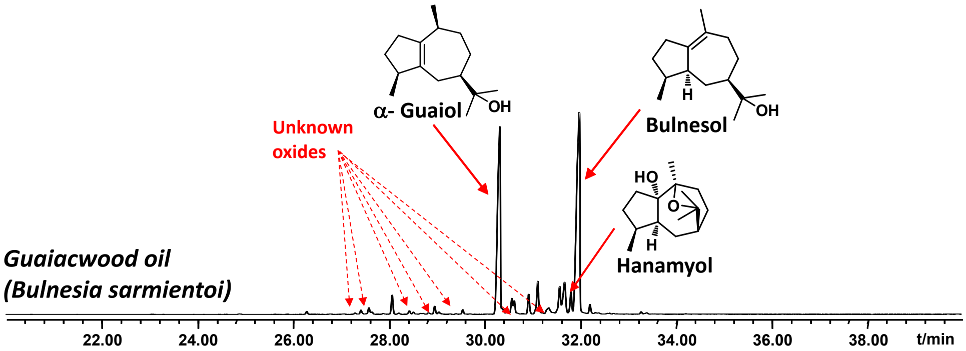
New sesquiterpenic ethers and alcohols from Guaiac wood oil

Loïc Tissandié[1], Hugues Brevard[2], Jean Jacques Filippi[1]
[1] *Institut de Chimie de Nice, Université de Nice Sophia Antipolis, UMR 7272 CNRS, Parc Valrose, 06108 Nice Cedex 2, France.*
[2] *Robertet S.A., 37 avenue Sidi Brahim, 06130 Grasse, France.*
E-mail : Jean-Jacques.FILIPPI@unice.fr

Keywords: *Bulnesia sarmientoi*, Guaiane sesquiterpenes, GC x GC, GC-Olfactometry

Guaiac wood oil is obtained by distillation of the wood of *Bulnesia sarmientoi* Lorentz ex. Griseb, an endemic tree from South Latin America. This oil is a common natural ingredient of the perfume industry used in both domestic and luxury fragrances for its highly appreciated rosy-woody odour, as well as its excellent fixative properties [1]. Despite its long and traditional use as perfume ingredient, Guaiac wood oil has been scarcely studied in the past [2, 3, 4, 5], so that a detailed investigation of its chemical composition is still missing.

We undertook the chemical characterization of its constituents by using a full array of GC-hyphenated techniques (GC-MS, GC×GC-MS, MDGC-MS and pc-GC) combined with conventional chemical fractionation. In the course of our work, we identified a complete series of new sesquiterpenic ethers and alcohols belonging to the Guaiane family.



Each isolated compound was fully characterized by means of spectroscopic methods (NMR, GC-FTIR). Their formation as natural constituents of Guaiac wood oil was investigated by performing simple hemisynthetic transformations of the main alcohols. On the overall, the specific chemical relationships observed between oxides and tertiary alcohols allowed us to get new insights in the chemistry of Guaiane sesquiterpenes.

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Effects of two volatile 2-oxazolidinethione derivatives from *Draba lasiocarpa* Rochel (Brassicaceae) and *Reseda lutea* L. (Resedaceae) on macrophage viability and nitric oxide production

Milica M. Todorovska[1], Niko S. Radulović[1], Dragan B. Zlatković[1],
Nikola M. Stojanović[2], Pavle J. Ranđelović[2], Mirjana Ilić[3]

[1]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia*
[2]*Faculty of Medicine, University of Niš, dr Zorana Đinđića 81, 18000 Niš, Serbia*
[3]*Pediatric Clinic, Laboratory of Biochemistry, Clinical Center and Medical Faculty of Niš, University of Niš, dr Zorana Đinđića 81, 18000 Niš, Serbia*
Corresponding authors : MT mimatod@gmail.com, NR nikoradulovic@yahoo.com

Keywords: *Draba lasiocarpa*, *Reseda lutea*, goitrogenic volatile products, macrophages

Two 2-oxazolidinethione derivatives, 5,5-dimethyl-2-oxazolidinethione (1) and 5-phenyl-2-oxazolidinethione (2), known goitrogens, were isolated from the autolyzates of the flowers of *Draba lasiocarpa* Rochel (Brassicaceae) and *Reseda lutea* L. (Resedaceae), respectively. The plant material (fresh roots, leaves, flowers and fruits) of the two taxa were collected from natural populations near the city of Niš, SE Serbia. Autolyzes of the homogenized plant organs were effectuated by endogenous myrosinases. After SiO_2 column chromatography of the crude flower autolyzate, the obtained pure samples of the two 2-oxazolidinethione were fully spectrally characterized, including 1D and 2D NMR. These goitrogenic volatile glucosinolate products were further screened for their effect on rat macrophage viability and nitric oxide production. The viability of macrophages was evaluated on rat peritoneal macrophages in a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, carried out after 24 h of cell incubation in the media containing the test compounds at increasing concentrations. An appropriate positive control, 5-fluorouracil, was also included in the assay. Nitric oxide concentration in the medium was assessed by the Griess reaction method. It was shown that, at concentrations 10^{-4}–10^{-6} moldm^{-3}, both compounds acted as cytotoxic agents. Compound 2 exerted prominent nitric oxide production-inhibiting properties at all tested concentrations, which may suggest an immunomodulatory activity of this 2-oxazolidinethione derivative, since NO is a well-known inflammation mediator. On the other hand, compound 1 had not reduced the amount of NO produced by lipopolysaccharide-activated macrophages.

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Classification of volatile component compositions of rosemary cultivars and physiological evaluation

Tomi K[1], Nagai K[2], Hayashi T[1]

[1]*Faculty of Agriculture, Kindai University, 3327-204, Nakamachi, Nara, Japan.*
[2] *ANBAS Corporation, 4-12-17 Toyosaki, Kita-ku, Osaka, Japan*
Corresponding author : tomi@nara.kindai.ac.jp

Keywords: *Rosmarinus officinalis*, principal component analysis, enantiomers

It has been reported that volatile component composition of herbs vary with genotypes, environmental factors and extraction method. Our previous study revealed the changes in rosemary volatiles caused by the difference of seasons and the extracts [1]. In this study, chemodiversity of rosemary cultivars and following aromachology effects were evaluated by using the most distinctive rosemary cultivars in volatile components.

Fifteen rosemary cultivars were applied to the experiments: ‘Rex’, ‘Prostratus’, ‘Santa Barbara’, ‘Dancing Waters’, ‘Mozart Blue’, ‘Fota Blue’, ‘Severn Sea’, ‘Majorca Pink’, ‘White’, ‘Miss Jessopp’s Upright’, ‘Marine Blue’, ‘Tuscan Blue’, ‘Blue Boy’, ‘Passion’ and ‘Arp’. Volatile component compositions of fresh rosemary leaves were analyzed by GC–FID and GC–MS. Volatile component compositions were different among the cultivars and classified as 6 types by the major volatile component; α-pinene (‘Marine Blue’ and ‘Passion’), *p*-cimene and eucalyptol (‘Prostratus’, ‘Santa Barbara’, ‘Miss Jessopp's Upright’ and ‘Blue Boy’), borneol (‘Rex’ and ‘Arp’), bornyl acetate (‘Dancing Waters’, ‘Mozart Blue’, ‘Fota Blue’ and ‘Severn Sea’), pinocarveol (‘Majorca Pink’ and ‘White’) and camphor (‘Tuscan Blue’). Enantioselective GC was also applied and revealed that the enantiomeric excess were different in camphor, linalool and borneol, among the cultivars.

Because difference of volatile component compositions in rosemary cultivars may influence on the physiological effects on humans, further investigation was carried by choosing the different cultivars with each other. The GC data was applied to principal component analysis and revealed that the most different cultivars in volatile component compositions were ‘Santa Barbara’ and ‘Severn Sea’. Fresh leaves of these two cultivars were hydrodistilled respectively and their extracts, essential oils and hydrosols, were used for further investigation. Physiological effects on humans were evaluated by means of power spectral analysis on R-R intervals on heart beats. There were no significant differences among the samples, suggesting that the effects of rosemary volatile inhalation on human autonomic nervous systems might be influenced by human emotion or experience. Physiological evaluation using mice are in progress.

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Essential oil composition of leaves of *Citrus tachibana* and its physiological effect in rats

Uchinami K[1], Tomi K[1], Nakamura M[1], Nagai K[2], Hayashi T[1]

[1]*Faculty of Agriculture, Kindai University, 3327-204, Nakamachi, Nara, Japan;*
[2]*ANBAS Corporation, 4-12-17 Toyosaki, Kita-ku, Osaka, Japan*
Corresponding author: tomi@nara.kindai.ac.jp

Keywords: *Citrus tachibana*, autonomic nervous system, physiological effect

Tachibana (*Citrus tachibana* (Makino) Tanaka) is one of familiar species from ancient times in Japan. Its leaves have not been practically used, while its fruits have been utilized as a perfume. Several researches of the petitgrain essential oil (EO) extracted from leaves of bitter orange trees have reported; its scent possesses a sedative effect [1], and its EO does an antioxidant activity [2]. These indicate a possibility of a practical use of the EO of tachibana leaves. In this study, EO extracted from tachibana leaves was subjected to examine volatile component composition and antioxidant property. Furthermore, its physiological effects were also evaluated in rats.

Tachibana leaves (200 g) were hydrodistilled with pure water (400 g), to obtain its EO. Analysis of volatile components in EO was performed by GC-FID and GC-MS. Quantitative evaluation was carried out by GC-FID and qualitative assessment was done by GC-MS. Enantioselective GC was also performed. The effect of the EO on the cutaneous arterial sympathetic nerve activity (CASNA) was also examined in rats.

The major volatile components in EO were linalool (24.2%), piperitone (21.6%), carene (15.5%), and γ-terpinene (14.5%). Among these volatile components, the (*R*)-form and (*S*)-form were determined for linalool and the ratio was (*R*) :(*S*) =2: 8. In this study, it was observed that olfactory stimulation with the scent of the EO suppressed CASNA in rats. Since a suppression of CASNA causes a dilatation of cutaneous arterioles [3], an increase in the cutaneous blood flow and a reduction in transepidermal water loss [4], the present results suggest that the scent of tachibana leaf EO might increase the cutaneous blood flow and water-retaining ability of the skin.

Our present study shows a possibility of a utilization of the tachibana EO to aromatherapy. Further studies are needed to reveal the stability of the EO.

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Determination of recently extended list of suspected cosmetic allergens in fragrance materials by using comprehensive gaschromatography

Margita Utczas[1], Mariosimone Zoccali[2], Sabastiano Pantò[2], Luigi Mondello[1,3]

[1]Chromaleont SrL, c/o Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali - Polo Annunziata - , University of Messina Viale Annunziata, 98168 – Messina, Italy.
[2] Leco European Application and Technology Center, Biotechpark, Max-Dohrn-Str. 8-10, Building B 5.2, 10589 Berlin, Germany
[3]Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali - Polo Annunziata - , University of Messina Viale Annunziata, 98168 – Messina, Italy.
Corresponding author : margita.utczas@chromaleont.it

Keywords: comprehensive gaschromatography, mass spectrometer, allergens.

In 1999, the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) identified a set of 24 fragrance contact allergens, for which label information should be provided to consumers in relation to their presence in cosmetics [1]. In 2012, the SCCS confirmed the previously-regulated allergens, and extended the attention to additional substances which have been shown to be skin sensitizers [2]. Specifically, 82 compounds were classified as contact allergens, of which 54 were single chemicals. As a consequence, a new European legislation, related to cosmetic products, is expected in the coming months. For such a reason, a comprehensive two-dimensional gas chromatography (GC×GC) approach has been developed, directed to the determination of the 54 compounds highlighted by the SCCS in cosmetic products. Operating parameters have been investigated in order to reach the highest separation of the analytical targets. Moreover, due to the solid or viscous nature of cosmetic products such as soap, lotions, and creams the direct injection into a GC is not possible. To overcome this problem, a thermal desorption technique was used. An entire analytical process, considering sample preparation, identification, and quantification was performed.

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Effect of edaphic factors on composition of essential oils of *Thymus pulegioides* growing wild in the east and south east of Lithuania

V.Vaičiulytė, K.Ložienė, R.Taraškevičius, R. Butkienė,

Nature Research Centre, Akademijos Str.2, LT- 08412 Vilnius, Lithuania
Corresponding author : vaiciulyte.vaida@gmail.com

Keywords: *Thymus pulegioides*, soil chemistry, geraniol, carvacrol, linalool.

Introduction. *Thymus pulegioides* (Lamiaceae) is essential oils bearing plant with strong antimicrobial and antioxidant properties [1]. The high infra-specific chemical variation in essential oils is characteristic of *T. pulegioides* growing wild in Lithuania: plants can accumulate different amountsof biologically active compounds such as thymol, carvacrol, geraniol, linalool. The climatic conditions, lightness can influence on the quantitative and qualitative composition of essential oils bearing plants Soil chemistry can also be important environmental factor of composition of essential oils [2]. The goal of study was to establish the influence of edaphic factors on quantitative and qualitative composition of essential oils of *T. pulegioides*.

Methodologies. The samples of plant material (at flowering stage) and topsoilwere collected from forty-four natural habitats. The essential oils from plant material were isolated by hydrodistillation and analysed by gas chromatography/mass spectrometry.The contents of microelements in topsoil were estimated by energy-dispersive x-ray fluorescence, mobile phosphorus and humus – photoelectrocolorimetrically, mobile potassium – by flame photometry, pH – electrometrically.

Main results and conclusion.The significant connections between quantitative composition of essential oil and soil pH, amount of humus, mobile phosphorus and mobile potassium were not established; however, an increase in amount of microelements aluminum, copper, iron, potassium and manganese in topsoil was accompanied by a decrease in amount of essential oil. Manganese and sulphur had the most influence on the qualitative composition on essential oils. Amount of manganese significantly ($p<0.05$) positively correlated with percentage of main compounds of essential oils of *T.pulegioides* geraniol chemotype– geranial ($r=0.32$), nerol ($r=0.34$) and neral ($r=0.33$), negatively – with carvacrol ($r=–0.33$), which is main compound of carvacrol chemotype. Sulphur positively influenced percentages of carvacrol ($r=0.33$) and linalool ($r=0.33$).The results showed that these topsoil's microelements can influence on processes of biosynthesis of essential oils in *T. pulegioides*.

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Characterization of two varieties of Patagonian Hops

C.M. van Baren[1], P. Di Leo Lira[1], D. Retta[1], A. Trochine[2], J. Burini[2], G. Reiner[2], D. Libkind[2], B. Gastaldi[3,4], F. Silva Sofrás[3], S. González[3] and A. Bandoni[1]

[1]*Cátedra de Farmacognosia-IQUIMEFA, FFyB, Universidad de Buenos Aires, Argentina*
[2]*Instituto en Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), CONICET- UNComahue, Bariloche, Argentina.*
[3]*Dpto. de Química, Facultad de Ciencias Naturales, UNPSJB - Sede Esquel, Argentina*
[4]*Consejo Nacional de Investigaciones Científicas (CONICET), Argentina*
Corresponding author: cbaren@ffyb.uba.ar

Keywords: Hop, *Humulus lupulus*, “Nugget”, “Cascade”, Patagonia

Hops flowers are used as ingredients in brewing to contribute to the bitterness and flavour. The main chemical components responsible for the aroma (and partly for the flavour) are part of the essential oils contained in the lupulin glands and the content of α-acids. The yield and chemical composition of essential oils may vary for different varieties of cultivar and because of external influences on the hop plant.

Nowadays, new aromatic profiles in hops are prized as a way to produce novel types of beer. In Argentina, “Nugget” is a variety characterized among the bitter ones (high humulones) and “Cascade” is more aromatic than bitter. For this reason, the volatile components of these varieties of hop cultivated in “El Bolsón” in the Patagonia, Argentina, were isolated by hydrodistillation and analyzed by GC-FID-MS. In addition, determination of α and β acids, as well as the α/β ratio and the HSI (Hops Storage Index) were done following the ASBC Methods. Besides, performance data comparing "flowers" and "pellets" for each variety is presented.

Essential oil yields were higher for the variety “Nugget” and always higher for flowers than for pellets. The overall volatile composition of the “Cascade” and “Nugget” varieties tested were similar to those found in other regions where it is cultivated, as USA and Spain. However, components as geranyl acetate, cis-α-bergamotene, neryl isobutanoate and E-β-farnesene were detected in larger percentages or exclusively in the “Cascade” variety; meanwhile, methyl nonanoate, 2-tridecanone and 4,7(11)-amorphadiene were only present in the “Nugget” variety. Values of α-acids in “Cascade” were greater than for other producing regions, like USA or New Zealand, though the α/β ratio obtained is greater with a value of 1.5 for the Patagonian variety regarding the 0.9-1.1 reported. Meanwhile, the α and β acids values for the Patagonian “Nugget”, as well as the α/β ratio, were similar to those reported for other producing regions (between 11-15% w/w). By comparing the hop flowers with pellets, values of α and β-acids were lower for pellets.

A complete comparative discussion about the values obtained in these Patagonian hops referred to other producing regions, is given.

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The galbanum odor of *Mulinum spinosum*

C.M. van Baren[1], P. Di Leo Lira[1], D. Retta[1], J. Riedel[1], V. Moscatelli[1], E. Dellacassa[2], A.L. Bandoni[1]

[1]Cátedra de Farmacognosia-IQUIMEFA(UBA-CONICET), FFyB, Universidad de Buenos Aires, Argentina
[1]Cátedra de Farmacognosia y Productos Naturales, FQ, Universidad de La República, Uruguay
Corresponding author: cbaren@ffyb.uba.ar

Keywords: Neneo, *Mulinum spinosum*, metoxipyrazines, aromagram

Mulinum spinosum (Cav.) Persoon (Apiaceae), commonly known as “neneo”, is a thorny divaricating shrub very widespread in the dry steppe of Patagonia in Argentina. It accumulates an oleogumresin (containing 12% essential oil) in stems and roots, which possesses a strong aromatic and characteristic smell which has been described as reminiscent of galbanum. These resin exudates after incisions or removal of above ground biomass. Galbanum oil is obtained of *Ferula galbaniflua* Boissier et Buhse (Apiaceae) and is widely used in perfumery to confer green notes and enhance fougére, chypre and oriental notes.

The chemical composition of the essential oils obtained by hydrodistillation from roots and aerial parts of “neneo” collected in 14 different localities of the Patagonia region in Argentina was analyzed by GC-FID-MS and NMR C^{13} showing a predominant monoterpene fraction. A total of 135 compounds accounting for 86.0-99.5% of the oils were identified. The main components were: α-tujhene (1.2-34.0%), α-pinene (tr-8.1%), 3-nonyne (0.8-30.6%), p-cymene (3.4-15.0%) and spathulenol (6.3 -36.0%). In addition, two 2-methoxy pyrazines were identified, probably responsible for its characteristic odour.

Galbanum oil is composed mainly of hydrocarbon monoterpenes such as α–pinene, β–pinene and δ–3-carene, but these components hardly contribute to their characteristic notes. Meanwhile, undecatrienes, methoxy-pyrazines, thiol ethers, sesquiterpenes alcohols and undecatrienes present in trace amounts were identified as the real contributors to the aroma. These compounds have very low threshold values (ng/g). Similarly, the essential oil of “neneo” has olfactory characteristics that are not directly attributable to the major compounds.

Comparative olfatometric evaluation between *M. spinosum* and commercially available galbanum oil from Iran was undertaken to describe the odour profiling of both. This study was done by GC-O and descriptive analyses. The aromagrams obtained of both samples showed defined segments with similar aroma descriptors that characterize the sensory profile of each: green, moisture, earthy. Methoxypyrazines in “neneo” were identified as the ones that conferred these attributes in the same segment were the pyrazines are present in galbanum.

“Neneo” essential oil and resin were also subjected to olfactory evaluation by local fragrance industry to evaluate specific possibilities with promising results. The results obtained in this study intend to motivate actions that promote regional developments using native natural resources.

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Critical comparison of selected olfactometric methods for the determination of impact odorants in essential oils: application to Timur (*Zanthoxylum armatum DC.)*

Clémence Vasseur[1], Esméralda Cicchetti[1]

[1] *Cosmo International Ingredients, R&D department, Analytical laboratory, 855 av. Maurice Donat, 06250 Mougins, France.*

Corresponding author: esmeralda.gonzales@cosmo-ingredients.com

Keywords: *Zanthoxylum alatum Hemsl., Zanthoxylum armatum DC.,* impact odorants, GC-Olfactometry, Intensity, Detection Frequency, Hybrid Method, odour classes.

GC-Olfactometry methods can be divided into four groups. The first one contains the dilution methods like AEDA and CHARM. Requiring few panelists, they are time consuming and often misused to determine impact odorants instead of perception thresholds. The second group includes the intensity methods like OSME and FSCM requiring trained assessors. The third group contains the detection frequency methods that do not require trained panelists but that don't allow the discrimination of the most potent odorants among the unanimous perceived ones. The last group is constituted of the hybrid methods between detection frequency and intensity methods such as the posterior intensity or VIDEO-Sniff® methods, the latter also taking into account the odour description. Solving respective limitations of the parent methods, they often differ in the scale used, number of panelists and the way the aromagrams are plotted: direct sum [1], average odour intensity [2,3], adjusted or modified frequencies (AF [4], MF [5]), standardized frequency (SF [4]) or total olfactory signal (TOS [6]), sometimes with colours depending on odour classes.

This poster will present a critical comparison of intensity, detection frequency and hybrid olfactometric methods for the determination of the most potent odorants of Timur (*Zanthoxylum armatum* DC.) essential oil. All the methods allowed the determination of methyl cinnamate to be responsible of the spicy odor of the oil, linalool and a series of aldehydes explaining its coriander aspect, limonene and myrcene for the citrus note, and several potent mercaptans for the grapefruit, blackcurrant and tropical facets. But according to the method, the impact odorants were not ranked the same in the oil. The aromagrams obtained with the different methods together with the determination of relevant olfactory classes to improve visual representation of the results will be discussed in the poster.

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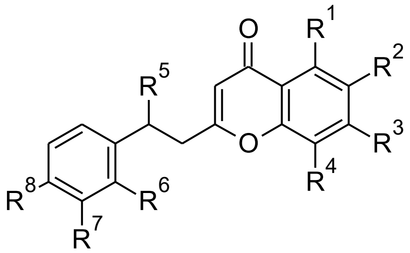
Characterization of Oud Oil (Agarwood, Aloeswood, Eaglewood, *Aquilaria* species) by GC-MS-FID and Derivatization

Juergen K. R. Wanner[1], Erich Schmidt[2], Leopold Jirovetz[3]

[1]*Kurt Kitzing GmbH, Hinterm Alten Schloss 21, 86757 Wallerstein, Germany.*
[2,3]*Department of Pharmaceutical Chemistry, University of Vienna, Austria.*
Corresponding author: juergen.wanner@kurtkitzing.de

Keywords: *Aquilaria* species, GC-MS-FID analysis, complex compositions, unknowns, separation, derivatization

Every now and then analytical chemists in the fragrance industry are confronted with the task to evaluate the authenticity of rare or unusual essential oils with complex compositions and unknown main constituents. This is, for instance, the case for the essential oils (EO) of *Aquilaria* species where a highly valued and unique smelling oil is obtained from the infected heartwood of these tropical trees. This once highest priced EO is now also produced from plants grown in plantations and therefore more available on the market than in earlier days where the wood was only collected in the wild from infected trees which are difficult to distinguish from healthy ones which resulted in the near extinction of these species. The dark brown, viscous EO is mainly composed of a complex mixture of sesquiterpenes, -terpenoids and the particularly characteristic 2-(2-Phenylethyl)-chromones **(I)** with unique structures for which analytical data or comparison chromatograms are hard to obtain. The necessary mass spectrometric data are in these cases not included in the standard (NIST, Wiley) or even special flavor and fragrance MS libraries and the analyst is reliant on original literature where single substances were isolated and identified. To assign certain chromatographic peaks to a specific chemical substance class it can be helpful to separate the EO into fractions and derivatize the oil with simple chemical reactions. In this presentation we want to show how to elucidate the composition of this oil by these means without the use of sophisticated instrumentation.



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Pollen diversity and volatile variability of Corsican blossom honey

Yin Yang, Marie-José Battesti, Jean Costa, Julien Paolini

Laboratoire Chimie des Produits Naturelle, Université de Corse, UMR CNRS 6134 SPE, 20250, Corte
Corresponding author : paolini@univ-corse.fr

Keywords: AOP-AOC « Miel de Corse – Mele di Corsica », melissopalynological analysis, volatile composition, SPME, chemical variability

The Corsican honey was certified by the AOC and AOP appellation "Miel de Corse-Mele di Corsica" and was classified in six varietal categories: "spring", "spring maquis", "honeydew", "summer maquis", "chestnut grove" and "automne maquis". The quality assessment of Corsican honey was essentially based on melissopalynological data taking into account of the entire pollen spectrum and completed by physico-chemical parameters [1-3]. The aim of this work was to characterize the volatile composition of Corsica blossom honey and to develop an interdisciplinary approach to complete the characterization of Corsican honey and the qualification of the botanical and/or geographical origin.

In this work, 195 nectar honeys were characterized by melissopalynological, physico-chemical and volatile analyses. Pollen analysis allowed the certification of Corsican origin and highlights the main nectariferous species and/or characteristic plant associations of each varietal range. The analysis of volatile fraction by HS-SPME, GC and GC/MS allowed the identification of 80 compounds amounting to 60.7 – 99.7 % of the total composition [4]. The volatile fraction of Corsican honeys was dominated by oxygenated compound (68.7 – 96.5 %) especially the aromatic compounds (32.9 – 56.4 %), except for "autumn maquis" honeys with isophorone derivatives (42.5 %) as major components class. Otherwise, some chemical markers of botanical origins were identified as 2-aminoacetophenone ("chestnut grove"); *p*-anisaldehyde and 4-n-propylanisole ("spring maquis"); isophorone and 3,4,5-trimethylphenol ("automne maquis"); isomers of lilac aldehydes and *p*-menth-1-en-9-al ("spring clementine") [4]. Finally, the "spring non-clementine" and "summer maquis" honeys were both dominated by phenylacetaldehyde and benzaldehyde.

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Composition and Antibacterial Activity of Essential Oils of Endemic Varieties of *Salvia sericeo-tomentosa*

Seçil Yazıcı-Tütüniş[1], Nur Tan[1], Yeter Yeşil[2], Betül Demirci[3], Emir Tan[4]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Istanbul University, 34116 Istanbul, Turkey
[2] Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, 34116 Istanbul, Turkey
[3] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey
[4] Department of Microbiology, Faculty of Pharmacy, Yeni Yuzyıl University, 34010 Istanbul, Turkey
Corresponding author: secilyaz@istanbul.edu.tr

Keywords: *Salvia*, essential oils, bioactivity

Salvia, the largest genus in the Labiatae family, represents more than 900 species throughout the world. The extracts and essential oils of *Salvia* species have been used widely in folk medicine. The experimental studies reports that these plants are known as "sage", have multiple pharmacological effects such as antimicrobial, antioxidative, anti-inflammatory, hypoglycemic, cardiovascular, anxiolytic, antitumor and sedative activities [1]. In this study, composition and antimicrobial activity of essential oils of *Salvia sericeo-tomentosa* var. *hatayica* Celep & Doğan (SH) and *Salvia sericeo-tomentosa* Rech. f. var. *sericeo-tomentosa* (ST) which are endemic varieties in Turkey were investigated. The essential oils were obtained by hydrodistillation from the aerial parts and were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The main constituents of oils were sabinyl acetate (79.9 - 80.1 %) and α-pinene (3.2 - 3.8 %). The antibacterial activity was determined against common Gram (+) and Gram (-) reference bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis,* methicillin resistant *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Proteus mirabilis*) by using standard microbroth dilution (modified with rezasurin) and disc diffusion methods [2]. Furthermore, the essential oil of ST showed better activity than essential oil of SH especially against *S. aureus*. No activity of both oils was observed against *K. pneumoniae, E. faecalis and P. mirabilis*.

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Chemical composition and antitumor activity of *Hedyosmum brasiliense* essential oils in different phenological phases

Maria C. M. Young[1], Cynthia Murakami[2], Marcos E. L. Lima[2], Ivana B. Suffredini[3], Paulo R. H. Moreno[4]

[1] Núcleo de Pesquisa em Fisiologia e Bioquímica, Instituto de Botânica, São Paulo, Brasil.
[2] Pós Graduação em Biodiversidade Vegetal e Meio Ambiente, Instituto de Botânica, São Paulo, Brasil.
[3] Núcleo de Pesquisas em Biodiversidade, Universidade Paulista – UNIP, São Paulo, Brasil.
[4] Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo – USP, São Paulo, Brasil.
Corresponding author: marxyoungmc@gmail.com

Keywords: *Hedyosmum brasiliense*, fenology, cytotoxicity, Brazilian Atlantic Rain Forest.

Hedyosmum brasiliense (Chloranthaceae) is a dioecious shrub commonly used in the Brazilian folk medicine [1]. The aim of this work was to compare the chemical composition and antitumor activity of *H. brasiliense* essential oils in different phenological phases obtained from plants collected at Paranapiacaba, an Atlantic Rain Forest reservation area. The essential oils from the different parts of male and female plants were collected during flowering, fruiting and vegetative phases, extracted by hydrodistillation and analyzed by GC/MS [2]. Cytotoxicity was tested towards tumor cell lines MCF-7 (breast adenocarcinoma) and PC-3 (prostate carcinoma), using doxorubicin as positive control [3, 4]. Statistical analysis was performed by one-way analysis of variance (ANOVA, $P<0.05$). Leaves and flowers showed similar yield (~0.20%) and the fruits 0.09%. All essential oils presented predominance of monoterpenes (60 – 73%), except for the flowers (36%). The major compounds of all leaves and flowers are sabinene (20-30% leaves, 10% flowers) and (Z)-α-bisabolene (7-10% leaves, 17% flowers), while in the fruits were found (E)-β-ocimene (14%) and cis-chrysanthenyl acetate (10%). Although the essential oil composition of flowers, fruits and leaves is different, the cytotoxicity was statistically similar, as the IC_{50} values ranged from 15±1 to 31±7 μg/mL against MCF-7 and from 25±0 to 99±25 μg/mL against PC-3. Considering that leaves are available all year round and presented similar cytotoxic potential as flowers and fruits, these results suggest that *H. brasiliense* leaves can be further studied for their antitumor potential during any phenological phase.

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Chemical composition and antimicrobial activity of the essential oil from *Teucrium kotschyanum*

Gizem Gulsoy-Toplan[1], Nur Tan[1], Gokalp Iscan[2], Çaglayan Gurer[1], Betul Demirci[2]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey
[2]Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir,Turkey
Corresponding author : eczgizemgulsoy@gmail.com

Keywords: *Teucrium kotschyanum*, Lamiaceae, Antimicrobial activity, Hemorrhoid plant

Turkey is a gene-centre for the family of Lamiaceae. The genus of *Teucrium*, one of the member of Lamiaceae, is represented by approximately 34 species in our country. This species are commonly known as 'hemorrhoid plant' due to treatment of hemorrhoid disease [1]. They have been used as a folk medicine for treatment of various diseases for more than 2000 years, mainly as diuretic, digestive, analgesic and antispasmodic agent and they also possess antibacterial, antifungal, antidiabetic, antihelmintic, antioxidative and anti-inflammatory activity [2]. *Teucrium* species are rich sources of diterpenoids and also contain monoterpenes, sesquiterpenes, triterpenes, iridoids, flavonoids and essential oil [3]. In this study, essential oil of *Teucrium kotschyanum* is obtained by hydrodistillation using Clevenger apparatus. The essential oil composition of the aerial parts of *Teucrium kotschyanum* collected from Izmir (Odemiş) was investigated by using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), simultaneously. The major compounds were identified as Germacrene D (20.0 %), α-cadinol (6.9 %), β-bourbonene (6.6 %), hexadecanoic acid (6.6 %), δ-cadinene (5.9 %), manool (4.1 %). Antimicrobial and anticandidal activity of the essential oil were also studied against six bacteria and six fungi, using a microdilution broth methods. The essential oil showed moderate to weak inhibitory effects. The minimum inhibitory concentration (MIC) values were also ranged from 125 µg/mL to 2000 µg/mL for all the tested microorganisms.

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Antimicrobial Activity and Chemical Composition of Essential Oils of *Inula germanica* L.

Nur Tan[1], Seçil Yazıcı-Tütüniş[1], Gökalp İşçan[2], Betül Demirci[2], Mahmut Miski[1]

[1] *Department of Pharmacognosy, Faculty of Pharmacy, Istanbul University, 34116 Istanbul, Turkey*
[2] *Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey*
Corresponding author: tannur01@gmail.com

Keywords: *Inula germanica*, essential oils, bioactivity

The genus *Inula* (Asteraceae) which is commonly known as “andızotu” in Turkey includes more than 100 species and mainly distributed in Europe, Africa, and Asia. Various extracts of some *Inula* species showed antimicrobial effect [1]. The chemical composition of essential oils obtained using a traditional (T) and modified (M1 and M2) hydrodistillation method from the aerial parts of *Inula germanica* was evaluated by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The major components of traditional hydrodistillated oil were showed to be α-bisabolol (30.1%), 12-carboxyeudesma-3,11 (13) diene (14.9%), hexadecanoic acid (5.7%), 2,3-didehydrocostic acid (5.5%), α-bisabolol oxide B (5.3%), the major components of M1 were α-bisabolol (6%), 12-carboxyeudesma-3,11 (13) diene (4.8%), 2,3-didehydrocostic acid (2%), 1,8-cineol (9.5%), trans-verbenol (9.5%), cis-chrysanthenyl acetate (9.3%), α-pinene (8.9%), menthone (7.1%), menthol (4.2%) and the major components of M2 were α-bisabolol (11.7%), 12-carboxyeudesma-3,11 (13) diene (24.3%), hexadecanoic acid (5.2%), 2,3-didehydrocostic acid (9.4%), β-caryophyllene (4.5%). Antibacterial and anticandidal effects of the samples were evaluated by using partly modified CLSI (formerly NCCLS) microdilution broth methods M7-A7 and M27-A2 [2,3], respectively. Chloramphenicol, ampicillin, amphotericin-B and ketoconazol were used as standard antimicrobial agents. The essential oils showed moderate to weak inhibitory effects against tested bacteria and *Candida* strains having MIC values of 125 to 2000 µg/mL. M1 showed better effects than the T and M2 between the concentrations of 125 to 500 µg/mL.

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Processing of *Cymbopogon nardus* into essential oil and non-volatile antioxidants by traditional and high pressure extraction methods

Petras Rimantas Venskutonis[1], Marie Elodie Clain[2], Chaker El Kalamouni[2], Renata Baranauskienė[1]

[1]Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų rd. 19, Kaunas, LT-50254, Lithuania

[2] Université de la Réunion, UM 134 Processus Infectieux en Milieu Insulaire Tropical (PIMIT), INSERM U1187, CNRS UMR9192, IRD UMR249. Plateforme Technologique CYROI, 97490 Sainte Clotilde, La Réunion, France.

Corresponding author: rimas.venskutonis@ktu.lt

Keywords: *Cymbopogon nardus*, antioxidant capacity, antibacterial capacity, supercritical fluid extraction, pressurized liquid extraction

Essential oil (EO) of *Cymbopogon nardus*, commonly called citronella, is well known for its citrus-like aroma and bioactivities, e.g. repellent activity against mosquitos, while non-volatile constituents of *C. nardus* have been poorly studied until now. Therefore, the aim of this study was to characterize other potential activities of extracts obtained by traditional and high pressure extraction techniques using supercritical CO_2 (SFE-CO_2) and pressurised liquids (PLE). For this purpose total phenolic content (TPC), antioxidant capacity using DPPH$^•$ and ABTS$^{•+}$ scavenging and oxygen radical absorbance capacity (ORAC) assays, and antimicrobial activities of plant extracts were measured.

The yields of extracts were in the range from 1.39 (EO) to 12.55% (freeze dried water extract obtained from the distillation liquid). More than 50 volatile constituents were identified. Citronellal (13.4%), citronellol (9.3%), geraniol (11.2%) and elemol (25.5%) were the main compounds in the EO. They were the same in the CO_2 extracts (the yield=1.71%) isolated at 50 MPa pressure and 50 ºC; however, citronellal, citronellol and geraniol were present at lower concentrations, 2.1, 7.4 and 7.2%, respectively.

TPC of the obtained products was in the range of 11-70 mg of gallic acid equivalents per g of dry extract weight (mg GAE/g dew); the highest TPC values were found for acetone (AE) and water extracts isolated from SFE-CO_2 residues by PLE, 70 and 64 mg GAE/g dew, respectively. The majority of the products obtained demonstrated strong antioxidant capacity; for instance, the highest radical scavenging capacity in ABTS assay possessed AE and ethanol extracts (EE), 816 and 727 µmol of trolox equivalents (TE/g dew), respectively. TPC values well correlated with antioxidant capacity data. Antibacterial activity of essential oil and extracts was tested by well agar diffusion method with various microorganisms; essential oil at 3% concentration showed antimicrobial capacity for almost all strains of bacteria, except for *S. aureus* and *S. typhimurium*. Minimal inhibitory concentrations (MIC) were in the range of 0.39 to 12.5 mg/mL for all microorganisms tested.

It may be concluded that *C. nardus* extracts may be considered as potential natural antioxidants and antibacterial agents and the source of (poly)phenolics: up to 19% of valuable bioactive substances may be isolated from *C. nardus* essential oil production residues by traditional and high pressure extraction methods.

Biorefining of essential oil bearing plants into valuable ingredients by using traditional and high pressure techniques

Petras Rimantas Venskutonis

Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų rd. 19, Kaunas, LT-50254, Lithuania
Corresponding author: rimas.venskutonis@ktu.lt

Keywords: supercritical fluid extraction; pressurised liquid extraction; antioxidants; phenolic compounds

Essential oils are valuable natural substances, which have been produced and used for various applications since medieval ages. Traditionally, they have been isolated from the essential oil bearing plants by various modifications of distillation using steam and water, while more recently other techniques such as supercritical fluid, ultrasound and microwave assisted extractions have been introduced. So far as essential oils constitute only a small fraction in the herbal materials (largely up to few percent), remarkable amounts of residues remain after their isolation. Such residues are discarded as a waste or being used rather inefficiently, mainly due to the absence of tailor-made technologies, which are based on comprehensive research, for their processing into other, nonvolatile high value substances.

This study is focused on the application of biorefining concept for better valorization of botanicals using traditional and high pressure extraction and fractionation techniques. The term '*biorefinery*' is defined as a facility integrating biomass conversion processes and equipment to produce fuels, power, heat, and value-added chemicals. Valorization of the residues after removing essential oil by hydrodistillation or its isolation together with other lipophilic substances by supercritical fluid extraction with carbon dioxide (SFE-CO_2) was of particular interest in this study.

Two main schemes were developed for the selected plant material: (1) traditional, consisting of hydrodistillation, drying of liquid and solid residues and further extraction of insoluble in water residue by using increasing polarity solvents; (2) high pressure, consisting of SFE-CO_2 and fractionation of the residues by the pressurised liquid extraction (PLE) using increasing polarity solvents. Antioxidant capacity of the fractions obtained was evaluated by several *in vitro* assays, while their phytochemical composition was monitored by various combinations of chromatographic and spectroscopic techniques (GC-FID/MS, UPLC-MS^n). The possibilities of biorefining will be demonstrated by the results of several case studies obtained with different plant materials, such as large-flowered calamint (*Calamintha grandiflora*), sweet cicely (*Myrrhis odorata*), black currant buds (*Riber nigrum*).

In general, application of biorefining concept enabled to increase the yield of valuable substances up to 25-35 %, including essential oils and several fractions of nonvolatile extracts possessing strong antioxidant capacity and containing various phytochemicals. For instance, the total content of polyphenolics in the extracts obtained was up to 350 mg gallic acid equivalents in one gram. The fractions contained various valuable phytochemicals, mainly belonging to the classes of phenolic acids and flavonoids.

Chemical composition and microbial activity of *Thymus ciliatus* harvested during two stages of development

Aïcha Touhami [1,2], Azzedine Chefrour [3] , Abbes Boukhari [1], Fadel Ismail [1,4]

[1] Laboratory of Organic Synthesis-Modeling and Optimization of Chemical Processes, Badji Mokhtar University, P.O. Box 12, 23000 Annaba, Algeria
[2] EPST-Annaba, P.O. Box 218, 23000 Annaba, Algeria
[3] Faculty of natural science and life, University of Mohamed Cherif Messaadia, P.O. Box 12, 41000 Souk-Ahras, Algeria
[4] Department of Process Engineering, Faculty of Engineering, Badji Mokhtar University, P.O. Box 12, 23000 Annaba, Algeria
Corresponding author: a.touhami@epst-annaba.dz

Keywords: *T. ciliatus*, two stages of development, GC- MS, oxygenated monoterpene compounds, antimicrobial activity.

The Thymus species being a part of the botanical family of Lamiaceae , include several developing around the Mediterranean sea, especially in North Africa it consist in 300-400 species .The essential oils obtained by steam distillation of the aerial parts of Thymus collected before and during the flowering period of eastern Algeria, was analyzed by gas chromatography coupled with mass spectrometry (GC -MS) type SHIMATZU QP2010, provided with an FID type detector, equipped with a capillary column OV 17, of 25 m length and 0.25 mm diameter, with a thickness of the movie of 0.25 µm.

The extraction yields of essential oil of T. ciliatus produced oil with yields of 1.002 % before flowering and 1.79 % during flowering. Chromatographic analysis showed the presence of 13 chemical compounds in the before flowering period with the dominance of thymol (25.08%), linalool (23.58%) and gamma terpinene (22.34%). while 54 chemical compounds were obtained during flowering period with dominance of: thymol (54.04%), linalool (8.55%) and carvacrol (7.09%), this oil is rich in oxygenated monoterpene compounds 54.07 % and 80.37 % corresponding to the two periods mentioned above respectively. The discs diffusions tested with 10 µl of the essential oils have proved the good results with reference bacteria microorganisms : *Escherichia coli 22 , Staphyllococcus 23 and Pseudomonas 53* with (7.3 to 50.8 mm) inhibition zones.

How the thin layer chromatography can secure the production of plant extracts and essential oils

Pierre Bernard-Savary[1]

[1] *Club de CCM, Pommiers-la Placette, France*
clubccm@hptlc.com

Keywords: HPTLC, Pharmacopeia, Identification, Detection, Cost-saving

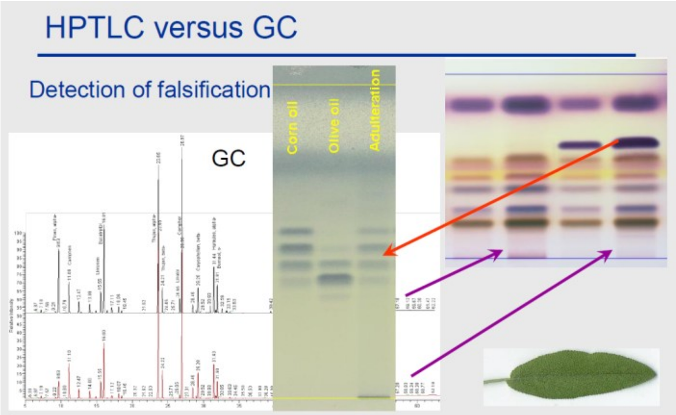
For many years, major players in the plant industry have trusted the thin layer chromatography (TLC) or, in its current version, the HPTLC for High Performance TLC for analysis of natural extracts in general, through identification called fingerprint. It is recognized in this by the Pharmacopeia all over the world (for instance chapter 203 of the USP). Therefore it is obvious that specialists have turned to this method for identifying plants at all stages of the process, and for the detection of falsification.

That is the reason why this method will become in the near future an excellent tool in the context of the changes of regulatory.

Through some examples, this poster will illustrate the various advantages of this method:

- the fast development of an analytical method.
- the importance of choice and the free availability of references and application notes in the field of plants, in particular.
- the reduced cost of the analysis.
- the massive standardization and reproducibility of analysis that enables an efficient and unambiguous analytical information flow.
- the various possibilities in term of detection which bring some answers not provided by other techniques [1]

Finally and to complete the range of possibilities offered by HPTLC, the international symposium (www.hptlc.com) is a database of the highest order. At the national level it is the Club TLC (www.clubdeccm.com) whose next edition will be held in October in Pierre Fabre Dermo-Cosmétique in Toulouse, which allows developing both skills through the training and industrial expert relations network in this domain.



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HPTLC : FASTER, CHEAPER AND MORE RELIABLE, AN ANALYTICAL MUST FOR ESSENTIAL OILS ACTIVITY PROFILE AND DIRECT ACTIVE COMPONENTS ASSAY BASED ON THEIR ACTIVITY

Pierre Bernard-Savary[1]

[1] *Club de CCM, Pommiers-la Placette, France*
clubccm@hptlc.com

Keywords: HPTLC, EDA, Bio-detection, Bioassay, Matrix, Flexibility

The aim of this poster is to show an overview of the current best solutions for direct detection of activity from components of essential oils based on HPTLC, coupled with various "bio-detections", called EDA.

HPTLC is the High-Performance version of Thin-Layer Chromatography. This liquid chromatography on plates is an off-line method. This means that the entire sample remains accessible during the chromatographic process. This gives several advantages including the possibility for running a bio reaction on the plate easily. The Effect Directed Analysis (EDA) is covering many different ways and media which may interact with the substances on the plate. This poster will review the possibilities offered by the HPTLC method regarding EDA, which may be switched in two branches [1]: biochemical staining when a substance, an enzyme for example, is used to react with the compounds, and microbiological when an organism, a bacteria for example, is used for the bioassay on the plate.

Besides this detection flexibility, HPTLC is running the samples in parallel, up to 20-30 on one side, or the double on both side, when a horizontal chamber is used for separation. A few consumption of solvent, speed of separation and the number of samples treated simultaneously add to the method a very good profile for being used in High Throughput Screening. The EDA-HPTLC method has the advantage of offering two steps in one: chromatography and bioassay.

Biochemical methods have shown a larger development because the precision and reliability are quite high in most cases. The capability to show directly the positive result towards a searched effect, toxic for example, gives strength to these methods.

This method benefits of the separated sample remaining on the plate. But for quantitative aspects and especially high sensitivity, high application volumes of any matrix combined with selective detection, may lead to unexpected low quantification limits.

The International Symposium for HPTLC last held in Lyon (July 2014), dedicated special sessions to bio-detection. Next issue will take place in Berlin, Germany in July 2017 (www.hptlc.com).

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Chemotaxonomy vs Genotaxonomy of Viola genus: Can native volatile compounds of flowers be genuine tracers for violets differentiation?

Justine Chervin[1,2,3], B. Dumas[2], M-T. Esquerre[2], N. Fabre[3], G. Marti[3], T. Talou[1,*]

1 Laboratoire de Chimie Agro-industrielle (LCA), Université de Toulouse, INRA, INPT, Toulouse, France
2 Laboratoire de Recherche en Sciences Végétales (LRSV), UMR 5546, CNRS, UPS, Toulouse, France
3 UMR 152 Pharma Dev, Université de Toulouse, IRD, UPS, Toulouse, France
Corresponding author: justine.chervin@ensiacet.fr

Keywords: Violet, Volatile compounds, HS-SPME-GC/O, Phylogeny, Chemotaxonomy.

Violets are odorous flowers known in the Mediterranean basin since Antiquity and were a part of the first perfumes created. Toulouse has an historical relationship with this flower which is the symbol of the town and the home of the famous Viola Tolosa, Parma of Toulouse variety (*Viola alba subsp. dehnhardtii*).

As a part of the regional "Viola Tolosa" project, a chemotaxonomy study of the hundred varieties/cultivars of violets belonging to the French Violet Conservatory hosted by the Toulouse Municipal Greenhouses will be set up. Based on headspace analysis of native volatile compounds emitted by flowers at maturity, dual chemical-sensory fingerprints were established for a rapid and efficient differentiation. A HS-SPME-GC/O method was used on two types of volatile samplings: an in-vivo and non-destructive analysis on the whole plant with the use of a bell-shaped glass covering to trap the volatiles as well as an ex-vivo analysis on the fresh cut flowers placed in a vial.

First, experimental analytical conditions were optimized by experimental design methodology in order to determine the best suited protocols to obtain the richest volatile cocktails. Then optimized methods were applied to a sampling of ten plants of the collection selected according to the colour of flowers and their geographical origins. In parallel, genetic analyses were conducted on the same plants to confirm the putative varieties or cultivars. Finally, all the results were combined to match the chromatographic profiles with the genetic affiliation to a group.

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Determination of antifungal effect of the essential oil combinations obtained by different methods

Musa Türkmen, Durmuş Alpaslan Kaya*

Department of Field Crops, Faculty of Agriculture, Mustafa Kemal University, 31034,
Antakya-Hatay, Turkey.
**Corresponding author : dak1976@msn.com.*

Keywords: *Sclerotinia sclerotiorum*, antifungal, *Foeniculum vulgare, Laurus nobilis*

In this study, *in vitro* antifungal efficacy of the essential oils obtained from different plant such as *Foeniculum vulgare* and *Laurus nobilis* plants, naturally growing in the different regions of Hatay province, Turkey, were investigated against soil-borne fungal (*Sclerotinia sclerotiorum)* disease agents.

Volatile and contact phase of essential oils at different concentrations inhibited the mycelial growth in a dose-dependent manner. Volatile phases of *L. nobilis* essential oils showed fungitoxic effect at 7.50 µl and *L. nobilis* essential oils showed fungicidal effect at 15 µl. *F. vulgare* essential oils showed fungitoxic effect at 5.0 µl concentration and *F. vulgare* essential oils show fungicidal effect at 7.50 µl. The two essential oils were mixed using different methods and they were coded as E (manually mixed oils), K (together distillation of oils) and H (using solvent and mixed oils) depending on the used methods. The mixed oils were tested against *Sclerotinia sclerotiorum* pathogens. The results showed that *L. nobilis* and *F. vulgare* obtained separately by manually mixing with appropriate combination of essential oils (E-R3D1) showed fungicidal effect at 5.0 µl; *L. nobilis* and *F. vulgare* essential oil obtained by distilling the mixture (K-R3D1) showed fungicidal effect at 3.75 µl concentrations and *L. nobilis* and *F. vulgare* essential oils with proper combination and mixture with hexane (H-R3D1) showed fungicidal effect at 3.75 µl.

Antimicrobial activities presented that the essential oils in proper combinations are more effective than their using alone against fungal disease agents. This demonstrate their potential to be used as a new alternative control measures against fungal diseases which is of high economically importance.

Anti-bacterial and antioxidant properties of essential oil of *Pterocarya fraxinifolia* L

Ahanjan Mohammad[1], Ebrahimzadeh M A[2], Fathi H[3]

1. Mazandaran pediatric infectious diseases research center, Mazandaran university of medical sciences, Sari, Iran

2. Faculty of pharmacy, Mazandaran university of medical sciences

3-Faculty of pharmacy, Mazandaran university of medical sciences

Corresponding Author: Mohammad Ahanjan

Email: ahanjan2007@gmail.com

Keywords: *Pterocarya fraxinifolia*, Anti-bacterial, antioxidant

Pterocarya fraxinifolia L. belongs to the family Juglandaceae. It is commonly known as the Caucasian wingnut or Caucasian walnut. It is native to the Caucasian region Armenia, Azerbaijan, Georgia, Iran, Russia, the Ukraine and Turkey. The tree is monoecious and grows to a height of < 30 m, the short, thick bole supporting widely spreading branches to form a rounded structure not unlike the Wych Elm. The pinnate leaves can exceed 60 cm in length, comprising 7 - 27 sessile leaflets. In the present work we determined phenolic and flavonoids content of *Pterocarya fraxinifolia* L. extracts and fractions and their antioxidant and anti-bacterial properties (1). Objectives: To evaluate the inhibition of antibacterial and antioxidant effects of the essential oil and fractions from *Pterocarya fraxinifolia* L. Methods: Antioxidant activity was evaluated with DPPH radical scavenging assay and anti-bacterial effect of fractions was measured by their inhibition potency on the human pathogen bacteria E.coli and Klebseilla pneumoniae. The GC-MS analysis of various organic crude extracts isolated from leaves of *Pterocarya fraxinifolia* L. was performed using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 500, USA) equipped with a VF-5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Results: The methanolic and ethanolic extracts exhibited a potent inhibition growth of tested bacteria. It also shows an antioxidant activity measured on DPPH with an IC50 of 100 mg/ml. and demonstrates a high antioxidant activity with an IC50 of 120 µg/m [2]. The major chemical compounds in hexane crude extract were found (fraction was the rich one on flavonoids and phenolic compounds (100 mg/g dry weight). Conclusion: Study conducted on *Pterocarya fraxinifolia* L. shows that inhibits efficiently the tested bacteria.

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Antibacterial activity chemical composition of the Alcoholic Extract of Marjoram against *Staphylococcus aureus, E.coli, Salmonella enterica* and *Pseudomonas aeroginosa*

Mohammad Ahanjan[1], M.Rahbar[2], R.Mehdizade[3], Soheila Ahanjan[4]

1- Traditional and Complementary Medicine Research Center, Mazandaran University of Medical Sciences, Sari, Iran

2- Microbiology Department, Mazandaran University of Medical Sciences

3-Microbiology Department, Islamic Azad university, Dameghan branch

4. Biotechnology student, Islamic Azad university, Babol Branch

Corresponding Author: Mohammad Ahanjan

E.mail: ahanjan2007@gmail.com

Keywords: Marjoram, Antibacterial activity, chemical composition

Herbs enjoy a unique value and importance in sustaining healthy communities in terms of disease prevention (1). In this regard, Marjoram is a plant of the mint family which has antibacterial properties (2). The current study aims to investigate the antimicrobial activity of the alcohol extracts of Marjoram plants on the bacteria of *Staphylococcus aureus* (ATCC: 25923) *E.coli* (ATCC: 25922), and *Salmonella enterica* (ATCC 13076) and *P. aeroginosa* through utilizing disk diffusion method. Also, the MIC and MBC concentration of ethanol and methanol extracts on *E.coli* were equal with 100 and 120 milligrams per milliliter, respectively. Moreover, the measurement of the MIC and of the of marjoram ethanol extraction on *Staphylococcus aureus* was reported to be 90 mg/ml 100 mg/ml, respectively. In addition, the amount of ethanol and methanol extracts on *Salmonella enteric* and *P. aeroginosa* was equal with 80 and 90 mg/ml, respectively. The results showed that Marjoram alcoholic extract have antibacterial properties. Also, among the alcoholic extracts, the ethanol extract has demonstrated to be the most effective extract on *Salmonella enterica* and *E. coli* and *P. aeroginosa.*

Acknowledgements: The authors are grateful to Mazandaran university of medical sciences for providing all chemicals and other expenses from their internal fund to carry out this project.

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Volatile glucosinolate autolysis products of three Serbian crucifers

Milica M. Todorovska[1], Niko S. Radulović[1]

[1]*Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia*
Corresponding author : mimatod@gmail.com

Keywords: *Draba lasiocarpa*, *Thlaspi praecox*, *Conringia orientalis*, glucosinolates, goitrogens, 5,5-dimethyl-2-oxazolidinethione

In the past few decades the importance of glucosinolates, once known as mustard oil glucosides, has increased further following the discovery of their potential as cancer-preventing agents, crop-protection compounds, and biofumigants in agriculture [1]. Hence, a detailed phytochemical profiling of plant species containing these secondary metabolites deserves attention. This work presents the first report on the glucosinolates present, inferred from the volatile products of natural autolysis (endogenous myrosinase), in three members of the family Brassicaceae from Serbia- *Draba lasiocarpa* Rochel, *Thlaspi praecox* Wulfen and *Conringia orientalis* (L.) Dumort. (*D. lasiocarpa* and *T. praecox* have never been investigated previously and *C. orientalis* for the first time from Serbia). A single goitrogenic volatile glucosinolate product, 5,5-dimethyl-2-oxazolidinethione, was detected by GC and GC-MS, suggesting the presence of only one glucosinolate, glucoconringiin (2-hydroxy-2-methylpropyl glucosinolate). 5,5-Dimethyl-2-oxazolidinethione was isolated in pure state by column chromatography and its structure confirmed by an extensive analysis of its 1D and 2D NMR spectra. All plant organs (flowers, leaves and roots) of *D. lasiocarpa* and *C. orientalis* contained this glucosinolate, but it was present only in the roots of *T. praecox*. Besides being previously reported from *C. orientalis* (and isolated for the first time) [2], glucoconringiin seems to have a restricted occurrence in the family Brassicaceae (previously identified only in *Draba aizoides* [3]), however also reported in other taxa of the order Capparales (*Reseda alba* [4] (Resedaceae), *Moringa stenopetala* [5] and *Moringa peregrina* [6] (Moringaceae)). Such a distribution may be of chemotaxonomic significance.

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AUTHOR INDEX

AUTHOR INDEX

LIST OF PARTICIPANTS

Surname	Name	Institution	Country	E-mail
Acs	Kamilla	University of Pécs	Hungary	kamilla.acs@gmail.com
Adrian-Scotto	Martine	Institut de Chimie de Nice	France	Martine.ADRIAN-SCOTTO@unice.fr
Ahanjan	Mohammad	Mazandaran university of medical sciences	Iran	tahanjan@mazums.ac.ir
Al-Saadi	Sahar		Iraq	saharmalik2010@gmail.com
Andreani	Stéphane	Institut de Chimie de Nice	France	andreani.stephane@gmail.com
Angelini	Eric	V. MANE FILS	France	eric.angelini@mane.com
Anglade	Juliette	V. MANE FILS	France	juliette.anglade@mane.com
Antoniotti	Sylvain	Institut de Chimie de Nice	France	sylvain.antoniotti@unice.fr
Asakawa	Yoshinori	Tokushima Bunri University	Japan	asakawa@ph.bunri-u.ac.jp
Asekun	Olayinka	University of Lagos	Nigeria	oasekun@unilag.edu.ng
Ashraf	Amir Reza	University of Pécs	Hungary	amirreza@gmail.com
Badalamenti	Filippo	Agrumaria corleone spa	Italy	filippo@agrumariacorleone.com
Baldovini	Nicolas	Institut de Chimie de Nice	France	baldovin@unice.fr
Barat	Nelly	Robertet	France	nelly.barat@robertet.com
Barata	Lauro	Federal University of the West Pará	Brazil	lauroesbarata@gmail.com
Barbu	Diana		France	diana.barbufr@yahoo.com
Baser	K.Husnu Can	Near East University	Cyprus	khcbaser@gmail.com
Belhassen	Emilie	Firmenich SA	Switzerland	emilie.belhassen@firmenich.com
Bellenot	Denis	ITEPMAI	France	denis.bellenot@iteipmai.fr
Bellin	Nelly	V. MANE FILS	France	nelly.bellin@mane.com
Berger-Gloaguen	Florence	Puressentiel	France	f.berger@puressentiel.com
Bertoli	Luca	MILESTONE SRL	Italy	extraction@milestonesrl.com
Bertolucci	suzan	Federal University of Lavras	Brazil	suzan@dag.ufla.br
Bettini	Fabienne	IFF	France	fabienne.bettini@iff.com
Bicchi	Carlo	Università degli Studi di Torino	Italy	carlo.bicchi@unito.it
Bicherel	Pascal	KREATiS	France	pascal.bicherel@kreatis.eu
Bizzo	Humberto	Embrapa Food Technology	Brazil	humberto.bizzo@embrapa.br
Blagojevic	Polina	University of Niš	Serbia	blagojevicpolina@gmail.com
Blerot	Bernard	IFF-LMR	France	bernard.blerot@iff.com
Bodifee	Han Paul	Pole de compétitivité PASS	France	hp.bodifee@pole-pass.fr
Bonhomme	Dominique	Institut de Chimie de Nice	France	Dominique.BONHOMME@unice.fr
Bosilcov	Alin	Brüder Unterweger GmbH	Austria	alin.bosilcov@unterweger-oils.com
Bouaza	Yasmine	DACHSER FRANCE	France	
Bouges	Hélène	Institut de Chimie de Nice	France	HELENE.BOUGES@unice.fr
Bouslama	Dorra	CADIMA PATHE	France	cadima@cadima.com
Brevard	Hugues	Robertet	France	hugues.brevard@robertet.com
Brouet	Jules	CMC Malongo	France	jbrouet@malongo.com
Brud	Wladyslaw	Pollena-Aroma Ltd	Poland	wsbrud@post.pl
Buchbauer	Gerhard	University of Vienna	Austria	gerhard.buchbauer@univie.ac.at
Bujara	Roman	Frey + Lau GmbH	Germany	rbujara@freylau.de
Bukowski	Nick	Markes International	UK	nbukowski@markes.com
Bushdid	Caroline	Institut de Chimie de Nice	France	caroline.bushdid@unice.fr
Cagliero	Cecilia	Università degli Studi di Torino	Italy	cecilia.cagliero@unito.it
Campos	Estefania	São Paulo State University	Brazil	vangelie_89@hotmail.com
Carbonell	Carolina	Eurofragance S.L.	Spain	ccarbonell@eurofragance.com
Carenini	Elise	ALBERT VIEILLE SAS	France	ecarenini@albertvieille.com
Carlson	Richard	Young Living	USA	richcarlson@youngliving.com
Cavailles	Margaux	V. MANE FILS	France	corinne.dalmasso@mane.com
Cavallero	Andrea	V. MANE FILS	France	andrea.cavallero@mane.com
Cazalilla	Florent	V. MANE FILS	France	florent.cazalilla@mane.com
Cerutti	Celine	IFF-LMR	France	celine.cerutti@iff.com
Cerveau	Laurent	Research Institute for Chromatography	France	laurent.cerveau@ric.eu
Chaintreau	Alain	Flavour and Fragrance Journal	Switzerland	alc.ffj@bluewin.ch
Charmeau	Carole	CEHTRA	France	carole.charmeau@cehtra.fr
Chervin	Justine	ENSIACET	France	justine.chervin@ensiacet.fr
Chizzola	Remigius	University of Veterinary Medicine, Vienna	Austria	Remigius.Chizzola@vetmeduni.ac.at
Cicchetti	Esmeralda	Cosmo International Ingredients	France	esmeralda.gonzales@cosmo-ingredients.com
Claud	Philippe	Pole de compétitivité PASS	France	p.claud@pole-pass.fr

Surname	Name	Institution	Country	E-mail
Cooper	Jane	Waters	UK	jane_cooper@waters.com
Corbi	Elise	CHANEL SAS, Laboratoire Parfums	France	elise.corbi@chanel-corp.com
Costa	Jean	Université de Corse	France	costa@univ-corse.fr
Courivaud	Alix	FRANCEAGRIMER	France	alix.courivaud@franceagrimer.fr
Csikos	Eszter	University of Pécs	Hungary	csiko18000@gmail.com
Danila	Elena	Leather and Footwear Research Institute	Romania	elena_only4u@yahoo.com
Davenne	Dominique		France	davenne.dominique@wanadoo.fr
David	Nathalie	CHANEL SAS, Laboratoire Parfums	France	nathalie.david@chanel-corp.com
David	Frank	Research Institute for Chromatography	Belgium	frank.david@richrom.com
de Panthou	Fabrice	IN EXTENSO INNOVATION	France	fabrice.de-panthou@inextenso-innovation.fr
Dekic	Milan	State University of Novi Pazar	Serbia	mdekic@np.ac.rs
delfino	carla	Imperial europe srl	italy	info@imperialeurope.it
Delsenne	Freddy	Waters	France	freddy_delsenne@waters.com
Demange	Luc	Institut de Chimie de Nice	France	Luc.DEMANGE@unice.fr
Demyttenaere	Jan	European Flavour Association	Belgium	jdemyttenaere@effa.eu
Djabou	Nassim	University of Tlemcen	Algeria	nassim_djabou@yahoo.fr
Dupouyet	Alice	CHARABOT	France	alice.dupouyet@charabot.com
Duroure	Leslie	Cosmo International Ingredients	France	leslie.duroure@cosmo-ingredients.com
Dutriez	Thomas	Givaudan	Switzerland	thomas.dutriez@givaudan.com
Ebibi	Nailee	CHANEL SAS, Laboratoire Parfums	France	nailee.ebibi@chanel-corp.com
Etienne	Jean-Jacques		France	jeanjetienne@aol.com
evrard	kevin	CHROMACIM	France	ke@chromacim.com
Ferreira	Vicente	University of Zaragoza	Spain	vferre@unizar.es
Ferse	Falk-Thilo	Waters	Germany	falk-thilo_ferse@waters.com
Feyaerts	Adam	VIB Department of Molecular Microbiology	Belgium	adamfeyaerts@gmail.com
Figueiredo	A. Cristina	FFCUL	Portugal	acsf@fc.ul.pt
Filipovic	Sonja	University of Niš	Serbia	sonjafilipovic86@yahoo.com
Filippi	Jean-Jacques	Institut de Chimie de Nice	France	Jean-Jacques.Filippi@unice.fr
Fodor	Csaba	Silvestris & Szilas Ltd	Hungary	csaba_fodor@silvestris.hu
Fourmentin	Sophie	UCEIV	France	lamotte@univ-littoral.fr
Franz	Chlodwig	Med'Arom	Austria	chlodwig.franz@vetmeduni.ac.at
Galmiche	Diane	EXPRESSIONS PARFUMEES	France	c.leja@epparfums.com
Garry	Pierre Philippe	BONTOUX SAS	France	pierre-philippe.garry@bontoux.com
Geyken	Nadia	drom fragrances international	Germany	nadia.geyken@drom.com
Giordano	Julien	Robertet	France	julien.giordano@robertet.com
Golebiowski	Jérôme	Institut de Chimie de Nice	France	Jerome.GOLEBIOWSKI@unice.fr
Gonzalez	Cécile	International Fragrance Association	Belgium	cgonzalez@ifraorg.org
Goursot	Jean-François	PRODAROM	France	jf.goursot@prodarom.fr
Green	Bob	Markes International	UK	bgreen@sepsolve.com
Grimato	Laura	Misitano & Stracuzzi SpA	italy	lgrimato@misitanoestracuzzi.com
Grolleau	Lucie	DACHSER FRANCE	France	lucie.grolleau@dachser.com
Guillamon	Nadine	Robertet	France	nadine.guillamon@robertet.fr
Guillard	Bruno	DACHSER FRANCE	France	bruno.guillard@dachser.com
Guillemain	Marine	ALBERT VIEILLE SAS	France	mguillemain@albertvieille.com
Hadiati	Septilia Wahyu	Badan POM	Indonesia	septilia.wh@gmail.com
Harlalka	Nikunj	C.G.Herbals	India	nikunj@cgherbals.in
Harlalka	Ramakant	C.G.Herbals	India	ramakant@nishantaromas.com
Hasegawa	Toshio	Saitama University	Japan	toshihas@mail.saitama-u.ac.jp
Hoeferl	Martina	University of Vienna	Austria	martina.hoeferl@univie.ac.at
Horvath	Györgyi	University of Pécs	Hungary	gyorgyi.horvath@aok.pte.hu
Hoshino	Tsutomu	Niigata University	Japan	hoshitsu@agr.niigata-u.ac.jp
Ilic	Ivan	University of Niš	Serbia	ilicko81@gmail.com
Jaquier	Alain	Firmenich SA	Switzerland	alain.jaquier@firmenich.com
Jesionek	Anna	Medical University of Gdansk	Poland	annajesionek@gumed.edu.pl
Join	Benoît	Symrise AG	Germany	benoit.join@symrise.com

Surname	**Name**	**Institution**	**Country**	**E-mail**
Jones	Helen	University of New England	Australia	gjones295apple@gmail.com
Jones	Graham	University of New England	Australia	gjones2@une.edu.au
Joulain	Daniel	SCBZ Conseil	France	
Juillard	Antonin	AZUR FAGRANCES	France	antonin.juillard@azur-fragrances.com
Kamkaen	Narisa	Faculty of Pharmacy, Rangsit university	Thailand	narisa.k@rsu.ac.th
Konopacka-Brud	Iwona	Pollena-Aroma Ltd	Poland	i.konopacka-brud@post.pl
Kormann	Karine	Robertet	France	karine.kormann@robertet.fr
Kuraya	Eisuke	National Institute of Technology, Okinawa College	Japan	kuraya@okinawa-ct.ac.jp
Lambin	David	V. MANE FILS	France	david.lambin@mane.com
Laurent	Caroline	BIPEA	France	claurent@bipea.org
Laurent	Manon	Robertet	France	manon.laurent@robertet.fr
Lavoine	Sophie	CHARABOT	France	sophie.lavoine@charabot.com
Le Borgne	Erell	Cosmo International Ingredients	France	erell.leborgne@cosmo-ingredients.com
Leko	Ivan	Bau Welt SC d.o.o.	Bosnia Herzegovina	ivan.leko@ao-leko.com
Lemasson	Yann	Firmenich SA	France	yann.lemasson@firmenich.com
Leonard	Coralie	Sigma Aldrich	Switzerland	coralie.leonard@sial.com
Levy	Florence	L'Oreal	France	flevy@rd.loreal.com
Llorca	Carlos	Eurofragance S.L.	Spain	cllorca@eurofragance.com
Llorens-Molina	Juan Antonio		Spain	juallom2@qim.upv.es
Lobo	Viviane	Technological Federal University of Paraná	Brazil	vivianelobo@utfpr.edu.br
Lochynski	Stanislaw	Wrocław University of Science and Technology	Poland	stanislaw.lochynski@pwr.edu.pl
Lopez	Estefania	Eurofragance S.L.	Spain	elopez@eurofragance.com
Loyer	Raymond	SPECTROCHROM	France	raymond.loyer@spectrochrom.com
Loziene	Kristina	Nature Research Centre	Lithuania	kristina.loziene@gmail.com
Ludwiczuk	Agnieszka	Medical University of Lublin	Poland	aludwiczuk@pharmacognosy.org
Magnan	Marie	COSMED	France	regulatory2@cosmed.fr
Maia	Beatriz	Federal University of Paraná	Brazil	bhsalesmaia@gmail.com
Marchini	Marie	ALBERT VIEILLE SAS	France	mmarchini@albertvieille.com
Marin	Christophe	EXPRESSIONS PARFUMEES	France	f.guerin@epparfums.com
Marpaung	Yuniar	Badan POM	Indonesia	yuniar_9664@yahoo.com
Martinet	Nadine	Institut de Chimie de Nice	France	nadine.martinet@inserm.fr
Martini	Sophie	Institut de Chimie de Nice	France	sophie.martini@unice.fr
Martins	Erika	University Paulista	Brazil	eramosmartins@gmail.com
Marx Young	Maria Claudia	Instituto de Botânica, São Paulo	Brazil	marxyoungmc@gmail.com
Masson	Jerome	Robertet	France	hubrevard@gmail.com
Mathé	Lotte	VIB	Belgium	lotte.mathe@mmbio.vib-kuleuven.be
Meierhenrich	Uwe	Institut de Chimie de Nice	France	uwe.meierhenrich@unice.fr
Mello	Romulo		Brazil	romulomm88@hotmail.com
Mercier	Jérémy	CHROMACIM	France	jm@chromacim.com
Meremans	Valérie	FLORAL CONCEPT	France	vmeremans@floral-concept.com
Mérida-Reyes	Max	University of San Carlos of Guatemala	Guatemala	maxmerida2050@yahoo.com
Merle	Philippe	Firmenich SA	Switzerland	philippe.merle@firmenich.com
Messineo	Elvire	BIPEA	France	emessineo@bipea.org
Miljkovic	Vojkan	University of Niš	Serbia	vojkanmm_serbia@yahoo.com
Miltojevic	Ana	University of Niš	Serbia	anamiltojevic@yahoo.com
Missitch	Christian	PERKINELMER	France	christian.missitch@perkinelmer.com
Moreno	Paulo	University of São Paulo	Brazil	prmoreno@iq.usp.br
Murakami	Cynthia	Instituto de Botânica, São Paulo	Brazil	cynthia.murakami@uol.com.br
Muratore	Agnès	V. MANE FILS	France	agnes.muratore@mane.com
Muselli	Alain	Université de Corse	France	muselli@univ-corse.fr
Nagai	Katsuya	ANBAS Corp. / Osaka University	Japan	knagai@anbas.co.jp
Nakamura	Mayu	Kindai University	Japan	1633650008a@nara.kindai.ac.jp
Ngom	Saliou	L'OREAL	France	sngom@rd.loreal.com
Niewiadomska	Maria		Poland	mrylka@gmail.com
Nikolic	Milica	University of Niš	Serbia	milica.nikolic.1990@gmail.com
Nyegue	Maximilienne A.	University of Yaoundé	Cameroun	maxy_nyegue@yahoo.fr

Surname	**Name**	**Institution**	**Country**	**E-mail**
Okoh	Sunday	University of Fort Hare	South Africa	sokoh@ufh.ac.za
Oliveira	Jhones Luiz	São Paulo State University	Brazil	jholuisoliveira@hotmail.com
Opruta	Tiberius	Terpena S.R.L.	Romania	tiberius.opruta@yahoo.com
Ozek	Gulmira	Anadolu University	Turkey	gozek@anadolu.edu.tr
Ozek	Temel	Anadolu University	Turkey	tozek@anadolu.edu.tr
Pacaud	yves	Agilent	France	y.pacaud@wanadoo.fr
Panero	ombretta		Switzerland	opanero@bacardi.com
Paolini	Julien	Université de Corse	France	paolini@univ-corse.fr
Pascoli	Monica		Brazil	monica.pascoli@outlook.com
Peix	Lorène	Puressentiel	France	l.peix@puressentiel.com
Peres	Christophe	CHANEL SAS, Laboratoire Parfums	France	christophe.peres@chanel-corp.com
Perez-Sabino	Francisco	Universidad de San Carlos de Guatemala	Guatemala	fpsabino@usac.edu.gt
Perichet	Christine	CHARABOT	France	christine.perichet@charabot.com
Peron	Marie-Christine	V. MANE FILS	France	marie-christine.peron@mane.com
Pesic	Marko	University of Niš	Serbia	marko.pesic89@yahoo.com
Phan	Tony	V. MANE FILS	France	tony.phan@mane.com
Picault	Aline	V. MANE FILS	France	aline.picault@mane.com
Pinto	Jose Eduardo	Universidade Federal de Lavras-UFLA	Brazil	jeduardo@dag.ufla.br
Plantier	Damien	BONTOUX SAS	France	benedicte.rozenblat@bontoux.com
Plantier	Damien	BONTOUX SAS	France	damien.plantier@bontoux.com
Plessis	Caroline	V. MANE FILS	France	caroline.plessis@mane.com
Poirieux	Nicolas	IPRA FRAGRANCES	France	poirieuxnicolas@gmail.com
Polatoğlu	Kaan	Istanbul Kemerburgaz University	Turkey	kaan.polatoglu@kemerburgaz.edu.tr
Poli	Jean-Pierre	Université de Corse	France	poli_jp@univ-corse.fr
Portier	David	PERFUM.COM	France	analyse@perfum.com
Protzen	Maren		Germany	maren.protzen@kaders.de
Qader	Karzan		Iraq	karzanqader2016@gmail.com
Radoias	Georges	Brüder Unterweger GmbH	Austria	labor.bu-oils@tirol.com
Radoias	Martha	Brüder Unterweger GmbH	Austria	martha.popovici@yahoo.com
Radulovic	Niko	University of Niš	Serbia	nikoradulovic@yahoo.com
Rosa	Mauricio		Brazil	mauriciofrosa@yahoo.com.br
Roudiere	Ingrid	IPRA FRAGRANCES	France	ingrid.roudiere@iprafragrances.com
Roy	Céline	ERINI	France	celine.roy@erini-institute.com
Rubiolo	Patrizia	Università degli Studi di Torino	Italy	patrizia.rubiolo@unito.it
Saint-Lary	Laure	PAYAN BERTRAND	France	lsaint-lary@payanbertrand.com
San Juan	Felipe	Eurofragance S.L.	Spain	fsanjuan@eurofragance.com
Santerre	Cyrille	ISIPCA	France	csanterre@isipca.fr
Santos	Marcio		Brazil	
Sarrazin	Elise	CHANEL SAS, Laboratoire Parfums	France	elise.sarrazin@chanel-corp.com
Schalk	Michel	Firmenich SA	Switzerland	Michel.Schalk@firmenich.com
Scheffer	Ella		Netherlands	ellascheffer@hetnet.nl
Scheffer	Hans (JJC)		Netherlands	jjc.scheffer@hetnet.nl
Schloss	haim	J.D. Schloss	Israel	info@jd-schloss.com
Schloss	Zipi	J.D. Schloss	Israel	zipi.schloss@gmail.com
Schmidt	Erich		Germany	info@artandfragrance.de
Schnaebele	Nicolas	CHARABOT	France	nicolas.schnaebele@charabot.com
Seijo Rigueira	Lorenzo	Institut de Chimie de Nice	France	lorenzoseijo@hotmail.com
Setzer	William		USA	wsetzer@chemistry.uah.edu
Sgorbini	Barbara	Università degli Studi di Torino	Italy	barbara.sgorbini@unito.it
Sharopov	Farukh	University of Central Asia	Tajikistan	sharopov@uni-heidelberg.de
Sherman	Zipi	J.D. Schloss	Israel	yddng5@gmail.com
Sieniawska	Elwira	Medical University of Lublin	Poland	elwira.sieniawska@gmail.com
Silva	Franceli		Brazil	franceli.silva@gmail.com
Simon	Yann	Thermo Fisher Scientific	France	yann.simon@thermofisher.com
Simsek	Gokhan	ERDOGMUS PARFUM SANAYI HUSEYIN ERGOGMUS	Turkey	gokhan@epsfragrances.com

Surname	**Name**	**Institution**	**Country**	**E-mail**
Sinche Ambrosio	Carmen Milagros		Brazil	carmen.milagros8@usp.br
Smith	Brett	Young Living Essential Oils	USA	btsmith@youngliving.com
Stappen	Iris	University of Vienna	Austria	iris.stappen@univie.ac.at
Stashenko	Elena	Universidad Industrial de Santander	Colombia	elenastashenko@gmail.com
Stojanovic	Nikola	University of Niš	Serbia	nikola.st90@yahoo.com
Strelow	Laurent	Société L&J	France	laurentstrelow@orange.fr
Strub	Daniel	Wrocław University of Science and Technology	Poland	daniel.strub@pwr.edu.pl
Suffredini	Ivana	ASSUPERO	Brazil	ibsuffredini@yahoo.com.br
Sulniute	Vaida	Kaunas University of Technology	Lithuania	vaida91@yahoo.com
Szemraj-Lochynska	Alicja		Poland	a.lochynska@gmail.com
Talou	Thierry	ENSIACET	France	Thierry.talou@ensiacet.fr
Tan	Nur	Istanbul University	Turkey	tannur01@gmail.com
Tan	Emir	Yeni Yuzyıl University	Turkey	etan57@gmail.com
Tartarin	Pascal	Thermo Fisher Scientific	France	pascal.tartarin@thermofisher.com
Tascone	Oriane	ASTIER DEMAREST	France	oriane.tascone@astierdemarest.com
Tayabali	Riad	BAROSYL	France	riad@barosyl.com
Teles	simone	Federal University of Recôncavo da Bahia/UFRB	Brazil	telessimone@gmail.com
Tetard	Nicolas	TOURNAIRE S.A.	France	nicolas.tetard@tournaire.fr
Thomas	Paul	KREATiS	France	paul.thomas@kreatis.eu
Tine	Yoro	Université Cheikh Anta Diop, Dakar	Senegal	yoro.tine@ucad.edu.sn
Tissandié	Loïc	Institut de Chimie de Nice	France	loic.tissandie@gmail.com
Todorovska	Milica	University of Niš	Serbia	mimatod@gmail.com
Tomi	Kenichi	Kindai University	Japan	tomi@nara.kindai.ac.jp
Tomi	Felix	Université de Corse	France	felix.tomi@univ-corse.fr
Topin	Jérémie	Institut de Chimie de Nice	France	Jeremie.TOPIN@unice.fr
Touhami	Aicha	Ecole préparatoire au sciences et techniques	Algeria	a.touhami@epst-annaba.dz
Tranchida	Peter Q.	University of Messina	Italy	peterquinto.tranchida@unime.it
Trestour	Sophie	PAYAN BERTRAND	France	strestour@payanbertrand.com
Truan	clement	ANALYTISS-UDA	France	contact@analytiss.com
Turkmen	MUSA	Mustafa Kemal University	Turkey	Turkmenmusa@hotmail.com
Uchinami	Kanae	Kindai University	Japan	1633650007a@nara.kindai.ac.jp
Uehara	Ayaka	Institut de Chimie de Nice	France	ayaka.uehara@etu.unice.fr
van Baren	Catalina Maria	Universidad de Buenos Aires	Argentina	cbaren@ffyb.uba.ar
Varshney	Vinay K.	FOREST RESEARCH INSTITUTE	India	vinay.varshney5@gmail.com
Vasilj	Hrvoje		Croatia	hrvoje.vasilj@gmail.com
Vasseur	Clémence	Cosmo International Ingredients	France	clemvasseur@orange.fr
Venskutonis	Petras Rimantas	Kaunas University of Technology	Lithuania	rimas.venskutonis@ktu.lt
Vey	Matthias	IFRA	Belgium	mvey@ifraorg.org
Wagemans	Jeroen	KU Leuven LRD	Belgium	jeroen.wagemans@kuleuven.be
Wanner	Juergen	Kurt Kitzing GmbH	Germany	juergen.wanner@kurtkitzing.de
Wassmer	Felix	LUZI AG	Germany	wassmer.felix@luzi.ch
Yang	Yin	Corsica university	France	yang@univ-corse.fr
Yazıcı-Tütüniş	Seçil	Istanbul University	Turkey	Secilyaz@istanbul.edu.tr
Yildirim	Asik Sureyya	EFECAN A.S.	Turkey	Sureyya@efecan.com.tr
Yildirim	Seyma	EFECAN A.S.	Turkey	Seyma@efecan.com.tr
Zhelijazkov	Valtcho	Goodwin	USA	Valtcho.Jeliazkov@oregonstate.edu