# ISEO 2021

# 51st International Symposium on Essential Oils (ISEO 2021)

*12-14 November 2021, Online*

**SYMPOSIUM PROGRAMME & ABSTRACT BOOK**

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**51st International Symposium on Essential Oils**

November 12-14, 2021, Online

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**Dear ISEO Permanent Scientific Committee Members,**
**Dear ISEO Participants,**
**Dear ISEO Sponsors,**

We hope in the covid period that all of you and your families and friends are doing well, safe and all are healthy…

We heartily thank you for all your feedback and guidance.

As you know, we have prepared initially for a "normal- physical" ISEO, since 2019… and we really hoped that the situation would be suitable in 2021 August, scheduled time for ISEO. However, today we are talking about the 3rd wave of covid, which forces us to an online-ISEO with a professional team. Thus, this has many drawbacks including finance, however, nothing is more important than the safety of all ISEO participants. With the agreement of the ISEO Permanent Committee we have decided on the new dates of the 51st ISEO as November 12-14, 2021

The symposium even online has associated costs as previously mentioned, however, the finance shall not be a hindrance for those who lack financial resources, we shall ask sponsors for support.

Our university is supporting online national and international symposia/ congresses and meetings, which I hope for other institutions as well.

We will soon make regular mailings and ask your continuous feedback and suggestions for the improvement of services and the symposium program. Also your advice regarding potential sponsors will indeed help for the overall quality of the meeting.

Stay well safe and healthy… kind regards

**Prof. Dr. K. Hüsnü Can Başer**
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## ISEO Medal of Honor


The idea of ISEO Medal was finally born in 2018 during the Permanent Scientific Committee meeting organized in Nis (Serbia). The first ISEO Medal of Honour was presented during the 50th anniversary of the ISEO symposia in 2019. This time, by decision of the all members of the ISEO Permanent Scientific Committee held in 2019 in Vienna, Professor Karl-Heinz Kubeczka (Würzburg, Germany) was elected to be the recipient of the ISEO Medal of Honor, and we have awarded this medal to him during 51st International Symposium on Essential Oils.
Unfortunately, in October 2021 we received information about the death of Prof. Kubeczka. We have lost a great scientist and friend. He was one of the founding fathers of ISEO meetings, as well as a valuable and highly respected member of ISEO symposia. ISEO meant very much to him, not only the science involved and the development of the different subjects, but the people he met there – his old friends, and also the younger generation of scientists. We all do appreciate his outstanding contribution in the field of essential oils, and feel sorry that we had to award the ISEO Medal to him posthumously. The medal and certificate will later be sent to Prof. Kubeczka's wife, Christa Kubeczka.

## A brief biography of Prof. Kubeczka

Prof. Dr. Karl-Heinz Kubeczka was born on March 31, 1935 in Ostrava, Czechoslovakia. Professor Kubeczka studied Pharmacy at the Technical University of Karlsruhe, Germany where he obtained his doctor degree (natural sciences) in 1967. After his habilitation in 1973 at the University of Hamburg he was appointed as a professor in Pharmaceutical Biology at the University of Wurzburg in 1974. Since 1989 he has been serving as Head of the Department of Pharmaceutical Biology at the University of Hamburg until his retirement in 2000. Besides university teaching his research has been directed in the area of essential oil research, especially the analysis of plants metabolites and the improvement of the analytical techniques. He was one of the founders of the International Symposium on Essential Oils (ISEO) in 1969 and organized or coorganized the following scientific meetings in 1976, 1979, 1981, 1982, 1989, 1995, 2000, and 2003. In 1991 he was awarded honorary member of the Societa Italiana di Fitochimica (Italian Society of Phytochemistry). He is the member of the editorial board of the Journal of Essential Oil Research and reviewer of several international journals on medicinal plant research, flavor and fragrances and phytochemistry. He is the author of more than 140 scientific papers related to essential oils, volatile terpenoids and analytical techniques.

Musical career; Karl-Heinz Kubeczka studied to play the flute from 1949 to 1960 at the first flutist Nicco Schnarr of the Baden State Orchestra and founded at the same time at the high school of Bruchsal, Germany the school orchestra as a conductor. In 1964 he played in the rococo palace of Bruchsal the concerto for flute and orchestra of Luigi Boccherini, as a soloist. From 1952 to 1962 and from 1979 to 1989 he acted as choirmaster and performed with his choir several masses in the churches near Bruchsal and Wurzburg. Up till now he plays regularly flute in a chamber ensemble.

On behalf of the ISEO Permanent Scientific Committee
Agnieszka Ludwiczuk

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# Scientific Programme

| | |
|---|---|
| **12 November (Fri)** | |
| 09.30-10.00 | Opening Speech - K. Hüsnü Can Başer ISEO President, Agniezska Ludwiczuk PSC-President |
| 10.00-10.30 | ISEO Medal of Honor |
| **Session 1 – Kubezcka Special** | |
| **Chairs: K. Hüsnü Can Başer, Agniezska Ludwiczuk** | |
| 10.30-11.00 | PL1 - Why are essential oils so important for the present and future renewability of the F&F industry?<br>**Alain Frix & Jonathan Bonello** |
| 11.00-11.20 | OP1 - Photo-protective effects of selected furocoumarins on D-(+)-limonene, β-pinene and γ-terpinene<br>**Hannes Bitterling** |
| 11.20-11.40 | OP2 - In vitro enzyme inhibitions of essential oils<br>**Fatih Demirci- K.H.C. Başer** |
| 11.40-12.00 | OP3 - Chemical and Bioinformatics Analyses of the Anti-Leishmanial and Anti-Oxidant Activities of Hemp Essential Oil<br>**Claudio Ferrante** |
| 12.00-12.20 | OP4 - Cinnamon essential oil encapsulation: controlled release for biosourced pesticides<br>**Chloe Maes** |
| 12.20-12.40 | OP5 - Pilot study: Which has the greater impact on instantaneous muscle power, simple olfactory stimulation or odour hedonics?<br>**Kei Sato** |
| 12.30-14.00 | Break |
| **Session 2** | |
| **Chairs: Jonathan Bonello, Yoshinori Asakawa** | |
| 14.00-14.30 | PL2 - Essential oils and nosocomial infections: challenge or reality?<br>**Györgyi Horváth** |
| 14.30-15.00 | PL3 - Contribution to the chemistry of agarwood volatiles<br>**Nicolas Baldovini** |
| 15.00-15.20 | OP6 - The current state of knowledge of essential oil in southern African Lamiaceae<br>**Ryan Rattray** |
| 15.20-15.40 | OP7 - Influence of drought stress on growth and essential oil yield of Ocimum species<br>**Sintayehu Mulugeta** |
| 15.40-16.00 | Break |
| **Session 3** | |
| **Chairs: Müberra Koşar, Györgyi Horváth** | |
| 16.00-16.30 | PL4 - Research Institute for Fragrance Materials (RIFM) program for comprehensive, risk-based safety assessments of natural complex substances used as fragrance ingredients<br>**Jim Romine** |
| 16.30-16.50 | OP8 - Study of patchouli essential oil chemical composition as a function of plant part, acid hydrolysis and distillation time<br>**Karen J. Ramirez** |
| 16.50-17.10 | OP9 - Effects of different preservation methods on the active compounds and organoleptic properties of Garden Sage (Salvia officinalis L.) leaves<br>Urbashi Hazarika |
| 17.10-17.30 | OP10 - Is 1D-GC really dead? Exploring the utility of a 4-parallel-columns GC-GC system for essential oils routine analysis<br>**Alexis St-Gelais** |
| 17.30-17.50 | OP11 - Regional Variability in pesticides in essential oils and extracts<br>**Noura Dosoky** |

# Scientific Programme

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| **13 November (Sat)** | |
| **Session 4** | |
| **Chairs: İhsan Çalış, Temel Özek** | |
| 10.00-10.30 | PL5 - Australian Essential Oils – Industry and Research<br>**Ian Southwell** |
| 10.30-11.00 | PL6 - Essential oils and volatiles in liverworts, mosses and hornworts<br>**Agnieszka Ludwiczuk** & Yoshinori Asakawa |
| 11.00-11.20 | OP12 - The Influence of Harvest Intensity and Season on Yield and Quality of the Essential Oil of Kunzea ambigua<br>**Chanjoo Park** |
| 11.20-11.40 | OP13 - *Cinnamomum cassia* and *Syzigium aromaticum* essential oils reduce the gut colonization capacity of *Salmonella typhimurium*, in an in vivo infection model using *Caenorhabditis elegans*<br>**Marie Lang** |
| 11.40-12.00 | OP14 -Improving the efficiency and antioxidant activity of essential oil extracted from Abies sachalinensis using underwater shockwave pretreatment<br>**Hideaki Kawai** |
| 12.00-12.20 | OP15 - Development of a new tool for pest management in fruit arboriculture using essential oils applied by trunk injection<br>**Pierre-Yves Werrie** |
| 12.20-12.40 | OP16 - Mutagenic, genotoxic, ecotoxic, and antimicrobial properties of low molecular oxime ethers<br>**Alicja Surowiak** |
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| **Session 5** | |
| **Chairs: Ben-Erik Van Wyk, Gökalp İşcan** | |
| 14.00-14.30 | PL7- Using essential oils to fight SARS-CoV-2 and treat COVID-19<br>**Adam F. Feyaerts** |
| 14.30-15.00 | PL8 - Innovative Approaches for Molecular Identification in the Flavor & Fragrances Field<br>**Luigi Mondello** |
| 15.00-15.20 | OP17 - Chemical composition, antifungal and antioxidant activity of essential oils from different subspecies of Helichrysum italicum grown in Italy<br>**Ylenia Pieracci** |
| 15.20-15.40 | OP18 - Farm to Pharmacy- The organic and sustainable supply of five essential oils in South Africa<br>**Karen Swanepoel** |
| 15.40-16.00 | Break |
| **Session 6 - IFEAT Young Scientists** | |
| **Chairs: Ben-Erik Van Wyk, Adam F. Feyaerts** | |
| 16.00-16.30 | YS1 - An exploratory analysis of the chemical composition and antibacterial activity of Citrus essential oils on Salmonella strains isolated from poultry litter<br>**Carmen M.S. Ambrosio** |
| 16.30-16.50 | YS2 - The effect of methyl jasmonate and salicylic acid on the essential oil of peppermint (Mentha piperita) and marjoram (Origanum majorana)<br>**Wafae Kandoudi** |
| 16.50-17.10 | YS3 - Essential Oils as Bioactive Secondary Metabolites against Bacterial and Fungal Mycetoma; One of the Most Neglected Tropical Diseases<br>**Shereen Abd Algaffar** |
| 17.10-17.30 | YS4 - The influence of plant matrix on the volatile organic compounds emission from medicinal and aromatic plants – the case study for various Mentha ssp.<br>**Jacek Łyczko** |
| 17.30-17.50 | YS5 - Accurate quantification of olibanic acids in several frankincense essential oils from diverse origin by stable isotope dilution assay.<br>**Kimberley Massei** |
| 17.50-18.10 | YS6 - Antibacterial Evaluation of β-Ionyl Acetate and The Bioconversion Metabolite<br>**Özge Özşen Batur** |

# Scientific Programme

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| **14 November (Sun)** | |
| **Session 7** | |
| **Chairs: Ana Cristina Figueiredo, Sibel Kılıç** | |
| 10.00-10.30 | PL9 - A global perspective on the diversity of aromatic plants<br>**Ben-Erik Van Wyk** |
| 10.30-11.00 | PL10 - Application of Phytochemicals from Bryophytes to Foods, Cosmetics, and Medicine<br>**Yoshinori Asakawa** |
| 11.00-11.20 | OP19 - The healing effect of Carvacrol on Nasal Septal Perforation<br>**Nilüfer Kuruca** |
| 11.20-11.40 | OP20 - Carvacrol have anti-apoptotic effects in rats with diabetic nephropathy<br>**Nilüfer Kuruca** |
| 11.40-12.00 | OP21 - Antibacterial Activities of Six Essential Oils and Carvacrol: Interaction of Selected Combinations<br>**Mehmet İlktaç** |
| 12.00-12.20 | OP22 -Which essential oils started to be used in ear diseases in Ottoman medicine and when?<br>**Serra Ağırman Yilmaz** |
| 12.20-12.40 | OP23 -Chemical composition and antibacterial activities of essential oils of different salvia species against plant bacterial disease agents<br>**Merve Kara** |
| 12.40-13.30 | Break |
| **Session 8** | |
| **Chairs: Eisuke Kuraya, Bülent Köse** | |
| 13.30-14.00 | PL11 – Green Extraction of Volatiles and Essential Oils<br>**Mustafa Z. Özel** |
| 14.00-14.20 | OP24 - Authentication of some essential oils using enantiomeric distribution of key components<br>**Prabodh Satyal** |
| 14.20-14.50 | Closing Ceremony<br>**K.H.C. Baser - Agnieszka Ludwiczuk -Daniel Strub** |


# Contents

## YOUNG SCIENTIST PRESENTATIONS

# Plenary Lectures

51st International Symposium on Essential Oils (ISEO 2021)
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# PL-1. Why are Essential Oils so Important for the Present and Future Renewability of the Flavour & Fragrance industry?

**Alain Frix[1], Jonathan Bonello[2]**

*[1]Chair of IFEAT Scientific Committee, [2]Chief Scientific Officer at IFEAT*

*Corresponding authors e-mail:* alain.frix@allchemix.com, cso@ifeat.org

## Abstract

It is estimated that the flavour and fragrance (F&F) industry consumes about 750'000 Mt of raw materials every year (weight of organic chemicals, excluded solvents or other additives) . Of these, about 500'000 Mt are of petrochemical origin while 250'000 Mt are obtained from natural and renewable sources. Of these approximately 250'000 Mt, over 110'000 Mt are derived from turpentine while another amount exceeding 100'000 Mt are Essential Oils and associated Natural Complex Substances (NCSs), split roughly 50:50 between citrus and non-citrus essential oils. The rest of the 250'000 Mt are composed primarily of other fruit and vegetable extracts, natural chemicals/compounds, and biotechnology products. Essential oils are not only renewable, they are also natural (unlike most turpentine derivatives) and as they provide work to millions of farmers across the globe, they represent by far the largest socio-economic contribution to our industry. Aside from flavour and fragrance applications, essential oils are increasingly used in aromatherapy, pharmaceutical, plant protection, nutritional supplements, food preservation among others. While biotechnology does provide a very promising future prospect, currently only a very small number of F&F, cosmetic and aromatherapy ingredients are produced in this way. There is therefore no doubt that essential oils, and associated NCSs are and will remain critical raw materials for such industries, and this for many decades.

In contrast, global industry, 1000 times larger than the F&F industry, very likely relies on about 750 million Mt of organic mass or hydrocarbons per year, mostly derived from fossil fuels. Among the largest consumers are, for example, plastics and other polymers, lubricants and coatings, plant protection products, detergents, automotive, textile, electronics, cosmetics, energy, and pharmaceutical industries. At present less than 10% of materials used in global industries are renewable and recycling is limited.

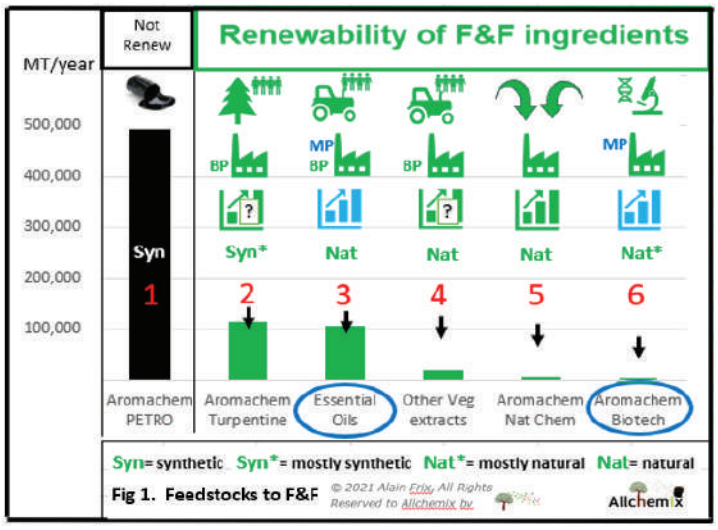


Fig 1. Feedstocks to F&F

Furthermore, today, most industries are seeking natural, renewable and sustainable raw materials, as in Figure1. Therefore, strong competition for biomass that complies with these objectives is very much a reality. As the global population grows, pressure on agricultural land and cultivation priorities as well as incentives in terms of crop production also becomes an important factor. Among all these competing forces in this so-called green gravitation, the strongest markets are likely to attract the larger proportions of renewable and sustainable hydrocarbons. Farmers and other producers of biomass are the backbone and therefore represent the future of our industry. The role of IFEAT, ISEO and other global and regional associations active in defending and promoting the essential oils and associated NCSs industry, becomes more relevant as well as more important in this scenario.

**Key words:** F&F, industrial applications, environmental issues, commercial aspects

**Acknowledgment:** The figures and data presented online during the plenary lecture are subject to copyright©.

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# PL-2. Essential oils and nosocomial infections: challenge or reality?

**György Horváth[1,*], Viktória Lilla Balázs[1], Eszter Csikós[1] and Béla Kocsis[2]**

*[1] Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs, 7624, Pécs, HUNGARY*

*[2] Department of Medical Microbiology and Immunology, Medical School, University of Pécs, 7624, Pécs, HUNGARY*

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## Abstract

The discovery of antibiotics was a revolutionary step in the treatment of infectious diseases. However, the microbiologists encountered resistant strains early. Alexander Fleming found a penicillin-G resistant *Staphylococcus aureus* strain as early as 1944. Methicillin was approved in 1958, and the first methicillin-resistant strain was isolated in 1961 [1]. The resistant strains have spread all over the world, mostly in hospitals where the so-called nosocomial poly-resistant or multidrug-resistant (MDR) strains are present. Nosocomial infections also referred to as healthcare-associated infections (HAI). According to the Centers for Disease Control and Prevention, the types of HAI include: (1) Central line-associated bloodstream infections (CLABSI); (2) Catheter-associated urinary tract infections (CAUTI); (3) Surgical site infections (SSI); (4) Ventilator-associated pneumonia (VAP). The broad spectrum of microorganisms, mainly bacteria, are responsible for nosocomial infections, e.g. methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-resistant *Enterococcus* (VRE), Enterobacteriaceae with extended-spectrum cephalosporin resistance consistent with extended-spectrum beta-lactamase (ESBL) production, carbapenem-resistant Enterobacteriaceae and *Acinetobacter* species, multi-drug resistant *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* [2]. There is no doubt that multiple antibiotic resistance of bacteria is a significant public health issue.

The popularity of essential oils (EOs) is growing, and a lot of EOs are recommended for treating different diseases, via aromatherapy or clinical aromatherapy. It is well known that EOs have antimicrobial potential. But, the question is: how this phenomenon of EOs can be utilized in a clinical atmosphere against nosocomial infections.

The plenary lecture will focus on the background of nosocomial infections, the types of organisms, main routes of transmission, prevention of nosocomial infections, EOs in health care system, evidences about EOs in the treatment of nosocomial infections, future outlook. Results coming from recently performed human trials will also be introduced.

Acknowledgment

György Horváth was supported by the NKFI (National Research, Development and Innovation Office) K 128217 grant. Eszter Csikós was supported by the ÚNKP-20-3-II New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

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**Keywords**: nosocomial infections, healthcare-associated infections, antibiotic-resistance, essential oil, clinical aromatherapy

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# PL-3. Contribution to the chemistry of agarwood volatiles.

**Nicolas Baldovini***
*Institut de Chimie de Nice, Université Côte d'Azur, CNRS, UMR 7272, Parc Valrose, 06108 Nice, FRANCE*
**Corresponding author. Email: Nicolas.Baldovini@unice.fr*

## Abstract

Agarwood (oud) is the resinous infected heartwood of Aquilaria trees (*Aquilaria* sp.), which grow in tropical forests of Southeast Asia. It has been used since almost 3000 years as an incense in Buddhist, Hindu, and Islamic cultures, and as a component of traditional Asian medicines for various effects. It also occupies a central role in the kôdô (香道), lit. « the way of the incense », the traditional Japanese art related to the ritual of burning incense. The very specific odor of agarwood has been described by Roman Kaiser, a renowned fragrance specialist from Givaudan Company, as « one of the most fascinating scent source of this world (…) a pervasive, mysterious, wonderful scent, bringing to mind the perfumes of all imaginable precious woods, balsams, and resins, as well as those of amber, musk, and castoreum and, somewhat hidden, even tender floral notes » (Kaiser, 2006). Nowadays, it is recognized unanimously as the most expensive wood, as well as the most prestigious natural raw material used in perfumery. Agarwood is widely employed in the Middle East, either burned as an incense or hydrodistilled to produce an essential oil (agarwood oil) used in fine perfume formulations. Because of the high interest of this material for the fragrance industry, its composition has been extensively studied and reported in many publications, which show that agarwood oils and extracts are extremely complex mixtures of hundreds of constituents (aromatics, chromones, sesquiterpenoids…) (Li, 2021). This communication will give an overview of the chemical compositions of agarwood essential oils reported in the literature, and of the analyses of agarwood smoke using various techniques for the characterization of smoke volatiles. It will be completed by a presentation of the compositions of commercial and lab made agarwood oils, and of the volatiles emitted by heating various samples of powdered agarwood in the conditions of the Kôdô (ca. 150°C). The analyses were performed by GC-MS and GC-FID, and the sample preparation technique for smoke analysis used a homemade Purge & Trap system which trapped agarwood smoke on Porapak Q in a glass tube.

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**Keywords**: Agarwood, *Aquilaria*, smoke analysis.

# PL-4. Research Institute for Fragrance Materials (RIFM) program for comprehensive, risk-based safety assessments of natural complex substances used as fragrance ingredients.

**James C. Romine, Anne Marie Api, Danielle Botelho, and Christen Sachse-Vasquez**
*Research Institute for Fragrance Materials (RIFM), Woodcliff Lake, NJ, 07677 USA*

**Abstract:**

The Research Institute for Fragrance Materials (RIFM) was founded in 1966 as a non-profit scientific organization missioned to establish acceptance and trust in the safe use of fragrance ingredients through applied science and research. Since 2014, RIFM has assessed and published over one thousand comprehensive safety assessments on individual fragrance ingredients. Recently, RIFM developed a systematic protocol for conducting safety assessments, covering seven human health and environmental outcomes for natural complex substances (NCS), most of which are essential oils. An overview of the RIFM NCS safety assessment methodologies is provided, along with the plan to evaluate approximately 850 NCS fragrance ingredients.

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# PL-5. Australian Essential Oils – Industry and Research

**Ian A. Southwell***
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*Corresponding author. Email: ian.southwell@scu.edu.au

## Abstract

Australia produces a number of essential oils for both domestic and international markets. Tea tree oil from *Melaleuca alternifolia* is the largest of these with more than 1000 tonnes produced annually. *Eucalyptus* (*E.polybractea*, *E. kochii* and others) for 1,8-cineole oils is next at 200 - 300 tonne. With sandalwood oil from traditional sources in short supply, Australian Sandalwood (*Santalum spicatum*) has been further developed to about 3 – 4 tonne and east-Indian sandalwood (*S. album*) from commercial plantations to about 1.5 tonne. Lemon myrtle (*Backhousia citriodora*) is being harvested as a rich source of citral for both dried leaf (eg lemon tea) and oil uses (eg source of citral, aromatherapy, medicinal uses). Lavender (*Lavandula augustifolia*) is grown for the export market and various lavandin variety oils distilled for domestic use principally in tourist outlets. In addition, there are many smaller crop initiatives, some already developed (eg Boronia (*Boronia megastigma*); Mountain Pepper (*Tasmannia lanceolata*)), and others either under development or showing potential for commercialisation.Such commercial production has encouraged essential oil research in many areas: (1) a tea tree (*Melaleuca alternifolia*) breeding project improving plantation yields for three decades[1]; (2) determination of the chiral ratios in the three main tea tree chemotypes[2] has led to the inclusion of enantiomeric composition specifications for principal component, terpinen-4-ol, in the ISO Standard for tea tree oil[3]; (3) examination of tea tree seedling extracts has revealed, for the first time, the presence of acylphloroglucinols similar in structure to those in other species with medicinal and plant growth regulatory properties; (5) a chemotaxonomic summary of the Australian mint-bush *Prostanthera* genus[4]; (6) the genetic basis for terpene chemotypes in *Melaleuca* and *Eucalyptus*[5]; (7) details of the taxonomy, essential oil composition, silvicultural characteristics, uses and natural distribution of nearly 300 species of *Melaleuca*[6] listed in one volume for use by people interested in developing an industry for commercial oil production. Much research has been aimed at identifying Australian native flora oil constituents to seek their commercialisation.

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**Keywords**: Australian essential oils, industry & research, oil production, new crops, development

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# PL-6. Essential oils and volatiles in liverworts, mosses and hornworts

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## Abstract

Bryophytes (mosses, liverworts and hornworts) represent a diverse group of lower plants. These are small, non-vascular, spore-forming plants, and the most basal group among the land plants. These tiny organisms contain numerous very interesting, from the chemistry point of view, terpenoids and other volatiles. Among the bryophytes, liverworts, in particular, are characterized by the presence of compounds that cannot be found in any other organisms (Asakawa et al., 2013; Asakawa & Ludwiczuk, 2018).

An enormous diversity of volatiles present in liverworts is used for chemotaxonomic studies. Compounds occurring in the essential oils and volatile extracts constitute a powerful tool for studying chemical differences between or within liverwort species, genera or families (Ludwiczuk & Asakawa, 2014). Terpenoids present in bryophytes are involved in many biochemical and ecological processes, especially as defenses against biotic stresses such as insects and microbial pathogens (Chen et al., 2018). The bryophyte components are also known to have interesting biological properties such as antibacterial, antifungal, antiviral, neurotropic, insect repellent, muscle relaxing, as well as cytotoxic, and apoptosis-inducing activities, among others **(**Asakawa & Ludwiczuk, 2018; **Ludwiczuk & Asakawa, 2019).**

This lecture will review current knowledge of terpenoid secondary metabolites and other volatile components present in bryophytes from the perspective of chemical diversity, chemotaxonomy, and biological functions.

**Acknowledgment**

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**Keywords**: bryophytes, liverworts, terpenoids, chemosystematics, biological functions

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# PL-7. Using essential oils to fight SARS-CoV-2 and treat COVID-19

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## Abstract

On March 11, 2020, the World Health Organization declared the outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) a pandemic (Cucinotta & Vanelli, 2020). The novel coronavirus SARS-CoV-2 can cause the potentially deadly coronavirus disease 2019 (COVID-19) in humans. In the early days of the pandemic, standard care for critically ill COVID-19 patients consisted mainly of advanced respiratory support, as no specific pharmaceutical treatments were available (Feyaerts & Luyten, 2020).

At the time, the global focus was mainly on prevention through personal non-pharmaceutical interventions (NPIs), such as staying at home in case of illness, and community NPIs such as social distancing ("Nonpharmaceutical Interventions (NPIs)," 2020). However, in an unprecedented effort, pharmaceutical interventions for prophylaxis, treatment or cure of COVID-19 were intensively sought ("Pharmaceutical Interventions," 2021). To date, no new small molecule drugs have been fully approved for the treatment of COVID-19 by the US Food and Drug Administration or the European Medicines Agency, although 66 countries have (un)officially adopted early treatments for COVID-19 ("Global adoption of COVID-19 early treatments," 2021), including the essential oil (EO) components (EOCs) citronellol, estragole, eugenol and limonene ("Other Early Treatments for COVID-19," 2021).

Several EO(C)s are known for their antiviral effects, especially against enveloped viruses such as coronaviruses (Wani, *et al*., 2021). Since SARS-CoV-2 spreads significantly by aerosols, and mainly infects the respiratory tract, we tested selected EOs *in vitro* using our vapour-phase-mediated susceptibility assay, adapted for testing against SARS-CoV-2 (Feyaerts *et al*., 2018). These pilot experiments show a clear radial effect of cellular toxicity and protection for all EOs tested. Some EOs show a mild antiviral effect, although none of the selected EOs provided complete protection against SARS-CoV-2. Based on our preliminary results, some benefits may be obtained from EOs in established COVID-19 infections, although the findings have yet to be confirmed in a clinical setting (Feyaerts, Baudoux, Neyts, & Luyten, 2021). Interrupting aerial transmission by EO(C)s that inactivate SARS-CoV-2 may prove more promising.

**Acknowledgment**

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**Keywords**: SARS-CoV-2, COVID-19, essential oil, essential oil component, vapor-phase-mediated susceptibility assay

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# PL8 – Innovative Approaches for Molecular Identification in the Flavor & Fragrances Field

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## Abstract

Gas chromatography coupled to mass spectrometry (GC-MS) represent the workhorse technique used in F&F application. However, in some cases the obtained information may not be enough for a reliable identification. To this concern, the use of Infrared Spectroscopy that provides molecular-level structural information, such as functional groups, bonding and conformation could be on hand (i.e. for the distinction among regio-isomers). Hyphenation of GC-MS to FTIR provide both vibrational spectra of chromatographically separated compounds and information about their retention behaviour.

A novel instrument configuration, consisting in a parallel coupling of a GC-MS and solid deposition (sd) FTIR detector was achieved by means of an external heated transfer-line developed in our laboratory. In detail, the exiting GC column flow was diverted to the two detectors by using capillaries of different length and size, to adjust the flow ratio, in order to meet the sensitivity requirements of both the detectors. Furthermore, the use of the solid-state interface, compared to a gas phase (light pipe) one, enabled to overcome the limitations related to the rather poor detection limits of GC-FTIR technique.

The effectiveness of this hyphenated instrument, in providing a single automated technique, for the identification of unknowns in F&F field is hereby demonstrated. The complementary information obtained and in addition, the use of Linear Retention Indices (LRI) strongly reduced the possibility of a misidentification.

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# PL-9. A global perspective on the diversity of aromatic plants

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## Abstract

The use of aromatic plants for cosmetic, medicinal and ritual purposes almost certainly originated in southern Africa, where the click-speaking San and Khoi people (the most ancient human cultures on earth, according to DNA evidence) used buchu powders (made from at least 50 species) mixed with sheep fat to anoint their bodies. Another ancient centre of diversity is Ethiopia, where frankincense and myrrh (*Boswellia* and *Commiphora* species) have been used in rituals and ceremonies for centuries. In modern times, essentials oils, extracts and resins have become industrialised, with large quantities used in the cosmetics and food industries. Prices for oils range from a few cents per gram for eucalyptus oil to more than the price of gold (currently ca. 58 dollars per gram) for pure agarwood essential oil (oud oil) from *Aquilaria* species.

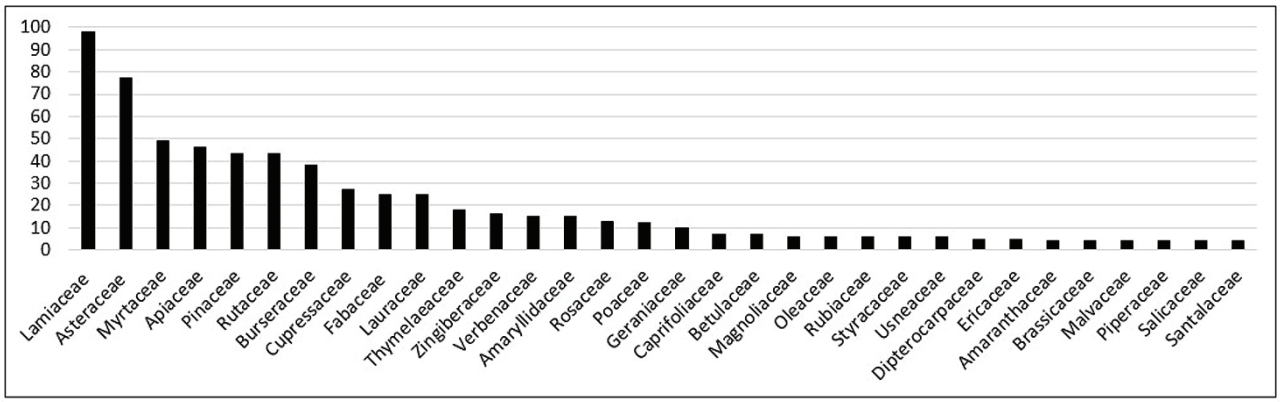


**Figure 1:** Approximate numbers of commercialised aromatic plant species (including infraspecific taxa) per family

The presence of essential oil in lichens, mosses and ferns suggests that the biosynthetic pathways for monoterpenoids and sesquiterpenoids have been establish early in the evolutionary history of vascular plants. The anatomical diversity of oil canals, oil glands and osmophores that give rise to volatile oils and aromatic resins raises interesting questions about the homology of chemical similarities between distantly related or unrelated plant groups. More than 600 aromatic plant species have been commercialised, with about 65% of the diversity represented by the top 10 plant families (Lamiaceae to Lauraceae, Figure 1). A different picture emerges when the percentage of commercialised species per family is considered. The Pinaceae and Cupressaceae then dominate, both with more than 16% of their species commercialised, followed by Burseraceae in a distant third position, with 6%. The commercial potential of large families of aromatic plants from temperate and arid regions such as the Lamiaceae, Asteraceae and Rutaceae remains poorly explored and a gradual increase in new essential oils for niche markets can be expected in the future. It is also likely that new sources of commercially relevant volatile compounds will be discovered.

**Acknowledgment**

I thank the organisers for inviting me to present this lecture. Financial support from the National Research Foundation of South Africa (grant no 84442 to the SARChI National Research Chair in Indigenous Plant Use) is gratefully acknowledged.

**Keywords:** Essential oils, commercialised aromatic plants, history of aromatherapy, plant families, resins, species diversity

# PL-10. Application of Phytochemicals from Bryophytes to Foods, Cosmetics, and Medicine

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Over several hundred new phytochemicals have been isolated from the bryophytes (three classes: liverworts, mosses and hornworts) and more than 50 new carbon skeletal terpenoids and aromatic compounds found in these classes. Most of liverworts elaborate characteristic odorous, pungent or, bitter tasting compounds many of which show antimicrobial, antifungal, allergenic contact dermatitis, neurotropic, insecticidal activity as well as farnesoid X-receptor activation, antitumor, and tubulin polymerization and NO production inhibitory activity [1a-c]. Several Marchantia species, such as M. polymorpha, M. paleacea subsp. diptera, M. tosana and M. debilis produce a large amount of marchantin-type bis-bibenzyls. Riccardia and Reboulia species contain riccardin-type bis-bibenzyls. The former bis-bibenzyls show antitrypanosomal and muscle relaxing activity and the latter one does anti-obesity activity [1a-c]. Almost all Radula species biosynthesize bibenzyl, prenyl bibenzyls and bis-bibenzyls. Especially R. perrottetii and R. marginata are pharmacologically quite interesting species because both species elaborate tetrahydrocannabinoid (THC) like compound, perrottetinene and its acidic derivative the former of which possesses exactly the same psycho- and potent anti-inflammatory property to those of THC [2,3]. When some liverworts, such as Conocephalum conicum or Marchantia paleacea subsp. diptera was cultured in high oxygen atmosphere, both species began to produce monoterpenoids or aromatic compounds which were not found in both species before cultivation. The former species produced very strong mushroomy odorous components whose contents are almost the same as those of the Japanese most expensive mushroom, Tricholoma matsutake [4]. The latter species elaborated (S)-(+)-perillaldehyde which is the most important flavor of Perilla frutescens var. crispata, the vegetable for JP cuisine, Sushi and Tempura [5]. In this paper, the application of phytochemicals found in liverworts to foods, cosmetics, and medicine will be discussed.

**Keywords:** liverworts, bibenzyl cannabinoids, perillaldehyde, bis-bibenzyls

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# PL-11. Green Extraction of Volatiles and Essential Oils

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## Abstract

Most companies in the food and pharma industries use conventional extraction methods such as those using solvents, or steam and hydro-distillation. Recent trends in extraction techniques have largely focused on minimizing the use of petroleum-based solvents. Recently, more efficient green extraction methods, such as subcritical water extraction (SWE) (Özel et al., 2003), supercritical fluid extraction (SFE) (Gao et al., 2021), and microwave assisted extraction (MAE) (Chemat and Cravotto, 2013) have been used for the extraction of volatiles and essential oils from various plants. These extraction techniques are not only cheaper and faster, but being considered environmentally-friendly, they would also enable these products to claim a green label. Having a green label is desirable to many customers.

SWE as a method, is non-toxic, non-flammable, fast, cheap, readily available, safe, environmentally friendly and uses a green solvent. The extraction of phenolic compounds, flavonoids, flavours, fragrances and essential oils has been carried out using SWE and both qualitative and quantitative results obtained. SFE (usually CO2) is relatively fast due to the low viscosity, high diffusivity, and tuneable solvent power of the supercritical fluid. SFE is also considered a green, environmentally-safe technology. MAE is a relatively new extraction technique that combines microwave and traditional solvent extraction methods. Microwaves are applied to heat the solvents and plant tissues during the extraction process, which increases the kinetics of extraction.

**Acknowledgment**

I would like to thank Professor Fahretin Gogus (Gaziantep University, Turkey), Professor Ally Lewis (York University, UK) and Dr Avtar Matharu (York University) for their collaborative work.

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**Keywords**: Green Extraction, Essential oil, Volatiles, Environmentally-friendly

# ORAL PRESENTATIONS

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# OP-1. Photo-protective effects of selected furocoumarins on D-(+)-limonene, β-pinene and γ-terpinene

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## Abstract

Furocoumarins constitute plant secondary metabolites mainly found in the *Apiaceae* and *Rutaceae* families, thus including all citrus fruits (Peroutka et al., 2007). During periods of stress, such as fungal, bacterial and herbivore attack plants may defend themselves by furocoumarin biosynthesis (Melough et al., 2018). Recovery of essential oils by cold-pressing usually goes along with transfer of furocoumarins into the oil. In an absorption range of 320 – 380 nm, furocoumarins are raised into a photo-excited state by electromagnetic radiation. Activated furocoumarins may decay into their ground state via fluorescence or phosphorescence emitting light of higher wavelengths up to 500 nm, or by reaction with substrate molecules. For this reason, they have been used in phototherapy to treat skin diseases such as vitiligo or psoriasis (Hee Paik & Chul Shim, 1991). However, upon exposure to sunlight, sunburn-like skin injuries, so called photo-dermatitis, may occur (Melough et al., 2018). However, photo-toxicity is very compound-specific or even non-existent for some furocoumarins (Scott et al., 1976). The aim of the present study was to investigate the influence of furocoumarins on terpene photo-oxidation upon UV-light irradiation at 366 nm. For this purpose, the reference terpenes α-pinene, γ-terpinene and *D*-(+)-limonene were spiked with 5% of the furocoumarins 8-methoxypsoralen, bergapten, bergaptol as well as bergamottin and were irradiated for ten days in the presence of atmospheric oxygen. The analysis of the samples was performed by GC/MS, GC/FID and TLC.
After ten days under UV-light, terpene degradation was decreased for all samples containing furocoumarins. The best protection was achieved for γ-terpinene by bergaptol addition. In this case, degradation was reduced by 73% compared to γ-terpinene control sample, when stored for ten days at 366 nm. For bergamottin (50%), bergapten (40%) and 8-methoxypsoralen (40%) the effect was also considerable. The protection of terpenes was even noticeable at a minimum concentration of 0.1% bergaptol. The main oxidation product formed from γ-terpinene was *p*-cymene. Both limonene and β-pinene chiefly showed hydroperoxide formation under UV-light. However, their generation was almost completely prevented when furocoumarins were added. This protective effect is presumably based on energy dissipation due to conversion of high energy UV-A radiation into visible light via fluorescence. Consequently, furocoumarins may be used in the future to increase shelf-life and avoid oxidative modifications of terpenes and essential oils.

**Acknowledgement**

Iris Klaiber is thanked for high resolution LC/MS measurements of bergamottin. Further thanks go to Dr. Jürgen Conrad for performing NMR analyses.

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**Keywords**: furocoumarin, terpene, photo-oxidation, fluorescence

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# OP-2. In vitro enzyme inhibitions of essential oils

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## Abstract

Essential oils are used for their broad spectrum biological and pharmacological effects since decades. In the course of our chemical - biological evaluation and screening of various *in vitro* biological activities of essential oils from different families like Lamiaceae, Asteraceae, Rutaceae bioassays were performed. Acetylcholine esterase (AChE), butrylcholine esterase (BuChE), lipoxygenase (LOX), cyclooxygenase (COX), mono amino oxidase (MAO), angiotensin converting enzyme 2 (ACE2) inhibitory activities among others were compiled in the present work. According to the *in vitro* enzyme inhibitory evaluation results of the tested essential oils and their constituents, potential central nervous system, insecticidal, antiinflammatory, cardiovascular as well as anti-SARS-CoV activities were observed using microplate based spectrophotometric, fluorescent assays. Also in the case of MAO inhibition activity, in silico evaluations were performed to complement the mode of action. The promising in vitro inhibition results need further in vivo investigations.

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**Keywords**: in vitro, enzyme, essential oil, bioactivity

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# OP-3. Chemical and Bioinformatics Analyses of the Anti-Leishmanial and Anti-Oxidant Activities of Hemp Essential Oil

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## Abstract

Industrial hemp is a multiuse crop that has been widely cultivated to produce fibers and nutrients. The capability of the essential oil (EO) from inflorescences as antimicrobial agent has been reported. However, literature data are still lacking about the hemp EO antiprotozoal efficacy in vivo. The present study aims to unravel this concern through the evaluation of the efficacy of hemp EOs (2.5 mL/kg, intraperitoneally) of three different cultivars, namely Futura 75, Carmagnola selezionata and Eletta campana, in mice intraperitoneally infected with *Leishmania tropica*. A detailed description of EO composition and targets-components analysis is reported. Myrcene, α-pinene and E-caryophyllene were the main components of the EOs, as indicated by the gas-chromatographic analysis. However, a prominent position in the scenario of the theoretical interactions underlying the bio-pharmacological activity was also occupied by selina-3,7(11)-diene, which displayed affinities in the micromolar range (5.4–28.9) towards proliferator-activated receptor α, cannabinoid CB2 receptor and acetylcholinesterase. The content of this compound was higher in Futura 75 and Eletta campana, in accordance with their higher scavenging/reducing properties and efficacy against the tissue wound, induced by *L. tropica*. Overall, the present study recommends hemp female inflorescences, as sources of bio-molecules with potential pharmacological applications, especially towards infective diseases.

**Acknowledgment**

The study was conducted within the joint project between Department of Pharmacy of "G. d'Annunzio" University and Veridia S.r.l. (Research Program 2020–2023).

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**Keywords**: *Cannabis sativa* L.; essential oil; scavenging/reducing activity; *Leishmania tropica*; bioinformatics

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# OP-4. Cinnamon essential oil encapsulation: controlled release for biosourced pesticides

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## Abstract

The long-term harmful effects of synthetic pesticides are one of the major controversies these days. Following the objective of reducing the use of these products without decreasing crop yield, essential oils (EOs) are a prime candidate for biocontrol. However, high volatility of EOs begets a challenge: increasing the duration of efficient activity of EOs.

In this project, an innovative green matrix for essential oil retention is proposed. Indeed, glycerol carbonate surface-modified polypropilenimine dendrimers (GD-PPIs) have shown their ability to encapsulate some metallic complexes and organic compounds (Balieu et al., 2013; Menot et al., 2015). *Cinnamomum zeylanicum* Blume EO has been chosen for their herbicide properties (Lins et al., 2019). After the optimization of the encapsulation of cinnamon EO with GD-PPI-3 (Maes et al., 2021), the release profile of the formulation was study in different environmental conditions with a volatile collect system (figure 1).

The optimal concentration of EOs encapsulation for germination inhibition of *Arabidopsis Thaliana* was first determined in closed and opened petri dishes: 0.125 and 3.6 mg per mL of GD-PPI-3 solution (2 mM) respectively. The release profiles of this optimized herbicide formulation express an initial burst followed by a controlled release of trans-cinnamaldehyde over 90 hours for all tested parameters (temperature, carrier matrix, storage). In addition, microscopy and granulometry showed a monodisperse droplet size distribution, which confirms the stability of the formulation. Finally, bioassays demonstrate the efficiency of the herbicide formulation on inhibition of germination of *Arabidopsis Thaliana* after a storage at room temperature in dark for 8 weeks (more than 60% inhibition) and on soil (60% inhibition).

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**Keywords**: biosourced dendrimer, essential oil, biosourced herbicide, encapsulation, slow release

# OP-5. Pilot study: Which has the greater impact on instantaneous muscle power, simple olfactory stimulation or odour hedonics?

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## Abstract

Athletes use Ammonia inhalant (AI) to enhance athletic performance. AI is known as smelling salt and was already existed as early as the 13th century. It is the mixture of ammonium carbonate ($(NH_4)_2CO_3H_2O$) which is the effective substance for increasing athletic performance and perfume or the preparation of ammonia, water, and alcohol. The medical kit of these mixtures is available for purchase. AI has been used as a stimulant or treatment of faint among athletes such as weightlifters, powerlifters, and football players, however, currently Branch *et. al* reported AI was not effective for deadlift performance. Although AI has been used among athletes for years, the detailed studies are very few and further research is demanded.

Our research group has reported the influence of aroma compounds on our mood and body. In some studies, the participants' odour preference was important factor to analyse the effect of aroma compounds. It seems that ammonia works not as odour but as stimulant for athletes, that is, it is assumed that the lining of nose is stimulated before feeling discomfort. Therefore, we examined whether instantaneous muscle power is enhanced by the simple irritant of nose and lungs or odour hedonics.

The back muscle strength was measured after inhalation of air (control), ammonium, fertilizer which was made of food waste as an unpleasant odour, and citrus perfume as a pleasant one, respectively. The fertilizer smells intensive putrid odour but the irritation of lining of nose is quite lower than ammonium. Fifteen participants joined this study. Due to the space of limitations the results of subject A and B are described here. When the subject A inhaled the citrus perfume, the back muscle strength increased 23 % compared to the control condition and after inhaling ammonium and fertilizer, the rate of increase of back muscle strength were 20% and 15 %, respectively. In the case of subject B, the back muscle strength of after inhaling ammonium, fertilizer, and citrus perfume were, -12%, -19%, and -10%, respectively. For subject A, ammonium was effective to put instantaneous muscle power, however, the citrus odour was slightly better. On the other hand, ammonium did not activate subject B and odours also decreased the back muscle strength. Although the results showed individual variation and some subject was decreased their performance by both of ammonium and odours, ammonium was effective to enhance back muscle strength for some participants and both of odours also showed the tendency of increasing it. It is considered that there is a relation between instantaneous muscle power and olfactory stimulants and even unpleasant odour is effective to enhance this power.

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**Keywords**: Instantaneous muscle power, ammonia inhalant, citrus perfume, putrid odour, odour hedonics

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# OP-6. The current state of knowledge of essential oil in southern African Lamiaceae

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## Abstract

The Lamiaceae is a large plant family encompassing 236 genera and ca. 7200 species, many of which produce essential oils (Karpi, 2020). These volatile oils have contributed to their medicinal use across the world, and subsequently become valued materials within the cosmetic, culinary, and pharmaceutical industries. The southern African Lamiaceae include 42 genera and 297 species, 105 of which are endemic to the subcontinent (Rattray & Van Wyk, 2021). Some of the region's most notable aromatic, edible and medicinal plant taxa belong to the Lamiaceae yet only 48 indigenous species within 17 genera have been explored for their essential oils, equating to 16% of the total southern African Lamiaceae flora (Figure 1). β-Caryophyllene is the most widely reported compound, followed by germacrene-D, and 1,8-cineole. A bird's-eye view of the current state of knowledge within the field of essential oil chemistry for indigenous southern African Lamiaceae is presented, and obvious knowledge gaps are highlighted for future investigation.

### Acknowledgment

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**Keywords**: Lamiaceae, southern Africa, essential oils, volatile oils, medicinal plants

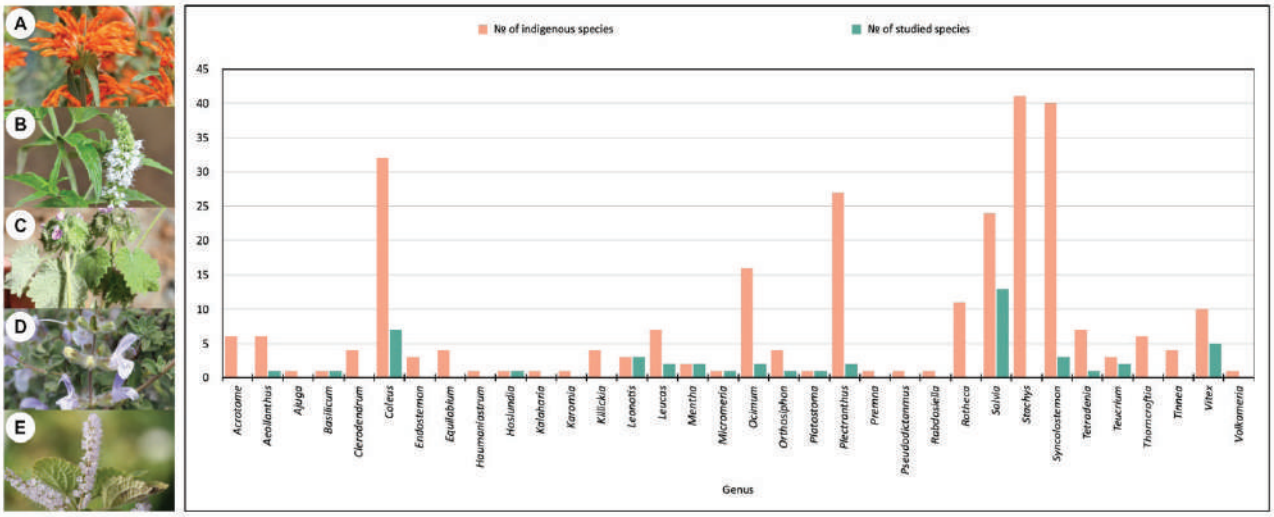


Figure 1: (Left top to bottom) Five popular and well-known essential oil-bearing southern African medicinal plants of the Lamiaceae. (A) *Leonotis leonurus*, (B) *Mentha longifolia*, (C) *Pseudodictamnus africanus*, (D) *Salvia africana* and (E) *Tetradenia riparia*. (Right) The number of indigenous species per genus (orange) and the number of those species investigated for essential oil (green).

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# OP-7. Influence of drought stress on growth and essential oil yield of *Ocimum species*

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## Abstract

The growth and essential oil production of basil cultivars are influenced by various environmental factors, drought stress being one (Simon *et al*., 1992). The effect of drought on different basil species is not well known except for few sweet basil cultivars. Studying the consequences of water supply on drug quantity and quality among commonly grown basil cultivars is paramount. Hence, a two-year pot experiment was conducted to assess the effect of drought stress on herb and essential oil yield of *Ocimum* species, in a semi-controlled greenhouse at Hungarian University of Agriculture and life sciences experimental field, Budapest-Soroksár (Hungary) in 2020 and 2021. The experiment was performed as a factorial based on a randomized complete design with three species of basil namely *O. basilicum* 'Genovese', *O. africanum*, and *O. americanum,* and three levels of soil water capacity (70, 50, and 30% which was determined by gravimetric method (Reynolds, 1970)), and replicated two times. The results of both years revealed that drought stress significantly influenced the growth and essential oil yield of *Ocimum* species (as indicated in table 1 below). On average, 50% and 66% fresh herb yield reduction were observed in the first and second years in low soil moisture treatments (30%), respectively. A significant essential oil yield reduction was also observed in both years and all basil species grown under low soil moisture. The reduction in a fresh herb and essential oil yield is associated with restricted morphological growth of basil plants under water deficit conditions. Among the species, *O. basilicum* 'Genovese' produced higher biomass while *O. africanum* accumulated higher essential oil yield. In conclusion, *Ocimum species* that are used under this investigation showed sensitivity to lower water supply. Thus, optimum water content is recommended for higher biomass and essential oil yield.

Table 1. Effect of drought on fresh herb yield and essential oil yield of *Ocimum species*

| Species | Fresh herb weight (g plant$^{-1}$) | | | Essential oil content (%) | | | Essential oil yield (ml plant$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Soil water capacity (%) | | | | | | | | |
| | 70 | 50 | 30 | 70 | 50 | 30 | 70 | 50 | 30 |
| | | | | 2020 | | | | | |
| *O. basilicum* 'Genovese' | 344.70Aa | 279.00Ab | 169.90Ac | 1.15Ba | 0.61Bb | 0.70Bab | 0.78Ba | 0.34Bb | 0.24Bb |
| *O. africanum* | 212.30Ca | 134.80Cb | 93.70Bc | 2.79Aa | 2.72Aa | 2.76Aa | 1.28Aa | 0.97Ab | 0.68Ac |
| *O. americanum* | 217.40Ba | 191.20Bb | 97.50Bc | 0.70Ba | 0.76Ba | 0.54Cb | 0.44Ca | 0.40Ba | 0.16Cb |
| | | | | *2021* | | | | | |
| *O. basilicum* 'Genovese' | 211.87ABa | 144.25Ab | 78.13Ac | 0.58C | 0.60C | 0.70B | 0.33Ca | 0.17Cb | 0.10Bc |
| *O. africanum* | 193.00Ba | 101.63Bb | 47.50Bc | 2.83A | 3.33A | 3.72A | 0.93Aa | 0.64Aab | 0.37Ab |
| *O. americanum* | 228.00Aa | 110.00Bb | 42.63Bc | 1.46Ba | 1.57Ba | 0.88Bb | 0.62Ba | 0.4Bb | 0.09Cc |

Different letters are for significantly different groups. Capital letters to differentiate between species under fixed drought stress treatment and small letters are used to differentiate drought stress under fixed species.

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# OP-8. Study of patchouli essential oil chemical composition as a function of plant part, acid hydrolysis and distillation time

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## Introduction

Patchouli (*Pogostemon cablin*, Lamiaceae) essential oil (EO) is one of the most important raw materials and has a high demand in the flavor and fragrance, food, pharmaceutic, cosmetics, and perfumery industries (Van Beek and Joulain, 2018). Patchouli EO quality is regulated by International Norm ISO 3757 (2002) (International Standard, 2002). In order to meet the standards of this norm, it is necessary to study chemical composition changes, which depend on the characteristics of the plant material and on the distillation process.

## Materials and Methods

In this work, patchouli plants were cultivated in experimetal plots, harvested and air-dried in the shade, at the CENIVAM Research Center in Bucaramanga, Colombia. The EO were obtained by microwave-assisted hydrodistillation (MWHD) and steam distillation (SD), from different plant parts (leaves, stems and their mixture), with an acid treatment, and taking oil fractions at different time intervals. The EO chemical composition was determined by GC/MS (AT GC 6890 Plus, AT MSD 5975), using the linear retention indices for each component measured on both polar and non-polar columns and by comparison of the experimental mass spectra with those from databases (Adams and NIST) and literature.

## Results and Discussion

The EO yields and chemical compositions showed variations according to the parameters studied. In all EOs, patchoulol was the major compound (34.0-42.0%). The EO obtained only from stems was rich in pogostone (31.0%). Patchouli oil yield increased with MWHD distillation time (45-150 min). The fractions of patchouli oil obtained by SD, every 30 min, allowed to understand the extraction process of volatile secondary metabolites as a function of time. Based on the analysis of the EOs analysed, it was determined that the plant under study was patchoulol-chemotype, with high patchouli alcohol content (>35%), which indicates the high quality EO and its commercial value.

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**Keywords**: Patchouli, Essential oil, GC/MS.

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# OP-9. Effects of different preservation methods on the active compounds and organoleptic properties of Garden Sage (*Salvia officinalis* L.) leaves

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## Abstract

Garden sage (*Salvia officinalis* L.) is a well known perennial shrub belonging to the *Lamiaceae* plant family with significant importance in culinary and herbal medicine. Leaves are the most important parts of the plant where EO are present along with many other active substances. This study aims to find the best preservation methods in order to conserve the active substance content and the organoleptic properties (color, taste) of sage leaves.

Sage variety named 'Extrakta' was used for the examinations. The sage plantation was established in the Research Field of the Department in Soroksár, Hungary. Approximately 5 kg of leaves were harvested at the beginning of August, after flowering. The homogeneous plant material was divided into ten parts for the different treatments. In our experiment the effect of sun drying, shade drying, oven drying at 40 and 60ºC, lyophilization, microwave drying at 250 and 700W, slow freezing and fast freezing was investigated compared to the freshly harvested plant material.

In raw sage leaves 1.90 ml/100 g EO accumulation level was measured, and almost all of the preservation techniques could keep this essential oil content. The highest values were recorded in the oven dried (40ºC) and lyophilized samples (2.11 and 2.09 ml/100g, respectively), but natural drying and freezing proved to be also very effective. However, in case of oven dried (60ºC) and microwave dried (250 and 700W) samples, the essential oil content significantly decreased (1.04, 0.67, 0.39 ml/100g, respectively).

According to GC-MS analysis, α-Thujone (33.5%), camphor (24.2%), manool (6.6%), viridiflorol (5.9%), 1,8-Cineol (5.8%) and β-Thujone (5.3%) were identified as main compounds in the essential oil of fresh sage leaves. Examining the essential oil composition of preserved samples, we found the same constituents, almost in the same ratio, except sage leaves dried at 60ºC, in which the ratio of α-Thujone decreased (15.6%) but the ratio of camphor increased (31.9%), and in microwave-dried samples. In case of microwaved sage leaves the EO composition completely changed: the proportion of monoterpenes decreased (evaporated) while the ratio of sesquiterpene viridiflorol and diterpene manool spectacularly increased.

Freezing and lyophilization could preserve the fresh sample's original color the best, but microwave dried samples also had very similar appearance properties. However, oven, sun and shade drying caused spectacular color degradation.

Based on e-tongue measurements we found that the taste of fresh sample **completely separated from the taste of preserved samples. Even the taste of sage leaves dried at 40ºC and dried with microwave at 700W were the most similar.**

In fresh leaves, 269.4 mg GAE/g total phenol content was measured. The highest TPC was recorded in leaves dried in shade, at 40ºC in oven and in the sun (549.0, 485.1 and 401.2 mg GAE/g, respectively). Whereas, TPC was decreased significantly in sample dried at 60ºC (133.2 mg GAE/g). In fresh sage leaves, 224.1 mg AAE/g total antioxidant capacity was measured. The significantly highest antioxidant capacity was recorded in microwave dried sample at 700W (403.6 mg AAE/g), while the lowest AC was observed in sage leaves dried at 60ºC (183.0 mg AAE/g).

**Acknowledgment**

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**Keywords**: sage, preservation, essential oil, organoleptic properties, antioxidant capacity

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# OP-10. Is 1D-GC really dead? Exploring the utility of a 4-parallel-columns GC-GC system for essential oils routine analysis

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## Abstract

In routine quality control of essential oils, gas chromatographic (GC) analyses should ideally be both quick and thorough, especially amongst minor constituents which have relevance as authenticity markers or indicators of contamination or adulteration. Abundant coelutions and partial overlaps often hamper the process on any given chromatographic phase, or even cause some compounds to escape detection entirely. Consequently, studies on essential oils have historically relied on parallel capillary columns 1D-GC setups (Bicchi et al., 1988), typically comprising a non-polar and a polar phase. More recently, GC × GC has emerged as featuring unprecedented separation power for complex volatile matrices (Tranchida et al., 2017). The latter highlights the shortcomings of 1D-GC by greatly increasing the number of observed peaks. However, GC × GC requires specialized instrumentation and software. This leaves to wonder whether there is a "third way" in scaling up the classical parallel columns to four phases to increase coelution solving power.

To assess the utility of a 4-parallel-columns system (GC-GC), DB-5, DB-Wax and 11 other columns phases (all 10 m x 0.10 mm) were assayed using identical parameters. Selectivity towards a mix of compounds, general chromatographic behaviour for a set of essential oils, and phase stability were evaluated. DB-624 (6%-cyanopropylphenyl polydimethylsiloxane) and SLB-IL60 (ionic liquid) columns were selected in addition to classical DB-5/ DB-Wax phases.

Three cases are examined using GC-GC. A comparison of approaches is done for guaiacwood essential oil. Published single-column GC-MS analyses report ≤30 compounds (e.g., Marongiu et al., 2007). A detailed GC × GC study lists 128 peaks (Tissandié et al., 2018). On the GC-GC system, 109 of those (and 26 additional unreported substances) can be observed, and 80% of assigned signals do not coelute or overlap on at least one phase. Furthermore, GC-GC usefully improves resolution of four closely eluting main constituents of amyris essential oil which bear relevance for ISO standardization of the species. GC-GC also more than triples the number of reportable peaks for the complex balsam poplar bud essential oil, compared to the 50 compounds previously observed using classical two-columns GC (Piochon-Gauthier et al., 2014). These examples suggest that GC-GC might be a viable approach to routine essential oils analysis.

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**Keywords**: Gas chromatography, chemical analysis, column phases, coelutions, GC-GC

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# OP-11. Regional Variability of Pesticides in Essential Oils and Extracts

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## Abstract

Despite their great benefits to the agri-food sector, pesticides pose a threat to human health and the environment. As the use of essential oils (EOs) continues to rise, a comprehensive assessment of pesticide residues in EOs is essential. In this study, a sensitive multiresidue LC-MS/MS method was used to quantify 226 pesticides in 2,676 essential oil samples from various origins from 2019 to 2021. Our results reflect the variability of pesticide use by country. Brazilian orange oil had a mixture of phosmet, diflubenzuron, propargite, malathion, chlorpyrifos, bifenthrin, triflumuron, pyraclostrobin, tebuconazole, and pyrimethanil while the Greek orange EO had phosmet, imazalil, and chlorpyrifos. Cumin oil from India had carbendazim, tricyclazole, azoxystrobin, hexaconazole, and tebuconazole while the Egyptian cumin EO had chlorpyrifos, diazinon, doramectin, malathion, and metalaxyl. The American grapefruit EO had carbaryl, chlorpyrifos, imazalil, phosmet, pyriproxyfen, and thiabendazole while the South African grapefruit EO had pyrimethanil, imazalil, thiabendazole, pyriproxyfen, pyraclostrobin, methoxyfenozide, chlorpyrifos, buprofezin, carbendazim, malathion, propiconazole, and trifloxystrobin. Ginger extracts from India had very high levels of mepronil, picoxystrobin, butocarboxim-sulfoxide, metalaxyl, chlorpyrifos, lufenuron, siduron, carbendazim, epoxiconazole, and propoxur. Rose oil from Bulgaria had copious amounts of spiroxamine, fenpropimorph, chlorpyrifos, followed by fenazaquin, mevinphos, nitenpyram, mepanipyrim, and cymoxanil. Chlorpyrifos and ethiofencarb are the major pesticides in Egyptian chamomile oil. Continued monitoring and further research are required to improve our understanding of pesticide use and effects.

## Acknowledgment

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**Keywords**: Pesticides, Essential oil, Chlorpyrifos, Malathion, Imazalil

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# OP-12. The influence of temperature and light on the colour and chemical profile of kunzea oil during storage

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## Abstract

Kunzea oil production is moving from wild-harvested to orchardised stands yet there have been no studies to establish optimal harvest management for maximising the oil yield. This study aimed to determine the influence of harvest intensity and season on 1. biomass accumulation and the recovery of non-structural carbohydrates (NSCs) levels and 2. variation in the quantity and quality of kunzea oil. The responses to the depth of cut at harvest (harvest intensity) of shallow (0.2m above ground) and deep-cut harvest (0.1m above ground), undertaken in early summer, were compared to uncut material (control). The cumulative above-ground biomass of shallow-cut was two-fold higher, relative to deep-cut treatment with a concomitant higher oil content in shallow-cut (1.84 ± 0.11% DW) compared to deep-cut treatment (1.54 ± 0.32% DW) in spring. Deep-cut treatment resulted in the plants having inadequate resources for re-growth and essential oil biosynthesis. There was no seasonal variation in essential oil content. An interactive effect of harvest intensity with season was found for α-pinene and bicyclogermacrene, whereby these components were elevated in both shallow-and deep-cut treatments in spring, relative to the control, possibly due to the plant defence response after defoliation. In relation to seasonal variation, autumn harvests produced the highest percentage of other bioactive constituents such as 1,8-cineole and viridiflorol. Therefore, the recovery of biomass post-harvest is optimised by shallow-cut harvests leaving the sufficient resources for both vegetative growth and essential oil biosynthesis. Further, autumn cropping maximises oil quantity, yielding premium kunzea oil with enhanced bioactive components.

**Acknowledgment**

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**Keywords**: Defoliation, harvest season, kunzea oil, plant defence

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# OP-13. *Cinnamomum cassia* and *Syzigium aromaticum* essential oils reduce the gut colonization capacity of *Salmonella typhimurium*, in an *in vivo* infection model using *Caenorhabditis elegans*.

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## Abstract

The presence of *Salmonella* sp. in poultry, swine, cattle, and other animals involves significant economic losses, but also contamination of the food processing chains (Bajpai et al., 2012). The regulation of intestinal colonization in livestock by using non-bactericidal feed additives is an important management lever for this zoonotic bacteria. *Caenorhabditis elegans* is proposed here as an *in vivo* model for the evaluation of five essential oils (EOs) as anti-colonization products against *Salmonella enterica* sv. Typhimurium. An infection methodology, adapted in liquid media from previously published work (Conery et al., 2014; Labrousse et al., 2000; Paulander et al., 2007), has been developed here for the study of lipophilic compounds such as EOs.

A preliminary evaluation of the toxicity of EOs against *C. elegans* showed $LD_{50}$ ranging from 74.5 ± 9.6 µg/ml for *Cinnamomum cassia* (CEO) to 271.6 ± 14.9 µg/ml for *Syzygium aromaticum* (SyEO). Both EOs significantly inhibited bacterial colonization in the digestive tract of *C. elegans,* with reduction of 0.88 and 0.70 log CFU/nematode at nontoxic concentrations of 50 µg/ml and 150 µg/ml, respectively. The minimal bactericidal concentrations of CEO and SyEO against *S.* Typhimurium being respectively 312.5 **µg/ml and 625 µg/ml, an antibacterial effect can be excluded to explain the inhibition of the bacterial load. The anti-colonizing activity of these two EOs could however be related to an inhibition of the swimming motility** of *S. typhimurium*, significantly reduced by 23.47 % for CEO at 50 µg/ml and 19.56 % for SyEO at 150 **µg/ml.**

This study shows the potential of *C. elegans* as a key *in vivo* model, to study the influence of essential oils on host-pathogen relationships. The results also demonstrate the ability of essential oils to modulate bacterial colonization of the gut through channels that differ from bactericidal activities.

**Acknowledgment**

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**Keywords**: *Salmonella typhimurium*, *Caenorhabditis elegans*, colonization assay, swimming motility, essential oil.

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# OP-14. Improving the efficiency and antioxidant activity of essential oil extracted from *Abies sachalinensis* using underwater shockwave pretreatment

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## Abstract

*Abies sachalinensis* (Sakhalin fir) is a conifer species widely distributed throughout Sakhalin Island, the southern Kurils (Russia), and northern Hokkaido (Japan). Abies sachalinensis essential oils have antifungal activity (Takashima et al., 2015) and effectively remove nitrogen dioxide (Ohira, 2015). In addition to improving air quality, essential oils have a relaxing effect. Underwater shockwaves generate instantaneous high pressure that reaches the entire cell and causes multiple cracks along the tracheids, causing the pit membrane to flake off through spalling destruction. These cracks function as permeation pathways, which improve essential oil extraction by subsequent steam distillation (Kawai et al., 2021). Moreover, these results indicate that the implementation of underwater shock wave treatment as a preprocessing step is useful for extracting functional components from biomass and is expected to improve the antioxidant activity of the obtained essential oils and water extracts. Herein, we introduce a novel application of this pretreatment process to improve the efficiency of essential oil extraction and antioxidant activity from A. sachalinensis leaves and branches. *Abies sachalinensis* leaves and branches were oven-dried (40–45 ºC) to a moisture content of ≤10% and were subjected to a shockwave pretreatment or left untreated before essential oil extraction by steam distillation. Chemical analysis was performed using gas chromatography-mass spectrometry. The essential oil yields of untreated dried leaves were 2.4 g/kg of leaf dry weight (DW). Upon application of a 3.0 kV, 3.6 kJ shockwave, the essential oil yield increased with increase in number of shockwave cycles. Compared to untreated dried leaves, the yield obtained (32.7 g/kg DW) increased by 13.6-fold, while the antioxidant activity of essential oil increased by more than 30-fold in content, after 10 cycles. The results suggest that instantaneous high-pressure treatment as a pretreatment for conventional steam distillation improves essential oil yield and the extraction of bioactive components.

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**Keywords**: *Abies sachalinensis* essential oil, underwater shockwave pretreatment, essential oil yield, antioxidant activity

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# OP-15. Development of a new tool for pest management in fruit arboriculture using essential oils applied by trunk injection

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## Abstract

Apple is among the first fruit crop worldwide, therefore orchards protection has always been of great agronomic and economic interest. The rosy apple aphid (RAA), *Dysaphis plantaginea* Passerini, causes severe plant injuries and shows increasing resistance to conventional pesticides. Essential oil (EO) based biopesticides are regarded as a potential alternative for insect management. However, phytotoxicity through generation of reactive oxygen species (ROS) is described in direct response to EO treatment (Werrie et al., 2020). To minimize EO application ratio trunk injection is considered as the delivering system. Therefore, the aim of this project is to establish treatment systematicity (I), insecticidal activity (II) and absence of EO phytotoxicity (III).

(I) Systemicity of *C. cassia* EO was investigated with (un)targeted volatile organic compounds (VOCs) analyses performed on VOCs leaf content and emissions (DHS-GC-MS and TDU-GC-MS) (Werrie et al., 2021).

(II) Population dynamic experiments and modelling were performed on aphids to test how the injection affects their survival and growth in controlled environment.

(III) Phytotoxicity was evaluated following biochemical and transcriptomic parameters. Photosynthetic pigments, malondialdehyde, glutathione redox status as well as 29 defence genes were monitored.

Results showed that trans-cinnamaldehyde, *C. cassia*'s major component, accumulated in leaves (mean concentrations of 350.54 ng $g_{DW}^{-1}$) but was not detected in the emission. Moreover, treatment results in increase of various VOCs.

The results are also conclusive in terms of efficiency against RAA, with a 60 % death rate after 5 days. A generalized linear model (GLM) highlights population development impairment with effects of time and injection (p-values <0.01).

Regarding phytotoxicity, an oxidative stress occurs as demonstrated by change in glutathione ratio without resulting in oxidative damage. Finally, clear activation of PR1 and PR14 defense genes (log2=5.82 and 6.42) suggest eliciting properties.

Extensive study may be considered to investigate the dose-effect relationship, to accurately determine the dose with sufficient insecticidal activity and low phytotoxicity. Field trials should be performed to establish efficiency as a biopesticide and the lack of harmful effect to beneficial insects. The absence of impact on apple quality or yield and on tree growth through long-term phytotoxicity should be established as well.

**Acknowledgment**

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**Keywords**: essential oil, biopesticide, Malus domestica, trunk-injection, Cinnamomum cassia

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# OP-16. Mutagenic, genotoxic, ecotoxic, and antimicrobial properties of low molecular oxime ethers

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## Abstract

The synthesis of new olfactory compounds contributes greatly to the cosmetics, perfumery, and pharmaceutical industries. Low-molecular carbonyl compounds, which occur most frequently in nature as secondary metabolites, are excellent raw materials for the creation of new fragrances. Series of new oxime ethers, derivatives of benzaldehyde, cinnamaldehyde, ionone, jasmine, citral, and *p*-menthane were synthesized. The selected compounds were then analyzed for their odorous properties and detection thresholds. Then, some of its biological activities were verified. In the antimicrobial test, it was observed that the *O*-alkylation reaction did not increase the activity. The best result was obtained for *p*- and *o*-anise *O*-ethyl oxime against *C. albicans* (MIC = 0.21 μM). In ecotoxicity tests on three different trophic levels compounds proved similar toxicity to other commonly used aromatic compounds. In studies with *Spirodela*, all compounds showed very acute toxicity (class V). In studies on algae, the *O*-methyl ether of β-cyclocitral oxime was the least toxic. As for the Daphnia tests, the ethers showed acute toxicity (class III), the *O*-methyl ether of *p*-tolualdehyde oxime was the least toxic. None of the tested compounds were mutagenic or genotoxic. Based on the test results, it can be concluded that these compounds cannot be considered as preservatives or antibiotics themselves but they might act as enhancers of other antimicrobial substances. Moreover, any of the tested compounds will not cause genetic diseases including cancer and can be safely used as fragrances in various industries.

**Acknowledgment**

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**Keywords**: oxime ethers, genotoxicity, ecotoxicity, antimicrobial activity

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# OP-17. Chemical composition, antifungal and antioxidant activity of essential oils from different subspecies of *Helichrysum italicum* grown in Italy

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## Abstract

*Helichrysum italicum* (Roth) G. Don (Asteraceae) is an aromatic, perennial subshrub, very common in the Mediterranean area (Najar et al., 2020). The chemical composition of its essential oil has been extensively evaluated by several authors and huge differences are reported. Geographical origin, plant growth stage and environmental conditions are considered the main factors affecting the EO volatile profile, along with the strong tendency of *H. italicum* to present anatomical and morphological polymorphisms (Ninčević et al., 2019)the interest in Helichrysum italicum scientific research and use has increased in the last couple of decades. Consequently, due to the increased industrial demand its plantation farming experienced a huge expansion in Eastern European Mediterranean countries. The aim of this review article was to present the available data on Helichrysum italicum and to identify the gaps in the knowledge, and openings for future potential investigations and contributions to the topic. The review of the Scopus database was performed and scientific studies from 1990 to the most recent publications in 2019 were summarized. The article includes the last reviewed taxonomy, detailed description of morphological characteristics, geographical distribution, uses, chemical diversity, biological activity (antimicrobial, antiviral, antioxidant, anti-inflammatory, repellent, and insecticidal activity. The different phenotype could reflect in the presence of various genotypes of the same species and subspecies, whose extracts presented deep chemical variability (Schipilliti et al., 2016)coming from Sicily and Corsica and growing up, at equal geographical area, at the same edaphic and climatic conditions, were investigated by GC-MS, GC-FID and gas chromatography carbon isotope ratio mass spectrometry (GC-C-IRMS. The aim of this work was to evaluate the chemical composition and the hydrodistillation yield of the essential oil (EO) obtained from the dried aerial parts of ten samples of *H. italicum* collected from different locations in Italy. Moreover, this study deals with the antimicrobial and antioxidant properties of the EOs obtained only from the two samples with the greatest differences in their chemical composition. The plant materials were hydrodistilled with a standard Clevenger-type apparatus, for 2h and the obtained EOs were analysed by gas chromatography coupled with mass-spectrometry (GC-MS) and then the composition was subjected to multivariate statistical analysis. The two selected EOs were further investigate by microdiluition assay for their antifungal activity against *Microsporum canis*, *Microsporum gypseum*, and *Aspergillus flavus.* Moreover, the total antioxidant activity of the essential oils was assessed by means of DPPH, ABTS, and FRAP methods. Considering the chemical composition of all the EOs, monoterpenes represented the main chemical class in almost all the samples, ranging from 43.6 to 94.3%. Among these compounds, the oxygenated ones were the most abundant, accounting for 90.8% in the EO of the sample from La Pelosa (Sardinia). The only exception was represented by the sample from Capraia Island (Tuscany), characterized by a predominance of sesquiterpenes (47.1%), both in their hydrocarbon and oxygenated forms. The EO of this sample was selected together with the sample from Stintino (Sardinia) for the antimicrobial and antioxidant investigations. These EOs presented a comparable effectiveness on *Microsporum gypseum* and *Aspergillus flavus,* whilst *Microsporum canis* resulted more sensitive to the EO of Capraia Isle sample. The EO of Stintino, instead, showed the highest free radical scavenging activity.

**Acknowledgment**: Claudio Cervelli (CREA-Sanremo), Andrea Primavera (F.I.P.P.O.) for providing the plant materials.

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**Keywords**: *Helichrysum* spp, GC-MS, neryl acetate, 1-*epi*-cubenol, biological activity

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# OP-18. Farm to Pharmacy- The organic and sustainable supply of five essential oils in South Africa

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## Abstract

The global pharmaceutical, food and beverage industry is undergoing gradual, yet significant transition concerning ingredients formulations. The growing consumer's inclination towards natural and organic products have made essential oils a crucial ingredient of choice for pharmaceutical and food industries. There has been a gradual increase in the consumer's understanding of the detrimental health effects of synthetic chemicals in medicine and for flavor applications. This will contribute significantly to the essential oils market growth, and create opportunities for South African producers. The essential oils market is majorly propelled by the robust trend of 'green' consumerism amidst the growing popularity of natural inputs for food preservation and sensory-related aspects.

Farmers and producers are cultivating the plants as organic and commercial crops and intercropping with existing crops to supply the ever-rising demand. Examples of such indigenous crops in South Africa now are; *Pelargonium var Rose, Lippia javanica, Eriocephalus* species, *Helichrysum* species, *Artemisia afra* to name but a few. Selecting the correct specie and chemotypes remain a challenge. Quality control is extremely important and the international tendency to encourage standards are now experienced and implemented.

The paper will highlight how quality awareness and sustainable supply through best agricultural and manufacturing practises of the mentioned species are applied by the producers.

**Keywords**: green consumerism, intercropping, indigenous crops, chemotypes, agricultural practises.

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# OP-19. The healing effect of Carvacrol on Nasal Septal Perforation

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## Abstract

We investigate the effect of topical carvacrol on the healing of nasal septal perforation in rabbits. Twenty-one male New Zealand rabbits were randomly divided into three equal groups. A five-mm biopsy punch was used to perforate the nasal septum behind the columella of each animal. A 99% carvacrol solution (Sigma-Aldrich W224511-100G-K; St Louis, MO) was diluted in olive oil to a concentration of 12.5%. For 14 days, bilateral gelatin sponges impregnated with carvacrol in group one (study group) and olive oil (vehicle group) in group two were placed by the perforation. Perforation only was performed in the control group. At the end of the 14th day, the animals were sacrificed and the nasal septum specimens were removed. The closure of perforation was measured and specimens were examined histopathologically (Hematoxylin&Eosin (H&E) and Masson's trichrome (MT) staining). Histopathologic parameters were scored as 0=none, 1=mild, 2=moderate, and 3=strong. The septum perforation closure in the carvacrol group was significantly greater compared with the other groups ($p<0.001$). Perforation diameter did not vary between the control and olive oil groups (Dunn test; $p=0.423$). Cartilage regeneration ($p=0.009$) and connective tissue density ($p=0.020$) were also found significantly higher in the carvacrol group compared to control groups. Topical application of carvacrol enhances wound healing in rabbit nasal septum perforation. It accelerated perforation closure by increasing cartilage regeneration and connective tissue.

**Keywords:** carvacrol; wound healing; nasal septal perforation; experimental study.

# OP-20. Carvacrol have anti-apoptotic effects in rats with diabetic nephropathy

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## Abstract

The aim of the study was to examine the anti-apoptotic effects of carvacrol on diabetic nephropathy in Wistar albino rats and to evaluate the results of the effects of this compound on kidney functions.

For this purpose, 40 adult male Wistar Albino rats that obtained from DEHAM (Ondokuz Mayis University Experimental Animal Application and Research Center) were used for this study. Rats were divided into four groups with 10 rats each. Except for those in the control (C) and the vehicle (olive oil) (V) groups, each animal received a single dose (50 mg/kg) of streptozotocin to induce diabetes. The animals with diabetes were divided to the diabetes (D) group and carvacrol (CAR) (Sigma-Aldrich W224511-100G-K; St Louis, MO) group (75 mg/kg/day).

At the end of 42 days, systemic necropsy of the rats was performed and kidney tissue samples were taken. Kidney samples from rats were fixed in 10% buffered formalin solution. After routine procedures, paraffin blocks, were prepared and sections with a thickness of 5 µm were taken for TUNEL staining for apoptosis. In 42 days STZ-induced diabetic rats, severe hyperglycemia and albuminuria were developed. TUNEL-positive cells were significantly more numerous in diabetic rats than other groups. Carvacrol significantly decreased the diabetes-induced apoptosis in the diabetic rat kidney. No significant changes were observed in the histopathology of the kidney tissues of control and vehicle groups.

Our results suggest that carvacrol could have a protective effect in kidney of diabetic rats.

**Acknowledgment**

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**Keywords**: Apoptosis, Carvacrol, Diabetic Nephropathy, Streptozotocin

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# OP-21. Antibacterial Activities of Six Essential Oils and Carvacrol: Interaction of Selected Combinations

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## Abstract

The continuous increase of antibiotic resistance and the lag in development of novel antibiotics are two important challenges in treatment of infectious diseases. Essential oils (EOs) and their combinations are promising alternatives for treatment of bacterial infections. The aim of the study was to determine volatile composition and investigate antibacterial activities of the EOs of *Origanum onites* L., *Thymus capitatus* L., *Eucalyptus radiata* A.Cunn. ex DC., *Mentha* x *piperita* L., *Thymbra spicata* L.,Cymbopogon winterianus Jowitt ex Bor. and their selected combinations.

The chemical compositions were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Antibacterial activities of carvacrol and EOs were investigated against *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 by broth microdilution method (CLSI, 2015). Ciprofloxacin was used as standard antimicrobial agent. Microdilution checkerboard assay was carried out to investigate the combined interactions of EOs of *O. onites*/*T. spicata*, *O. onites*/*C. winterianus*, *O. onites*/carvacrol, and *T. capitatus*/carvacrol (Humphries RM, 2016).

EOs of *E. radiata* and *M. x piperita* showed weak antibacterial effect. EO of *C. winterianus* had moderate and weak effect against Gram negative and Gram positive bacteria, respectively. EOs of *O. onites, T. capitatus,* and *T. spicata* and the compound carvacrol revealed moderate activity against both Gram positive and Gram negative bacteria (Table 1). The major component of *O. onites, T. capitatus,* and *T. spicata* was carvacrol (>46%). The combination of EO of *O. onites* at 0.015 mg/ml with that of *T. spicata* at 0.03 mg/ml resulted in synergism against *K. pneumoniae*. All of the other combinations revealed indifferent effect. Initial findings suggest further evaluation of antibacterial effect of especially more than two combinations of *O. onites*, *T. capitatus*, *T. spicata* and carvacrol.

Table 1: Minimum inhibitory concentrations of EOs*.

| | *O. onites* | *T. capitatus* | *E. radiata* | *M. piperita* | *T. spicata* | *C. winterianus* | Carvacrol | Ciprofloxacin* |
|---|---|---|---|---|---|---|---|---|
| *S. aureus* | 0.0625 | 0.5 | 16 | 8 | 0.0625 | 2 | 0.0315 | 0.125 |
| *E. faecalis* | 0.0625 | 0.5 | 1 | 4 | 0.0315 | 4 | 0.0625 | 0.5 |
| *E. coli* | 0.125 | 0.5 | 16 | 8 | 0.125 | 0.125 | 0.125 | 0.007 |
| *K. pneumoniae* | 0.125 | 0.5 | 16 | 16 | 0.1252 | 0.125 | 0.125 | 0.015 |

*Minimum inhibitory concentration of EOs is expressed in mg/mL where as that of ciprofloxacin is expressed in mg/L.

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**Keywords**: Antibacterial, combination, essential oil, synergy

# OP-22. Which essential oils started to be used in ear diseases in Ottoman medicine and when?

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## Abstract

**Introduction:** With the invention of the distiller by the founder of modern chemistry, Jabir b Hayyan, distillation systems have been developed and continued to be used since the 8th century. Although distillation was invented in the early period, it took a certain time to use these systems in medical applications in the 16th century, with the influence of Paracelsus in Europe, distillation systems were added to the methods of preparation of medicinal drugs. The aim of the study is to indicate when this effect reached Ottoman Medicine and to detail its use in ear diseases.

**Materials and methods:** The Ottoman medical manuscripts between the 13th and 19th centuries, which were transcribed, were examined within the scope of the purpose. Although, essential oils that started to be used for ear diseases in the Tıbb-ı Jadid period, the properties of these oils as far as written in the texts of the history of medicine and the monographs of the oils will be mentioned.

**Results:** As a result of our examination, we can start the use of essential oils obtained by distillation systems in Ottoman medicine with the 17th century. These oils were also used in the treatment of ear diseases in the works of the 18th century. The essential oils used are Rosemary oil, Bay berry oil, caraway seed oil, Clove oil, Marjoram oil, Fennel seed oil, Psoriasis Oil, Selime oil, Cinnamon is oil.

**Discussion:** This study is valuable because of the scarcity of information in the literature about the Tıbb-ı Jadid period and because it explains the first use of essential oils in this period. But dates and information may become invalid due to manuscripts to be transcribed later.

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**Keywords:** essential oils, Tıbb-ı Jadid, distillation systems, Ottoman Medicine, ear diseases.

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# OP-23. Chemical composition and antibacterial activities of essential oils of different salvia species against plant bacterial disease agents

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## Abstract

Plant diseases caused by certain bacterial pathogens are reported as one of the major factors which cause enormous yield losses in agriculture production on a global scale. *Salvia* (sage) is the largest genus of the Lamiaceae family, which is native of the Mediterranean area and includes about 900 species (Walker et al., 2018; Karik et al., 2018). Essential oils (EOs) of different *Salvia* spp. possess antimicrobial activities against (Yousefzadi et al., 2007; Mirzania et al., 2018). The aim of this study was to investigate chemical composition of EOs obtained from *Salvia tomentosa* Mill., *S. fruticosa* Mill., *S. aramiensis* Rech. fil., and *S. officinalis* L., and to evaluate their antibacterial activities against plant pathogenic bacterial disease agents. Chemical compositions of the EOs were identified by Gas Chromatography coupled with Mass Spectrometer detector (GC-MS). According to GC-MS analysis, 38 components (99.94%) were identified in *S. fruticosa* EO, 31 components (99.61%) in *S. officinalis* EO and 29 components (99.72-99.95%) in *S. aramiensis* and *S. tomentosa* EOs, respectively. The main components determined in EOs of *S. officinalis* EO, were camphor (32.45%), δ-thujone (25.15%) and cineole (%16.12). Cineole (59.65%), β-pinene (11.07%) and borneol (4.85%) were determined in *S. aramiensis* EO. In the EO of *S. tomentosa*, cineole (54.42%), β-pinene (21.34%) and camphor (9.17%) were determined as the main components. Cineole (52.15%), camphor (28.35%) and α-terpineol (8.13%) were the predominant compound in the EO of *S. fruticosa*. Antibacterial activities of EOs were determined against five different economically important plant bacterial disease agents such as bean halo blight disease agent *Pseudomonas syringae* pv. *phaseolicola*, potato soft rot and black leg disease agents *Pectobacterium carotovorum* subsp. *carotovorum*, Dic*keya chrysanthemi, Pectobacterium parmentieri* and walnut bacterial blight disease agent *Xanthomonas arboricola* pv. *juglandis* by using disc diffusion assay. Among the EOs tested, the mean strongest antibacterial activity (recorded as inhibition zones in mm) was shown by *S. tomentosa* EO (14.8 mm) followed by *S. fruticose* (14.33 mm), *S. aramiensis* (11.0 mm) and *S. officinalis* (7.87 mm) EOs, respectively. Based on inhibition zone diameter values, the highest antibacterial activities were displayed by *S. tomentosa* and *S. fruticosa* EOs against *P. carotovorum* subsp. *carotovorum* (17.33-17.67 mm), followed by *D. chrysanthemi* (13.67-16.33 mm) and *P. parmentieri* (12.33-16.33 mm), *P. syringae* pv. *phaseolicola* (14.33-12.67 mm) and *X. arboricola* pv. *juglandis* (13.67-11.33 mm), respectively. Among the tested EOs, the weakest antibacterial activity was displayed by *S. officinalis* EO. Essential oil of *S. officinalis* did not displayed antibacterial activity against *X. arboricola* pv. *juglandis.* Based on our results, the essential oil of sage plant collected from Hatay province has a potential to be applied as biopesticides against important plant bacterial disease agents.

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**Keywords** Antibacterial, Plant pathogen, Essential oil, *Salvia* spp, Sage

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# OP-24. Authentication of some essential oils using enantiomeric distribution of key components

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## Abstract

Due to the lack of reliable authentication techniques and regulations, adulteration of natural products is rampant worldwide. Approximately 80% of commercially available essential oils are adulterated in one way or another. Chiral GCMS is one of the most useful and practical instruments for the authentication of essential oils. Since the same plant species from any origin produce essential oils with the same enantiomeric ratio, it can be used as means to authenticate essential oils. Despite sharing the same physicochemical properties, the d- and l- isomers have distinctly different biological and organoleptic properties. In this study, chiral GCMS results showed that lavender has 95% l-linalool, basil, and oregano EOs have 100% l-linalool whereas coriander has 84% d-linalool. Bergamot and orange oils have 99% d-limonene while fir needle, holy basil, and pimento leaf EOs have 100% l-limonene. Rosemary oil has 99% d-α-pinene, 100% d-verbenone, and 100% l-bornyl acetate. We can conclude that chiral GCMS analysis can be useful in detecting adulteration by addition of oils from other species or isolated compounds.

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**Keywords**: Lavender, Coriander, Bergamot, Fir needle, Chiral GCMS

# YOUNG SCIENTISTS

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# YS-1. An exploratory analysis of the chemical composition and antibacterial activity of *Citrus* essential oils on *Salmonella* strains isolated from poultry litter

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## Abstract

In the poultry sector, to grow-out multiple flocks of chicken on the same litter is a common practice to avoid a rise in the cost of chicken and egg production. However, this practice can cause disease outbreaks in chickens as re-used litters contain a high load of bacterial pathogens such as *Salmonella* sp. Thus, re-use of poultry litter can pose food safety issues due to the possible transmission of *Salmonella* through the poultry processing chain. Approaches such as house cleanout to reduce *Salmonella* in poultry litters are often carried out, which include the use of disinfectants. Essential oil-based disinfectants could be a safe, non-toxic and eco-friendly alternative to combat this pathogen. *Citrus* essential oils (EOs) are a by-product of *Citrus* processing industries and there is a huge availability of these EOs in the global market. Therefore, *Citrus* EOs can be a feasible and sustainable alternative to control the occurrence of *Salmonella* sp. in poultry litter. The aim of this study was to evaluate the antibacterial activity of six commercial *Citrus* EOs on four *Salmonella* strains isolated from poultry litters as well as to determine the chemical composition of these *Citrus* EOs. Initially, a screening by disk diffusion method was performed, followed by the determination of the Minimal Inhibitory Concentration (MIC) by microdilution method and the Minimal Bactericidal Concentration (MBC) by plating. Next, bacterial growth kinetic was studied by absorbance reading hourly at several EO concentrations (from 0.116 to 14.8 mg/ml) and without EO (control), and modeling was performed using the modified Gomperzt Model (Zwietering, Jongenburger, Rombouts, Van ', & Riet, 1990)Gompertz, Richards, Schnute, and Stannard. The chemical composition profile of the EOs was identified by GC-MS and the relative amount of each compound was analysed by GC-FID. The screening results showed that five of the six *Citrus* EOs had an antibacterial activity on all *Salmonella* strains, however, the EO called Orange Peel Oil (OPO) stood out by exhibiting the highest antibacterial activity ($P<0.05$) presenting inhibitory zone diameters > 17.4 mm. Moreover, among the *Salmonella* strains, *S. minnesota* was the most resistant strain to the activity of the *Citrus* EOs. MIC and MBC results for OPO on *S.* Minnesota were 3.7 and 7.4 mg/mL. Furthermore, OPO caused disturbances on the normal growth kinetics of *S.* Minnesota at sub-MICs. The action was dose-dependent on the maximal culture density (A) and the lag phase duration (λ) of this strain. The highest sub-MIC (1.85 mg/mL) extended λ in 3.8 times and reduced A by 78.5%. Limonene was detected as the major compound in the six *Citrus* EOs (63.9-95.1%). However, specific minor compounds were detected in a higher relative amount or exclusively in OPO. Therefore, these minor compounds are most likely responsible for the antibacterial activity of OPO.

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**Keywords**: By-product, poultry production, essential oil, bacterial growth kinetics

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# YS-2. The effect of methyl jasmonate and salicylic acid on the essential oil of peppermint (*Mentha piperita*) and marjoram (*Origanum majorana*)

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## Abstract

Peppermint (Mentha piperita) and sweet Marjoram (Origanum majorana L.) from the family of Lamiaceae are widely used in traditional medicine, in perfumery, pharmaceutical and cosmetics industries and as flavoring agents in gastronomy (Tamakou et al.,2017), due to their bioactive compounds that contribute to their biological activities, such as antimicrobial, antioxidant, cytotoxic and antiviral properties (Raja,2012).

Several strategies were studied to increase the secondary metabolites (SMs) of medicinal and aromatic plants in the last few years, including elicitation (Radman et al.,2003). The application of elicitors on plants triggers defense reactions, which might lead to an increase in the production of SMs including volatile components (Klessig et al.,2018).

In this study, we are focusing on the exogenous application of two of the most prominent phytohormones as elicitors, methyl jasmonate (MeJa) and salicylic acid (SA) on peppermint (Mentha piperita L.) and sweet marjoram (Majorana hortensis Mönch.) with the objective to evaluate the effect of these elicitors on the essential oil (EO) yield and chemical composition.

The experiments were carried out in open field plots in Budapest, Hungary. Two dosages (0.1mM and 2mM) of each elicitor were applied twice with an interval of one week for both species in a completely randomized block design with three replications for each treatment. After a week from the second treatment, arial parts of the plants were harvested and air dried in a shady place with ambient temperature. The essential oil of the dried materials from each sample were hydro-distilled in a Clevenger type apparatus and investigated by GC/MS. Components were identified by comparision of their linear retention indices with scientific and mass spectral library references.

Both phytohormones were able to increase the EO yield in marjoram regardless of the applied dosage while there were no chemical differences in the main components except for cis-sabinene-hydrate that was decreased with the concentration 0.1mM of SA. In case of peppermint, the highest yield was obtained by the non-treated sample, moreover, a variation in the percentage of the two main components was observed. The concentrations 0.1mM and 2mM of MeJa were able to increase the menthol but decrease the menthone ratios. Our results suggest that the effect of the elicitation does not only depend on the type of the elicitor and the dosage but also on the plant species. Further studies are in force which will allow us to understand the mechanisms involved.

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# YS-3. Essential Oils as Bioactive Secondary Metabolites against Bacterial and Fungal Mycetoma; One of the Most Neglected Tropical Diseases

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## Abstract

Mycetoma is a subcutaneous mycosis, characterized by large tumor-like swellings located mainly in the extremities. It can be caused by taxonomically diverse microorganisms, both of bacterial (actinomycetoma) and fungal origin (eumycetoma). World-wide the bacterium *Actinomadura madurae* is the most common causative agent of actinomycetoma and the fungus *Madurella mycetomatis* of eumycetoma. The mainstay therapy for actinomycetoma are the sulfonamides and aminoglycosides, and for eumycetoma the triazoles, yet with limited efficacy and high morbidity rates (van de Sande *et al*, 2014, Zijlstra *et al*, 2016). Hence, there is an urgent need to identify novel, efficacious, safe, and affordable treatments. Essential oils (EOs) are volatile lipophilic secondary metabolites composed mainly of mono- and sesquiterpenoids of complex chemical structure with prominent activity against pathogenic microorganisms among other myriad medicinal properties. Twenty-eight EOs of taxonomically diverse aromatic medicinal plants belonging to twelve botanical families including Asteraceae, Burseraceae, Cyperaceae, Euphorbiaceae, Lamiaceae, Myrtaceae, and Poaceae were extracted by hydrodistillation followed by GC/MS analysis. All EOs were screened *in vitro* against *A.madurae* and *M.mycetomatis* at concentrations ranging from 0.25-0.0039%v/v employing resazurin viability assay (AbdAlgaffar *et al*, 2021, Mahmoud *et al,* 2020). The aminoglycoside, amikacin and the triazole itraconazole were used as positive controls towards *A.madurae* and *M.mycetomatis,* respectively. Selected oils were then subjected to *in vivo* toxicity in *Galleria mellonella* larvae model (Kloezen *et al*, 2015). Most of the EOs exhibited remarkable *in vitro* antimycetomal activity (MIC 0.125- 0.0078%v/v) with no toxicity at 0.5-1%v/v (Figure 1). GC/MS analysis identified diverse monoterpenes and sesquiterpenes associated with antifungal activity. The most active essential oils were distilled-derived from leaves of *Croton zambesicus* Muell Part, resin of *Commiphora myrrha*, flower buds of *Syzygium aromaticum* (L.) Merr. et Perry and *Boswellia papyrifera* (Del.) Hochst. Some of the identified pure compounds (β-caryophyl, p-cymene, sabinene, 1,8-cineole, linalool, thymol and borneol) previously exhibited varying degrees of antimycetomal activity as single compounds (unpublished data). GC/MS analysis coupled with the antimycetomal activity (chemometrics study) revealed that these monoterpenes, terpinen-4-ol, n-octyl acetate, p-cymene, 1,8-cineole, α-terpineol and linalool are among the most bioactive compounds against both bacterial and fungal mycetoma.

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**Keywords:** Mycetoma, Resazurin assay, Essential oils, *Galleria mellonella* model

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# YS-4. The influence of plant matrix on the volatile organic compounds emission from medicinal and aromatic plants – the case study for various *Mentha* ssp.

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## Abstract

The quality of medicinal and aromatic plants (MAPs) commonly is evaluated on the basis of total essential oil yield or particular bioactive compounds content. Such an approach is applicable in numerous cases, however, it is insufficient for determination of the sensory quality of MAPs. Therefore, to improve the possibilities of the prediction of MAPs sensory quality based on chemical analyses, without incorporating time-consuming and expensive sensory panels, studies on the relationship amongst MAPs essential oil composition, volatiles emitted to headspace and plant matrix are highly needed.

In this study, the comprehensive investigation on the various Mentha (namely, linalool chemotype, carvone chemotype and menthol chemotype) was carried out. The harvested plants were divided into four groups: (i) fresh reference material; (ii) subjected to convective drying (CD) at 40 °C; (iii) subjected to CD at 50 °C and (iv) subjected to CD at 70 °C. The obtained dried materials, with forced changes in essential oils composition and plant texture changes were used for essential oils hydrodistillation with Deryng apparatus, which were subjected to GC-MS analysis. In addition, headspace Arrow solid-phase microextraction (HS-SPME Arrow) followed by GC-MS analysis was carried out for all objects (fresh plants, dried plants and clear essential oils).

The obtained in the study results had shown the influence of plant matrix on the emission of particular volatile organic compounds present in mint essential oils. It was clearly presented that the composition of essential oils does not directly translate to the plants volatile profile. Therefore, new data for establishing useful protocols for MAPs sensory quality prediction based on chromatographical analyses were obtained.

**Acknowledgment**

The research was supported by the National Science Centre (Poland) grant "Odour sensory quality of herbs as an outcome of volatile constituents composition and plant matter correlation – described on Mentha spp." (2019/35/N/NZ9/01237).

**Keywords**: quality, aroma profile, HS-SPME Arrow, mint, essential oils

# YS-5. Accurate quantification of olibanic acids in several frankincense essential oils from diverse origin by stable isotope dilution assay.

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## Abstract

Cis- and trans-olibanic acids are responsible for the typical church-like base note of frankincense. These compounds are present in a relatively low concentration (several hundred of ppm) in the oils, and an accurate and sensitive method is required for their precise quantification. Deuterated cis- and trans-olibanic acids were synthesized and used for the quantification of both isomers of olibanic acids by stable isotope dilution assay (SIDA). This method was validated by determining linearity, recovery, accuracy, matrix effect, repeatability, intermediate reproducibility, LODs and LOQs. It showed an excellent linearity, and proved to be very sensitive, accurate and reproducible. It was then applied to the analysis of essential oils of *Boswellia sacra* from diverse geographical origins, and we could show the importance of the geographical origin concerning the distribution of cis- or trans-olibanic acids. African samples of *B. sacra* essential oil were found to possess significantly higher amounts of trans-olibanic acids than *B. sacra* essential oil samples from the Arabian Peninsula, while this situation is much less clear for the cis- isomer which is known to be a more potent odorant.

**Acknowledgment**

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**Keywords**: Frankincense oil, GC-MS, olibanic acids, quantification, SIDA

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# YS-6. Antibacterial Evaluation of β-Ionyl Acetate and The Bioconversion Metabolite

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## Abstract

Flavor and fragrance compounds have a wide range of use in food, beverage, pharmaceuticals and create a world wide industry. Biotechnological processes such as microbiological transformations are used for production of fragrant terpenes which are used as a starting material for biotechnological production of new compounds (Carroll, Desai, & Atsumi, 2016).

β-Ionyl acetate is an acetyl derivative of β-ionone which is a cyclic terpenoid (in nature) derived from the degradation of carotenoids (Lopez et al., 2015). Like β-Ionone and its derivatives are added in many daily products of soap, detergents, creams and especially perfumes as fragrance chemicals (Belsito et al., 2007; Longo & Sanroman, 2006; Marumoto, Shimizu, Tanabe, Okuno, & Miyazawa, 2017).

In this study, bioconversion of *β*-ionyl acetate, was carried out using different microorganisms. It was found out that biotransformation of *β*-ionyl acetate with *Aspergillus alliaceus* NRRL 317 resulted in metabolites evident from GC/MS analyses. The formed metabolite was purified by column chromatography with 22,4% yield. Metabolite structure 4β-Hydroxy-β-ionol was elucidated by using spectroscopic methods. The antibacterial activities of β-Ionyl acetate and its derivative obtained were carried out for our continuing research directed towards the identification of new candidates that may be valuable for designing new, potent, selective antibacterial agents.

**Acknowledgements**

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**Keywords**: Aroma substances, antibacterial activity, terpenes, biotechnology

# POSTER PRESENTATIONS

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# PP-1. Activity profiling of essential oils and aromatic extracts on SARS-CoV-2 key enzymes

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## Abstract

Coronaviruses (fam. *Coronaviridae*) are crown-like enveloped viruses comprising single-stranded, positive-sense RNA. Host targets for coronaviruses are animals and humans. Before the current outbreak HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV, and MERS-CoV were known to be able to infect humans [1]. The first four are the cause of (in most cases) mild illnesses (common cold), whereas infections with SARS-CoV and MERS-CoV can develop into severe respiratory disease that can be fatal [2]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for the current outbreak of the disease called COVID-19, is the seventh coronavirus known to infect humans [3, 4].

Two of the very well characterized and promising drug targets are the main protease ($M^{pro}$, $3CL^{pro}$) and the papain-like protease ($PL^{pro}$), which have a key role in viral replication and transcription [5-7]. In the course of our studies, we wanted to address the question whether common flavor and fragrance materials are inhibitors or have the potential to be the source for effective inhibitors of the SARS-CoV-2 main and papain-like proteases. Over 400 samples of essential oils and aromatic extracts were evaluated on the inhibitory activity on SARS-CoV-2 key enzymes. Details of these preliminary studies will be presented. As essential oils are volatile products, it could provide an interesting subsidiary inhalation therapeutic strategy in the long term.

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**Keywords**: SARS-CoV-2, COVID-19, essential oils, flavors, fragrances

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# PP-2. Chemical composition of essential oils from native plants of Philippines

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## Abstract

Indigenous plant species are considered as potential sources of new phytochemicals such as those present in essential oils (EOs) [1]. These plants are commonly found in biodiversity dense locations around the world. The Philippines, a tropical archipelago in Southeast Asia, is one of the top biodiversity hotspots in the world [2]. Some of the flora species found in the country are EO-bearing plants which are likely sources of EOs such as species belonging to the Lauraceae, Myrtaceae, Piperaceae, and Zingiberaceae families. The enormous variety of plant species in the country suggests various EOs with possible significant economic and commercial importance. In this study, we aimed to determine the chemical composition of volatile compounds present from six Philippine indigenous plant species, namely *Alpinia elegans* (C.Presl) K.Schum. (seeds), *Alpinia haenkei* C. Presl (pericarp), *Cinnamomum iners* Reinw. (leaves), *Litsea leytensis* Merr. (leaves), *Piper philippinum* Miq. (leaves) and *Xanthostemon verdugonianus* Náves (leaves). The EOs were collected via hydrodistillation and analyzed using dual-column/dual-detector system gas chromatography-mass spectrometry (GC/MS). Analysis of the EOs revealed that D-limonene (16.8/15.4% w/w), methyl cinnamate (86.99/88.39% w/w), caryophyllene (21.0/34.9% w/w), caryophyllene oxide (15.7/11.7% w/w), ishwarane (26.9/24.9% w/w), and α-gurjunene (32.3/19.5% w/w) are major components of *A. elegans* (seeds), *A. haenkei* (pericarp), *C. iners* (leaves), *L. leytensis* (leaves), *P. philippinum* (leaves), and *X. verdugonianus* (leaves), respectively. The results are contributing to the knowledge of the phytochemistry of Southeast Asian plant taxa, especially on the chemical composition of essential oil-bearing plant species and their volatile compounds.

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**Keywords:** chemical composition, GC/MS, native plants, Southeast Asia, volatile compounds

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# PP-3. Evaluation of antibacterial effect of essential oils from Indian medicinal plants using new broth macrodilution volatilization method.

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## Abstract

Lower respiratory tract infections, which include acute bronchitis, bronchiolitis, influenza, and pneumonia, are a significant cause of morbidity and mortality worldwide (Feldman and Richards, 2018). Typical causative species include *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* (Zafar et al., 2016). Besides traditional systemic routes of drug delivery, inhalation therapy is a possible way of treatment as the drug is directly delivered at the site of the diseases, so it maximizes its efficacy (Traini and Young, 2009). For this reason, *in vitro* antimicrobial activity of volatile essential oils (EOs) has been studied intensively using various methods based on disc volatilization (Kloucek et al., 2012). However, most of them are labour and material-consuming qualitative methods and some require special equipment which is not commonly available (Houdkova and Kokoska, 2020). Therefore, with the aim intending to overcome the problems associated with previously developed methods (Kloucek et al., 2012) and for the better optimization of volatile plant compounds, we designed a novel macrodilution volatilization assay that combines the principles of broth microdilution volatilization (Houdkova et al., 2017) and standard broth macrodilution methods (CLSI, 2012). Additionally, this assay is performed in commercially available microtubes (Eppendorf, Hamburg, DE) which allow fast, easy, cost, and labour effective screening of volatile compounds. With aim to verify the validity of this method, three EOs, obtained from the commonly known Indian medicinal plant species i.e., *Cymbopogon citratus* (DC.) Stapf, *Cyperus scariosus* R.Br. and *Trachyspermum ammi* (L.) Sprague were tested against the bacteria causing respiratory infections namely *H. influenzae* ATCC 49247, *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619, and *S.pyogenes* ATCC 19615 in liquid and solid media. The highest antibacterial activity was observed for *T. ammi* seeds EO against *H. influenzae* with respective minimum inhibitory concentration (MIC) values of 128 and 256 µg/mL in liquid and vapour phase, followed by growth-inhibitory effect against *S. aureus*, *S. pneumoniae*, and *S. pyogenes* with MIC values 512 µg/mL for both phases. *EOs of C. citratus* (leaves) *and C. scariosus* (rhizomes) exhibited the same level of antibacterial activity in liquid and vapour phases against all bacterial strains tested except *H. influenzae* which was more susceptible with MIC values of 256 and 512 µg/mL. The results demonstrate the practical applicability of this assay for the screening of volatile agents. Moreover, this study also suggests the potential of Indian EOs (e.g. *T. ammi* seeds EO) for application in the inhalation therapy; however, further research on the cytotoxicity and in vivo evaluation is necessary to verify its practical use.

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**Keywords**: Antimicrobial activity, *Cymbopogon citratus*, *Cyperus scariosus*, *Trachyspermum ammi*, vapour phase.

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# PP-4. HS-SPME-GC/MS analysis of volatile secondary metabolites of *Varronia curassavica* Jacq. and *Satureja viminea* L. leaves

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## Abstract

The volatile compounds, emitted by aromatic plants, have diverse biological functions such as chemical defense against herbivores, pathogens and microorganisms, also as pollinator attractants (Tholl et al., 2006). The aim of this work was to determine the chemical composition of the volatile fractions obtained by headspace solid-phase microextraction (HS-SPME) from leaves of two species *Varronia curassavica* (Boraginaceae) and *Satureja viminea* (Lamiaceae). Both are aromatic subshrubs, which grow in subtropical regions (Gupta, 1995).

HS-SPME technique was performed, as indicated by Stashenko et al., 2009, with a poly (dimethylsiloxane) -divinylbenzene fiber, PDMS/DVB (65 µm). The fresh leaves of *V. curassavica* (100 mg) and *S. viminea* (1.30 g), separately, were heated (60 °C, 10 min). The fiber was exposed to the vapor phase of the plant material (30 min) and then was desorbed in the injection port of a gas chromatograph (250 °C, 15 min). The volatile compounds were analyzed by gas chromatography coupled to mass spectrometry (GC/MS) using polar DB-WAX and apolar DB5-MS columns with temperature programming, 50 °C -250 °C, at 4 °C/min.

The fragrances of the leaves of two species studied had in common *trans*-β-caryophyllene (23±2% for *V. curassavica* and 18.0±0.80% for *S. viminea*). This compound plays an important role in the pharmaceutical industry as a non-steroidal anti-inflammatory and in the flavour and fragrance industry. *V. curassavica* contained α-pinene (24±1%), β-pinene (7.7±0.5%) and germacrene D (7.1±0.6%) among other compounds; while *S. viminea*, *p*-ment-3-en-8-ol (14.6±0.25%), pulegone (10.6±0.21%) and aromadendrene (10.5±0.34%).

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**Keywords**: *Varronia curassavica*, *Satureja viminea*, volatile fraction, HS-SPME, GC/MS.

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# PP-5. Chemical Diversity and Anti-fungal Action of Kanuka (Kunzea ericoides from different geographical locations in New Zealand

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## Abstract

Kānuka oil, Kunzea ericoides is an endemic tree throughout New Zealand. A volatile oil is commercially produced from the leaves and branches by steam or vacuum distillation in various geographical locations around New Zealand Islands. Whilst the tree is closely related to Mānuka, Leptospermum scoparium, however chemically it is quite diverse. In commercially available oil, little attention is paid to understanding where the oil has come from geographically or the chemical diversity. This presentation will highlight the range of differences in the chemistry of commercially available oils from different locations, where the key constituents, alpha pine and linalool vary significantly. In addition, minor constituents are also present or absent in different locations (Maddocks, 2021). Following this a series of studies were undertaken on two common pathogenic fungi, T. rubric and M. canis. These are implicated in a number of minor skin conditions. The in-vitro studies found that kānuka oil from Great Barrier Island in the north was the most effective against both organisms, even though it had a lower alpha pinene content. Alpha pinene in isolation has been found to be effective in treating M. canis (Silva et al 2017). The age of the branches also were important. These effects were dose dependent. The oil with the highest alpha pinene had the lowest anti-fungal effect. This work suggests that there are key actions of the minor constituents which contribute to the overall effect, rather than just one or two major constituents. Kānuka oil is considered a safe and non irritating oil for skin use and a further study is underway to test for allergic reactions in the various percentages.

**Acknowledgment**

The studies were funded by a research allocation to Wendy Maddocks. The fungal laboratory work was conducted by Associate Professors Garrill and Research Associate Ayelen Tayagui, assisted by two undergraduate science students, Briar Collins and Olivia Williams for some of the preliminary investigations. All work was carried out at the University of Canterbury

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**Keywords**: Kānuka oil, anti fungal, essential oil, New Zealand,

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# PP-6. Study of essential oil obtained from *Salvia aratocensis* (Lamiaceae) cultivated in Colombia

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## Abstract

The *Salvia aratocensis* species belongs to the Lamiaceae family and grows in the Eastern Cordillera in the states of Santander and Boyacá, Colombia (Fernández-Alonso, 2003). *S. aratocensis* is a shrub with a height of up to 1.5 m, it spreads very easily on trails damaged by livestock and adapts easily to areas with a high concentration of nitrogen (Fernández-Alonso, 2003). The purpose of this work was to determine the chemical composition of the secondary metabolites present in the *S. aratocensis* essential oil (EO).

The plant material was collected from an experimental plots at the CENIVAM research center (Bucaramanga, Colombia). The essential oil was obtained by a Clevenger-type microwave-assisted distillation (MWHD) apparatus, equipped with Dean-Stark trap. The analysis was performed on a GC 7890 gas chromatograph (AT, Palo Alto, CA, USA), equipped with a mass selective detector AT 5975C (with electron ionization, 70 eV), split/splitless injector (split 30: 1) and a ChemStation data system, G1701-DA, which included the ADAMS, NIST and WILEY spectral libraries. A fused-silica capillary apolar DB-5MS column [(J&W Scientific) of 60 m × 0.25 mm id, coated with 5% phenyl poly(dimethylsiloxane), 0.25 µm film thickness] and polar DB-WAX column [(J&W Scientific) of 60 m × 0.25 mm id, coated with poly(ethyleneglycol), 0.25 µm film thickness] were used.

The *S. aratocensis* EO yield was 0.23%. The following were the major compounds identified by GC/MS in the EO: (*E*)-calamenene (8%), α-cadinene (8%), α-humulene (10%), (*E*)-β-caryophyllene (20%), and germacrene D (33%). (*E*)-β-Caryophyllene has antimicrobial (Öztürk *et al*., 2009), antifungal (Moreira *et al*., 2010), antioxidant, and anti-inflammatory (Miguel, 2010) activities.

**Acknowledgments**

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**Keywords**: *Salvia aratocensis*, essential oil, GC/MS.

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# PP-7. Antibacterial Activity of *Thymus vulgaris* L. Essential Oil in the Vapour Phase and its Headspace GC/MS Analysis.

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## Abstract

Acute lower respiratory infections and particularly pneumonia are still the world's most deadly communicable diseases. While aerosolized antibiotics remain the recommended treatment, difficulties to implement optimal nebulization techniques have limited their widespread adoption (Houdková, 2018). In this context, the volatile constituents of essential oils (EOs) appear as promising alternatives in the development of novel inhaled antibiotic therapy **(Netopilova, 2020)**. Currently, EO inhalations, and more particularly *Thymus vulgaris* EO, are approved as treatments for moderate respiratory infections by the Herbal Medicinal Product Committee (HMPC) of the European Medicine Agency (EMA). In this study, we aimed to determine the in vitro antibacterial activity of the vapour-phase of three *Thymus vulgaris* EO samples against respiratory pathogens including *Staphylococcus aureus*, *Streptococcus pyogenes* and *Haemophilus influenzae* and explore their gaseous phase chemical profile in a series of GC/MS analyses using headspace sampling techniques.

The antimicrobial activity of EO liquid and vapour phases were evaluated using broth-microdilution volatilization (BMV) method which is a simple, fast, cost, and labour effective technique that provides reproducible and quantitative results (Houdková and Kokoška 2020). Vapour of EOs extracted from the dried aerial part of three different commercial sources of *Thymus vulgaris* were tested against bacterial pathogens causing lung infections including *Haemophilus influenzae, Staphylococcus aureus*, and *Streptococcus pyogenes*. Subsequently, the three EO samples were characterized using GC-MS analysis using both a non-polar (HP-5MS) and polar (DB-HeavyWAX) columns. Eventually, a time series headspace analysis of vapours above a mixture of Mueller-Hinton growth medium and *T. vulgaris* EO were performed using two different sampling methods: Solid Phase Microextraction (HS-SPME) and a Syringe Headspace sampling technique (HS-GTS).

All samples exhibited significant inhibitory effect with minimum inhibitory concentration (MIC) values ranging from 512 to 1024 µg/ml in both liquid and vapour phase. The three EO samples were characterized as thymol chemotypes with peak percentage areas for thymol ranging in between 38.42 and 48.65 % across the three EO samples. Moreover, results show a different distribution of volatile compounds in the headspace using either HS-SPME or HS-GTS techniques. For instance, thymol was found in an unusually low amount with peak percentage area lower than 5.27 % (HS-SPME) and 0.60 % (HS-GTS) across the three EO samples. Multiple factors could explain these results such as parameters related to the use of a fibre coating assembly but also the experimental conditions such as the incubation temperature and the matrix used (Adam, 2009).

**Acknowledgment**

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**Keywords**: Essential oils, Thymus vulgaris, antimicrobial activity, vapor phase, headspace analysis,

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# PP-8. *Caenorhabditis elegans* as an *in vitro* model for the evaluation of anthelmintic activities of essential oil blends.

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## Abstract

Parasitism is a major concern in livestock, and the appearance of resistance to anthelmintics encourages the development of new alternatives. The search for new actives involves the evaluation of many compounds by screening methods, which are not compatible with the use of parasitic nematodes, requiring passage through the host during their larval development (Burns et al., 2015; Katiki et al., 2011). The use of *Caenorhabditis elegans*, a free-living nematode isolated from soil, is proposed here as a model to evaluate, and discriminate between pure and blended essential oils (EOs).

Anthelmintic activities of five pure EOs (*Cinnamomum cassia, Origanum vulgare, Satureja hortensis, Thymus vulgaris* and *Syzygium aromaticum*) were first established on *C. elegans* through an egg hatching inhibition assay, and by monitoring their toxicity on L4 stage larvae. *Cinnamomum cassia* EOs was the most toxic EO on eggs and L4 larvae, with $EC_{50}$ of 8.45 µg/ml and 74.75 µg/ml respectively. *Syzygium aromaticum* EO was the less active, with an $EC_{50}$ of 329.56 µg/ml in the egg hatching inhibition assay and an $EC_{50}$ of 260.10 µg/ml when tested on L4 larvae. Two blends of essential oils (EOB1 and EOB2) were then formulated, and their activities were successively evaluated on *C. elegans* and on *Haemonchus contortus*, a parasitic nematode that is recurrent in small ruminants. According to the egg hatching inhibition assay, the best activity was obtained with EOB1, on both worms: an $EC_{50}$ of 19.57 µg/ml was observed on *C. elegans,* against an $EC_{50}$ of 92.00 µg/ml on *H. contortus.*

The EOs and blends exhibited comparable *in vitro* activities in parasitic and non-parasitic nematodes. These results suggest the possibility of using *C. elegans* as a pre-assessment system for anthelmintic activities of natural products. The compatibility of *C. elegans* with liquid media, as well as with the use of surfactants, allows the evaluation of lipophilic compounds such as EOs. The democratization of this cost-efficient model will certainly support the identification of new actives, which until now requires the use of laboratory animals, necessary for the multiplication of parasites.

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**Keywords**: *Caenorhabditis elegans*, anthelmintic, gastrointestinal nematodes, essential oil.

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# PP-9. *Salvia apiana In Vitro* Shoot System as a source of unique volatile fraction

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## Abstract

White sage (*Salvia apiana* Jepson) is an endemic species typical for the *chaparral* plant formation of mild, Mediterranean-type climate area of the California Floristic Province in North America. *S. apiana* has been used as a traditional medicinal and ritual plant by the Native North American Chumash people for a long time. Aerial parts of *S. apiana* were used in folk medicine to treat inflammation and as a calmative agent (Krol et al., 2021).

Research to date has shown that aerial parts of white sage are rich source of pharmacological active terpenoids, including volatile fraction. The endemic nature of the species and the absence of effective methods for its cultivation constitute a major obstacle to further research into its chemical composition and biological activity. Given the above limitations, *in vitro* cultures of *S. apiana* could become an alternative source of high quality plant material.

In view of the above, white sage was used to obtain *in vitro* shoot system capable of constant production of volatile oil with analgesic and anti-inflammatory properties. *S. apiana* biomasses were cultured in the large laboratory scale, in the prototype and commercial bioreactors (the temporary immersion or spray systems). The phytochemical investigations included qualitative and quantitative GC/FID analyses of volatile oils obtained by hydrodestillation (Clevenger apparatus) from the *in vitro* biomasses, received at all stages of biotechnological experiments, and from the ground plant material of white sage. Moreover, in order to further justify the selection of white sage for this biotechnological project, comparative chromatography was conducted on the volatile fraction obtained from common garden sage (*S. officinalis* L.), for comparison.

The microshoot culture was initiated from shoot tips of aseptically germinated seedlings of *S. apiana*. They were grown on Schenk – Hildebrandt (SH) agar medium supplemented with 6-benzylaminopurine (BAP) 2 $mgl^{-1}$ and thidiazuron (TDZ) 0,22 $mgl^{-1}$. The initial microshoots were subcultered every 3 weeks for about 7 months. After this time, a continuous culture was obtained, capable of supplying plant material for further phytochemical and biological experiments. *S. apiana* microshoots were cultured in the prototype spray bioreactor and in temporary immersion bioreactor systems RITA and PLANTFORM. The largest biomass accumulation (fresh weight = 290.0 g $l^{-1,}$ growth index = 583.3, dry weight = 18.2 g $l^{-1}$) and essential oil content (1.27% *v/m*) were achieved with application of prototype spray bioreactor for 4 weeks. The volatile fraction was rich in 1,8-cineole, ***α***-pinene, ***β***-pinene, camphor and did not contain thujone.

**Acknowledgments**

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**Keywords**: Lamiaceae, terpenoids, chemical composition, ethnomedicine, essential oil

# PP-10. *Cymbopogon nardus* (Poaceae) essential oil steam distillation and fractionation

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## Abstract

*Cymbopogon nardus* (family Poaceae) is an aromatic plant, native from India and Sri Lanka, cultivated in tropical and subtropical areas. *C. nardus* essential oil (EO) is of commercial interest for its insect repellent activity and applications in the treatment of rheumatism, menstrual problems, fever, and intestinal parasites (Kaur et al., 2021).

*C. nardus* EO was obtained by steam distillation in a 0.1 $m^3$ stainless still. Three levels of bed density and steam flow were evaluated. The bed height (0.7 m) and distillation time (1.5 h) were constant. Condensate was collected every 5 and 10 min during the distillation time. The yield was calculated as the total mass of EO obtained over the mass of treated plant material.

The yields obtained in the present work (0.37-0.55%) were similar to those reported by Chong et al. (2015). The highest yield was obtained under the following conditions: 240 kg/$m^3$ (bed density) and 200 mL/min (steam flow). The EO was characterized by GC/MS and the major components in the fractions were quantified by GC/FID. The composition of the EO was similar to that reported by Rastuti et al. (2020). The major compounds were citronellal (12-72%), citronellol (9-36%), geraniol (0.2-15%), and elemol (1-17%). A principal component analysis (PCA) was performed for the major compounds in the 12 EO fractions and their grouping into four different fractions was determined.

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**Keywords**: *Cymbopogon nardus*, essential oil, fractionation, GC/MS.

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# PP-11. Characterisation of trichomes morphology and density in the genus *Ocimum* L.

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## Abstract

Non-glandular and glandular trichomes (GTs) found on the stem and leaves of many plants act as a physical barrier against biotic and abiotic stresses. GTs store and secrete various materials including essential oils, which are of great interest in food, cosmetic and pharmaceutical industries (Rehman et al., 2016) Members of Lamiaceae, including *Ocimum* L. (basil), produce essential oils which have potent antioxidant, antiviral, and antimicrobial properties which could be used for treating various ailments (Shahrajabian et al., 2020). In this comparative study, *Ocimum*'s trichomes ultra-structure, pattern and distribution on leaves have been investigated, at different developmental stages of several *Ocimum* species, varieties, cultivars, and types, using Scanning Electron Microscopy (SEM). In the genus, both capitate and peltate types of GTs were present which were densely distributed on the leaf surface as compared to hairy non-glandular trichomes. Moreover, in the young leaves the density of GTs was very high compared to the mature leaves. It has been reported that basil oil' concentration is controlled by density, size, and relative proportion of both types of GTs (Maurya et al., 2019). This study provides the basis for further investigation of the genetic and chemical mechanisms involved in the production of essential oils in *Ocimum*.

**Acknowledgments**

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# PP-12. Chemical composition and antimicrobial activity of essential oils against plant pathogens in vapour phase.

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Abstract

Diseases caused by plant pathogens lead to significant losses to most perishable food crops in controlled atmosphere such as green houses or storages. Plant diseases in closed environment are currently controlled with fungicides, however, reliance on this single strategy leads to problems such as harmful residues and health issues of workers. Therefore there is an increasing pressure to find more natural methods of disease control in special environment. One of the prospective methods could be the use of essential oil (EO) vapors [1]. EOs from aromatic plants have previously demonstrated antimicrobial and antifungal activity against number of plant pathogens. Despite the great antimicrobial potential EOs, growth-inhibitory effects of their vapors have poorly been investigated against microorganisms causing spoilage of agriculture products [1]. In this study we determined chemical composition and antimicrobial activity of EOs and their vapors against significant plant pathogens such as *Aspergillus niger, Fusarium oxysporum* and *Pectobacterium carotovorum* using broth microdilution volatilization method [2, 3]. EOs of five plants namely *Allium sativum, Cinnamomum zeylanicum, Citrus sinensis*, *Syzygium aromaticum* and *Thymus vulgaris* were obtained from dried plant material using hydrodistilation. Subsequently, minimum inhibitory concentrations (MICs) were determined [3]. The results showed that the *C. zeylanicum, T. vulgaris* and *S. aromaticum* EO produced highest antimicrobial activity in vapor or broth phase at MICs 256-1024 (µg/mL). The chemical composition of the most effective EOs and their vapors have been determined using GC-MS. The major components were thymol (*T. vulgaris*), (E)-cinnamaldehyde (*C. zeylanicum*), eugenol (*S. aromaticum*). In conclusion, above mentioned EOs could be used for the development of new products for control of pathogenic microorganisms causing spoilage of agricultural products e.g. in form of controlled atmosphere or fumigation.

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**Keywords**: Antimicrobial activity, vapor phase, essential oils, plant pathogens

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# PP-13. *Ambrosia peruviana* (Asteraceae) essential oil from Colombia and its chemical characterization

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## Abstract

*Ambrosia peruviana* Willd. (Asteraceae family), is an herbaceous plant, known as altamisa, artemisa, altamiz, alcanfor, *Ambrosia Silvestre*, and Maki. It is native to Peru and is distributed from Mexico to South America (Ecuador, Bolivia, Colombia, Antilles). Traditionally, cooking the roots and/or leaves (also their infusion) has been used as a hypotensive, antispasmodic, depurative, diaphoretic, as well as in the treatment of headaches, fever, ulcers, skin spots, scars, varicose veins, and for menstrual disorders (Zapata et al., 2010).

The essential oil (EO) was obtained by microwave-assisted hydrodistillation using a Clevenger equipment, with a Dean-Stark trap, and a microwave oven (Samsung, 1000 W, 2.45 GHz), operated at 60% of its capacity. EO analysis was performed on a gas chromatograph (Agilent Technologies 6890 Plus, Palo Alto, CA, USA), coupled to a mass selective detector (AT MSD 5973 Network, full scan mode), split/splitless injector (split 30: 1) and a ChemStation data system, G1701-DA, which included the ADAMS, NIST and WILEY spectral libraries. Fused-silica capillary columns DB-5MS [(J&W Scientific) of 60 m x 0.25 mm id, coated with 5% phenyl poly(dimethylsiloxane), 0.25 µm flim thickness] and DB-WAX [(J&W Scientific) of 60 m x 0.25 mm id, coated with poly(ethyleneglycol), 0.25 µm flim thickness] were used. The GC oven temperature was programed from 45 °C to 150 °C (5 min) at 4 °C/min, then to 250 °C (5 min) at 10 °C/min and to 275 °C (15 min) at 10 °C/min.

More than 60% of all EO compounds were positively identified in the EO, in relative amounts >0.1%. 16 components were detected, among them there were *ar*-curcumene (10.8%), germacrene D (10.6%), γ-curcumene (10.5%), chrysanthenone (9.2%), bicyclogermacrene (6.2%), myrcene (2.7%), β-bisabolene (2.1 %), (*E*)-β-caryophyllene (1.8%), phytol (1.1%), and linalool (1.1%). Santolina triene (1.0%), (*E*)-β-farnesene (1.0%), β-pinene (0.8%), caryophyllene oxide (0.7%), α-humulene (0.6%) and (*E*)-β-bergamotene (0.5%) were found in *A. peruviana* EO in smaller amounts.

**Acknowledgments**

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**Keywords**: *Ambrosia peruviana*, Asteraceae, essential oil, GC/MS.

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# PP-14. Chemical composition of the essential oil of *Hyptis colombiana* (Lamiaceae)

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## Abstract

*Hyptis colombiana* (Lamiaceae family) is a shrub native to the Colombian and Venezuelan Andes (Ayers and Boufford, 1988). The genus *Hyptis* is traditionally used for the treatment of digestive disorders, menstrual disorders and respiratory diseases (McNeil et al., 2011).

The plant material was collected from experimental plots at the CENIVAM research center (Bucaramanga, Colombia). The essential oil (EO) was obtained in a Clevenger-type microwave-assisted hydrodistillation (MWHD) equipment, with a Dean-Stark distillation reservoir; adapted to a conventional microwave oven (SAMSUNG model MS-1242zk, 1200 W). The EO analysis was carried out on a GC 7890 Plus gas chromatograph (Agilent Technologies, AT, Palo Alto, CA, USA) coupled to a mass selective detector AT MSD 5973 with electron ionization (70 eV). Capillary apolar column DB-5MS [60 m x 0.25 mm (id) x 0.25 µm] with stationary phase of 5%-phenyl-poly(dimethylsiloxane) and polar column DB-WAX [60 m x 0.25 mm (id) x 0.25 µm] with stationary phase of poly (ethylene glycol) were employed. The temperature of the GC oven was programmed from 45 °C for 5 min, then at 5 °C/min to 250 °C. The EO compound identification was based on comparison of their mass spectra with those from databases (ADAMS, NIST02, Wiley), and using linear retention indices measured on both columns.

The essential oil yield was 0.09% (w/w).The main compounds identified in the EO were (*E*)-β-caryophyllene (21.3%), germacrene D (17.5%) and caryophyllene oxide (13.9%). This composition was similar to that of the H. *colombiana* EO obtained in Venezuela: (*E*)-β-cariofileno (29.5%) germacrene D (22.2%) and caryophyllene oxide (3.5%) (Flores et al., 2015). The amount of each compound in the EOs from Colombia and Venezuela differed possibly due to climatic and environmental conditions, the phenological plants status of the plants, and geographic location. Flores et al., (2015) indicated that these compounds may have antimicrobial activity, based on previous tests he conducted.

**Acknowledgments**

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**Keywords**: *Hyptis colombiana*, essential oil, GC/MS.

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# PP-15. Essential oils extracted from organic propolis residues: an evaluation of their antibacterial and antioxidant properties and determination of their chemical volatile compounds

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## Abstract

Crude propolis processing to obtain ethanolic extract, a commercialized form of propolis, generates residues. Essential oils (EO) from propolis residues could be a potential source of natural bioactive compounds to replace antibiotics and synthetic antioxidants in pig production. Thus, this study aimed to evaluate the antibacterial and antioxidant activities of EOs from crude organic propolis (EOP) and from propolis residues, moist residue (EOMR) and dried residue (EODR), and determine their chemical volatile profile. The EOs were extracted by hydrodistillation and their chemical volatile profile was identified by GC-MS. Next, their antibacterial activity, phenolic content, and antioxidant capacity (by ABTS, DPPH and FRAP) were determined. Results showed that EOs had an antibacterial effect on *Escherichia coli* and *Lactobacillus plantarum* as they caused disturbances on the normal growth kinetics of both bacteria. However, EODR stood out for having a selective antibacterial activity, higher activity on *E. coli* than on *L. plantarum*, as it caused higher reduction of the maximal culture density (A) of *E. coli* (86.7 %) than *L. plantarum* (46.9%). Additionally, EODR exhibited certain antioxidant activity. Conversely, EOMR displayed the highest antioxidant activity in term of ABTS (0.90 µmol TE/mg), FRAP (463.97 µmol Fe2+/mg), and phenolic content (58.41 mg GAE/g). The chemical composition results showed each EO had a different chemical volatile profile, however, α-pinene and β-pinene were detected as the major compounds in EOP, EOMR and EODR. Interestingly, some minor compounds such as ethyl benzoate, limonene, (E)-caryophyllene, n-decanal, α-thujene, γ-terpinene, n-nonanal, α-terpinene, sabinene, ethyl decanoate and zonarene were detected in a higher relative abundance in EODR compared to EOP and EOMR. In case of EOMR, thuja-2,4(10)-diene, p-cymene, myrcene, acetophenone and n-octanal and α-copaene were present in higher abundance in this EO compared to EOP and EODR. Thus, due to the differential biological properties and chemical composition of the EOs from propolis residues, those minor compounds detected in higher abundance could be more associated in conferring the biological activities of EOMR and EODR.

**Acknowledgments**

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**Keywords**: *Escherichia coli*, HS-GC/MS, Lactobacillus, pig production, feedstuff, bee product

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# PP-16. Essential Oil Composition and Ethnobotanical Profile of Some Selected Species of *Eucalyptus* Growing in Cyprus

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## Abstract

The Genus *Eucalyptus* consists of tall, evergreen trees with leathery leaves and belongs to the family Myrtaceae. They are indigenous to Australia and contain about 900 species and subspecies. There are about 62 species of *Eucalyptus* found in Cyprus, of which 47 are classified but 15 are not classified or hybrid. The aim of this study is to investigate the traditional uses of eucalyptus trees growing in Cyprus and to analyse essential oil constituents. The leaf essential oils of two *Eucalyptus* species (*E. camaldulensis* and *E. torquata*) growing in Cyprus were isolated by hydro-distillation and analysed by GC and GC–MS, simultaneously. Also the existing ethnobotanical research literature was reviewed. As a result, *E. camaldulensis* oil yield was 2.4%, and major compounds were identified as α-phellandrene (10.3%) and β-phellandrene (30.6%), respectively, followed by *p*-cymene (8.2%), bicyclogermacrene (6.1%) and spathulenol (9.3%). On the other hand, the oil yield of *E. torquata* was relatively lower (1.6%), with major compounds being α-pinene (18.6%), 1,8-cineole (18.8 %), β-eudesmol (10.3%) and torquatone (29.2%). According to the literature of traditional usage of *Eucalyptus* species in Cyprus, *E. camaldulensis* leaves are used orally and topically for abortion, after-labor tonic, cold / flu symptoms, decongestant, urinary tract antiseptic and syphilis (Yöney et al., 2010). The Cypriot people use to treat flu, cold, and other upper respiratory tract infections by inhaling the steam of the boiled leaves of *E. camaldulensis*. Additionally, the liquid obtained from this boiling process is used externally for antirheumatic effect (Kaya Yıldırım, 2010; Ozan, 2011). In another study, the same species was recorded for respiratory tract disorders, musco-skeletal disorders and skin disorders (González-Tejero et al., 2008). However, no such ethnobotanical usage information of *E. torquata* exists in Cyprus. In conclusion, the essential oil composition of *E. torquata* was reported for the first time in addition to the *E. camaldulensis* essential oil composition. The research showed that there are chemical variations in the essential oil composition of *E. camaldulensis* growing in Northern Cyprus. We assume that the essential oil of *E. camaldulensis* belongs to the chemotype rich in phellandrene (α- and β-), *p*-cymene, spathulenol with the absence of 1,8-cineole. On the other hand, the essential oil composition of *E. torquata* growing in Cyprus showed variations with the literature data. Further research is in progress in order to reveal chemical variations including the biological activities of the essential oils of other *Eucalyptus sp.* growing in Cyprus.

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**Keywords**: *Eucalyptus camaldulensis*, *Eucalyptus torquata*, Essential oil, Ethnobotany, Cyprus

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# PP-17. Anti-inflammatory activity and chemical compositions of *Prangos platychlaena* essential oil and fractions

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## Abstract

In the pharmaceutical and agrochemical industries, Apiaceae family members are important sources. Apiaceae is represented by 511 taxa in Turkey, with about 485 species. There are 181 endemic taxa among them. *Prangos platychlaena* Boiss. ex Tchihat (Apiaceae) is a Turkish endemic perennial herbaceous plant known locally as **çağşır**, **çakşır**, kirkor, and korkor (Başer and Kırımer, 2014; Tabanca et al., 2018). In the present work, the essential oil (EO) of *P. platychlaena* aerial parts was obtained by hydrodistillation using a Clevenger type apparatus for 3h. Obtained essential oil was separated into fractions with different polarities using column chromatography. Essential oil and the fractions were analysed both by GC-FID and GC-MS, simultaneously.

Nona-3,5-diyne-2-yl acetate (46%) and 3,5-Nnonadiyne (13.5%) were found as the main constituent of the EO of *P. platychlaena*. The *n*-hexane fraction was characterized as 3,5-nonadiyne (45.6%) and germacrene B (16.4%), while main components of the methanol fraction were nona-3,5-diyne-2-yl acetate (59.6%) and 3,5-nonadiyne-2-ol (25.9%). Also, *in vitro* anti-inflammatory activity was evaluated by 5-lipoxygenase (5-LOX) inhibitory effect of the essential oil and the fractions, spectrophotometrically. Anti-inflammatory activity of the essential oil, *n*-hexane fraction and methanol fraction were determined as 70.98±**1.**7%, 67.10±2.5% and 50.11±4.8 in 100 **µg**/mL, respectively.

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**Keywords**: *Prangos platychlaena*, Apiaceae, essential oil, column chromatography, anti-inflammatory activity

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# PP-18. Anti-inflammatory activity of essential oils of two *Echinophora* L. species

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## Abstract

Six species of the genus *Echinophora* (Apiaceae), including three endemics, are found in Turkey's flora. In Turkish, *E. tenuifolia* L. is called **çörtük**, **çördük**, tarhana otu or turşu otu while *E. tournefortii* L. is called dikenli **çörtük**. They have traditionally been utilized for wound healing, and their 5% infusions are used to treat stomach ulcers (Davis, 1972; Genç and Ecevit-Genç, 2014).

In this study, the essential oils (EOs) of *Echinophora tenuifolia* and *E. tournefortii* aerial parts were obtained by hydrodistillation using a Clevenger type apparatus for 3h. Essential oils were analysed both by GC-FID and GC-MS, simultaneously. *(Z)*- ***β***-Ocimene (21.2%), *α*-pinene (20%) and ***β***-pinene (16.2%) were found as the main constituent of the EO of *E. tenuifolia*. The essential oil of *E. tournefortii* was characterized with ***δ***-3-Carene (48.6%) and methyl eugenol (34.6%). The *in vitro* anti-inflammatory activity was evaluated by 5-lipoxygenase (5-LOX) inhibitory effect of the essential oils spectrophotometrically. The anti-inflammatory activity of the essential oils of *Echinophora tenuifolia* and *E. tournefortii* were determined as 70.98±1.7%, 67.10±2.5% and 50.11±4.8 in 100 **µg**/mL, respectively. The anti-inflammatory activity of essential oil of *E. tournefortii* is lower than the EO of *E. tenuifolia* (74.72±3.83, in 130 **µg**/mL).

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**Keywords**: *Echinophora tenuifolia*, *Echinophora tournefortii,* Apiaceae, essential oil, anti-inflammatory activity

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# PP-19. Chemical profile and anticholineesterase power of *Artemisia* volatile oils from Croatia and Bosnia and Herzegovina

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## Abstract

Artemisia genus is one of the largest of the Asteraceae family. This genus is consists of large number of species which are widespread throughout Europe, Asia, Africa, and North America. Many Artemisia plants is often used in traditional medicine as antimalarial, antiviral, antitumor, spasmolytic, and others agents (Tan et al, 1998).

In this work the volatile oils of six different Artemisia species (*A. absinthium, A. abrotanum, A. annua, A. arborescens A. verlotiorum* and *A. vulgaris*) from Dalmatia (Croatia) and Bosnia and Herzegovina were tested on chemical composition and anticholinesterase power. Artemisia volatile oils were isolated by hydrodistillation and their chemical compositions were determined by GC-MS/FID. Volatile oil of *A. absinthium* was characterized by high amounts of *Z*-sabinyl acetate and *Z*-epoxy-ocimene; *A. abrotanum* oil was characterized by *Z*-davanone and piperitone; A. annua oil was characterized by camphor and artemisia ketone; *A. arborescens* oil was characterized by camphor and chamazulene; *A. verlotiorum* oil was characterized by *Z*-thujone; *A. vulgaris* oil was characterized by *E*-thujone. Anticholinesterase (antiAChE and antiBuChE) power was tested by Ellman method (Ellman, 1961). All of the tested volatile oils showed moderate anticholinesterase power comparing with huperzin A, as good anticholinesterase agent. AChE inhibition power of tested *Artemisia* volatile oils (1 mg/mL) were in the range of 29.5-55.6 %, while BuChE inhibition power of tested oils were in the range of 11.4-49.0 %.

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Generally, the last paragraph of the paper is the place to acknowledge people (dedications), places, and financing (you may state grant numbers and sponsors here).

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**Keywords**: Artemisia, volatile oils, GC-MS/FID, AChE, BuChE

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# PP-20. Essential oil composition of *Capsella bursa-pastoris* (L.) Medik herba

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## Abstract

*Capsella* Medik. genus belongs to Brassicaceae family and is represented by 4 species in Turkey. Among them, *C. bursa-pastoris* (L.) Medik. is a cosmopolite species and grows naturally throughout Turkey (Mutlu, 2012). There are a few studies on the essential oil composition of different parts of the plant (Miyazawa et al., 1958, Lee and Choi, 1996; Choi et al., 2006; Gao et al., 2009; Kamali et al., 2015), and as far as we are concerned, essential oil composition of the plant growing in Turkey has not been studied previously. Thus, in this study we aimed to isolate and analyse the essential oil composition of the aerial parts of *C. bursa-pastoris* growing naturally in Ankara, Turkey.

Plant material was collected from Ankara University Tandoğan Campus in April 2021 and voucher specimen is kept in AEF with herbarium number 30718. Essential oil of the aerial parts was obtained by hydrodistillation using a Clevenger type apparatus for 3h and analyzed both by GC-FID and GC-MS, simultaneously.

Essential oil yield was 0.2% and 90.2% of the essential oil was identified, corresponding to 21 components. Major components of the oil were determined to be nonacosane (19.6%) phytol (19.3%), pentacosane (13.5%), heptacosane (9.9%), hexadecanoic acid (9.9%). Phytol was also found to be the main component of the essential oil of the leaves and aerial parts (16.34% and 13.14, respectively) in the study by (Lee et al, 1996).

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**Keywords**: *Capsella bursa-pastoris*, Brassicaceae, essential oil, aerial

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# PP-21. Isolation and identification of acylphloroglucinols in the seedlings of the medicinal plant, *Melaleuca alternifolia* (Australian tea tree)

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## Abstract

*Melaleuca alternifolia* (tea tree), family Myrtaceae, is endemic to the northern rivers of NSW, Australia. Since 1925, the volatile components of the steam-distilled oils of the leaves have been studied in detail. However, the less-volatile compounds have not been investigated. Using an ethanolic extract of the seedling leaves, the non-volatile components were studied using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Four of these less-volatile components were isolated from young seedling leaves and identified as the acylphloroglucinols 1-(2,6-dihydroxy-4-methoxy-3-methylphenyl)-2-methylpropan-1-one, callisalignone A, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-methylbutan-1-one and pulverulentone B described here for the first time from *M. alternifolia*. Potential uses for these components are discussed.

**Keywords:** *Melaleuca alternifolia*, tea tree, acylphloroglucinols, prep-HPLC, 2D NMR

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# PP-22. Essential Oil Profile and Yield of Corolla, Calyx, Leaf, and Whole Flowering Top of Cultivated *Lavandula angustifolia* Mill. (Lamiaceae) From Utah

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## Abstract

*Lavandula angustifolia* Mill. (lavender) is an essential oil bearing plant in the Lamiaceae family. Volatile oil produced through the steam distillation of lavender was examined to establish the essential oil yield and aromatic profile from each portion of the plant—namely, the corolla, calyx, leaf, and whole flowering top. The resulting essential oils were analyzed by GC-FID and GC-MS. The different plant parts generally shared similar compounds but in varying relative percentages. Aromatic profiles of the whole flowering top and calyx were similar, with prominent compounds being linalool acetate (34.3%, 32.0%), linalool (26.5%, 32.9%), lavandulyl acetate (5.6%, 4.9%), terpinen-4-ol (5.3%, 7.0%), and (*Z*)-β-ocimene (4.5%, 5.4%), respectively. Aromatic profiles for the corolla and leaf were unique. Prominent aromatic compounds of the corolla included linalool acetate (18.4%), linalool (10.8%), epi-α-cadinol (10.0%), borneol (7.3%), and lavandulyl acetate (6.3%). Prominent aromatic compounds of the leaf included epi-α-cadinol (19.8%), γ-cadinene (11.0%), borneol (6.0%), caryophyllene oxide (4.9%), and bornyl acetate (4.8%). Complete profiles and essential oil yields of corolla, calyx, leaf, and whole flowering top were established. This study establishes the influence the corolla, calyx, and leaf exert on the aromatic profile of the whole flowering top and provides insight into authentication of lavender essential oil.


**Figure 1.** Portions of *Lavandula angustifolia* used: (**A**) corolla, (**B**) calyx, (**C**) leaf, (**D**) flowering top.

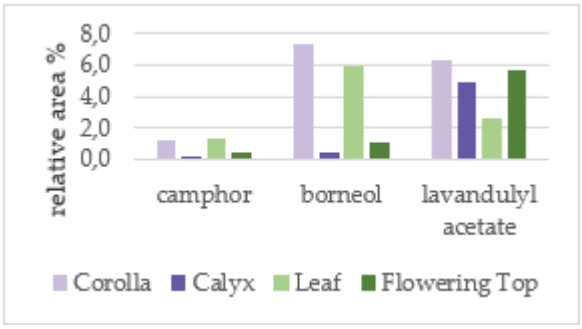


**Figure 2.** Relative area % of camphor, borneol, and lavandulyl acetate in each portion of *L.* Angustifolia, namely the corolla, calyx, leaf, and whole flowering top. The profiles of the calyx and whole flowering top are similar. Despite the low yield of the corolla and leaf, both portions impact the profile of the whole flowering top.

**Acknowledgment**

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**Keywords**: *Lavandula angustifolia*, corolla, calyx, leaf, flowering top

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# PP-23. Essential oil, insect, and microbe relationship with *Juniperus osteosperma* trees killed by wildfire.

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## Abstract

In the state of Utah (USA), pinyon-juniper forests are common and are primarily comprised of three essential oil-bearing tree species: *Juniperus osteosperma* (Utah juniper), *J. scopulorum* (Rocky Mountain juniper), and *Pinus edulis* (Pinyon pine). Previous work has shown antimicrobial properties of volatile oil extracted from Utah juniper heartwood and bark/sapwood (Clark, 1990). Pinyon-juniper forests are often destroyed by wildfire, and it was hypothesized that essential oils are retained within dead trees and that their antimicrobial properties would slow the rate of decomposition. To determine if burnt, dead trees could be identified through their essential oils alone, live trees of *J. osteosperma, J. scopulorum,* and *P. edulis* were harvested, processed, distilled, and the resulting oils analysed by GC-FID and GC-MS. *J. osteosperma* trunk essential oil was high in α-pinene, δ-3-carene, cis-thujopsene, and cedrol (Wilson, 2019). *J. scopulorum* trunk essential oil was high in α-pinene, (E)-caryophyllene, widdrol, cedrol, and allo-aromadendrene epoxide (Poulson, 2021). *P. edulis* trunk essential oil was high in α-pinene, δ-3-carene, ethyl octanoate, longifolene, and germacrene D (Poulson, 2020). Utilizing wildfire data from 1998-2018, sixteen samples of dead Utah juniper trunks were harvested. Since conventional voucher samples could not be created from burnt plant material, chemical profiling of steam distilled plant material was used to distinguish species. The trunk of each tree was separated into different sections to characterize fungal and bacterial species colonizing the dead trees, to catalogue insect activity, and for essential oil distillation. Microbial results showed 1258 bacterial and 326 fungal taxa. Years since burn did not have an apparent effect on fungal diversity or composition, but it did influence bacterial diversity and community composition. The combination of α-acorenal and β-acorenal was a statistically significant and negative predictor of fungal diversity [GLM; P = 0.0171]. No predictable effect of these factors on discrete microbial taxa was found. Insect bore holes were found in all samples, with older trunks displaying an incrasing number of holes. The trunk essential oil of dead Utah juniper had a similar composition to living Utah juniper, except the ratios of monoterpenes to sesquiterpenes differed. Live Utah juniper trunk contains nearly 60% α-pinene (Wilson, 2019). Dead Utah juniper essential oil showed a great deal of variation but was highest in α-cedrene (2.1% - 34.1%), cis-thujopsene (8.7% - 30.7%), widdrol (1.4% - 15.5%), and cedrol (5.8% - 38.1%), and low in all monoterpenes. Yields ranged from 0.1% to 1.0%. While there were no clear trends in essential oil composition or yield from year to year, results show that essential oils can be retained in dead trees for over twenty years.

**Acknowledgments**

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**Keywords**: *Juniperus osteosperma,* wildfire, essential oil, bacteria, fungi

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# PP-24. Portuguese monofloral honeys – the importance of volatile profile in botanical source differentiation

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## Abstract

Honey's aroma depends on the present volatile compounds, mostly in the nectar, which help to discriminate honey types from different botanical and geographical origins. Mainland Portugal and the Azores and Madeira Islands are characterised by a rich and varied honey flora, contributing to the production of a great diversity of local monofloral honeys.

The volatile profile of 51 samples from 12 monofloral-labelled Portuguese honey types were assessed. Honeys of bell heather, carob tree, chestnut, eucalyptus, incense, lavender, orange, rape, raspberry, rosemary, sunflower and strawberry tree were collected from several regions from mainland Portugal and from the Azores Islands. When available, the corresponding flower volatiles were comparatively evaluated. Honey volatiles were isolated using solid-phase microextraction (SPME) and hydrodistillation (HD).

Agglomerative cluster analysis of honey HD volatiles evidenced two main clusters, one of which had nine sub-clusters. Components grouped by biosynthetic pathway defined alkanes and fatty acids as dominant, namely *n*-nonadecane, *n*-heneicosane, *n*-tricosane and *n*-pentacosane and palmitic, linoleic and oleic acids. Oxygen-containing monoterpenes, such as *cis*- and *trans*-linalool oxide (furanoid), hotrienol and the apocarotenoid α-isophorone, were also present in lower amounts. Aromatic amino acid derivatives were also identified, namely benzene acetaldehyde and 3,4,5-trimethylphenol. A fully grown classification tree allowed the identification of the most relevant volatiles for discriminating the different honey types. Twelve volatile compounds were enough to fully discriminate eleven honey types (92%) according to the botanical origin.

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**Keywords**: Portuguese monofloral honeys, volatile profile, honey type discrimination, botanical origin determination

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# PP-25. Volatile composition, biological properties and potential uses of hydrolates

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## Abstract

Hydrolates (also known as hydrosols, floral waters, herbal waters, or essential waters) are distillates, that is, products of condensation obtained after the essential oils distillation procedure (ISO 9235:2013). Hydrolates are colloidal suspensions composed of a continuous phase, the distilled water, and a dispersed phase, the emulsion of essential oil droplets and water-soluble components, namely oxygen-containing compounds (Lei et al., 2018). The movement towards a circular economy has increased the interest in the essential oil industry co-products, such as hydrolates. Hydrolates composition has been mostly studied on their volatile constituents. Moreover, they have shown a diverse range of biological properties, with potential application in food, beverages, cosmetic and pharmaceutical industries, as well as in the agroforest sector (D'Amato et al., 2018). Nevertheless, the information is fragmented and dispersed under the different hydrolates designations. The main volatile components of 333 hydrolates obtained from 186 species from 49 families are described, and, whenever reported, the information on the corresponding essential oil main components is detailed. Additionally, the methodologies of extraction and analysis are surveyed, as well as hydrolates characteristics and traditional uses. The different biological properties that have been attributed to hydrolates and, according with those properties, their potential uses, are also described.

**Acknowledgments**

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**Keywords**: Hydrolate, hydrosol, aromatic water, volatiles, biological activity

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# PP-26. Allelopathic effects of *Satureja hortensis* and its essential oil on the germination of selected species

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## Abstract

The genus *Satureja* includes about 800 species (Siedelmann, 2005). Two species, winter savory (*Satureja montana* L.) and summer savory (*Satureja hortensis* L.) are grown in the Czech Republic. The flowering stem of savory contains mainly thymol, cymene, carvacrol, terpinene, triterpenes and flavonoids. Most essential oils and aromatic substances are contained in young leaves or flowers at stage of flowering. In addition to the known use in the food industry, savory can also be used in the plant protection. Savory essential oil has been effective against pests or phytopathogenic fungi such as peach aphid (*Myzus persicae*), potato beetle (*Leptinotarsa decemlineata*) (Navarro-Rocha et al., 2019) or fungus *Botrytis cinerea* (Boyraz and Özcan, 2006). Carvacrol obtained from winter savory also prevented the germination of many plant species e.g. *Chenopodium album, Portulaca oleracea, Echinochloa crus-galli, Raphanus sativus L.*, and *Capsicum annuum* (Angelini et al. 2003).

In this study, allelopathic effects of winter savory (seeds, flowers and essential oils) were investigated against 8 species (*Sinapis alba* L., *Lactuca sativa* L., *Trifolium repens* L., *Lolium multiflorum* Lam., *Satureja hortensis* L., *Thymus vulgaris* L., *Melissa officinalis* L. and *Salvia officinalis* L.). The effect of different concentrations (10, 50 and 100 µl µl) of the essential oil (company Salus) or fresh flowers (0, 500 and 1000 mg) on germination, the root and shoot length and the dry weight selected species was determined in vitro conditions (25±1°C) after 6 days cultivation. The essential oil or flowers was applied to the source well in a tissue culture experiment plate (BIOFIL)., the seeds were put on the filter paper with 0.5 ml of distilled water in a well at distance 4 cm and 8 cm. Each well contained 6 seeds of the tested species. In the seed test, 30 seeds of winter savory were placed on filter paper with 3 ml of distilled water in a Petri dish alternately with 30 seeds of the test plant species. Only savory seeds were used as control. The experiments consisted of 4 replicates. The results were evaluated by analysis of variance in the program Statistica 12.

The insignificant effect of germinating seeds of winter savory on the growth and germination of most of the tested species suggests that winter savory uses as allelopathics mainly volatile substances that are formed in the plant during the growth. The presence of winter savory flowers had a positive effect on germination in *S. alba, L. multiflorum*, *M. officinalis,* and *S. officinalis*. The highest inhibition of germination and seed growth of most tested plants had essential oil. This proves the strong effect of savory essential oil and its possible use as a bioherbicide.

**Acknowledgments**

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**Keywords**: savory, allelopathy, seed, essential oil

# PP-27. Analysis of the chemical composition and stability of aromatic hydrosols

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## Abstract

Aromatic hydrosols (AH), i.e., water-based plant extracts usually obtained during steam distillation of essential oils, differ fundamentally in chemical composition from corresponding essential oils [1]. Rigorous control of the chemical stability of commercial AHs is mandatory to determine appropriate storage conditions and reasonable expiry dates. However, as for the chemical composition of many AHs neither standardised analysis methods nor normative guidelines have been published yet [2]. Thus, we aimed at developing an analytical method allowing for the monitoring of key indicators of chemical stability of AHs.

Commercial melissa (lemon balm; *Melissa officinalis* L.) and lavender (*Lavandula angustifolia* L.) AH samples were stored at 20 °C (RT) and 2–8 °C (CS), respectively, for 12 months. Chemical composition of each sample was analysed at 1, 3, 6, 9, and 12 months after purchase. Samples were double extracted by means of a self-developed hexane-based LLE-method and analysed using tailored GC-MS- and GC-FID-techniques. In addition, *p*H of the samples was measured at each analysis point.

*p*H of both AHs remained more or less unchanged during storage independent of temperature. In melissa AH, in line with [3] we observed a profound decline of monoterpene aldehydes, specifically citral, and formation of *p*-menthadienol isomers. Changes were more pronounced at RT than at CS. Lavender AH was more stable than melissa AH at both temperatures, although monoterpene alcohols, particularly linalool [4], degraded. However, a-terpineol content increased.

In conclusion, chemical stability of AHs seems to be specific and depends on both storage conditions and the reactivity of AH components.

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**Keywords**: Aromatic hydrosol; chemical composition; chemical stability; GC-MS/FID

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# PP-28. Anticandidal Activity and Chemical Composition of the Essential Oils from Aerial Part, Inflorescence, and Roots of Endemic *Prangos abieticola* Aytaç & H.Duman

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## Abstract

The genus Prangos Lindl. comprises approximately 35 species worldwide, which are widespread in the Irano-Turanian phytogeographic region, Turkey, Iran and Central Asia (Kurkcuoglu, 2016; Bagci et Dogan, 2015). There are 20 taxa, 12 of which are endemic, which are important medicinal and aromatic plants in Turkey (Aćimović et al., 2015). The present study aimes to analyse the chemical composition and anticandidal activity of essential oils (EOs) obtained by using hydrodistillation from different parts of the *Prangos abieticola* Aytaç & H.Duman, a recently described endemic in Turkey. EOs were characterized by GC and GC/MS analyses. Forty compounds constituting 59.3% of aerial parts of the oil, thirty-eight compounds constituting 88.0% of the inflorescence oil and twenty compounds constituting 97.8% of the root oil have been identified. The major constituents of aerial parts were **β**-elemene (8.8%), **α**-selinene (11.0%) and caryophyllene oxide (7.9%). The main compounds of inflorescence oil were found as **α**-pinene (10.9%), germacrene D (9.0%) and hexadecanoic acid (6.5%) while the main compounds of roots oil were **α**-pinene (11.3%), **β**-pinene (11.7%), **δ**-3-carene (26.7%), **α**-phellandrene (7.3%) and β-phellandrene (16.6%). The essential oils were screened for their anticandidal activity using broth microdilution method. All the essential oils showed weak activity against tested Candida strains. The anticandidal activity and essential oil compositions of P. abieticola are reported for the first time here.

**Acknowledgments**

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**Keywords**: Anticandidal activity; Apiaceae; Endemic; Essential oil; *Prangos abieticola*.

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# PP-29. *In vitro* ACE2 Enzyme Inhibition of Different *Salvia* Essential Oils

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## Abstract

In this present study, *Salvia triloba* L., *Salvia officinalis* L., and *Salvia sclarea* L. essential oils were evaluated for their *in vitro* angiotensin converting enzyme 2 (ACE2) inhibitory activity. The *S. triloba, S. officinalis*, and *S. sclarea* essential oils composition was confirmed both by GC-FID and GC/MS. Main components of the *S. triloba* essential oils were characterized as 1,8-cineole (22.8%), camphor (17.15%), α-thujone (15.18 %), β-caryophyllene (11.43 %), and α-humulene (3.0 %). α-thujone (28.46 %), camphor (20.58 %), 1,8-cineole (10.95%), α-humulene (5.0 %), and camphene (4.89 %) were found in the *S. officinalis* essential oil. Whereas linalyl acetate (56.8 %), linalool (21.06%), α-terpineol (6.05 %), geraniol (4.99%), and β-caryophyllene (3.35%) were the major components of the *S. sclarea* essential oil. Activity studies were performed at concentrations of 20 µg/mL of essential oils. The essential oils were evaluated using a fluorometric multiplate based enzyme inhibition kit, where the ACE2 inhibitions of *S. triloba, S. officinalis,* and *S. sclarea* essential oils were 50.07%, 60.45%, and 72.12%, respectively. As a result, three different *Salvia* essential oils may have antiviral potential applications against coronaviruses due to ACE2 enzyme inhibition with anti-inflammatory effects. Further *in vivo* studies are needed to confirm the efficacy of the results.

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**Keywords**: *Salvia,* essential oil, ACE2, antiviral

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# PP-30. The Influence of Temperature and Light on the Colour and Chemical Profile of Kunzea oil during Storage

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## Abstract

Essential oil extracted from the leaves and twigs of *Kunzea ambigua* is in high demand for its 'medicinal and soothing aroma' and claimed muscle relaxant properties. The study investigated the changes in colour and chemical composition of kunzea oil over eight months of storage in a freezer (-20°C), a refrigerator (4°C) and at room temperature (20°C) with and without light. The oil colour was measured by a colourimeter and chemical compositions were analysed using Gas Chromatography (GC) Mass Spectrometry (MS) and Flame Ionization Detection (FID).

The colour difference ($\Delta E^*$) was highest in oils stored in the light at room temperature for eight months (5.60 ± 0.03%) whilst sample kept in the freezer was most stable (1.08 ± 0.17%). Stored kunzea oil darkened, becoming less yellow and decreasing in greenness, with extended storage periods. Significant interactions between storage period and temperature/light were recorded for bicyclogermacrene, α-humulene, α-campholenal and linalool, showing a decrease in oils under all conditions during extended storage period, though changes were minimal in frozen oil. The oil stored under light showed a significant decrease in germacrene D, β-caryophyllene and α-humulene, owing to thermal isomerization. Especially, kunzea oil stored under the light for more than three months may have reduced antimicrobial and antifungal properties owing to significantly reduced amounts of bicyclogermacrene, so that the oil may only be used for its fragrance. Overall, the colour and chemical components of kunzea oil remained stable and kept its primary quality when stored in a freezer or refrigerator.

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**Keywords**: Bicyclogermacrene, chemical alteration, kunzea oil, storage

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# PP-31. Antioxidant/Antityrosinase Activities and Volatile Composition of *Peucedanum alkaliniae* Essential Oil

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## Abstract

In this study, volatile composition and antioxidant and antityrosinase activities of *Peucedanum alkaliniae* essential oil were evaluated. *Peucedanum alkaliniae* was collected from Antalya, Gazipasa, Turkey, on August 09, 2021. The air-dried herbal parts were subjected to hydrodistillation, using a Clevenger-type apparatus for 3h. Volatile constituents were identified using GC/MS and GC-FID, simultaneously. The essential oil yield was calculated as 0.4 %. The major compounds were identified as myrcene (15.4 %), *p*-cymene (13.1 %), α-phellandrene (10.5 %), α-pinene (9.5 %), β-pinene (6.7 %), limonene (6.4 %) and β- phellandrene (5.1 %). Essential oil showed DPPH radical scavenging activity 68.79 ± 2.36 % at 14 mg/mL concentration and inhibited the enzyme tyrosinase 9.22 ± 0.77 % at 1 mg/mL concentration.

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# PP-32. Volatile Constituents and *In Vitro* Antioxidant and Antityrosinase Activities of the *Prangos munzurensis* Fruits and Roots Essential Oils

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## Abstract

This study was aimed to determine volatile composition and their antioxidant and antityrosinase activities of *Prangos munzurensis* essential oils. *Prangos munzurensis* was collected on July 05, 2021 from Salordek village, Pülümür, Tunceli, Turkey. The air-dried roots and fruits of *P. munzurensis* **were subjected to** hydrodistillation using a Clevenger-type apparatus for 3h. Volatile constituents were identified using GC/MS and GC-FID, simultaneously. Furthermore, DPPH scavenging activity and potential tyrosinase inhibition of the essential oils were evaluated. The essential oil yield of the fruits was calculated as 1.52 %. Essential oil from the roots were obtained in trace amounts. The major compounds in the fruit essential oil were identified as 2,3,6-trimethylbenzene (29.7 %), *trans* chrysanthenyl acetate (11.0 %), unknown-1 (11.4 %), γ- terpinene (10.3 %), α-pinene (8.6 %) and *trans* verbenol (6.6 %), respectively. In the root essential oil, chrysanthenyl isobutyrate (25.8 %), hexadecanoic acid (17.1%), 2,3,6-trimethyl benzaldehyde (16.2 %), unknown-2 (12.0 %), (*Z*)-β-ocimene (6.6 %) and *trans* chrysanthenyl acetate (5.2 %) were identified as major constituents. Fruit essential oil showed DPPH radical scavenging activity with the $IC_{50}$ value 5.14 ± 0.14 mg/mL and inhibited tyrosinase 22.25 ± 0.26 % at 1 mg/mL concentration.

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# PP-33. Lentisk - *Pistacia lentiscus* L. Leaf Essential Oil Variations and Applications

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## Abstract

*Pistacia lentiscus*, the Lentisk of Anacardiaceae is a shrub of the natural habitat of Turkey with well-known and documented historical medicinal and aromatic use. The plant material which was selected as leaves, twigs and fruits of the present study, was collected during different vegetative seasons from various sites in Urla, İzmir, Turkey. The plant material was subjected to classical hydrodistillation as well as industrial scale distillation where the main components were identified as **α**-pinene (19,4-22,7%) and limonene (13,3-17%) during the 3rd vegetative period whereas the fruit essential oil was characterized as myrcene (21,1%), limonene (18,6%) and **α**-pinene (13,8%) as major components.

*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium*, Bacillus subtilis *Streptococcus mutans, S. sanguinis, S. sobrinus, S.mitis, Corynebacterium striatum, Porphyromonas gingivialis, Bacteroides fragilis, Prevotella nigrescens, Fusobacterium nucleatum*, *Candida albicans*, *C. parapsilosis, Aspergillus niger, Penicillium chrysogenum, Fusarium culmorum* human pathogens were used for antimicrobial evaluation of different oils using in vitro microdilution methods. In addition, antioxidant, anti-inflammatory 5-LOX inhibitory assays were performed for the *in vitro* evaluation and utilization potential of the oil and its preparations.

**Acknowledgment**

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**Keywords**: *Pistacia lentiscus*, essential oil, biological activities

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# PP-34. Olfactive Comparison of *Vitex agnus castus* L. Seed Essential Oils from Turkey

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## Abstract

*Vitex agnus-castus* L. (Verbenaceae), the caste tree is a shrub characteristic with its volatiles within the natural habitat in Turkey. In this present study, *Vitex* seeds were collected from their natural habitats at 3 different provinces of North Aegean and Mediterranean Regions of Turkey. The essential oils from were obtained from the air-dried seeds by hydrodistillation and analyzed for their chemical compositions. GC/MS analyses revealed α-pinene, sabinene, 1,8-cineol, and (*E*)-β-farnesene, and bicyclogermacrene as main constituents, respectively. In addition, HS-SPME-GC/MS analyses were performed to evaluate the volatiles. The olfactory evaluation showed characteristic clove, fresh and balsamic sweety, spicy, nature suggesting their uses especially in female's fragrances and cosmetics. .

**Acknowledgment**

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**Keywords**: *Vitex agnus-castus L.*, Seed essential Oil, Olfactory

# TALYA


RELAX
Bitkisel Karışım
İçeren Takviye Edici Gıda
60 Kapsül / Capsules
37.2 g
Melissa
Valerian Root
St John's Wort
Lavender
Passiflora
TALYA
Collagen
Hidrolize
Kollajen
İçeren Takviye Edici Gıda
60 Tablet
60 g
Coenzyme Q10
L-Ascorbic Acid
Hyaluronic Acid
Grape Seed
Pomegranate Fruit Peel
TALYA
Korean Red
Ginseng
Kırmızı Kore
Ginsengi
60 Tablet
60 g
TALYA
Kekik
Yağı
Oregano
Oil
TALYA
Sarı Kantaron
Yağı
St. John's Wort
Oil
TALYA
Lavanta
Yağı
Lavender
Oil
TALYA
Tatlı Badem
Yağı
Sweet Almond
Oil
TALYA
Çörekotu
Yağı
Black Cumin
Seed Oil
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