Special Issue of Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

55th International Symposium on Essential Oils

Book of Abstracts

INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS
~ I S E O ~

UNIVERSITY OF SARAJEVO
FACULTY OF SCIENCE

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Zmaja od Bosne 33-35, BA-Sarajevo
Bosnia and Herzegovina
Phone: +387-33-279-918
Fax: +387-33-649-359
E-mail: glasnik@pmf.unsa.ba
glasnikhtbh@gmail.com

EDITORIAL BOARD

Faculty of Science Sarajevo
Zmaja od Bosne 33-35, BA-Sarajevo
Bosnia and Herzegovina
Phone: +387-33-279-904 (Administration)
+387-33-279-911 (Executive Editors)
Fax: +387-33-649-359

E-mail: glasnik@pmf.unsa.ba
glasnikhtbh@gmail.com

The journal is published semiannual, and full text version of the papers published are available free of cost at http://www.pmf.unsa.ba/hemija/glasnik.

Bulletin of the Chemists and Technologists has been licensed for indexing in:

Emerging Sources Citation Index (Web of Science, Clarivate Analytics)

CAPlus (Chemical Abstracts Plus):

Academic Search Complete (EBSCO)

Book of Abstracts prepared by: Anela Topčagić & Jelena Ostojić

ISEO 2025
BOOK OF ABSTRACTS

55th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

7-10. September 2025

SARAJEVO, BOSNIA AND HERZEGOVINA

ISEO 2025

iseo2025@ffsa.unsa.ba

UNIVERSITY OF SARAJEVO
FACULTY OF PHARMACY

ORGANIZERS:

University of Sarajevo

University of Sarajevo, Faculty of Pharmacy

Association of Criminalists, Criminologists and Security Managers (KKMS)

EDITORS:

Hurija Džudžević - Čančar, University of Sarajevo, Faculty of Pharmacy

Amra Alispahić, University of Sarajevo, Faculty of Pharmacy

Alema Dedić, University of Sarajevo, Faculty of Pharmacy

Photo: Hurija Džudžević-Čančar and Osman Hrnjica

Graphical Design: Ranija Džudžević, Amra Alispahić and Hurija Džudžević-Čančar

ISEO History

The idea for an International Symposium on Essential Oils (ISEO) was initiated in 1968 at a GA meeting in Würzburg, Germany, during discussions by Professor Anders Baerheim Svendsen, Karl-Heinz Kubeczka, Friedrich Wilhelm Hefendehl and Dr. Jan Karlsen. It was proposed to hold a discussion group on all aspects of essential oils (terpene separation, chemistry, chemotaxonomy or other relevant topics regarding the constituents of essential oils).
In 1969, an informal meeting was organized by F. W. Hefendehl (University of Freiburg, FRG) and K.-H. Kubeczka (Technical University of Karlsruhe, FRG). Dr. J. Karlsen and Professor A. Baerheim Svendsen (both Leiden University, The Netherlands).
The first symposia, organized from 1971 to about 1980, were called Symposium Ätherische Öle, and were later changed to the International Symposium on Essential Oils (ISEO) by which it is known in the world of essential oil science and profession today.

Edition	Year	Venue
1	1969	Leiden, The Netherlands
2	1971	Freiburg, Germany
3	1972	Helsinki, Finland
4	1973	Freiburg, Germany
5	1974	Freiburg, Germany
6	1975	Leiden, The Netherlands
7	1976	Würzburg, Germany
8	1977	Freiburg, Germany
9	1978	Münster, Germany
10	1979	Würzburg, Germany
11	1980	Groningen, The Netherlands
12	1981	Marburg, Germany
13	1982	Würzburg, Germany
14	1983	Freising-Weihenstephan, Germany
15	1984	Leiden, The Netherlands
16	1985	Holzminden/Neuhaus, Germany
17	1986	Bad Bevensen, Germany
18	1987	Leiden, The Netherlands
19	1988	Zürich-Greifensee, Switzerland
20	1989	Würzburg, Germany
21	1990	Lahti, Finland
22	1991	St. Vincent, Italy
23	1992	Auchincruive, UK
24	1993	Berlin, Germany
25	1994	Grasse, France
26	1995	Hamburg, Germany
27	1996	Wien/Vienna, Austria
28	1997	Eskisehir, Turkey
29	1998	Frankfurt, Germany
30	1999	Leipzig/Miltitz, Germany
31	2000	Hamburg, Germany
32	2001	Wroclaw, Poland
33	2002	Lisbon, Portugal
34	2003	Würzburg, Germany
35	2004	Giardini Naxos, Italy
36	2005	Budapest, Hungary
37	2006	Grasse, France
38	2007	Graz, Austria
39	2008	Quedlinburg, Germany
40	2009	Savigliano, Italy
41	2010	Wroclaw, Poland
42	2011	Antalya, Turkey
43	2012	Lisbon, Portugal
44	2013	Budapest, Hungary
45	2014	Istanbul, Turkey
46	2015	Lublin, Poland
47	2016	Nice, France
48	2017	Pecs, Hungary
49	2018	Nis, Serbia
50	2019	Vienna, Austria
51	2021	Nicosia, N. Cyprus (On-line)
52	2022	Wroclaw, Poland
53	2023	MIlazzo, Italy
54	2024	Balatonalmádi, Hungary
55	2025	Sarajevo, Bosnia and Herzegovina

GENERAL INFORMATIONS

Symposium venue

Hotel Holiday - Sarajevo, Bosnia and Herzegovina (Address: Zmaja od Bosne 4, Sarajevo 71000, Bosnia and Herzegovina) https://www.hoteleuropegroup.ba/ba/holiday

ISEO 2025 - Organizing Committee

Chairperson

Hurija Džudžević-Čančar, University of Sarajevo, Bosnia and Herzegovina

Coordinators

Amra Alispahić, University of Sarajevo, Bosnia and Herzegovina
Alema Dedić, University of Sarajevo, Bosnia and Herzegovina
Mirela Mujović, KKMS Association. Bosnia and Herzegovina

Members

Elma Omeragić, University of Sarajevo, Bosnia and Herzegovina
Alija Uzunović, Agency for Medicinal Products and Medical Devices of Bosnia and Herzegovina
Emina Boškailo, Herzegovina University, Bosnia and Herzegovina
Sabina Begić, University of Sarajevo, Bosnia and Herzegovina
Lejla Klepo, University of Sarajevo, Bosnia and Herzegovina

Ph.D. Students, young researchers at University of Sarajevo-Faculty of Pharmacy

Emina Korić
Irma Gušić
Merima Šahinović
Emina Bečić
Irmela Ivazović
Hana Helać

Technical assistants (IT)

Osman Džudžević, Indiana University, USA
Osman Hrnjica, University of Sarajevo,

Students, University of Sarajevo, Bosnia and Herzegovina

Ismet Fatih Čančar
Medin Omerspahić,
Asim Halač,
Ajna Avdić
Adin Pašić

ISEO 2025 - Honorary Committee

Prof. Fahir Bečić, DSc, PhD, Dean of University of Sarajevo-Faculty of Pharmacy
Prof. Tarik Zaimović, DSc, PhD, Rector of University of Sarajevo

ISEO Permanent Scientific Committee (PSC)

The PSC is ISEO's main scientific and professional advising and consultative body. It consists of experienced scientists and professionals, as well as promising young researchers, from around the world in the fields of essential oils.
The aim of PSC activities is to promote the cooperation among scientists, experts, industry, and policymakers to enhance essential oil research and comprehension as well as contribute to the field's dynamic growth.
Under the new leadership elected for the triennium 2025-2027, the PSC is committed to maintaining ISEO's tradition of excellence.

PSC members:

Luigi Mondello ***(President)***,	University of Messina, Italy
Györgyi Horváth *(vice-President)*	University of Pécs, Hungary
Humberto Bizzo *(Executive Secretary)*	Embrapa Food Technology, Brazil
Yoshinori Asakawa	Tokushima Bunri University, Japan
Nicolas Baldovini	University Côte d'Azur, France
Hüsnü Can Baser	Near East University, North Cyprus
Carlo Bicchi	University of Turin, Italy
Jonathan Bonello	IFEAT, UK
Fatih Demirci	Anadolu University, Turkey
Jan Demyttenaere	European Flavour & Fragrance, Belgium
Paola Dugo	University of Messina, Italy
Hurija Džudžević-Čančar	University of Sarajevo, Bosnia and Herzegovina
Adam F. Feyaerts	KU Leuven, Belgium
Ana Cristina Figueiredo	University of Lisbon, Portugal
Toshio Hasegawa	Saitama University, Japan
Michael Keusgen	The Philipps University of Marburg, Germany
Eisuke Kuraya	National Institute of Technology, Okinawa College: Nago, Japan
Stanisław Lochyński	Wrocław University of Science and Technology, Poland
Agnieszka Ludwiczuk	Medical University of Lublin, Poland
Johannes Novak	University of Veterinary Medicine, Austria
Niko Radulović,	University of Niš, Serbia
Patrizia Rubiolo	Universtity of Turin, Italy
Iris Stappen	The University of Vienna, Austria
Daniel Strub	Wrocław University of Science and Technology, Poland
Sandy Van Vuuren,	University of the Witwatersrand, South Africa
Alvaro Viljoen	Tshwane University of Technology, South Africa
Éva Zámboriné-Németh	Corvinus University of Budapest, Hungary
Honorary Members	
Anders Baerheim-Svendsen †	University of Oslo, Norway
Karl-Heinz Kubeczka †	University of Hamburg, Germany
Jan Karlsen †	University of Oslo, Norway
Junior Members	
Marta Pavarino	University of Turin, Italy

ISEO MEDAL OF HONOUR

The tradition of awarding the Medal of Honor, sponsored by Merck, continues on ISEO 2025. The 2025 Medal of Honor award selection by the ISEO Permanent Scientific Committee was announced at https://www.iseoils.com. The medal will be presented in Sarajevo, Bosnia and Herzegovina, during the ISEO 2025 event.

The first promoters of the ISEO Medal were Giovanni Dugo and Luigi Mondello, Chairmen of the 35th ISEO organized in Giardini Naxos, Italy, in 2004. The idea of the ISEO medal came up again in 2017, when the Permanent ISEO Scientific Committee has decided to create an honorary medal to be attributed every year to a scientist that during his scientific carrier has contributed significantly and innovatively to promote the development of the scientific knowledge in the essential oil field. The scientific topics to be considered for the award must illustrate one or more aspects of the multitask discipline including botany and cultivation, chemistry, biological activity, and applications in food, cosmetic, pharmaceutical and other fields.

The ISEO Medal was officially instituted in 2019, on the occasion of the 50th anniversary of ISEO, held in Vienna, Austria. With the help of the members of the ISEO Permanent Scientific Committee and an artist from Messina, as well as the Merck company, official sponsor of the medal, the ISEO Medal of Honour was created.

Previous ISEO Medal of Honour Recipients

2019: Prof. Ian Karlsen, University of Oslo, Norway (50th ISEO, Wien, Austria)
2021: Prof. Karl Heinz Kubeczka, University of Wurzburg, Germany (51st ISEO, Turkey, on-line)
2022: Prof. Carlo Bicchi, University of Turin, Italy (52nd ISEO, Wroclaw, Poland)
2023: Prof. Dr. Kemal Hüsnü Can Başer, University of Nicosia, N. Cyprus and
Prof. Giovanni Dugo, University of Messina, Italy (53rd ISEO, Millazzo, Messina, Italy)
2024: Prof. Dr. Yoshinori Asakawa, Institute of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan
Prof. Dr. Chlodwig Franz, University of Veterinary Medicine Vienna, Austria (54th ISEO, Balatonalmadi-Hungary)

2025: Laureate of ISEO Medal of Honor for 55th ISEO, in Sarajevo, Bosnia and Herzegovina:

Prof. Dr. Gerhard Buchbauer, Emeritiert, University of Vienna, Faculty of Life Sciences, Department of Pharmaceutical Sciences, Vienna, Austria

Prof. Dr. Ana Cristina Figueiredo, University of Lisbon, Faculty of Sciences of Lisbon, Lisbon, Portugal

Professor Gerhard Buchbauer, Emeritiert
Institut für Pharmazeutische Wissenschaften,
Fakultät für Lebenswissenschaften der Universität Wien, Wien, ÖSTERREICH

Gerhard Buchbauer (born 1943) studied pharmacy at the University of Vienna and has dedicated his entire scientific career to odors, essential oils, and their constituents. He created the issue of odor- and aroma compounds in its entirety (synthesis, analysis, computer-aided fragrance design, biological impact in animal models and humans) as a distinct discipline in Vienna's Pharmacy and was affiliated to worldwide research groups ranging from Australia, Canada, and Europe to Japan. He wrote over 470 articles (book chapters and peer-reviewed manuscripts, 270 of which were on essential oils) and is co-editor of "Handbook of Essential Oils" (Taylor & Francis, 2010, 2016, 2020) and "Aromatherapie in Wissenschaft und Praxis" (Stadelmann Verlag, 2013, 2023). Gerhard Buchbauer has held positions in several scientific organizations, including IFEAT, ÖGwA, AG Lebensmittelchemie, GÖCH, ISEO, ICEOFF, ICEIRM, Forum Cosmeticum, and others, and was also a member of the editorial boards of the Journal of Essential Oil Research, the Flavour and Fragrance Journal, the Journal of Essential Oil-Bearing Plants, and the International Journal of Essential Oil Therapeutics. In 1992, he received the IFEAT Medal Lecture Award. He initially visited ISEO in 1985 and since then returned every year from 1987 till 2016, serving on the ISEO PSC from 1996-2016. In 1996, he co-organized the 27th ISEO in Vienna and served as a senior adviser for the 50th ISEO in 2019.

Professor Ana Cristina Figueiredo
Faculdade de Ciências da Universidade de Lisboa, Departamento de Biologia Vegetal, CE3C, Lisboa, Portugal

Ana Cristina Figueiredo is full Professor at the Departamento de Biologia Vegetal da Faculdade de Ciências da Universidade de Lisboa (FCUL), and head of the plant biotechnology group at the Center for Ecology, Evolution and Environmental Changes (CE3C) and Global Change and Sustainability Institute (CHANGE).
As a professor at FCUL, she is involved in several Bachelor's, Master's and Doctorate courses, and is, or was, responsible for supervising Internships, Master's Degrees and Scientific Research Fellows. She was, or is, responsible for supervising national and foreign PhD Students and is the co-author of pedagogical publications. She has been engaged in knowledge transfer, by being, since 2014, head of the Technical Committee for Standardization 5 (CT5), Essential Oils, in Portugal, coordinated by the Instituto Português de Qualidade (IPQ). The scientific activity has focused on the areas of plant biotechnology, and phytochemistry, being involved, in intra- and inter-institutional national and international collaborations, in diverse topics of plant biotechnology research. She has been involved in securing funding through over 30 projects of different types. These studies let to over 200 publications on peer-reviewed journals, 46 on conference proceedings, 16 book chapters, in addition to participations in national and international conferences.

SPONSOR of the ISEO Medal of Honour

MERCK

IFEAT YOUNG SCIENTISTS FELLOWSHIP

The International Federation of Essential Oils and Aroma Trades (IFEAT) Executive Committee provided financial support for 38 young scientists who were chosen to participate in the 55th International Symposium on Essential Oils ISEO 2025 in Sarajevo, for which the ISEO 2025 Organizing Committee is extremely grateful. As a result, following a rigorous examination and selection process, the honorees are:

Akyüz Beyzanur	University of Ataturk - Faculty of Pharmacy, Erzurum, Türkiye
Amato Giuseppe	University of Salerno, Fisciano (SA), Italy
Baberowska Kinga	Wrocław University of Science and Technology, Wrocław, Poland
Bacińska Zuzanna	Wrocław University of Science and Technology, Wrocław, Poland
Badalamenti Natale	University of Palermo, Palermo, Italy
Bečić Emina	University of Sarajevo, Sarajevo, Bosnia and Herzegovina
Bella Bella Yean Marie	University of Douala, Rue Ange Raphael, Douala, Cameroon
Bogevski Krume	Goce Delcev University, Štip, North Macedonia
Boškailo Emina	Herzegovina University, Mostar, Bosnia and Herzegovina
Čamdžić Ajka	University of Sarajevo, Sarajevo, Bosnia and Herzegovina
Cannizzaro Francesca	University of Messina, Messina, Italy
Civaş Ayşe	University of Ataturk - Faculty of Pharmacy, Erzurum, Türkiye, Iğdır University-Tuzluca Vocational School, Iğdır, Türkiye
Danila Elena	National Research and Development Institute for Textiles and Leather, Bucharest, Romania
Dedić Alema	University of Sarajevo, Sarajevo, Bosnia and Herzegovina
Gupta Abhishek	Banaras Hindu University, Lucknow, India
Gusinac Amina	University of Niš, Niš, Serbia
Helać Hanna	University of Sarajevo, Sarajevo, Bosnia and Herzegovina
Huang Yeqin	Bologna University of Bologna, Bologna, Italy
Imamović Berina	University of Sarajevo, Sarajevo, Bosnia and Herzegovina
Irrera Elisa	University of Messina, Messina, Italy
Ivazović Irmela	University of Sarajevo, Sarajevo, Bosnia and Herzegovina
Janeiro Alexandre	University of the Azores, Angra do Heroísmo, Portugal
Kahya Sümeyye Elif	Istanbul Medipol University, Istanbul, Türkiye
Karakaş Şeydanur	University of Ataturk - Faculty of Pharmacy, Erzurum, Türkiye
Khvalbota Liudmyla	University of Technology in Bratislava, Bratislava, Slovakia
Cucinotta Lorenzo	University of Messina, Messina, Italy
Maphanga Veronica	Tshwane University of Technology, Pretoria, South Africa
Meseldžić Neven	University of Sarajevo, Sarajevo, Bosnia and Herzegovina
Mishra Tripti	CSIR-National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram, India
Mohanty Debajani	Centre for Biotechnology, Siksha O Anusandhan UNI.,Kalinga Nagar, India
Mušinbegović Sarah	University of Sarajevo, Sarajevo, Bosnia and Herzegovina
Ormai Edit	University of Pécs, Pécs, Hungary
Özer Elif Beyza	University of Ataturk - Faculty of Pharmacy, Erzurum, Türkiye / Ondokuz Mayis University, Samsun, Türkiye
Salihu Abubakar Siddiq	University Pendidikan Sultan Idris (UPSI), Malaysia/Nigeria
Sönmez Hatice Rümeysa	University of Ataturk - Faculty of Pharmacy, Erzurum, Türkiye
Sotade Ayopo	University of Ibadan, Nigeria
Surowiak Alicja	Wroclaw University of Science and Technology, Wrocław, Poland
Touyama Akiko	Okinawa Flora, Okinawa, Japan

PLENARY LECTURES (PL)
KEY NOTE LECTURES (KNL)

Carvacrol and Its Biological Activities: An Updated Review

Başer Kemal Hüsnü Can [a]*, Haskoloğlu I.C.[b] and Erdağ E.[c]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Near East University, Nicosia, N. Cyprus*
[b]*Department of Pharmacology, Faculty of Pharmacy, Near East University, Nicosia, N. Cyprus*
[c]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Near East University, Nicosia, N. Cyprus*

Corresponding author:khcbaser@gmail.com; kemalhusnucan.baser@neu.edu.tr

Keywords*: biological activity, carvacrol oregano, monoterpenic phenol.*

Objective: Carvacrol, a monoterpenic phenol found abundantly in essential oils of Origanum, Thymus, Satureja, Thymbra, and Lippia genera, is recognized for its extensive range of biological and pharmacological activities. This bioactive compound, primarily responsible for the health-promoting properties of oregano essential oil, exhibits diverse functionalities, including antimicrobial, antitumor, antimutagenic, analgesic, anti-inflammatory, antioxidant, and neuroprotective effects. Its therapeutic applications extend to managing gastrointestinal ailments, reducing oxidative stress, and serving as an insecticidal agent. Furthermore, carvacrol has demonstrated potential as a feed additive and in honeybee breeding. Advances in encapsulation and nanotechnology have enhanced its stability and bioavailability, broadening its utility across food, pharmaceutical, and agricultural industries. **Conclusion:** This review synthesizes the evidence for carvacrol's biological activities and explores its possible in vivo mechanisms of action, emphasizing its promise as a natural therapeutic agent.

Insight into the chemical diversity of volatile organic compounds from chosen macroalgae compared to plants

Jerković, Igor [a,b]*

[a]*Faculty of Chemistry and Technology, University of Split, R. Boškovića 35, 21000 Split, Croatia*
[b]*Mediterranean Institute for Life Sciences, University of Split, Šetalište I. Meštrovića 45, 21000, Split, Croatia*

***Corresponding author**: igor@ktf-split.hr; igor.jerkovic@unist.hr*

***Keywords**: brown, green and red macroalgae, gas chromatography and mass spectrometry, headspace, hydrocarbons; lower aliphatic compounds, terpenes, volatile oil.*

Objective: More than 30,000 volatile organic compounds (VOCs) are released from terrestrial plants. Prior to 66, only dimethyl sulfide (DMS) was identified as a VOC from the seaweeds. Afterwards, the number of identified marine algae VOCs has been constantly growing, particularly in the last decade [1]. Marine VOCs are small, low-molecular compounds with low to moderate hydrophilicity and high vapor pressure and can cross cell membranes to be released freely into the environment. However, a limited number of chemical profiling studies have been carried out on macroalgae from the Adriatic Sea, and it is known that environmental factors (light, temperature, nutrition conditions, and abiotic stress) are affecting algae VOC emissions. Therefore, the research goals were to unlock this gap for targeted brown (e.g., *Cystoseira corniculata* (Turner) Zanardini 1841, *Ericaria amentacea* (C.Agardh) Molinari and Guiry 2020, *Taonia atomaria* (Woodward) J.Agardh, 1848, and *Padina pavonica* (Linnaeus) Thivy, 1960), green (e.g., *Codium bursa* (Olivi) C. Agardh), and red (e.g., *Amphiroa rigida* J.V. Lamouroux, *Asparagopsis taxiformis* (Delile) Trevisan 1845) macroalgae, and to: 1) determine their headspace VOC composition; 2) analyze their volatile oils; 3) compare the chemical biodiversity of found VOCs with other marine algae with the same, similar, or related compounds; 4) determine the air-drying impact on the VOC composition; and 5) discuss the possible biosynthetic origin of identified VOCs from the literature data. **Methods:** Low volatile compounds were extracted by headspace solid-phase microextraction (HS-SPME) with different fibers: DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) or PDMS/DVB (polydimethylsiloxane/divinylbenzene). The volatile oils were obtained by hydrodistillation (HD) in a Clevenger-type apparatus with the solvent trap. All isolates were analyzed by gas chromatography and mass spectrometry (GC-MS) on an HP-MS capillary column**. Results:** Lower aliphatic compounds [2] were dominant in the headspace (HS) of *C. corniculata,* followed by monoterpenes (β-cyclocitral, β-citral, and β-cyclohomocitral) and sesquiterpenes (cubenol), while fatty acids and their derivatives were the most abundant in HD, followed by sesquiterpenes ((*E*)-geranylgeraniol and cubenol). Lower aliphatic compounds prevailed in *E. amentacea* HS, while pentadecane (C15), heptadecane (C17), pentadecanal, and hexan-1-ol were predominant in the HD. *T. atomaria* HS and oil composition [3] were quite similar (containing mainly germacrene D, epi-bicyclosesquiphellandrene, β-cubebene, and gleenol). *P. pavonica* HS and oil composition differed significantly (dimethyl sulfide, octan-1-ol, and octanal dominated in the HS, while the oil contained mainly higher aliphatic alcohols, *trans*-phytol, and pachydictol A). Dimethyl sulfide was the major HS compound of *C. bursa,* and the HD contained heptadecane and docosane among the major compounds [4]. *A. rigida* HS was dominated by C17 and C15, and the predominant compound in the HD was (*E*)-phytol [5]. Tribromomethane and dibromiodomethane were the major VOCs in *A. taxiformis*. **Conclusions:** When VOC components of macroalgae and terrestrial plants are compared, the algae were found to be different, containing a various VOCs, including lower aliphatic compounds, hydrocarbons, monoterpenes, sesquiterpenes, diterpenes, benzene derivatives, halogenated compounds, and others. Drying significantly influenced the VOCs composition (e.g., within benzene derivatives, terpenes) that could be important for their accurate chemotaxonomy.

REFERENCES

[1] Cikoš, A.-M., Jurin, M., Čož-Rakovac, R., Gašo-Sokač, D., Jokić, S., Jerković, I. (2021) Update on sesquiterpenes from red macroalgae of the Laurencia genus and their biological activities (2015–2020), Algal Research, 2021, 102330.
[2] Radman, S., Jerković, I. (2020) Volatile Organic Compound Profiles of Cystoseira corniculata (Turner) Zanardini 1841 and Ericaria amentacea (C. Agardh) Molinari and Guiry 2020 (ex. Cystoseira amentacea (C. Agardh) Bory de Saint-Vincent, 1832), Molecules, 27, 7131.
[3] Jerković, I., Kranjac, M., Marijanović, Z., Roje, M., Jokić, S. (2019) Chemical Diversity of Headspace and Volatile Oil Composition of Two Brown Algae (Taonia atomaria and Padina pavonica) from the Adriatic Sea, Molecules, 24, 495.
[4] Jerković, I., Kranjac, M., Marijanović, Z., Šarkanj, B., Cikoš, A.-M., Aladić, K., Pedisić, S., Jokić, S. (2019) Chemical Diversity of Codium bursa (Olivi) C. Agardh Headspace Compounds, Volatiles, Fatty Acids and Insight into Its Antifungal Activity, Molecules, 24, 842.
[5] Cikoš, A.-M., Flanjak, I., Bojanić, K., Babić, S., Čižmek, L., Čož-Rakovac, R., Jokić, S., Jerković, I. (2021) Bioprospecting of Coralline Red Alga Amphiroa rigida J.V. Lamouroux: Volatiles, Fatty Acids and Pigments, Molecules, 26, 520.

The significance of the essential oils in the treatment of postinfection syndromes: Long Covid, Chronic fatigue syndrome and Late Lyme syndrome: neurocardiological approach and experience

Milovanović, Branislav [a,b]*, Marković, N.[a], Petrović, S.[a], Petrović, N.[a], Petrović, M.[a], Bojić, M.[a,b]

[a]*Institute for Cardiovascular Diseases Dedinje, University of Belgrade, Medical Faculty*
[b]*Medical Faculty, University of Belgrade, Belgrade, Serbia*

Corresponding author: profdrbranislavmilovanovic@gmail.com

***Keywords**: Antiinflamatory, essential oil, neurocardiological, postinfection syndromes, syncope, Thymus serpyllum.*

Objective: Dysautonomia encompasses a group of disorders characterized by autonomic nervous system (ANS) dysfunction and is a common underlying mechanism in patients with syncope, especially those with orthostatic hypotension (OH), as well as postinfection syndromes: Post Covid Syndrome, Chronic Fatigue Syndrome, and Lyme disease. *Thymus serpyllum* (wild thyme), a plant with known antioxidant and anti-inflammatory properties, has shown potential in modulating cardiovascular and autonomic function in animal models. To investigate the effects of *Thymus serpyllum* essential oil in tablet form on autonomic nervous system parameters and selected biochemical markers in patients with post-infection syndromes (Post Covid Syndrome, Chronic Fatigue Syndorme, Lyme disease) and syncope. **Methods:** A retrospective-prospective study was conducted on 15 adult patients with a history of syncope and a diagnosis of Post Covid/ Chronic Fatigue/ Chronic Lyme, excluding those with cardiogenic syncope, epilepsy, volume depletion, or primary autonomic neuropathies. Patients were treated with Thymus serpyllum essential oil capsules for three months. Beat-to-beat blood pressure and heart rate variability (HRV), 24-hour Holter ECG, ambulatory blood pressure, and laboratory parameters were measured before and after therapy. **Results:** After three months, beat-to-beat analysis revealed increased low-frequency HRV components (LFnu) and a higher LF/HF ratio, indicating enhanced sympathetic tone with parasympathetic withdrawal. No significant changes in heart rate or blood pressure were noted. Biochemically, AST and C-reactive protein levels were significantly reduced, suggesting hepatoprotective and anti-inflammatory effects. Other biochemical and cardiovascular parameters showed no statistically significant changes. **Conclusion:** *Thymus serpyllum* essential oil may modulate autonomic balance by increasing sympathetic activity in patients with syncope and postinfection syndromes, particularly those with OH. Its hepatoprotective and anti-inflammatory effects may provide additional therapeutic benefits.

Essential Oils and Their Role in Designing Active Packaging, Controlled Release of Bioactives, and Food Safety: An Overview

Jasim, Ahmed [a,*]

[a]*Environment & Life Sciences Research Center, Kuwait Institute for Scientific Research, Kuwait City, Kuwait*

***Corresponding author:** jaahmed@kisr.edu.kw; jahmed2k@yahoo.com*

***Keywords:** Antimicrobial activity, antimicrobial packaging, cyclodextrin, food safety, encapsulation, essential oil.*

Objective: Essential oils (EO) are plant-derived secondary metabolites that have been used in traditional medicine since ancient times for healing wounds, as flavoring agents in culinary applications, and in cosmetics. Due to their excellent antimicrobial and antioxidant properties, EOs such as cinnamon, clove, thyme, garlic, black pepper, and tea tree have gained interest in food packaging. However, the solubility and high volatility of the EOs present real challenges when used in this context. Additionally, the low compatibility of EOs with polymers restricts their application in this field. To enhance their usability, EOs can be entrapped in suitable wall materials, a process commonly referred to as "encapsulation." Both β- and γ-cyclodextrin are considered the most suitable wall materials for EO encapsulation, achieving over 90% encapsulation efficiency while retaining high levels of antimicrobial activity. We have fabricated both single-layer and multilayer antimicrobial packaging of biodegradable polylactides using extrusion technology drawn from our decade-long research. The central layer contained antimicrobial agents in the three-layer films with controlled release.

Conclusion: Antimicrobial films with EOs are suitable for perishable food (e.g., poultry, cheese, and meat) packaging, maintaining food safety at optimal levels during refrigerated storage for a month. In the lecture, various aspects of antimicrobial packaging, including their fabrication, properties, controlled release, food safety, and future directions, will be discussed.

Current status and the need to improve the Standardization of Essential Oils intended for the Production of Food Supplements

Jašić, Midhat [a,]*, Spaseska-Aleksovska, E.[a], Džudžević-Čančar, H.[b], Dautović, E.[a]

[a]Faculty of Pharmacy, University of Tuzla, Tuzla, Univerzitetska 8, Bosnia and Herzegovina
[b]University of Sarajevo-Faculty of Pharmacy, University of Sarajevo, Zmaja od Bosne 8, Sarajevo 71000, Bosnia and Herzegovina

***Corresponding author:** jasic_midhat@yahoo.com*

***Keywords:** Essential oils, food supplements, standardization.*

Objective: The purpose of essential oil standardization is to ensure the quality, safety, and efficacy of their use in the production of food supplements, as well as in other pharmaceutical and cosmetic products. The current situation is characterized by fragmented standards, inconsistent quality control practices, and insufficient legislative regulation, which hinders their broader and safer application. The aim of this paper is to define the current state and the need for improving the standardization of essential oils intended for the production of food supplements. **Methods:** To define the current state, data on the standardization of essential oils intended for the production of food supplements were collected, systematized, and analyzed. **Results:** Standardized essential oils or isolated active components, most commonly from peppermint, oregano, lemon, lavender, cinnamon, clove, basil, and others, are often used in food supplement formulations. Due to the potential toxicity at higher doses, they are applied with great caution. Nevertheless, certain essential oils are approved and widely used owing to their positive effects on general health, immunity, digestion, the respiratory system, and in reducing inflammation and stress. They are available in the form of capsules, emulsions, or syrups. Numerous in-house, national, and international standards are currently used to define the quality of essential oils. Standardization is most commonly based on pharmacopoeial monographs (e.g., Ph. Eur., USP), ISO standards (ISO/TC 54), Codex Alimentarius, GRAS status (FDA, USA), and European regulations (EFSA). Regulation (EC) No 1334/2008 defines the use of flavorings, including essential oils, in food and food supplements, while Regulation (EC) No 1925/2006 regulates the addition of biologically active substances to food. Manufacturers of food supplements, in accordance with GMP and HACCP principles, must meet clearly defined requirements for essential oils. Although certain standards and statuses such as ISO and GRAS exist, they still do not encompass the full complexity and variability of essential oils as functional ingredients. Improvement requires more efficient and coordinated cooperation among scientific, regulatory, and industrial stakeholders to achieve greater safety, quality, and transparency in the market. **Conclusion:** The quality and use of essential oils in the production of food supplements are regulated by a set of standards and guidelines aimed at ensuring product safety, efficacy, and purity. However, a significant portion of the market remains outside the scope of standardization, which favors the presence of unregulated and adulterated products and highlights the need for better harmonization among various regulatory approaches.

Cracking the Code of Plant Extracts: The Business Side of Regulatory Classification

Bonello, M. Jonathan [a,*]

[a]*IFEAT Scientific Platform Coordinator, IFEAT - International Federation of Essential Oils & Aroma Trades*

***Corresponding author:** sciplatform_coordinator@ifeat.org*

***Keywords**: EFEO-IFEAT Scientific Program, EU regulation for EO, expertise, EU market, natural substances.*

Objective: By December 2029, the European Commission must deliver a pivotal scientific report, potentially with legislative consequences, on substances containing more than one constituent (MOCS) derived from plants. This development could significantly reshape the regulatory environment for essential oils and other natural extracts within the EU, with far-reaching business implications.
The EFEO-IFEAT Scientific Program stands at the forefront of this challenge, advocating that essential oils, owing to their natural complexity and unique intrinsic properties, cannot be equated with synthetic mixtures of isolated components. Through an alliance of industry leaders, academic institutions, and scientific experts in toxicology, environmental fate, analytical chemistry, and regulatory policy, the program is generating high-quality, EU-aligned data to support sound, science-based classifications and defend the regulatory status of natural extracts. We are calling for targeted expertise; your participation can directly influence the scientific and regulatory outcomes that will define the future of natural substances in the EU market. **Conclusion:** Join us to discover how science meets regulation to protect the business of natural extracts and how your support can help lead the way.

Methods and strategies to evaluate the safety of essential oils as natural complex substances

Bascoul, Cecile [a,b]*

[a]*dōTERRA International, 389 S. 1300 W., Pleasant Grove, Utah, USA*
[b]*Esseterre, Dobrich, Bulgaria*

***Corresponding author:** cbascoul@doterra.com*

***Keywords:** Essential oil, natural complex substance safety, risk assessment, toxicology.*

Objective: The growing global use of essential oils (EOs) across wellness, cosmetic, and food applications has heightened the importance of understanding their safety. While many EOs are rooted in long-standing traditional use, this historical familiarity does not guarantee safety, particularly in new contexts of application or emerging usage patterns. As such, a comprehensive safety assessment is a critical step in the responsible development of EO-based products. EOs are inherently complex natural substances, characterized by a rich diversity of constituents and variability in chemical composition. This variability arises not only from botanical factors such as species, chemotype, and plant part but also from agricultural conditions and manufacturing processes. These complexities pose unique challenges in safety evaluation, particularly when compared to single-compound substances. A multifaceted strategy is preferred to accurately assess EO safety. Traditional toxicological testing remains a cornerstone, encompassing endpoints such as acute toxicity, dermal irritation and sensitization, phototoxicity, genotoxicity, and repeated-dose toxicity. These studies offer a foundational insight into potential health hazards and are essential for setting safe usage parameters. Evaluating complex mixtures also demands complementary approaches. By integrating chemical characterization with toxicological data on individual essential oil constituents, it becomes possible to identify potentially hazardous components and gain a deeper understanding necessary for informed risk management decisions. The field is also advancing toward more ethical and human-relevant testing methodologies. *In vitro* alternatives, such as reconstructed human epidermis models and human cell-derived assays, are increasingly used to reduce reliance on animal testing while improving the relevance of results to human exposure scenarios. Additionally, real-world safety data from clinical trials, case reports, and retrospective analyses provide valuable insights. These data help contextualize controlled study results, though limitations in study design, dosing consistency, and reporting quality must be carefully weighed in the final assessment. **Conclusion:** Ultimately, the toxicological assessment of essential oils benefits from a holistic and integrative approach. By combining classical toxicology, modern analytical techniques, and ethical testing innovations, researchers and industry stakeholders can ensure essential oils are used safely and effectively. Continued vigilance is necessary, particularly in vulnerable populations and across diverse routes of exposure, to support the safe evolution of EO applications in an ever-expanding marketplace.

ACKNOWLEDGMENTS
The work is supported by dōTERRA International. The author gratefully acknowledges the contributions of the Product Safety team including Joe Dawson, Dakota Carter, Diana Saavedra and Sarah Shah; and collaborators at dōTERRA, Dr. Russ Osguthorpe, Prabodh Satyal, Ambika Poudel, Nicole Stevens; at Esseterre, Todor Nikolaev; at the National Center for Natural Products Research, Shabana Khan and Ikhlas Khan; at the American Botanical Council, Stefan Gafner; at the Botanical Safety Consortium, Connie Mitchell and Julie Krzykwa; and at SenzaGen, Tim Lindberg.

Halal Essential Oils: A Rising Trend in the Global Market

Kamaruddin, Nur Latifah [a,*]

[a]*Borneo Essentials Global, SD2, Jalan MHH 2/5, Melaka Halal Hub, Jasin 77300, Melaka, Malaysia*

***Corresponding author:** nlatifahk@gmail.com*

***Keywords**: Clean-label, ethical aromatherapy, halal essential oil certification, extraction, green methods, regulatory bodies, traceability.*

Objective: The demand for Halal-certified products is accelerating worldwide, particularly in the cosmetics, personal care, and wellness sectors. Essential oils, a core ingredient in these industries, are increasingly scrutinized not only for their purity and therapeutic potential but also for their compliance with Halal principles. This trend is driven by increasing global consumer demand and a wider movement towards ethical, sustainable, and traceable sourcing practices. Consequently, researchers face the challenge of discovering new solutions that span the cultivation of plant materials to the production processes of essential oils and related products. The application of green methods in these processes, as well as the application of nanoparticles, are just some of the changes introduced to meet the extremely demanding standards for halal product certification. The study also explores the significance of halal certification in essential oil production and its impact on market access, regulatory compliance, and consumer trust. The certification process ensures that essential oils are free from prohibited substances and contamination during all stages of production, including distillation, handling, and packaging. These measures are particularly crucial in sectors such as food manufacturing, pharmaceuticals, and cosmetics, where product safety and integrity are essential. In addition to traditional uses in aromatherapy and skincare, Halal-certified essential oils are now being integrated into broader applications, including Halal-compliant food formulations and functional health products. Emerging research also highlights their potential as supportive therapies in disease management. Innovations in traceability systems, quality control, and green extraction technologies further enhance their credibility in the global market. **Conclusion:** By analyzing research developments, industrial practices, and current market trajectories, this paper emphasizes the role of halal essential oils as a growing category that merges faith-based compliance with scientific and commercial value. The topic holds significant relevance for stakeholders in academia, industry, and regulatory bodies looking to align with evolving consumer expectations and international standards.

Palmarosa oil – from field to ISO standard

Protzen, D. Maren [a,*]

[a]*Paul Kaders GmbH, Hamburg, Germany*

Corresponding author: maren.protzen@kaders.de; m.protzen@voegele-ingredients.de

Keywords*: Cymbopogon martini (Roxb.) Will.Watson, ISO standard, Palmarosa oil, standard development.*

Objective: ISO (International Organization for Standardization) is an independent, non-governmental international organization with a membership of 173 national standards bodies. As one of the oldest non-governmental international organizations, ISO has enabled trade and cooperation between people and companies all over the world since 1946. The more than 250 thousand International Standards published by ISO serve to make lives easier, safer, and better. Experts in 828 technical committees are developing standards dealing with anything from making a product to managing a process. [1] ISO/TC 54 "Essential oils" was already established in 1947 and has published 138 standards so far. The standardization of essential oils has significantly bolstered the global trade of key essential oils used in the food, perfumery, and cosmetics industries, contributing not only to enhancing the safety profiles of these essential oils but also to establishing stringent quality benchmarks. [2]**Conclusion:** The revision of the ISO Standard 4727 Essential Oil of Palmarosa (*Cymbopogon martini* (Roxb.) Will. Watson var. *motia)* will serve as an example for the work of ISO TC 54 “Essential oils” and the contribution of participating and observing member organizations (i.e., DIN in Germany). The structure of ISO will be elucidated and workflows presented. The results of analytical studies of commercial Palmarosa Oils, considering the production process and biosynthetic pathways in Cymbopogon martini plants [3], will illustrate the development of an international standard for essential oils in the corresponding committee [4].

ACKNOWLEDGMENTS

Essential oil department of Joh. Vögele KG for samples and analytical data; Paul Kaders GmbH for analytical data and exemption for ISO/DIN expert work; A. Dierberger for production pictures in Paraguay; H. Naina for production pictures in India.

REFERENCES

[1] https://www.iso.org/structure.html (11.08.2025)
[2] IFEATWORLD 2024 - ISSUE 63
[3] Dubey V.S., Luthra R. (2001) Biotransformation of geranyl acetate to geraniol during palmarosa (Cymbopogon martinii, Roxb. wats. var. motia) inflorescence development, Phytochemistry 57 pp. 675–680
[4] Protzen, M.D. (2020) Ätherisches Palmarosaöl – Vom Ursprung zur Anwendung, Forum 43 pp. 43 – 46

Essential oil from Eucalyptus globulus leaves: The (un) expected potential

Santos, A. O. Sónia [a,*]

[a]*CICECO-Aveiro Institute of Materials, Department of Chemistry, Campus de Santiago 3810-193, Aveiro, Portugal*

***Corresponding author:** santos.sonia@ua.pt*

***Keywords:** Cineole, essential oil, Eucalyptus globulus, high-value applications, triterpenic compounds.*

Objective: *Eucalyptus globulus* leaves have been intensively exploited as a source of essential oil (EO), normally obtained by hydrodistillation, which is mainly composed of the monoterpenic compounds 1,8-cineole (CO), also known as eucalyptol (17.2–86.5%), α-pinene (2.8–52.7%), and limonene (6.6–28.0%), as well as the sesquiterpenic compounds aromadendrene (3.7–7.1%) and globulol (2.4–9.8%). Due to its medicinal properties, E. globulus EO is used in pharmaceutical, sanitary, agricultural, cosmetic, and food applications. In addition, E. globulus leaves are also rich in pentacyclic triterpenic compounds, betulonic, betulinic, oleanolic, ursolic, 3-acetyloleanolic, and 3-acetylursolic acids, and β-amyrin. In this work we developed a process in which *E. globulus* EO (and with its major component, CO, for comparative purposes) is used to extract triterpenic compounds from the leaves after hydrodistillation [1, 2]. **Methods:** Fresh *Eucalyptus globulus* Labill. leaves from Portugal were subjected to hydrodistillation to collect the EO. Dried samples of hydrodistilled leaves (EgHDL) (~7 g) were extracted with E. globulus EO or with CO (solid-liquid ratio of 1:4 w/v, dw EgHDL) at room temperature, protected from light and with stirring for up to 24 h. The biomass and the liquid fraction (TTAs-enriched extracts dissolved in EO or CO, from now on denominated as EO or CO crude extracts, respectively) were separated by pressing. The extracts were prepared in triplicate. Extracts were characterized by GC-MS, and their cell viability and anti-inflammatory activity were evaluated. **Results:** EO obtained from *E. globulus* leaves by hydrodistillation was for the first time used as a bio-based solvent in the subsequent extraction of TTAs from hydrodistilled leaves. UA, OA, BoA and BA were successfully extracted with EO and, for comparison purposes, with its major component, CO. No significant differences in the yields obtained with EO and CO were observed, and so EO can be used advantageously (no energy consumption in the purification steps to obtain pure CO). The cytotoxicity assays on cells of the innate immune system (macrophages) showed that the non-toxic concentrations of the EO and CO crude extracts are less than or equal to 0.04 and 0.08 mg mL^{-1}, respectively. Additionally, the CO crude extract showed higher anti-inflammatory activity than the EO crude extract, and lastly, these extracts exhibited higher activity than their respective mixture of TTAs, which is indicative of the potential beneficial synergistic effects of EO and CO with TTAs. **Conclusions:** This study demonstrates how *Eucalyptus globulus* essential oil can be transformed from a traditional aromatic product into a value-added, multifunctional solvent for biorefinery applications. Acting as a bio-based medium for recovering TTAs, EO, and its main component, CO, proved highly efficient in extraction. Effective, sustainable, and non-toxic, EO not only extracts bioactive compounds but also gains new market potential in nutraceutical, cosmetic, and pharmaceutical applications.

ACKNOWLEDGMENTS

This work was carried out under the Project InPacTus – Innovative products and technologies from eucalyptus, Project No. 21874 (POCI-01-0247-FEDER-021874), funded by Portugal 2020 through the European Regional Development Fund (ERDF) in the frame of COMPETE 2020 no. 246/AXIS II/2017, and projects CICECO–Aveiro Institute of Materials (UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020), LAQV-REQUIMTE (UIDB/50006/2020 & UIDP/50006/2020) and CIBB (UIDB/04539/2020 & UIDP/04539/2020), financed by national funds through the FCT/MEC (PIDDAC). FCT is also acknowledged for the research contract under Scientific Employment Stimulus to S. Santos (2021.03348.CEECIND).

REFERENCES

[1] Oliveira C., Moreira P., Cruz M. T. et al., (2023). An integrated green process for the extraction of triterpenic acids from Eucalyptus globulus leaves after hydrodistillation, *RSC Sustainability*, 4, 1016-1024

[2] Integrated process for extracting bioactive compounds from eucalyptus biomass, (2022). European Patent EP4186575B1

Aromatic plants and perfumery in India- The legacy revisited

Rameshkumar, K. B. [a,*]

[a]*Phytochemistry and Phytopharmacology Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute (KSCSTE-JNTBGRI), Palode, Thiruvananthapuram-695562, Kerala, India*

***Corresponding author:** kbrtbgri@gmail.com*

Keywords: *Aromatic plants, spice, Brihat Samhita, India, parfimery, volatile aroma compounds.*

Objective: India is a center of aromatic and spice plants and has a rich and diverse heritage of perfumery among the various ancient civilizations. Perfumery was considered as one among the sixty-four arts in ancient India, and Varahamihira, the famous Indian astrologer of the 6th century A.D., in his monumental work *Brihat Samhita,* devotes an entire chapter to perfumery under the title 'Gandhayukti.' *Acorus calamus* (Vayambu), *Aglaia wallichii* (Priyangu), *Alpinia galanga* (Galangal), *Aquilaria malaccensis* (Oud), *Boswellia serrata* (Kunduruka), *Catunaregam spinosa* (Malankara), *Cinnamomum camphora* (camphor), *Coriander sativum* (Coriander), *Cyperus rotundus* (Musta), *Hedychium spicatum* (Shati), *Kaempferia galanga* (Kacholam), *Myristica fragrans* (Jatiphala), *Ocimum tenuiflorum* (Tulsi), *Pimpinella aromatica* (Satapushpa), *Santalum album* (Sandal), *Valeriana jatamansi* (Jatamamsi), and *Vettiveria zizanoides* (Ramacham) were the common aromatic plants used for perfumery in ancient India. India, with 4 biodiversity hotspot regions, hosts around 200 highly aromatic species and is also the center of origin and diversity of several spice crops. Further, India has an established aromatic plant industry of sandalwood, lemongrass, palmarosa, vetiver, rose, and jasmine. The family Lamiaceae is the major aromatic plant group, followed by Rutaceae, Zingiberaceae, Asteraceae, Oleaceae, and Lauraceae. In addition, there exists wide genetic diversity among the aromatic plants in the region. The volatile aroma compounds isolated through conventional hydrodistillation/steam distillation or using supercritical fluid extraction and headspace sampling through SPME were analysed through conventional GC-MS, headspace GC-MS, chiral GC-MS, or using supercritical fluid chromatography to characterise the exact enantiomeric aroma compounds.

Conclusion: Merging the ancient wisdom in perfumery with modern science and technology may yield astonishing results. The emerging field of herbal technology holds great potential for economic development, and the usage of aromatic plants in flavoring, perfume, cosmetics, toiletries, medicinal, and allied industries is ever increasing.

Alchemy of Scent: Essential oils at the cross-roads of medicine, perfumery and innovation

Çelebi Babaç, Rana [a,*]

[a]*International School of Medicine, Istanbul Medicine University, Istanbul, Turkey*

Coresponding author: rana.babac@medipol.edu.tr

Keywords: Essential oils, healing, medicine, neurocosmetics, perfumery, renaissance alchemy, ritual, rose water.

Abstract: This keynote unveils the profound intersections of essential oils, perfumery, and medicine, revealing scent as a vital force in healing, ritual, and innovation across millennia. From ancient Mesopotamian and Egyptian rituals [1] to the distilled elixirs of Greco-Roman and Islamic medicine [2], fragrance has bridged body, soul, and cosmos. Drawing on textual sources, materia medica, and archaeological evidence [3], we trace essential oils' evolution through sacred rites, Renaissance alchemy [4], Enlightenment chemistry, and modern aromatherapy [5]. Key milestones—Avicenna's rose water distillation, medieval apothecary waters, and 19th-century fragrance compounds—highlight scent's enduring role in health and sensory perception. Today, essential oils are redefining wellness at the nexus of biotechnology, neurocosmetics (the study of cosmetics' neurological effects [6]), and sustainable design, with applications in mood modulation and multisensory experiences like wellness tourism [7]. This talk invites attendees to reimagine scent not merely as a chemical or aesthetic phenomenon but as a dynamic interface between tradition and transformation, nature and science and past and future. By exploring these connections, we uncover how essential oils continue to shape human well-being and inspire interdisciplinary innovation in health, culture, and design.

REFERENCES

[1] Manniche, L. (1999). Sacred Luxuries: Fragrance, Aromatherapy, and Cosmetics in Ancient Egypt. Ithaca, NY: Cornell University Press.
[2] Levey, M. (1973). Early Arabic Pharmacology: An Introduction Based on Ancient and Medieval Sources. Leiden: Brill.
[3] Day, J. (2013). "Botanical Remains and Perfumery in Mesopotamia." In The Perfume of Mesopotamia: Aromatics in Ritual and Daily Life, edited by A. Butz & J. Day, 25–40. Berlin: De Gruyter.
[4] Moran, B. T. (2005). Distilling Knowledge: Alchemy, Chemistry, and the Scientific Revolution. Cambridge, MA: Harvard University Press.
[5] Tisserand, R., & Young, R. (2014). Essential Oil Safety: A Guide for Health Care Professionals (2nd ed.). Edinburgh: Churchill Livingstone.
[6] Herz, R. S. (2009). "Aromatherapy Facts and Fictions: A Scientific Analysis of Olfactory Effects on Mood, Physiology, and Behavior." International Journal of Neuroscience, 119(2), 263–290.
[7] Spence, C. (2021). "Scent in the Context of Multisensory Design: A New Frontier for Product Experience." Journal of Multisensory Research, 34(5), 487–512.

ORAL LECTURES (OP)

Fractionation of essential oils towards better antimicrobial activity

Ludwiczuk, A.[a,*], Lach-Wójtowicz, K.[a], Giancola, F.[a], Biernasiuk, A.[a]

[a]*Department of Pharmacognosy with the Medicinal Plant Garden, Medical University of Lublin, Chodzki Street, 20-093 Lublin, Poland*
[b]*Department of Pharmaceutical Microbiology, Medical University of Lublin, 1 Chodzki Street, 20-093 Lublin Poland*

Corresponding author: agnieszka.ludwiczuk@umlub.pl

Keywords*: Antifungal activity, DCVC fractionation, geraniol and nerol, Lippia alba.*

Objective: Essential oils (EOs) are increasingly becoming a subject of interest as an alternative for combating bacteria, fungi, and viruses that pose a threat to humans. The attractiveness of oils as antimicrobial substances stems from the fact that no mechanisms for microbial resistance to their components have been identified. A review of the literature also indicates that both biological activity and the associated chemical composition pertain to the essential oil as a whole. There is a lack of data indicating which chemical compounds present in the essential oil are responsible for specific pharmacological properties. Authors of the research studies often attribute the biological activity of the studied EOs to the main compounds contained within them. Therefore, the aim of the presented research was to answer the question of which compounds are responsible for fighting pathogenic microorganisms. The second question we asked was whether fractionating EOs would contribute to obtaining better microbiological properties. **Methods:** The subjects of the present study were six commercial essential oils from *Cymbopogon citratus* (DC.) Stapf. (leaf), *Lavandula angustifolia* Mill. (flowering tops), *Lippia alba* (Mill.) N.E.Br. ex-Britton & P. Wilson (leaf/stem), *Mentha x piperita* L. (leaf/stem), *Rosmarinus officinalis* L. (leaf), and *Salvia lavandulifolia* Vahl. (herb). All EOs were fractionated using the DCVC (dry column vacuum chromatography) method using silica gel as a stationary phase. Elution was carried out in a gradient of the mobile phase using a mixture of n-hexane and diethyl ether. The EOs and the fractions obtained were analyzed for their chemical composition using the GC-MS technique, and their antimicrobial activity was tested towards reference bacteria belonging to *Staphylococcus aureus* and *Escherichia coli* and fungi from *Candida* spp. These studies were performed using the broth microdilution method and a checkerboard technique. **Results:** The fractionation method used divided the EO components based on their polarity. The first fractions contained mono- and sesquiterpene hydrocarbons (e.g., α- and β-pinene, camphene, limonene, p-cymene, germacrene D, and β-caryophyllene), while the subsequent fractions were dominated by more polar compounds, such as alcohols, esters, and ketones. The tested EOs and fractions showed potential antibacterial activity with a minimal inhibitory concentration (MIC) in the range of 0.312-20 mg/mL and anticandidal effect with MIC = 0.078-10 mg/mL. Studies have shown that, compared to the EOs themselves, their fractions exhibited stronger activity. The first fractions were usually the most active. Of all the fractions, the fourth fraction obtained from *Lippia alba* proved to be the most active. It was characterized by the presence of geraniol and nerol. Moreover, the interactions between this fraction and the antimycotics, fluconazole and nystatin, showed synergistic or additive effects (FICI = 0.373-1.000) towards *Candida* strains. **Conclusions:** Fractionation of EOs enhanced their antimicrobial activity, with certain fractions showing significantly stronger effects than the whole oils. The most active fraction, rich in geraniol and nerol, also demonstrated synergistic or additive effects with antifungal drugs against *Candida* strains. The obtained results are promising and encourage further research.

ACKNOWLEDGMENTS
This research was funded by the statutory funds DS25 of the Medical University of Lublin (Poland).

Development and validation of liquid matrix volatilization methods for antimicrobial susceptibility testing of plant-derived volatile agents in vapor phase

Kokoska, L.[a,*]

[a]*Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamycka 129, 165 00 Prague, Czech Republic*

***Corresponding author:** kokoska@ftz.czu.cz*

***Keywords:** Antimicrobial activity, plant-derived volatiles, susceptibility testing method, vapour phase, volatile agent, volatilization matrix.*

Objective: The lack of appropriate methods for evaluation of antimicrobial activity of volatile agents in vapor phase is one of the main limiting factors for development of innovative plant-derived pharmaceutical, food, and agricultural products. Due to their high volatility and hydrophobicity, conventional laboratory methods face specific problems in the research of volatiles, including affection of the results of standard biological assays. In the past decades, several methods have been developed with the aim to study the potential of vapors of volatile agents to inhibit the growth of pathogenic microorganisms. Methods based on the solid matrix volatilization principle (e.g., disc volatilization assay) are simple to carry out, but they usually provide only qualitative results and require high amounts of material and labor [1]. The main aim of the study was to develop and validate simple and rapid quantitative methods for antimicrobial susceptibility testing of plant-derived volatile agents in the vapor phase. allowing a cost- and labor-effective high-throughput screening of essential oils, extracts, and volatile compounds. **Methods:** A broad spectrum of volatile compounds, hydrodistilled essential oils, and extracts obtained by supercritical CO_2 extraction were tested against pneumonia and food spoilage causing bacteria using broth micro- and macro-dilution volatilization assays [2, 3]. Anti-staphylococcal activity of various combinations of volatile compounds and essential oils was evaluated using the broth volatilization checkerboard method [4]. The chemical composition of essential oils, supercritical CO_2 and their vapors was characterized using a dual-column/dual-detector gas chromatograph system and headspace sampling. **Results:** Among all volatile agents tested in the gaseous phase, the vapors of thymoquinone and 8-hydroxyquinoline (both from Sigma-Aldrich, Prague, Czech Republic) were the most effective against *Haemophilus influenzae* (ATCC 49247) and *Staphylococcus aureus* (ATCC 29213) with MIC 8 µg/mL. In the case of complex mixtures of volatiles, the essential oil of *Alpinia oxymitra* K.Schum. pericarp from Cambodia containing β-pinene and caryophyllene epoxide as main constituents was the most effective against *H. influenzae* with MIC 32 µg/mL and *trans*-cinnamaldehyde-rich supercritical CO_2 extract obtained from commercially purchased *Neolitsea cassia* (L.) Kosterm. [syn. *Cinnamomum cassia* (L.) J.Presl] bark was the most active against *Listeria monocytogenes* (ATCC 7644) with MIC 256 µg/mL. The best combinatory anti-staphylococcal effect was found in the vapor phase against *S. aureus* (ATCC 25923) in the combination of carvacrol and thymol (ΣFIC=0.51). **Conclusions:** In a series of experiments, liquid matrix volatilization assays have been developed and validated to be suitable for simple and rapid susceptibility testing of microbial pathogens to volatiles and their combinations in the liquid and the vapor phase. They allow a cost- and labor-effective high-throughput screening of volatile agents using microtubes or microplates. In the future, these methods can be used for research and development of new agricultural (e.g., fumigants), food (e.g., modified atmosphere packaging agents), pharmaceutical, and medicinal (e.g., inhalation drugs and disinfecting fogging agents) products.

ACKNOWLEDGMENTS

This work was financially supported by the Technology Agency of the Czech Republic (project no. TQ11000051).

REFERENCES

[1] Houdkova M., Kokoska L. (2020). Volatile antimicrobial agents and *in vitro* methods for evaluating their activity in vapour phase: A review. *Planta Medica*, 12, 857-822.

[2] Houdkova M., Rondevaldova J., Doskocil I., Kokoska L. (2017). Evaluation of antibacterial potential and toxicity of plant volatile compounds using new broth microdilution volatilization method and modified MTT assay. *Fitoterapia*, 118, 56-62.

[3] Houdkova M., Chaure A., Doskocil I., Havlik J., Kokoska L. (2021). New broth macrodilution volatilization method for antibacterial susceptibility testing of volatile agents and evaluation of their toxicity using modified MTT assay *in vitro*. *Molecules*, 26, 4179.

[4] Netopilova M., Houdkova M., Rondevaldova J., Kmet V., Kokoska L. (2018). Evaluation of *in vitro* growth-inhibitory effect of carvacrol and thymol combination against *Staphylococcus aureus* in liquid and vapour phase using new broth volatilization chequerboard method. *Fitoterapia*, 129, 185-190.

Chemical diversity of medicinal and aromatic plants from the Portuguese flora

Machado, A.M.[a,*], Lopes, V.[b], Barata, A.M.[b], Póvoa, O.[c, d], Farinha, N.[c, d], Figueiredo, A.C.[a]

[a]*Centre for Ecology, Evolution and Environmental Changes (CE3C) & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, Lisboa, Portugal*
[b]*Banco Português de Germoplasma Vegetal (BPGV), Instituto Nacional de Investigação Agrária e Veterinária, Quinta de S. José, S. Pedro de Merelim, Braga, Portugal*
[c]*VALORIZA—Centro de Investigação para a Valorização de Recursos Endógenos, Instituto Politécnico de Portalegre, Praça do Município 11, Portalegre, Portugal*
[d]*Instituto Politécnico de Portalegre, Praça do Município 11, Portalegre, Portugal*

Corresponding author: ampmachado@fc.ul.pt

Keywords: *Apiaceae, chemical descriptors, chemotypes, essential oils, Lamiaceae, medicinal and aromatic plants.*

Objective: Mainland Portugal, together with the Azores and Madeira archipelagos in the middle of the Atlantic Ocean, constitutes a geographically distinctive area that comprises three major biogeographical regions: the Atlantic, the Mediterranean, and Macaronesia. These unique conditions allow for the development of a rich biodiversity, with 3,800 described species, 500 of which have aromatic and/or medicinal potential [1, 2]. This work aims to contribute to the knowledge on the chemical variability of Portuguese medicinal and aromatic plants (MAPs) from the Lamiaceae and Apiaceae by giving potential essential oils (EOs) chemical descriptors, based on EO chemotypes, to go along with the currently available morpho-agronomic traits. For this, EOs from *Origanum vulgare* subsp. *virens*, *Coriandrum sativum* L., and *Foeniculum vulgare* Mill were evaluated for the presence of chemotypes. Together, chemical and morpho-agronomic traits allow monitoring biodiversity in agricultural ecosystems, contribute to *in* situ and *ex situ* conservation, and contribute to decision-making. **Methods:** EOs were isolated by hydrodistillation from accessions (ACC) obtained in different regions in mainland Portugal from i) oregano flowering aerial parts (38 ACC), ii) fruits and vegetative aerial parts of coriander (11 ACC), and iii) fruits of fennel (19 ACC). The EOs were analyzed by gas chromatography (GC) and GC coupled to mass spectrometry (GC-MS), and their composition was used in the sample's chemical correlation evaluation, as in [3]. **Results:** From the obtained data, the putative oregano chemotypes include carvacrol, thymol, and linalool, with γ-terpinene, *p*-cymene, and *cis*- and *trans*-ocimene also contributing as chemical descriptors [3]. The EOs of coriander showed no chemotypes but varied according to the plant part; the fruits were dominated by linalool, while the EOs of vegetative aerial parts evidenced 2-*trans*-decenal and *n*-decanal as the main compounds [4]. Fennel fruits' EOs showed chemical variability, suggesting that their descriptors should include fenchone and estragole in addition to *trans*-anethole [4]. **Conclusions:** The results obtained highlighted the occurrence of chemotypes, thereby underlining the importance of preserving their genetic biodiversity from invasive species, overexploitation, diseases, or climatic and environmental changes. The information gathered supports the relevance of clarifying the chemical variability of MAPs and provides additional indicators (chemical descriptors) to the conventional traits, helping in recognizing which plants are best suited to market demands.

ACKNOWLEDGMENTS
Thanks are due to PDR2020-7.8.4-FEADER-042741 and FCT/MCTES financial support through CE3C UID/00329/2025, and & CHANGE.

REFERENCES

[1] Barata A. M., et al., (2018). Plantas Aromáticas, Instituto Nacional de Investigação Agrária e Veterinária, Oeiras.
[2] Barata A. M., Rocha F., Lopes V., Bettencourt E., Figueiredo A. C., (2011). Medicinal and aromatic plants-Portugal. In *Medicinal and Aromatic Plants of The World, Encyclopaedia of Life Support Systems (EOLSS)*, Eolss Publishers, Oxford.
[3] Machado A. M., Lopes V., Barata A. M., Póvoa O., Farinha, N., Figueiredo A. C., (2023). Essential Oils from *Origanum vulgare* subsp. *virens* (Hoffmanns. & Link) Ietsw. Grown in Portugal: Chemical Diversity and Relevance of Chemical Descriptors, *Plants*, 12, 621.
[4] Machado A. M., Lopes V., Barata A. M., Póvoa O., Farinha, N., Figueiredo A. C., (2023). Chemical Variability of the Essential Oils from Two Portuguese Apiaceae: *Coriandrum sativum* L. and *Foeniculum vulgare* Mill., *Plants*, 12, 2749.

Is Linalyl Anthranilate Indeed Found in Plant Samples? GC-MS Misidentifications in the Scientific Literature

Pokajewicz K.[a,*], Biernat T.[b], Wieczorek P. P.[a]

[a]*Department of Analytical Chemistry, Faculty of Chemistry and Pharmacy, Opole University, ul. Oleska 48, Opole, Poland*
[b]*SynthPharm, ul. Witkacego 14, Skierniewice, Poland*

***Corresponding author:** katarzyna.pokajewicz@uni.opole.pl*

***Keywords:** Linalyl anthranilate, retention index.*

Objective: Linalyl anthranilate (LNA) is a compound that has been repeatedly detected in many plant extracts and essential oils, including lavender, thyme, mint, marjoram, and coriander, by various authors using gas chromatography-mass spectrometry (GC-MS). In consequence of multiple reports of its presence in various herbs and associated biological activity studies, it is depicted in the scientific literature as an antimicrobially active secondary plant metabolite. However, the elution order of reported LNA presented in publications in GC composition tables does not correspond with retention behavior, as should be expected regarding its linear retention index (LRI) for column type "5" in the NIST database [1]. The objective of this research project was to collect accurate retention data for linalyl anthranilate to verify whether the reports of the presence of LNA in natural products from plants are correct or are the result of GC-MS misidentifications. **Methods:** To accomplish this, linalyl anthranilate was synthesized using a novel two-step procedure. The resulting product was authenticated using 1H NMR, 13C NMR, and MS. The retention indices for linalyl anthranilate were then determined on the three most commonly used GC stationary phases: polydimethylsiloxane, 5% diphenyl-95% polydimethylsiloxane, and polyethylene glycol. The study confirmed the accuracy of NIST's determination of the linear retention index for the "5" type column [2]. **Results:** However, the putative linalyl anthranilate, reported in the scientific literature in plant-derived samples, had a much lower retention index, approximately 1000-1400, strongly suggesting that these reports were the result of GC-MS misidentifications. A systematic review of the existing literature did not provide any reliable evidence of the actual presence of linalyl anthranilate in natural samples. **Conclusion:** All indications suggest that these were misidentifications of linalyl acetate due to the occurrence of an erroneous spectrum in the older versions of the NIST mass spectra database. Consequently, anthranilate cannot be considered a secondary plant metabolite [2].

REFERENCES

[1] NIST. 1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate. NIST Chemistry WebBook, SRD 69.
[2] Pokajewicz K., Biernat T., Wieczorek P. P., Is Linalyl Anthranilate Indeed Found In Plant Samples? GC-MS Misidentifications in the Scientific Literature, *Journal of Natural Products*, 87, 2839-2846.

Investigation of Quality and Yield Parameters in Growing Japanese Quails (*Coturnix Coturnix Japonica*) Feed with Microencapsulated *Origanum Onites* Oil

Avşar, A.[a], Yavuz, M.[a,*], Korkmaz, M.A.[b], Sarıkaya, H.S.[b], Orhan, H.[c]

[a]*Isparta University of Applied Sciences, Agriculture Faculty, Animal Science Department, Isparta, Türkiye*
[b]*Burdur Mehmet Akif Ersoy University, Veterinary Medicine Faculty, Burdur, Türkiye*
[c]*Isparta Süleyman Demirel University, Faculty of Medicine, Department of Basic Medical Sciences, Isparta, Türkiye*

***Corresponding author:** musayavuz@isparta.edu.tr

Keywords: *Japanese quail, microencapsulation, Origanum onites L., performance, quality, yield.*

Objective: The first aim of this study was to obtain a product resistant to environmental conditions that can be used in animal husbandry by applying the microencapsulation technique of *Origanum onites* L. oil (OO), and the second aim was to investigate the effect of the produced product on yield and quality parameters in growing quails in long-term feeding. **Methods:** The study was approved by the Isparta University of Applied Sciences Animal Experiments Local Ethics Committee, numbered E-77211729-804.01-18969.336. Chicks were obtained from the Isparta University of Applied Sciences, Agriculture Faculty, Education, Research and Application Farm's Poultry Unit. Japanese quail (*Coturnix coturnix japonica*) hybrid lines were used. The experimental design was established as a control group (C) fed only with basal ration, and seven groups were formed with microencapsulated *Origanum onites* Oil (MOO) and OO at doses of 100, 300, and 600 mg/kg (C, MOO100, MOO300, MOO600, OO100, OO300, OO600). The experimental group had 3 replications, comprising 21 subgroups with 16 animals. **Results:** There were no statistically significant differences among the groups in blood parameters, carcass yield, relative organ weights, meat quality, or sensory analysis results ($P > 0.05$). However, the lowest feed conversion ratios were observed in the following groups: 2.46% in OO600 (2nd week), 2.80% in OO100 (3rd week), 4.59% in MOO600 (4th week), 6.84% in MOO300 (5th week), and 10.38% in MOO100 (6th week). Although the differences in hot and cold carcass yields were not statistically significant, the highest hot carcass yield (74.07%) was found in the MOO300 group, while the lowest (71.58%) was in the OO300 group. Similarly, the highest cold carcass yield (73.23%) was observed in the MOO300 group, and the lowest (70.49%) in the OO300 group. Pathological evaluations revealed that high doses of OO negatively affected liver and testicular tissues. The most notable finding was that the number and size of fat vacuoles in hepatocyte cytoplasm increased proportionally with the OO dose. Although individual variation was observed in testicular tissue, a general dose-dependent decrease in spermiogenesis was noted. **Conclusions:** The use of microencapsulated *Origanum onites* oil at doses of 100–300 mg/kg during the growing period can be recommended for producers to support performance without negative health impacts.

Sleeping Genes - The Essential Oil Compounds of a Species Cross

Novak, J.[a,*], Raskovic, J.[a], Lukas, B.[b]

[a]*Centre for Veterinarian System Transformation and Sustainability, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria*
[b]*Institute of Botany, BOKU University, Gregor-Mendelstr. 33, A-1180 Vienna, Austria*

***Corresponding author:** johannes.novak@vetmeduni.ac.at*

***Keywords:** Cymyl pathway, hybridisation, ocimyl pathway, O. majorana, O. vulgare ssp. vulgare, sabinyl pathway.*

Objective: This study aimed to examine the impact of hybridization between *O. majorana* ('marjoram', OM) and *O vulgare* ssp. *vulgare* ('wild marjoram,' OV) on plant secondary metabolites. Within the monoterpenes of the genus *Origanum*, two predominant pathways are identified: the sabinyl pathway, characterized by marjoram-associated sabinene, *cis*- and *trans*-sabinene hydrates, and their acetates, and the cymyl pathway, which includes γ-terpinene, *p*-cymene, thymol, and carvacrol, typical of oregano. The phenotype of the OV individuals utilized in this work is not predominantly characterized by sabinyl or cymyl compounds; rather, it is defined by *cis*- and *trans*-ocimene ('ocimyl type'), alongside monoterpenes from the sabinyl pathway. **Methods:** OM and OV were hybridized using a cytoplasmic male sterile breeding line to regulate the crossover. The regulated species cross produced viable seeds of the first filial generation (F1). Leaves from 65 F1 plants, aged three months, were collected and extracted using dichloromethane prior to analysis via GC/MS. The regions of the resulting monoterpenes were normalized to the total monoterpene content. **Results:** Four individuals from each parent (OM and OV) and 65 F1 plants were analyzed. OM included a high concentration of sabinyl compounds (81% of all monoterpenes), whereas OV had 33% of sabinyl compounds. OV included a significant concentration of *cis*-*trans*-ocimene as well as allo-ocimene (together referred to as 'ocimyl compounds,' comprising 37%), whereas OM contained these compounds only in minimal amounts. Both OM and OV had modest levels of cymyl compounds (4% and 15%, respectively), with thymol and carvacrol missing in OM and present in minimal quantities in OV (1.4% and 1%, respectively). The F1 generation exhibited an average of 58% sabinyl compounds and 10% of ocimyl compounds, with cymyl compounds slightly above OV (20%) and an average of 6% carvacrol. The carvacrol content of some individuals of the F1 generation increased to 54%. **Conclusions:** Numerous terpene molecules in the F1 generation conformed to the anticipated pattern, exhibiting intermediacy between the two phenotypically disparate parents. In certain F1 individuals, the dormant cymyl-pathway was revived.

REFERENCES

[1] Skoula, M.; Harborne, J. (2002). The Taxonomy and Chemistry of *Origanum*: In: Kintzios, S. (ed.) Oregano, CRC Press, Boca Raton.

Evaluation of Chemical Composition and Biological Activities of *Salvia veneris* Hedge essential oil

Ödüncüoğlu, G.[a,*], Şanlitürk, G.[a], Hanoğlu, A.[b], Başer, K.H.C.[b]

[a]*Department of Pharmacognosy, Bahcesehir Cyprus University-Faculty of Pharmacy, Nicosia, Cyprus*
[b]*Department of Pharmacognosy, Near East University-Faculty of Pharmacy, Nicosia, Cyprus*

***Corresponding author:** goksu.oduncuoglu@baucyprus.edu.tr*

Keywords: *antibacterial activity, anti-inflammatory activity, GC-MS, MRSA, Salvia veneris*

Objective: The *Salvia* genus, which belongs to the Lamiaceace family, includes valuable species that are rich in essential oils. *Salvia veneris* Hedge is known as Krythean Sage, endemic to Cyprus, and consumed as herbal tea by local people. The rise of antibiotic resistance presents a significant global health challenge, highlighting the need for the development of novel pharmaceutical agents to combat resistant pathogens. This study aimed to identify the chemical composition of *Salvia veneris* Hedge (Kythrean sage) leaf essential oil (WD) from Cyprus and to evaluate antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 33591). **Methods:** Aerial parts of *Salvia veneris* Hedge were collected at flowering time from the mountains of Kyrenia, Northern Cyprus, in April 2025. The essential oil from air-dried leaves of the plant was obtained by hydrodistillation for 3 h, using a Clevenger apparatus. The essential oil was analyzed by gas chromatography-mass spectrometry (GC–MS). The anti-inflammatory activity of essential oil was determined through the bovine serum albumin denaturation method. The antibacterial activity against MRSA (ATCC 33591) was assessed using the agar well diffusion method and minimum inhibitory concentration assay. **Results:** GC–MS analysis of the essential oil identified the existence of different constituents. The essential oil showed significant anti-inflammatory activity with a dose-dependent increase in the percentage inhibition of protein denaturation in the range of 21.23% and 46.90% at 50–400 µg/ml concentrations, respectively. Essential oil of *S. veneris* demonstrated moderate antibacterial activity against MRSA with a 12.30 mm inhibition zone and MIC > 10 mg/ml. **Conclusions:** The results suggest that the essential oil of *Salvia veneris* possesses significant anti-inflammatory and antibacterial potential, particularly against MRSA.

Fractionation of *Cryptomeria japonica* cones essential oil via hydrodistillation: antioxidant and cytotoxic properties

Arruda, F.[a,b]*, Lima, A.[c], Surowiak, A.[d], Janeiro, A.[b], Baptista, J.[a,c], Rosa, J.S.[b,e], Lima, E.[a,c]

[a]*Institute of Agricultural and Environmental Research and Technology, University of the Azores, Angra do Heroísmo, Portugal*
[b]*Department of Biology, Faculty of Science and Technology, University of the Azores, Ponta Delgada, Portugal*
[c]*Department of Physics, Chemistry and Engineering, University of the Azores, Ponta Delgada, Portugal*
[d]*Department of Chemical Biology and Bioimaging, Faculty of Chemistry, Wroclaw University of Science and Technology, Wroclaw, Poland*
[e]*Biotechnology Centre of Azores, University of the Azores, Ponta Delgada, Portugal*

***Corresponding author**: filipe.mp.arruda@uac.pt*

***Keywords:** Antioxidant, Cryptomeria japonica, cytotoxicity, essential oil fractionation, waste valorisation.*

Objective: *Cryptomeria japonica* (Thunb. ex L.f.) D. Don (Cupressaceae) is the most economically important tree in the Azores Islands, Portugal. Azorean *C. japonica* immature female cones (Az-CJIFC) are a particularly rich source of essential oil (EO) with multiple bioactivities [1]. To promote the optimal use of this valuable resource, the present study aimed to fractionate the Az-CJIFC EO and to evaluate the antioxidant and cytotoxic activities of the resulting fractions. **Methods:** Simultaneous hydrodistillation–fractionation (HD–FR) was performed for 4 h in a Clevenger apparatus in order to obtain EO from Az–CJIFC, which was fractionated into six EO fractions (Frs. 1–6), collected at sequential HD timeframes (HDTs: 0–2, 2–10, 10–30, 30–60, 60–120, and 120–240 min). The obtained EO samples (crude EO and fractions) were evaluated for their chemical composition through GC–FID/GC–MS analyses as described in [2]. The EO samples were investigated for their *in vitro* antioxidant properties by DPPH, ABTS, and FRAP assays, and for cytotoxicity using the *in vitro* MTS assay on HaCaT cells and the *in vivo* brine shrimp (*Artemia salina*) lethality assay. **Results:** EO fractions (Frs. 1–6) collected during the HD process varied significantly in the concentration (%) of their major terpene compounds. Particularly, monoterpene hydrocarbons were highest at the beginning of HD (97% in Fr1), gradually decreasing over distillation time. In contrast, oxygenated sesquiterpenes were predominant in the final fractions, constituting 71% of Fr6. Oxygenated monoterpenes (mainly terpinen-4-ol) reached maximum concentration in mid-distillation on Frs. 3 and 4 (18% and 17%, respectively). Regarding bioactivities, Fr5 and Fr6 exhibited the highest antioxidant activities (DPPH and ABTS IC50 values: ≈0.3 mg/mL; FRAP: >30.0 mg Trolox equivalents/g EO). These fractions also showed, interestingly, the highest cytotoxicity in both assays (HaCaT IC_{50}: 0.06–0.1 mg/mL; *A. salina* lethality IC_{50}: 0.22–0.04 mg/mL). The observed bioactivities are closely linked to chemical composition, with Fr5 and Fr6 enriched in sesquiterpenoids, notably elemol and α, β, and γ eudesmol isomers. These findings highlight their potential for further investigation, particularly regarding anticarcinogenic properties. **Conclusions:** Fractionation of Azorean *C. japonica* cones EO by the HD method was revealed to be an effective method to obtain EO fractions with enhanced bioproperties, highlighting their potential for diverse industrial applications.

ACKNOWLEDGEMENTS
Filipe Arruda acknowledges his PhD scholarship (ref. M3.1.a/F/008/2021) from FRCT. This work is supported by funds from DRCT, under the project M1.1.C/PROJ.EXPLORATÓRIOS/003/2022-PotBioCJap. Este projeto, com a referência [M3.3.A/PART.R.C./003/2025], recebeu financiamento da Direção Regional da Ciência, Inovação e Desenvolvimento, através do Sistema de Incentivos PROSCIENTIA.

REFERENCES
[1] Janeiro A., Lima A., Arruda F., Wortham T., Rodrigues T., Baptista J., Lima, E. (2024). Variations in Essential Oil Biological Activities of Female Cones at Different Developmental Stages from Azorean *Cryptomeria japonica* (Thunb. ex L.f.) D. Don (Cupressaceae). *Separations*, 11, 102.
[2] Figueiredo A. C., Moiteiro C., Rodrigues, M. C. S., Almeida, A. J. (2021). Essential oil composition from Cryptomeria japonica D. Don grown in Azores: Biomass valorization from forest management. *Natural Product Communications*, 16(8).

Exploring the Scent of Dune Pepper: New Aromas from Quebec

St-Gelais, A.[a,*], Baldovini, N.[b], Villeneuve, J.-C.[c], Cartau, R.[d], Roger, B.[e], Baker, K.[a]

[a]*Laboratoire PhytoChemia, 628 boul. du Saguenay Ouest, Chicoutimi (QC), Canada*
[b]*Institut de Chimie de Nice, Université Côte d'Azur, 28 avenue Valrose, Nice, France*
[c]*BoreA Canada, 142 rue de la Cogénération, Chapais (QC), Canada*
[d]*Posthumous; Distillerie Boréalis, 259 rue Jacques-Cartier, Rivière-au-Tonnerre (QC), Canada*
[e]*AlChemia Solutions, 628 boul. du Saguenay Ouest, Chicoutimi (QC), Canada*

***Corresponding author:** a.st-gelais@phytochemia.com*

Keywords: *Alnus alnobetula subsp. crispa, essential oil variability, eudesmyl acetates, GC-olfactometry, structural elucidation, (Z)-isogeranyl acetate.*

Objective: The winter dormant male catkins of *Alnus alnobetula* subsp. *crispa* (Aiton) Raus, a characteristic shrub of boreal landscapes thriving in rocky or sandy soils in Quebec, are rather erect, sessile, golden-brown to yellow, slightly resinous, and delicately scented [1-2]. They are becoming increasingly popular as a spice called "poivre des dunes" (dune pepper, or alder pepper), which is hand-picked from wild populations during the winter and is especially used in fine restaurants, in specific spirits, and amongst local culinary enthusiasts [2]. Given the distinctive scent of this spice, in recent years, several commercial essential oil distillers have independently attempted small productions of dune pepper essential oil (DPEO), whose composition, to the best of our knowledge, has never been detailed in scientific literature. When applied to a smelling strip or the skin, DPEO develops a very pleasant characteristic note, which one could describe as ripe, fruity, velvety woody, and slightly reminiscent of clean vetiveryl acetate, and which lingers for hours to days. This collaborative study aims to assess the chemical variability of DPEO, to improve the characterization of some of its constituents, and to provide insights with regard to its enticing aroma. **Method:** Sixteen batches of DPEO were obtained as follows: Three lots of handpicked catkins were distilled using a Clevenger apparatus by PhytoChemia*; 9 commercially distilled samples were procured (1 from Groupe Boréaressources, 1 from Distillerie Boréalis*, 1 from Noblessence, and 6 from BoreA Canada); and four bulk batches of commercially-picked catkins* provided by BoreA Canada were subjected to pilot-scale distillation by AlChemia Solutions using a Nano stainless steel apparatus. Oils were analyzed using both GC-MS and GC-FID, with the inclusion of a tetradecane internal standard for quantitation [3] as % m/m, on DB-1, DB-5, and DB-Wax columns. GC-olfactometric assays were conducted by A. St-Gelais and N. Baldovini to locate high-impact odorants. A pooled sample of 26 g of DPEO was subjected to repeated fractionation over silica gel and silver-impregnated silica gel using gradients of diethyl ether and ethyl acetate in petroleum ether to produce fractions enriched in compounds of interest, unidentified constituents, and aroma-bearing targets. Compounds were further purified by preparative GC on a DB-1 column and characterized by NMR. ** A voucher specimen was deposited at the Louis-Marie Herbarium, Université Laval, Quebec*. **Results:** DPEO is dominated by sesquiterpenoids, with only linalool (0.1–1.7%), (*Z*)-isogeraniol (0.1–3.9%), and (*Z*)-isogeranyl acetate (0.2–3.0%) exceeding 1% m/m amongst monoterpenoids; the latter was isolated and characterized by NMR. Major sesquiterpene hydrocarbons were γ-cadinene (4.0–11.0%) and δ-cadinene (1.6–11.5%) in all samples, while the profile of oxygenated sesquiterpenoids was variable. In particular, eudesmols and eudesmyl acetates could range from absent to major constituents of the oil. GC-olfactometric assays and purification suggested that α-eudesmyl acetate (0–12.0%) and one other sesquiterpenyl acetate (still under examination at the time of submission) were important contributors to the fruity-woody notes of DPEO. Further fractionation led to the identification of several minor sesquiterpenoids and isolation of a mixture of two related minor compounds bearing the characteristic aroma of DPEO. **Conclusions:** The present work documents for the first time the original composition of DPEO, including the large variability of content of eudesmyl acetates and the characterization of previously unreported sesquiterpenoids. It also provides insights regarding the unique aroma of this essential oil and paves the way for further studies.

CONFLICTS OF INTEREST & ACKNOWLEDGMENTS

J.C. Villeneuve is a commercial producer of essential oils and could benefit financially from the sales of DPEO. We thank R.H. Fontaine for the collection of a sample of catkins, R. Baldovini for assistance with GC-olfactometry, and Groupe Boréaressources (now discontinued) for providing a DPEO sample. R. Cartau passed away during the conduction of this study and is listed posthumous as a contributor.

REFERENCES

[1] IRIIS Phytoprotection (2025) Aulne crispé, [consulted on 2025-04-27], URL: https://beta.iriisphytoprotection.qc.ca/fr/mauvaises-herbes/mauvaises-herbes-des-cultures/aulne-crispe.

[2] Villeneuve, S., Chainé, M., Cartier, P. (2019) Une épice venue du froid, *Couvert Boréal*, spring 2019, 22-23.

[3] Tissot, E., Rochat, S., Debonneville, C. *et al.* (2012) A rapid GC-FID quantification technique without authentic Samples using predicted response factors: rapid GC-FID quantification technique, *Flavour Fragr. J.*, 27, 290-296.

Sniff Away Your Stress: The Effect of Essential Oil Inhalation on Oxidative Stress

Buranits, S.[a], Stappen, I.[a,*]

[a]*Department of Pharmaceutical Sciences, University of Vienna-Faculty of Life Sciences, Vienna, Austria*

***Corresponding author:** iris.stappen@univie.ac.at

Keywords: *Aromatherapy, FORT, FORD, hedonic valence, inhaler pen.*

Objective: Oxidative stress is characterized by an imbalance between the generation and accumulation of reactive oxygen species (ROS) in the body and the organism's capacity to neutralize these products. This imbalance may result in cell and tissue damage due to detrimental effects on critical biological components, including lipids, proteins, and nucleic acids, and is associated with the initiation or progression of numerous diseases [1]. Despite the recognized benefits of essential oils (EO) against depression, anxiety, and sleep disorders after inhalation [2], few studies report on their impact on biomarkers of oxidative stress [3]. To address this issue, the effect of melissa and valerian EO on oxidative stress was investigated in healthy humans in a randomized controlled trial. **Methods:** A between-subjects design with repeated measures was utilized. 61 pharmacy students, comprising 50 women, volunteered and were assessed for oxidative stress levels (FORT), defense levels (FORD), and blood pressure (BP). Further, a mood questionnaire was completed. Subsequently, participants were characterized into four groups: *Neg-Controls* (no intervention), *Sniff-Controls* (inhaler pen devoid of odor), *Melissa Group* (positive control; inhaler pen with melissa essential oil), *Valerian Group* (inhaler pen with valerian essential oil) and were directed to sniff the odor twice daily for a duration of nine weeks. Ultimately, FORT, FORD, BP, and mood were reassessed, and the odors were evaluated for pleasantness, familiarity, intensity, and anticipated effect. **Results:** Due to the ongoing investigation until mid-July, only preliminary findings can be presented in this abstract. Around 50% of the subjects had increased oxidative stress levels (> 230 FORT), which were significantly higher in women using hormonal contraceptives (6 females) compared to men and other women ($p < 0.001$) [4]. Nonetheless, based on the literature regarding the calming effects of inhaling melissa and valerian oils [5], we anticipate a reduction in FORT levels, particularly among students with excessive levels, following EO inhalation. Moreover, FORD levels may rise over time. We hypothesize that FORD levels will decline more in individuals who perceive the odor as pleasant [6]. **Conclusions:** If our hypothesis is accurate, an inhaler pen administered EO may serve as a safe, straightforward, and effective tool for reducing oxidative stress when used over a longer period.

ACKNOWLEDGMENTS

The authors want to thank Anca Cristurean and Marie-Susan Reindl for their support in data acquisition and calculation, Brüder Unterweger GmbH for valerian essential oil, Nicola Stojanovic for melissa essential oil, Johannes Novak for fruitful discussions, and all students who volunteered for the study.

REFERENCES

[1] Pizzino G., Irrera N., Cucinotta M., et al. (2017). Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 2017, Article ID 8416763.

[2] Heuberger E., Stappen I., Rudolf von Rohr R., (2017). Riechen und Fühlen, Fischer & Gann, Munderfing.

[3] a) Murbach Teles andrade B. F., Braga C. P., dos Santos K. C., et al. (2014). Effect of inhaing *Cymbopogon martinii* essential oil and gerankols on serum biochemistry parameters and oxidative stress in rats. *Biochemistry Research International*, 2914, Article ID 493183. b) Aponso M, Patti A., Bennett L. E. (2020). Dose-related effects of inhaled essential oils on behavioral measures of anxiety and depression and biomarkers of oxidative stress. *Journal of Ethnopharmacology*, 250, 112469.

[4] Cauci S., Xodo S., Buligan C., et al. (2021). Osidative stress is increasedin combined oral contractptives user and is positifely associeated with high-sentisivity C-reactive protein. *Molecules*, 26, 1070.

[5] a) Watson K., Hatcher D., Good A. (2019). A randomized controlled trial of lavender (L. angustifolia) and lemon balm (M. Officinalis) essential oils for the treatment of agitaed behaviour in older people witha and without dementia. *Complememtary Therapies in Medicine,* 42, 366-373. b) Wang W., Wang Y., Guo Q., et al. (2022). Walerian essential oil for treatint insomnia via the serotonergic synapse pathway. *Frontiers in Nutrition*, 9, 927434.

[6] Jellinek S. (1997). Psychodynamic odor effects and their mechanisms: Failure to identify the mechanism can lead to faulty conclusions in odor studies. *Cosmetics & Toiletries*, 112, 61-71.

Impact of Aroma/Floriculture/Citrus Mission on Essential Oil Production in India

Harlalka, R.[a,*]

[a]*Department of Chemistry in Pharmacy, Institute of Chemical Technology, Mumbai, Nathalal Parekh Marg, Matunga East, Mumbai, Maharashtra, India*

Corresponding author: ramakant@nishantaromas.com

Keywords: *Aroma mission, citrus oils, cultivation, essential oils, floriculture, India.*

Objective: This study aims to explore the transformative impact of the Government of India's Aroma/Floriculture/Citrus Mission (Phase 3) on the essential oil production sector. Launched under the visionary leadership of Prime Minister Shri Narendra Modi, the mission targets the systematic cultivation of 50,000 hectares of aromatic and medicinal crops, especially in regions impacted by climate-induced or natural disasters. The broader goal of the mission is to strengthen India's position as a global leader in essential oil production by reducing dependency on imports of high-value oils like Geranium, Vetiver, and Lavender and promoting indigenous oils such as Palmarosa, Lemongrass, Peppermint, Basil, Citriodora, Tagetes, and Vetiver. **Methods:** The methodology employed includes a comprehensive policy review and historical analysis of Aroma Mission Phases 1 through 3. Primary data were obtained through structured interviews with farmers, field experts, and industry representatives, while secondary data were gathered from government publications and reports issued by CSIR and the Ministry of Science & Technology. The analysis also incorporated regional surveys and case studies from pilot areas involved in the mission. **Results:** The combined implementation of Aroma Mission Phases 1 and 2 has yielded a notable increase of approximately 1000 metric tons in essential oil production across targeted zones. This expansion resulted in a revenue generation of nearly US$50 million for farmers and associated industries. Phase 3 of the mission introduces sustainability metrics and emphasizes local employment generation through decentralized processing units. Notably, the cultivation of high-demand crops like lemongrass and palmarosa has doubled in several states, leading to the emergence of new rural micro-enterprises. **Conclusions:** The Aroma/Floriculture/Citrus Mission has become a cornerstone initiative in reshaping India's essential oil industry. By aligning scientific research, farmer education, and industry needs, the mission has set a precedent for agricultural development with an industrial focus. With dedicated involvement from experts, the mission has not only enhanced domestic production but also elevated India's reputation in the global essential oils market. It stands as a prime example of how policy-driven initiatives, backed by research and industrial expertise, can create sustainable growth in niche agricultural sectors.

ACKNOWLEDGMENTS

The author acknowledges the Government of India and the monitoring committee of the Aroma Mission for their continuous support and data access.

REFERENCES

[1] Harlalka, R., (2025). The Impact of Government Missions on Essential Oil Production, Journal of Aroma Studies, 14(2), 45–52.
[2] Ministry of Science & Technology, (2023). Annual Report on Aroma Mission, Council of Scientific & Industrial Research, New Delhi.

Portuguese lavenders as a source of promising skin anti-aging compounds: A focus on cellular senescence

Alves-Silva, J.[a], Domingues, J.[b,c], Delgado, F.[b,c], Girão, H.[a], Salgueiro, L.[d,e], Zuzarte, M.[a,d,e]*

[a]*Univ Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal*
[b]*Research Centre for Natural Resources, Environment and Society (CERNAS), Polytechnic Institute of Castelo Branco (IPCB), Castelo Branco, Portugal*
[c]*Plant Biotechnology Center of Beira Interior (CBPBI), Castelo Branco, Portugal*
[d]*Univ Coimbra, Faculty of Pharmacy, Coimbra, Portugal*
[e]*Univ Coimbra, Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, Coimbra, Portugal*

***Corresponding author:** mzuzarte@uc.pt

Keywords: *Essential oils, fibroblasts, β-galactosidase, lavandula, γH2AX foci, SASP.*

Objective: The development of strategies to promote healthy aging is attracting increasing attention, particularly with regard to skin health, as a crucial factor in overall wellness. Considering that chronic low-level inflammation - known as inflammaging - can accelerate aging by promoting cellular senescence, and bearing in mind the reported anti-inflammatory properties of *Lavandula pedunculata* (Mill.) Cav. and *L. luisieri stoechas* subsp. *luisieri* (Rozeira) Rozeira essential oils [1], this study aimed to evaluate their anti-senescent potential. **Methods:** Essential oils were extracted from the flowering aerial parts of plants collected in the Castelo Branco region (Portugal) by hydrodistillation, and their chemical composition was characterized using gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). Cellular senescence was induced in NIH/3T3 fibroblasts using etoposide, a DNA-damaging chemotherapeutic agent. To assess the protective effects of the essential oils, multiple senescence-associated markers were evaluated. These included β-galactosidase activity, nuclear accumulation of γH2AX as an indicator of DNA damage, Ki67 expression to assess cell cycle arrest, modulation of the p53/p21 signaling pathway, and the secretion of proinflammatory mediators associated with the senescence-associated secretory phenotype (SASP). Furthermore, transmission electron microscopy observations enabled an overall ultrastructural assessment. **Results:** The essential oil of *L. pedunculata* was predominantly composed of fenchone (63.8%) and camphor (20%), while *L. stoechas* subsp. *luisieri* was characterized by high levels of *trans*-*α*-necrodyl acetate (18.8%), lavandulyl acetate (13.3%), camphor (8.6%), and 1,8-cineole (6.0%). Etoposide-induced senescence in NIH/3T3 fibroblasts in a time-dependent manner, with senescence markers becoming more prominent seven days post-treatment. These included increased X-galactosidase staining, enlarged cell and nuclear size, and a higher number of γH2AX foci per nucleus, indicating persistent DNA damage. Notably, treatment with both essential oils resulted in a concentration-dependent decrease in the percentage of X-galactosidase-positive cells. Other senescence-associated features, including γH2AX foci, nuclear deformation, and nuclear enlargement, were also attenuated following essential oil treatment. In addition, a reduction in the secretion of SASP-associated pro-inflammatory factors was observed, alongside enhanced cell proliferation, as indicated by an increase in Ki67-positive cells. These anti-senescent effects may be mediated, at least in part, through modulation of the p53/p21 signalling pathway, with both protein levels found to be downregulated in treated cells. Ultrastructural analysis further supported these findings, showing pronounced morphological alterations in etoposide-treated cells that were markedly reduced in the presence of the essential oils. **Conclusion:** Overall, this study highlights the promising anti-senescent potential of both essential oils, with *L. stoechas* subsp. *luisieri* standing out due to its effectiveness at remarkably low concentrations (6.25 µg/mL). The unique chemical composition of this species, particularly its richness in necrodane derivatives, which are rare in the plant kingdom, further underscores its scientific and therapeutic interest. When considered alongside previously reported anti-inflammatory properties [1], these findings support the potential application of these essential oils in cosmeceutical formulations aimed at promoting skin health and mitigating age-related skin deterioration.

ACKNOWLEDGMENTS
La Caixa Foundation and Portuguese Foundation for Science and Technology supported this work (project PD21–00003).

REFERENCES
[1] Zuzarte, M., Sousa, C., Cavaleiro, C., Cruz, M. T., Salgueiro, L. (2022). The Anti-Inflammatory Response of *Lavandula luisieri* and *Lavandula pedunculata* Essential Oils. Plants, 11(3), 370.

Development and Incorporation of a Multifunctional Essential Oil Blend into a Pullulan-Based Film for Enhanced Acne Management

Pavarino, M.[a,b,c,*], Oliveira, A.S.[b], Ferreira, E.[b,c], Ramos, A.[c], Marengo, A.[a], Luís, Â.[b,c], Palmeira-de-Oliveira, A.[b], Rubiolo, P.[a], Sgorbini, B.[a], Duarte, A.P.[b]

[a]*Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125 Turin, Italy*
[b]*RISE-Health, Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200-506, Covilhã, Portugal*
[c]*Departamento de Química, Faculdade de Ciências, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201–001 Covilhã, Portugal*

Corresponding author: m.pavarino@unito.it

Keywords *Acne vulgaris, antimicrobial activity, essential oil blend, pullulan-based films, tyrosinase inhibition.*

Objective: *Acne vulgaris* is a widespread chronic inflammatory condition of the pilosebaceous unit that is often accompanied by post-inflammatory hyperpigmentation (PIH). In this sebum-rich microenvironment, factors such as altered keratinization and inflammation contribute to follicular obstruction, creating favorable conditions for the proliferation of *Cutibacterium acnes*, a key microbial factor in acne pathogenesis. In this context, natural substances such as essential oils (EOs) are gaining increasing attention in the treatment of acne due to their combined antimicrobial and anti-pigmentation properties [1]. The present study focused on a blend of EOs—including *Litsea cubeba* (Lour.) Pers., *Pinus mugo* Turra, and *Cymbopogon winterianus* Jowitt ex Bor—developed in a previous study, which demonstrated promising tyrosinase inhibitory activity [2]. This combination was specifically designed to reduce the concentration of citral—an important active component with known anti-pigmentation activity but also listed as a potential allergen—in order to minimize the risk of skin sensitization while maintaining its functional activity. Building on this, the study further investigates the antimicrobial efficacy of the EO blend, develops a pullulan-based topical delivery system, and evaluates its physicochemical properties and retention of antimicrobial activity. **Methods:** The EOs and their blend were chemically characterized by GC-MS/FID. Tyrosinase inhibition was evaluated using an *in vitro* colorimetric assay optimized by the authors [2]. Antimicrobial activity was evaluated *in vitro* against *Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* using Clinical and Laboratory Standards Institute microdilution methods to determine the minimum inhibitory concentration (MIC). Minimum bactericidal activity (MBC) was also determined. Bioactive films were prepared by solution casting with 3% (w/v) pullulan, 15% (w/w) glycerol, and the EO blend at the highest MIC observed among the three tested microorganisms. Film characterization included measurements of grammage, thickness, mechanical, and optical properties. EO incorporation was confirmed by FTIR and DSC analyses. The wettability and surface free energy were evaluated through contact angle measurements. The antimicrobial activity of the final films was assessed via a solid diffusion assay on inoculated agar plates. **Results:** The EO blend showed significant tyrosinase inhibition and antimicrobial activity against all tested strains, exhibiting a bactericidal effect against *C. acnes* and *S. aureus* and a bacteriostatic effect on *S. epidermidis*, determined by the MBC/MIC ratio. *S. epidermidis* is a commensal species increasingly recognized for its role in modulating *C. acnes* growth and maintaining skin microbiome balance [3]. The pullulan-based film functionalized with the EO blend exhibited favorable physicochemical properties suitable for topical application and a good aesthetic appearance, supporting potential consumer acceptance. Antimicrobial assays confirmed that the EO blend's activity was maintained after incorporation into the film. **Conclusions:** This study highlights the potential of a multifunctional essential oil blend—applied in this work for both antimicrobial and tyrosinase inhibitory activity—and its successful incorporation into a biodegradable pullulan-based delivery system. The results suggest that it is a sustainable and user-friendly skin care approach targeting both the microbial and pigmentation aspects of acne. Future studies will focus on evaluating the formulation's ecotoxicity and further exploring its pigment-modulating effects using advanced *in vitro* skin tissue models.

REFERENCES

[1] Deyno S., Mtewa, A. G., Abebe, A., hymete, A., Makonnen, E., Bazira, J., Alele, P. E., (2019). Emerging Insights into the Applicability of Essential Oils in the Management of Acne Vulgaris, *Complement Ther Med*, *28*, 6395.
[2] Capetti, F., Cagliero, C., Argenziano, M., Cavalli, R., Dianzani, C., Pavarino, M., Bicchi, C., Rubiolo, P., Sgorbini, B., (2024). A New Blend of Litsea Cubeba, Pinus Mugo, and Cymbopogon Winterianus Essential Oil Active as an Anti-Tyrosinase Ingredient in Topical Formulations, *Planta Med*, *90*, 267–27.
[3] Claudel, J. P., Auffret, N., Leccia, M. T., Poli, F., Corvec, S., Dréno, B., (2019) Staphylococcus Epidermidis: A Potential New Player in the Physiopathology of Acne? *Dermatology*, *235*, 287–294.

ORAL PREZENTATION - IFEAT Young Scientist Fellowship Award

Initial Toxicological Assessment of Selected Essential Oils

Surowiak, A.K.[a,*], Arruda F. [b,c], Poręba, M.[a], Strub, D.J.[a]

[a]*Department of Biological Chemistry, Wroclaw University of Science and Technology, Wroclaw, Poland*
[b]*Institute of Agricultural and Environmental Research and Technology, University of the Azores, Angra do Heroísmo, Portugal*
[c]*Department of Biology, Faculty of Science and Technology, University of the Azores, Ponta Delgada, Portugal*

***Corresponding author:** alicja.surowiak@pwr.edu.pl*

***Keywords:** Cytotoxicity, essential oils, keratinocytes, MTS assay*

Objective: Natural complex substances (NCSs), including essential oils (EOs), are widely utilized across various sectors—such as food, cosmetics, and pharmaceuticals—due to their diverse biological properties. EOs are frequently incorporated into cosmetic formulations as active ingredients, fragrances, and agents in aromatherapy. Given their widespread use, particularly of EOs, in consumer products, it is essential to evaluate their safety profiles. The aim of this study was to assess the cytotoxicity of 15 commercial essential oils by examining their impact on the viability of immortalized human keratinocytes (HaCaT cells) and to preliminarily evaluate their general ecotoxicity toward marine fauna. **Methods**: The following commercially available EO samples were evaluated: Petitgrain Lemon – *Citrus limon* (L.) Osbeck (Spain, Bordas S.A.); Ravintsara – *Cinnamomum camphora* Ness et Eberm.; Clove Leaf EO and Clove Bud EO – *Syzygium aromaticum* (L.) Merr. & L. M. Perry; Ylang Ylang I EO – *Cananga odorata* Hook.f. & Thomson forma *genuina* (Madagascar, Jacarandas International); Hiba – *Thujopsis dolabrata* (Thunb. ex L.f.) Siebold & Zucc. (Japan); Tea Tree – *Melaleuca alternifolia* (Maiden & Betche) Cheel (China); Sandalwood – *Santalum album* L. (India, Kallin International Ltd.); Lemongrass – *Cymbopogon flexuosus* (Nees ex Steud.) W.Watson; Jasmine – *Jasminum grandiflorum* L.; Peppermint – *Mentha × piperita* L. (Egypt, A. Fakhry & Co.); Australian Blue Cypress – *Callitris intratropica* Baker & H.G.Sm. (Australia, Essentially Australia); Patchouli Oil Sumatra Iron-Free Min 32 PA – *Pogostemon cablin* (Blanco) Benth. (Indonesia, PT Van Aroma); White Oud EO – *Aetoxylon sympetalum* (Indonesia, Hermitage Oils SRL); Wild Carrot Seed – *Daucus carota* subsp. *sativus* (Macedonia, Vessel Essential Oils); and Opoponax Extra – *Commiphora erythraea* var. *glabrescens* Engl. (Somalia, Payan Bertrand S.A.). An *in vitro* cell viability assay (MTS assay) was conducted to assess HaCaT cell viability following 24-hour exposure to test substances at a concentration of 100 µg/mL. To evaluate ecotoxicity, an *in vivo* Brine Shrimp Lethality Assay (BSLA) using *Artemia salina* was performed to determine the median inhibitory concentration (IC_{50}) values. **Results:** Among the 15 tested EOs, 8 significantly reduced cell viability (by 20–31%), 1 exhibited a moderate effect (~40% reduction), and 6 showed only slight reductions in cell viability (ranging from 80–96%). In the BSLA test, IC_{50} values ranged from 34.90 to 433.10 µg/mL. Variations were observed between the *in vitro* and *in vivo* toxicity results (see Table 1).

Table 1. Results of MTS Assay and BSLA

Name	IC_{50} BSLA [µg/mL]	Cell viability at 200 µg/mL [%]	Name	IC_{50} BSLA [µg/mL]	Cell viability at 200 µg/mL [%]
Hiba	120.2	24.94	Petitgrain Lemon	363.9	30.67
Tea Tree	234.2	90.95	Australian Blue Cypress	269.2	25.80
Sandalwood	145.9	23.16	White Oud	244.0	23.32
Ylang Ylang I	128.0	24.97	Clove Bud	34.9	93.45
Lemongrass	117.8	43.48	Clove Leaf	41.3	84.07
Patchouli Oil Sumatra Iron Free Min 32 PA	133.7	21.15	Peppermint	387.8	95.90
			Oppoponax Extra	433.1	24.64
Wild Carrot Seed	268.8	92.34	Jasmine	127.2	91.64

Conclusions: This study demonstrated that commercial EOs exhibit varying levels of cytotoxicity toward human keratinocytes and general ecotoxicity toward aquatic invertebrates. In most of the cases its impossible to correlate results of BSLA assay with cytotoxicity results. These findings highlight the importance of conducting comprehensive safety assessments before incorporating EOs into consumer products, including more advanced *in vitro* and *in vivo* studies.

ACKNOWLEDGMENTS
We would like to thank representatives of the EOs industry and members of IFEAT. A Fakhry and Co., Bordas S.A., International, Essentially Australia, Kallin International Ltd., PT Van Aroma, Vessel Essential Oils and Payan Bertrand S.A., Hermitage Oils SRL for the donation of the samples of essential oils for this study.
First author is awarded by the IFEAT within the YSFA at ISEO 2025.

Thymoquinone in the Spotlight: An Essential Oil-Derived Antioxidant Targeting Lipid Peroxidation and Ferroptosis

Huang, Y.[a,*], Li, H.[b], Jin, Z.[a], Shi, L.[b], Amorati, R.[a]

[a]*Department of Chemistry "Giacomo Ciamician", University of Bologna, Via Piero Gobetti, Bologna, Italy*
[b]*State Key Laboratory of Plant Diversity and Specialty Crops, Institute of Botany, Chinese Academy of Sciences, Xiangshan street, Beijing, China*

Corresponding author: yeqin.huang2@unibo.it, huangyeqin1996@gmail.com

Keywords: *Antioxidant activity, essential oils, 1,4-cyclohexadiene derivatives, ferroptosis, lipid peroxidation, thymoquinone.*

Objective: Many essential oils (EOs) are widely used in food, cosmetics, and medicinal applications due to their notable antioxidant properties, traditionally attributed to phenolic compounds. Beyond phenolics, structurally related classes such as 1,4-cyclohexadiene derivatives have also attracted attention for their potential antioxidant activity. This study aimed to compare the antioxidant potential of representative 1,4-cyclohexadiene-derived components found in essential oils, including γ-terpinene (a monoterpene hydrocarbon abundant in *Cuminum cyminum* L. and *Petroselinum crispum* (Mill.) Fuss EOs), carvacrol and thymol (phenolic compounds predominant in *Origanum vulgare* L. and *Thymus vulgaris* L. EOs), and thymoquinone (a quinone derived from *Nigella sativa* L. seed EO), with the goal of identifying compounds with superior anti-lipid peroxidation activity and further investigating their mechanisms of action. **Methods:** Antioxidant activity was first evaluated *in vitro* using lipid oxidation models, including AAPH-induced oxidation in micelle systems and DTUN-induced oxidation in liposome systems. The most active compound, thymoquinone, was further investigated using a ferroptosis model in 3T3-L1 mouse preadipocytes treated with RSL-3. Intracellular levels of reactive oxygen species (ROS), malondialdehyde (MDA), and glutathione peroxidase (GPx) activity were measured to assess oxidative stress and membrane damage. In addition, lipid peroxidation was specifically visualized using the fluorescent probe C11-BODIPY 581/591, which enables the detection of oxidized lipids in live cells. Furthermore, proteomic and lipidomic analyses were conducted to examine changes in redox-related protein expression and lipid distribution patterns, oxidative lipid profiles, and membrane lipid remodeling. **Results:** Among the tested compounds, thymoquinone exhibited the strongest anti-lipid peroxidation activity in both chemical and cellular models. In RSL-3-treated 3T3-L1 cells, thymoquinone significantly reduced intracellular ROS and MDA levels, mitigated membrane lipid oxidation, and preserved GPx activity. Proteomic analysis revealed upregulation of key antioxidant and redox-regulating enzymes, including NAD(P)H quinone dehydrogenase 1 (NQO1), cytochrome P450 oxidoreductase (POR), superoxide dismutase (SOD1), glutathione S-transferase P1 (GSTP1), and several peroxiredoxins (PRDX1, PRDX2, PRDX6), as well as fatty acid synthase (FASN). Lipidomic analysis indicated that thymoquinone attenuated lipid peroxidation and helped preserve membrane integrity by reducing the levels of oxidative lipid markers such as LPE(O-18:0), LPE(O-16:0), and PE(16:1_20:4), while also preventing the accumulation of diacylglycerols like DG(O-22:2) and DG(O-18:0) and protecting structural components such as DG(33:0), DG(O-15:1_22:6), and ceramide species like Cer(d24:0). **Conclusions:** This study highlights thymoquinone as a potent non-phenolic antioxidant that effectively attenuates oxidative stress and ferroptosis in 3T3-L1 cells. Its protective effects are primarily mediated through the enhancement of cellular antioxidant defenses and, notably, the preservation of lipid homeostasis and suppression of lipid peroxidation—both critical in ferroptosis regulation. These findings not only expand the current understanding of the antioxidant capacity of non-phenolic essential oil constituents but also provide novel insights into lipid-targeted antioxidant mechanisms, supporting the development of essential oils as functional agents for combating lipid oxidative damage.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES
[1] Huang, Y., Ding, M., Wang, D., et al. (2024). Chemical diversity and antimelanoma potential of rosemary essential oils: Unveiling mechanistic insights through quantitative proteomics, *Industrial Crops and Products*, 215, 118652.
[2] Jin, Z., Mollica, F., Huang, Y., et al. (2024). Pro-aromatic natural terpenes as unusual "slingshot" antioxidants with promising ferroptosis inhibition activity, *Chemistry – A European Journal*, 30(67), e202403320.
[3] Daci, M., Berisha, L., Mercatante, D., Rodriguez-Estrada, M. T., Jin, Z., Huang, Y., & Amorati, R. (2024). Advancements in biosensors for lipid peroxidation and antioxidant protection in food: A critical review, *Antioxidants*, 13(12), 1484.
[4] Schneider-Stock, R., Fakhoury, I. H., Zaki, A. M., El-Baba, C. O., & Gali-Muhtasib, H. U. (2014). Thymoquinone: Fifty years of success in the battle against cancer models. *Drug Discovery Today*, 19(1), 18–30.

Fragrant Frontiers in Herbal Medicine: GC-MS/MS Analysis and Enzyme Inhibitory Activities of *Salvia albimaculata, Salvia viridis,* and *Salvia tomentosa*

Mahab, M.M.M.[a], Özer, E. B.[a,e,*], Bilge, A.[b], Yuca, H.[a], Demirci, B.[c], Karakaya, S.[d]

[a]*Department of Pharmacognosy, University of Ataturk-Faculty of Pharmacy, Erzurum, Türkiye*
[b]*Department of Pharmacognosy, University of Erzincan Binali Yıldırım-Faculty of Pharmacy, Erzincan, Türkiye*
[c]*Department of Pharmacognosy, University of Anadolu -Faculty of Pharmacy, Eskişehir, Türkiye*
[d]*Department of Pharmaceutical Botany, University of Ataturk-Faculty of Pharmacy, Erzurum, Türkiye*
[e]*Department of Pharmacognosy, University of Ondokuz Mayıs-Faculty of Pharmacy, Samsun, Türkiye*

***Corresponding author:** elifbeyza.ozer@omu.edu.tr*

***Keywords:** Enzyme Inhibitory, essential oil, Salvia temontosa, S. albimaculata, S.viridis.*

Objective: Diabetes mellitus (DM) and Alzheimer's disease (AD) are chronic disorders associated with aging and oxidative stress. AD involves cognitive decline, while type 2 DM is characterized by insulin resistance and increased oxidative damage, especially in the elderly [1]. This study aimed to comprehensively evaluate the biological activities of essential oils and methanol and water extracts—obtained via maceration—from the flowering aerial parts of *Salvia albimaculata* Hedge & Hub.-Mor., *Salvia viridis* L., and *Salvia tomentosa* Mill., collected from the Karaman region, including one endemic species. Additionally, essential oil components were analyzed via gas chromatography–mass spectrometry (GC-MS). A further aim was to explore a potential therapeutic approach for these chronic diseases. **Methods:** Essential oils were extracted by hydro-distillation using a Clevenger-type apparatus. Their chemical composition was analyzed using GC-MS/MS and identified through commercial spectral libraries (Wiley GC/MS Library, MassFinder 4.0) [2,3] and an internal spectral database ("Başer Library"). Enzyme inhibition assays were conducted on essential oils and methanol (MeOH) and water extracts targeting α-amylase, α-glucosidase, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), collagenase, and tyrosinase. Antioxidant activity was evaluated using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging assays. [1] **Results:** *S. albimaculata* essential oil exhibited the highest antioxidant activity ($ABTS^{\bullet+}$: 2.66%; $DPPH^{\bullet}$: 3.64%), though lower than standard antioxidants. For α-amylase inhibition, *S. albimaculata* oil was most effective (41.92%), approaching the efficacy of acarbose (67.87%). Among extracts, *S. tomentosa* MeOH extract showed the strongest inhibition (19.58%). Regarding α-glucosidase inhibition, *S. tomentosa* oil demonstrated the highest effect (23.71%), and its MeOH (80.30%) and water (77.82%) extracts exhibited stronger activity than acarbose (74.72%; IC_{50}: 2434 µg/mL), with IC_{50} values of 353 and 198 µg/mL, respectively. In AD-related enzyme assays, *S. viridis* oil showed the highest AChE inhibition (13.13% at 5 µg/mL), while all oils exhibited low BChE activity. Among extracts, *S. albimaculata* had the highest AChE inhibition (26.81% at 100 µg/mL), and *S. tomentosa* MeOH extract showed the strongest BChE inhibition (31.65% at 1000 µg/mL), though both were weaker than donepezil. GC-MS analysis revealed caryophyllene oxide as the major component of *S. albimaculata* oil (32.0%), with caryophyllenol II at 8.5%. Similarly, *S. tomentosa* oil was rich in caryophyllene oxide (15.8%) and caryophyllenol (4.6%). **Conclusions:** The tested *Salvia* species exhibited notable biological activities, especially enzyme inhibition relevant to DM and AD. The MeOH and water extracts of *S. tomentosa* demonstrated higher α-glucosidase inhibition than acarbose, highlighting its potential as a natural antidiabetic agent. Although other samples showed lower inhibition compared to standard drugs, their biological potential remains promising. These findings support further pharmacological investigations of these species as valuable natural resources.

ACKNOWLEDGMENTS
The authors sincerely acknowledge the support provided by Atatürk University.
Coresponding author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Aydın, B., Alkuyruk, S. B., Tekman, E., Yuca, H., Karadayı, M., Gülşahin, Y., Çeçen, Ö., Ekşi Bona, G., Demirci, B., Bona, M. & Karakaya, S. (2024). Investigating the phytochemical profile, biological potentials, morphological, and anatomical characteristics of *Cyclotrichium origanifolium* (Labill.) Manden. & Scheng. (Lamiaceae) from Turkey. *Protoplasma*, *1*-20.
[2] McLafferty F.W., Stauffer D.B., (1989). The Wiley/NBS Registry of Mass Spectral Data, J Wiley and Sons: New York.
[3] Hochmuth D. H., (2008). MassFinder 4.0, Hochmuth Scientific Consulting, Hamburg, Germany.

Exploring Nutraceutical and Therapeutic Potential of *Origanum heracleoticum* Essential Oils from Different Italian Regions

Amato, G.[a,*], De Martino, L.[a], Caputo, L.[a], Nazzaro, F.[b], Fratianni, F.[b], Gruľova, D.[c], Tkačikova, L.[d], Conforti, F.[e], De Feo, V.[a,b]

[a]*Department of Pharmacy, University of Salerno, Fisciano (SA), Italy*
[b]*Institute of Food Sciences (CNR-ISA), Avellino, Italy*
[c]*Department of Ecology, Faculty of Humanities and Natural Sciences, University of Presov, Presov, Slovakia*
[d]*Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Kosice, Slovakia*
[e]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende (CS), Italy*

***Corresponding author:** gamato@unisa.it*

***Keywords:** Antibiofilm, cytotoxicity, metabolic disorders, neurodegenerative disorders, Origanum heracleoticum.*

Objective: *Origanum* belongs to the Lamiaceae family and is characterized by great morphological and chemical diversity [1]. Species of this genus are widely used in the food industry and in traditional medicine in several countries for the treatment of various diseases [2]. The aim of this work was to clarify the complex chemotaxonomy that characterizes this genus through the evaluation of the chemical composition of five essential oils (EO) obtained from the leaves of *O. heracleoticum* L. from different locations in southern Italy (Puglia, Sardinia, Campania, and Calabria). Furthermore, a possible use of these samples in the nutraceutical field was evaluated through the evaluation of different activities. The antioxidant activity was evaluated by different assays (DPPH, FRAP, ABTS). A possible activity against enzymes involved in both metabolic disorders (α-amylase, α-glucosidase, and lipase) and neurodegenerative disorders (acetylcholinesterase, butyrylcholinesterase, and tyrosinase, evaluating both the monophenolase and diphenolase reactions) was evaluated. In addition to these, the antibiofilm activity of different bacterial strains (*Acinetobacter baumanni, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa,* and *Staphylococcus aureus)* and the cytotoxic activity on tumor (CACO2) and healthy cells (IPEC-J2) were also evaluated. **Methods:** The essential oil was obtained by hydrodistillation and analyzed by GC/MS. The antioxidant and enzymatic activities were evaluated by spectrophotometric assays. The antibiofilm activity was evaluated by the MTT and crystal violet assays. The cytotoxic activity was evaluated through the MTT assay. **Results:** EO 'Puglia' was mainly composed of thymol, showing greater antibiofilm activity against *L. monocytogenes* and *E. coli*. It also showed an increase in metabolic activity in healthy IPEC-J2 cells. The EO 'Sila' (Calabria) was mainly composed of thymol (34.24%) and carvacrol (29.28%). It was the most active EO against lipase with an IC_{50} of 10.7 μg/mL, also resulting in cytotoxicity for CACO2 tumor cells. EO 'Grimaldi' (Calabria) was the most active sample against both acetylcholinesterase (IC_{50}: 31.66 μg/mL) and tyrosinase in the diphenolase reaction (503 μg/mL). This EO was mainly composed of thymol (28.96%) and γ-terpinene (23.66%). It also showed antibacterial action against *A. baumannii,* showing a biofilm inhibitory activity higher than 50%. EO 'Sardegna' was mainly composed of carvacrol (56.98%), resulting in the most active sample against tyrosinase for the monophenolase reaction with an IC_{50} of 290.5 μg/mL. It also showed a positive effect on healthy IPEC-J2 cells by increasing their proliferation. Finally, EO 'Campania' was mainly composed of carvacrol (23.48%) and thymol (20.77%), resulting in the most active sample against butyrylcholinesterase (IC_{50}:191 μg/mL). It also showed promising activity both on lipase with an IC_{50} of 10 μg/mL and against tumor CACO2, resulting in cytotoxicity. **Conclusions:** The five EOs of *O. heracleoticum* presented different chemotypes. The results obtained can help, as much as possible, the identification of the different chemotypes present in the species *O. heracleoticum*. Furthermore, the biological activities performed suggest a possible use of this sample for the treatment of metabolic disorders such as diabetes and obesity and neurological diseases such as Alzheimer's and Parkinson's. Furthermore, these samples were toxic for CACO2 tumor cells but not for healthy IPEC-J2 cells.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES
[1] M., Statti G.A., Conforti F., (2018). *Origanum* spp.: an update of their chemical and biological profiles. *Phytochemistry Reviews,* 17, 873–888.
[2] Erenler R., Meral B., Sen O., Elmastas M., Aydin A., Eminagaoglu O., Topcu G., (2017) Bioassay-guided isolation, identification of compounds from *Origanum rotundifolium* and investigation of their antiproliferative and antioxidant activities. *Pharmaceutical Biology*, 55(1), 1646-1653.

One step ahead in the enantioselective separation of terpenes in *Citrus* essential oils by exploiting conventional and tandem chiral columns

Cucinotta, L.[a,*], Cannizzaro, F.[a], Sciarrone, D.[a], Mondello, L.[a,b]

[a]*Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Messina, Italy*
[b]*Chromaleont s.r.l., c/o, Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Messina, Italy*

****Corresponding author:*** lcucinotta@unime.it

Keywords: *Carrier gas selection, chromatographic parameters optimization, enantiomeric separation, tandem chiral phases, terpenes.*

Objective: This research aimed to evaluate the performance of nitrogen and hydrogen as carrier gases, in place of helium, in the field of chiral gas chromatography. In this field, maintaining optimal resolution is paramount, as co-elution directly impacts the accuracy of enantiomeric ratio determination and, consequently, the reliability of analytical data. To these aims, a comprehensive investigation was carried out by analyzing a bergamot essential oil on a conventional cyclodextrin (CD) stationary phase and subsequently on tandem chiral phases. **Methods**: In the first part of the research study, the 2,3-di-O-ethyl-6-O-tert-butyldimethylsilyl-β-CD stationary phase was used to compare the performance of helium, nitrogen, and hydrogen at varying linear velocities, from 10 to 60 cm/s. In the second part, tandem chiral columns were exploited by joining different segments of the 2,3-di-O-ethyl-6-O-tert-butyldimethylsilyl-β-CD and other chiral phases. **Results:** The analysis revealed distinct chromatographic profiles, underscoring the importance of selecting the appropriate carrier gas and chromatographic conditions for specific separation challenges. Notably, hydrogen demonstrated superior performance compared to helium, exhibiting higher resolution at elevated linear velocities. However, the analysis of a real sample highlighted significant limitations, including co-elution of chiral components with each other and with achiral compounds. To address these issues, the separation was optimized by joining two chiral stationary phases. The strategic arrangement of chiral selectors within this tandem phase column facilitated enhanced separation of critical enantiomeric pairs. This approach yielded significant improvements in enantiomer resolution, both amongst themselves and in relation to achiral compounds, compared to the conventional column. **Conclusions:** These findings offer insights into the design and application of tandem chiral stationary phases for the analysis of chiral components in essential oils. The ability to fine-tune separation selectivity through the strategic combination of chiral selectors, optimization of chromatographic parameters, and judicious selection of carrier gas represents a significant advancement. This comprehensive approach provides a powerful tool for tackling challenging separations and holds broad implications for the quality control and authentication of essential oils and related products.

ACKNOWLEDGMENTS
The authors thank MEGA srl, Shimadzu Corp. and Merck KGaA for their support.
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

Yield and chemical composition of essential oil from twigs of *Cryptomeria japonica* (Cupressaceae) from the Azores

Janeiro, A.[a,*], Arruda, F.[a], Wortham, T.[b], Rodrigues, T.[a], Lima, A.[a], Lima, E.[a]

[a]*Faculty of Science and Technology (FCT) and Institute of Agricultural and Environmental Research and Technology, University of the Azores, Azores, Portugal*
[b]*The Perfumery, 621 Park East Blvd. New Albany, IN, USA*

Corresponding author: alex-19961917@hotmail.com

Keywords: *Cryptomeria japonica twigs, essential oil, GC/MS analysis, sustainable circular bioeconomy, waste biomass valorization.*

Objective: *Cryptomeria japonica* (Thunb. ex L.f.) D. Don is currently the most important commercial forest tree in the Azores Archipelago (Portugal). The economical exploitation of this tree species generates large amounts of biomass residues, which can be converted into eco-friendly, high added value products, such as essential oils (EOs), with social, environmental, and economic impacts. However, the existing literature on the chemical composition of EOs from various *C. japonica* parts, such as twigs, remains scarce [1]. Thus, the present study aimed to investigate, for the first time, the yield and chemical composition of EO hydrodistillated from Azorean *C. japonica* twigs (Az–CJT). **Methods:** The aerial parts of *C. japonica* were collected in January 2022 (winter season) in Achada (37°48'51.1"N, 25°14'31.6"W), located on São Miguel Island (Azores archipelago). The fresh plant material was separated into different parts, with the twigs being the only plant part used to extract EO. After being dried at room temperature in a well-ventilated area, the twigs were hydrodistillated, using a Clevenger-type glass system, according to Lima et al. [2], with the EO yield being calculated on a dry weight (DW) basis. The EO chemical composition was ascertained by GC-FID/MS, as detailed in [2]. **Results:** The Az–CJT EO exhibited a dark yellow color, a yield of 0.33% (*v/w*, DW), and 94 identified compounds, accounting for 94% of the total EO composition. It was lowest in sesquiterpenes (3%) and richest in sesquiterpenoids (44%), mainly α-eudesmol (20%), elemol (12%), and γ-eudesmol (5%). Diterpenes (21%), mainly phyllocladene (15%), were the second major class, followed by diterpenoids (13%), mainly nezukol (11%); monoterpenes (8%), mainly α-pinene (4%); and monoterpenoids (5%), mainly terpinen-4-ol (5%). A study on CJT EO from Taiwan revealed a similar yield and a dominance of sesquiterpenoids (74%) and α-eudesmol (25%) but a remarkably lower diterpene content (<1%) [1]. In addition, among the studied Azorean *C. japonica* plant parts (leaves, foliage, cones, sawdust, and bark), the sawdust revealed the most similar results, i.e., a yield of 0.27% (v/w, DW) and a preponderance of sesquiterpenoids (67%) [2]. **Conclusions:** The obtained results will help to better characterize EOs from biomass residues of Azorean *C. japonica* and support this conifer EO industry in meeting diverse market demands.

ACKNOWLEDGMENTS
This work is supported by funds from DRCT, under the project M1.1.C/PROJ.EXPLORATÓRIOS/003/2022 – PotBioCJap. Alexandre Janeiro also acknowledges FRCT for Doctoral Research Scholarship (M3.1.a/F/029/2024).
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Ho, C.L.; Wang, E.I.; Yu, H.T.; Yu, H.M.; Su, Y.C. Compositions and antioxidant activities of essential oils of different tissues from Cryptomeria japonica D. Don. Q. J. Chin. For. 2010, 32, 63–76.
[2] Lima, A.; Arruda, F.; Wortham, T.; Janeiro, A.; Rodrigues, T.; Baptista, J.; Lima, E. Chemical Compositions and In Vitro Antioxidant Activities of the Essential Oils of Sawdust and Resin-Rich Bark from Azorean *Cryptomeria japonica* (Cupressaceae). *Antioxidants* 2024, 13, 728.

POSTER PRESENTATIONS (PP)

1-Oxo-bisabolone-rich *Pulicaria burchardii* Hutch. subsp. *burchardii* essential oil: Spectroscopic characterization and biological effects

Castagliuolo, G.[a], Pio, S.[a], Di Napoli, M.[a], Troiano, A.[a], Antonini, D.[a], Badalamenti, N.[b,c,*], Bruno, M.[b,c], Ilardi, V.[b], Varcamonti, M.[a], Zanfardino, A.[a]

[a]*Department of Biology, University of Naples, Federico II, Via Cinthia, 80126, Naples, Italy;*
[b]*Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), Uni-versity of Palermo, Viale delle Scienze, 90128, Palermo, Italy;*
[c]*NBFC-National Biodiversity Future Center, Piazza Marina 60, Palermo, 90133, Italy.*

Corresponding author: natale.badalamenti@unipa.it

Keywords: *Antimicrobial activity, antigerminative activity, bisabolone, NMR, Pulicaria burchardii, SARS-CoV-2.*

Objective: The *Pulicaria burchardii* species is known for its medicinal properties, but comprehensive studies on its biological activity are still limited. This study examines the properties of the aerial parts of *P. burchiardii* subsp. *burchardii* (PB) essential oil (EO), collected in Morocco. The focus is on its antimicrobial, anti-germinative, antioxidant, and antiviral activities. **Methods:** The fresh aerial parts of PB were collected near Chichaoua (Morocco) in May 2023 and were subjected to hydrodistillation by Clevenger apparatus. The NMR spectra were acquired with a Bruker Avance II instrument. EO was analyzed by GC-MS. Antimicrobial properties were investigated using the agar diffusion method; Cell viability assay in HaCat cell lines was determined by a rapid colorimetric assay; the MTT assay was performed to evaluate the EO cytotoxicity; and the antiviral properties were investigated against HSV-1 and SARS-CoV-2. **Results:** GC-MS and NMR analyses revealed that the EO is rich in oxygenated sesquiterpenes (72.59%), with 1-oxo-bisabolone being the predominant component (65.09%). 1-Oxo-bisabolone and 6-hydroxybisabol-2-en-1-one were isolated, and their structures were correctly determined by 2D-NMR and circular dichroism spectra. The antimicrobial activity was tested against various Gram + and Gram – bacteria, demonstrating significant inhibition of bacterial growth, particularly against *Bacillus subtilis* (MIC value of 0.6 mg/mL). For antiviral activity, the EO was tested against several pathogenic viruses, including SARS-CoV-2 and HSV-1, showing an effective broad-spectrum reduction in viral replication *in vitro*. **Conclusions:** This exploratory study revealed the therapeutic potential of *Pulicaria burchardii* Hutch. subsp. *burchardii* EO, rich in oxygenated sesquiterpenes (65.09%,) such as 1-oxo-bisabolone. The antimicrobial activity shows the highest activity against the *B. subtilis* strain (MIC 0.6 mg/mL). The antibacterial capabilities against the *Bacillus* genus were also confirmed by the excellent anti-germinating properties already at the 0.6 mg/mL dose, with a deceleration in germination confirmed for up to 90 minutes of treatment against *B. subtilis* bacteria. For antiviral activity, the essential oil was tested against some pathogenic viruses, including SARS-CoV-2 and HSV-1. In the co-treatment assay, at the highest sub-toxic concentration tested (0.31 mg/mL), the EO achieved 69% and 65% viral inhibition rates for HSV-1 and SARS-CoV-2, respectively, and it showed antiviral effects in the extracellular environment, suggesting inhibition of viral contact and fusion with the host cell. The marked antibacterial and antiviral effects suggest, in a future perspective, an in-depth analysis of 1-oxo-bisabolone to confirm all the observations made so far.

ACKNOWLEDGMENTS
Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP B73C22000790001, Project title "National Biodiversity Future Center - NBFC".
Corresponding author is awarded by the IFEAT within the ISEO 2025 – YSFA.

Therapeutic Evaluation of Spice Essential Oils for Anti-Inflammatory and Bronchodilatory Activity

Jolly, A.M.[a], Jaymurthi, P.[a,b], Kumaran, A.[a,b], Chanda, D.[b,c], Anirban, P.[b,c], Mishra, T.[a,b,*]

[a]*Agro and Food Processing and Technology Division, CSIR – National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram 695019, India*
[b]*Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India*
cBioprospection and Product Development, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, India

Corresponding Author: triptimishra@niist.res.in, triptimishra18@gmail.com

Keywords: *Essential oil, bronchodilation, GC-MS, MTT assay, respiratory distress, spices.*

Objectives: The study comprises essential oil extracted from four selected spices, namely ginger (*Zingiber officinale Roscoe*) rhizome, fennel (*Foeniculum vulgare Mill.*) seed, and cinnamon (*Cinnamomum verum J. Presl*) bark, from India. Oil was extracted by hydrodistillation using the Clevenger apparatus. Major bioactive compounds were identified using a Gas Chromatograph Mass Spectrometer (GC-MS). The anti-inflammatory and bronchodilatory activities of the essential oil were evaluated in a dose-dependent manner. **Methods:** The essential oil was analyzed by Shimadzu Gas Chromatograph Mass Spectrometer (QP2020C NX) to identify its major bioactive constituents. Cytotoxicity of the oil was evaluated in vitro using the MTT assay on RAW 264.7 macrophage cells. Anti-inflammatory potential was assessed by quantifying pro-inflammatory cytokines, including TNF-α and IL-6, using an enzyme-linked immunosorbent assay (ELISA) and a lipopolysaccharide-stimulated macrophage model [1]. In vivo studies were conducted using a murine model to evaluate the bronchodilator and airway relaxation effects of the oil in carbachol-preconstructed bronchial rings isolated from Swiss albino mice, wherein changes in airway responsiveness and respiratory parameters were monitored following administration [2]. Behavioral assessments were carried out at six predetermined time intervals up to 24 hours. The mice were observed for changes in the following parameters: general movement, fur condition, paw licking, eye changes (such as lacrimation), nostril changes (such as erythema), presence of secretions or excretions, gait, posture, and grooming behavior. **Results:** Cinnamon essential oil exhibited the most pronounced inhibitory effect on proinflammatory cytokines, IL-6 and TNF-α secretion, at a concentration of 10 µg/mL. No significant deviations from normal behavior were observed in any of the assessed parameters, indicating that the essential oil administration did not induce observable toxicity or adverse behavioral effects in mice up to 24 hours post-exposure. Cinnamaldehyde is the major component identified by GC-MS (83.70%). **Conclusion:** Our findings show that cinnamon oil has shown significant activity regarding pro- and anti-inflammatory cytokines IL-6 and TNF-α at 10 µg/mL, bronchodilation, and animal behavior study after exposure to the oil.

ACKNOWLEDGEMENTS
The authors thank the Director of CSIR-National Institute for Interdisciplinary Science and Technology in Thiruvananthapuram, India, for the facilities and encouragement received. The authors express their gratitude to the Spices Board, India, for the financial support provided for the project (GAP-129739).
Corresponding author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Bhatt D, Kumar S, Kumar P, et al (2021) Rutin Ameliorates Malaria Pathogenesis by Modulating Inflammatory Mechanism: An In-Vitro and In-Vivo Study.
[2] Secher T, Rodrigues Coelho F, Noulin N, et al (2012) Enhancement of methacholine-evoked tracheal contraction induced by bacterial lipopolysaccharides depends on epithelium and tumor necrosis factor. Wiley Online Library 2012:1–10.

Comparative characteristics of the effect of modification of the structure of lily of the valley alcohol on sensory properties and skin cell toxicity

Baberowska, K.[a,*], Surowiak, A.K.[a], Živković Stošić, M.Z.[b], Radulović, N.S.[b], Strub, D.J.[a]

[a]*Department of Chemical Biology and Bioimaging, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, Wrocław, Poland*
[b]*Department of Chemistry, University of Niš, Višegradska 33, Niš, Serbia*

***Corresponding author:** kinga.baberowska@pwr.edu.pl*

Keywords: *Cell viability, fragrance compounds, keratinocytes, lily of the valley alcohol derivatives, pro-inflammatory potential.*

Objective: Fragrance compounds inspired by the scent of lily of the valley are highly valued in the perfumery industry. However, many traditionally used substances have come under scrutiny due to concerns regarding their potential toxicity, allergenic effects, and environmental impact. These challenges have driven demand for novel compounds that retain desirable olfactory characteristics while offering improved toxicological profiles [1,2]. The objective of this study was to synthesize new fragrance compounds - specifically, derivatives of lily alcohol - with the aim of developing safer and more stable alternatives to currently used synthetic aromatic substances. The research aimed to integrate fragrance chemistry and skin toxicology, providing insights into structure-scent and structure-activity relationships of new compounds. **Methods**: The synthesis of the new fragrance compounds was conducted in three stages: (1) synthesis of acid derivatives, (2) esterification, and (3) subsequent reduction of the resulting esters to the corresponding alcohols. The biological effects of the newly synthesized substances, along with those of commercially available lily-of-the-valley fragrance compounds, were evaluated at concentrations ranging from 200 to 800 μM using a human keratinocyte cell line (HaCaT). Cell viability was assessed using the MTS assay, which measures cellular metabolic activity. The pro-inflammatory potential of the compounds was evaluated by quantifying levels of the inflammatory cytokine IL-1β, reflecting the cellular inflammatory response to the tested substances. **Results:** Preliminary data on keratinocyte viability indicated that the newly synthesized compounds, with herbal and floral scent profiles, exhibited similar effects to those of commercially available substances within the tested concentration range. More detailed results regarding cell viability and pro-inflammatory responses will be presented. **Conclusions:** Preliminary toxicological evaluations using human keratinocyte cells demonstrated that the new compounds maintained comparable cell viability to existing commercial lily-of-the-valley fragrances at the tested concentrations. Further studies are required to confirm their toxicological and olfactory profiles and to better understand their potential for safe application in fragrance formulations.

ACKNOWLEDGMENTS

The work was supported by the project Minigrants for doctoral students of the Wrocław University of Science and Technology.
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Kraft P. *et al.,* (2000). Odds and Trends: Recent Developments in the Chemistry of Odorants, *Angewandte Chemie International Edition,* 39, 2980-3010.
[2] Elterlein F. *et al.,* (2024). Sniffing Out the Sustainable Future: The Renewability Revolution in Fragrance Chemistry, *Chemistry - A European Journal*, 30, e202400006.

Chemical Diversity and Antibacterial Potency of Cupressaceae Essential Oils: Machine Learning Insights into Component Synergy

Huang, Y.[b,*], Wang, G.[a], Amorati, R.[b], Li, H.[a], Shi, L.[a]

[a]*State Key Laboratory of Plant Diversity and Specialty Crops, Institute of Botany, Chinese Academy of Sciences, Xiangshan street, Beijing, China*
[b]*Department of Chemistry "Giacomo Ciamician", University of Bologna, Via Piero Gobetti, Bologna, Italy*

***Corresponding author:** yeqin.huang2@unibo.it, huangyeqin1996@gmail.com*

***Keywords:** Antibacterial activity, chemical composition, Cupressaceae essential oils, machine learning, seasonal variation, synergistic interaction.*

Objective: This study aimed to investigate the chemical composition and antibacterial potential of Cupressaceae essential oils (CEOs) derived from 11 species—*Calocedrus macrolepis, Chamaecyparis hginsii*, *C. obtusa*, *Cupressus funebris*, *Platycladus orientalis*, *Thuja occidentalis*, *T. sutchuenensis*, *Thujopsis dolabrata*, *Juniperus chinensis*, *J. rigida*, and *J. sabina*—collected from China (Beijing, Yunnan, Guizhou) during spring and autumn. Apical branches (stems and leaves) were harvested, shade-dried, and subjected to hydro-distillation. The study further aimed to (i) identify seasonal and chemotypic variation in CEO composition, (ii) evaluate their antibacterial activity against *Escherichia coli* CMCC 44113 and *Staphylococcus aureus* ATCC 33591, and (iii) predict and validate synergistic interactions among CEO constituents using machine learning and molecular docking. **Methods**: CEOs were isolated via hydro-distillation and chemically profiled by GC–MS. Antibacterial activity was assessed through disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Structural disruption was visualized by SEM, while protein, nucleic acid, and ATP leakage were quantified spectrophotometrically. A chemical–bioactivity dataset was used to build and evaluate 12 machine learning models with 10-fold cross-validation. SHAP values and interaction matrices were used to interpret feature importance and synergy. Key combinations were experimentally validated using checkerboard assays. Major components were docked against nine bacterial protein targets using AutoDock Vina. **Results:** The 22 CEOs exhibited four distinct chemotypes (α-pinene, sabinene, thujone, and bornyl acetate types) with marked seasonal differences. Spring-harvested CEOs showed higher yields and stronger antibacterial effects, especially against *E. coli*. *C. obtusa* and *T. occidentalis* EOs exhibited the largest inhibition zones and lowest MICs. Among major components, thujone had the strongest activity, followed by terpinen-4-ol. Machine learning (notably XGBoost and CatBoost) identified thujone and terpinen-4-ol as top contributors to antibacterial activity. SHAP interaction analysis predicted synergism between these two compounds, confirmed experimentally. Molecular docking supported multi-target binding of thujone and terpinen-4-ol, especially against *E. coli* GyrB, MurA, and FabI. **Conclusions:** Seasonal and genetic factors strongly influence the chemical profile and antibacterial efficacy of CEOs. Spring-harvested thujone-type CEOs exhibited the greatest activity against *E. coli*, linked to thujone content and membrane-disruptive effects. Machine learning enabled the identification and prediction of synergistic interactions, notably between thujone and terpinen-4-ol. These findings underscore the potential of CEOs as natural antibacterial agents and highlight machine learning as a powerful tool for synergy prediction in essential oil research.

Suppression of bacterial growth with lavender essential oils (*Lavandula angustifolia and Lavandula latifolia*)

Džudžević-Čančar, H.[a,*], Alispahić, A.[a], Tursunović, A.[b], Dedić, A.[a], Imamović, B.[c], Hamidović, S.[c], Uzunović, A.[d]

[a]*Department of Chemistry in Pharmacy, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[b]*Apotheke Walch, Germesheim, Germany*
[c]*Department of Plant Protection and Food Products, University of Sarajevo, Faculty of Agriculture and Food, Sarajevo, Bosnia and Herzegovina*
[d]*Agency for Medicinal Products and Medical Devices of Bosnia and Herzegovina*

***Corresponding author:** hurija.dzudzevic-cancar@ffsa.unsa.ba*

Keywords: *Essential oils, Escherichia coli, inhibitory activity, Lavandula angustifolia, Lavandula latifolia, Salmonella enteritidis.*

Objective The increasing use of chemical preservatives and antibiotics in food production, transportation, and processing has contributed to the emergence of resistant bacterial strains, particularly within the family Enterobacteriaceae, posing a significant threat to public health. As a result, there is growing interest in safe and effective natural alternatives. This study aimed to investigate the in vitro inhibitory activity of essential oils derived from *Lavandula angustifolia Mill*. and *Lavandula latifolia Medik.* (Lamiaceae), as well as the hydrolat of *L. angustifolia*, against food-derived strains of *Escherichia coli* and *Salmonella enterica subsp. enterica serovar Enteritidis*. **Methods:** Fresh flowers of *L. angustifolia* and *L. latifolia* were collected in June 2025 in Sarajevo, Bosnia and Herzegovina. Essential oils were obtained by hydrodistillation using a Clevenger-type apparatus, while the hydrolat of *L. angustifolia* was collected as a by-product during the same distillation process. The antimicrobial activity was evaluated using the disk diffusion method according to CLSI guidelines (CLSI M02-A12, 2015), with necessary adjustments for testing natural extracts. Bacterial test strains (*E. coli* and *S. enteritidis*) were isolated from food and cultured to a concentration of 1×10^8 CFU/mL (0.5 McFarland). Mueller-Hinton agar plates were inoculated using a sterile swab, and sterile 6 mm paper disks were impregnated with 20 µL of each tested substance. A standard antibiotic disk (Hemomycin) served as a positive control. Plates were incubated at 37 °C for 24 hours. Inhibition zones were measured in millimeters. All tests were performed in duplicate. **Results:** The essential oil of *L. angustifolia* demonstrated the strongest antimicrobial activity, with inhibition zones of 27.5 mm against E. coli and 22.5 mm against *Salmonella enteritidis*. The essential oil of *L. latifolia* showed moderate inhibition of both test organisms (21.5 mm). The hydrolat of *L. angustifolia* did not show any antibacterial effect (0 mm). These results indicate that the composition and origin of the essential oils significantly influence their antimicrobial potential. **Conclusion:** Essential oils of lavender, particularly from *L. angustifolia*, exhibit promising antimicrobial activity against Gram-negative foodborne pathogens such as *E. coli* and *S. enteritidis*. Hydrolat, however, did not exhibit measurable inhibition under the tested conditions. These findings support further investigation into the use of lavender essential oils as potential natural antimicrobial agents for food safety applications.

Inhibition of *Escherichia coli* and *Salmonella enteritidis* growth by essential oils of Caraway (*Carum carvi L.*) and Fennel (*Foeniculum vulgare Mill.*)

Imamović, B.[a], Turković, S.[b], Turković, I.[c], Hamidović, S.[a,*]

[a]*Department of Plant Protection and Food Products, University of Sarajevo, Faculty of Agriculture and Food, Bosnia and Herzegovina*
[b]*"Health Insurance Institute of Sarajevo Canton", Sarajevo, Bosnia and Herzegovina*
[c]*Faculty of Medicine, University of Sarajevo, Bosnia and Herzegovina*

***Corresponding author:** s.hamidovic@ppf.unsa.ba*

***Keywords:** Antibacterial activity, Carum carvi, essential oils, Escherichia coli, Foeniculum vulgare, Salmonella enteritidis.*

Objective: *Escherichia coli* and *Salmonella enterica subsp. enterica serovar Enteritidis* are among the most common foodborne pathogens, particularly in fresh or undercooked food. Due to increasing resistance to conventional antibiotics, numerous studies have focused on identifying plant-based antimicrobials. This study aimed to evaluate the inhibitory effect of essential oils derived from caraway (*Carum carvi L.,* Apiaceae) and fennel (*Foeniculum vulgare Mill.,* Apiaceae) on the growth of *E. coli* and *S. enteritidis* under in vitro conditions. **Methods:** Essential oils were extracted by hydrodistillation using a Clevenger apparatus from fresh leaves of the respective plant species collected in Bosnia and Herzegovina. Antibacterial activity was assessed by a modified Kirby-Bauer disk diffusion method (Bauer et al., 1959). Sterile paper disks (6 mm) were impregnated with essential oils and placed on Mueller-Hinton agar inoculated with standardized bacterial suspensions (0.5 McFarland ≈ 1×10^8 CFU/mL). Inhibition zones were measured in millimeters after 24 hours of incubation at 37 °C. **Results:** The essential oil of fennel demonstrated a stronger inhibitory effect on both bacterial species compared to caraway oil. The highest inhibition was observed with fennel oil against *S. enteritidis* (28.6 mm) and *E. coli* (26.3 mm). Caraway oil showed a moderate effect, with inhibition zones of 25.2 mm for *S. enteritidis* and 20.6 mm for *E. coli*. **Conclusions:** Compared to the EUCAST reference inhibition zone for gentamicin (15 mm), both essential oils exhibited significantly higher antibacterial activity against the tested strains. These results suggest that fennel and caraway essential oils could serve as potent natural alternatives to conventional antibiotics in controlling foodborne pathogens.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

A novel *cut and sew* procedure for the natural reconstitution of essential oils prior to biological assays

Cucinotta, L.[a,*], Pavarino, M.[b], Cannizzaro, F.[a], Sgorbini, B.[b], Rubiolo, P.[b], Sciarrone, D.[a], Mondello, L.[a,c]

[a]*Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Viale G. Palatucci snc 98168, Messina, Italy*
[b]*Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125 Turin, Italy*
[c]*Chromaleont s.r.l., c/o, Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Viale G. Palatucci snc 98168, Messina, Italy*

***Corresponding author:** lcucinotta@unime.it

Keywords: *Biological assays, bioguided fractionation, menthol, preparative gas chromatography.*

Objective: Menthol is the most representative molecule in *Mentha × piperita* L. essential oils (EOs), accounting for between 30 and 55% of the entire chemical composition. A literature review by Kamatou *et al.* extensively described its bioactivity, reporting more than ten different biological properties [1]. Of course, the presence of menthol plays a prominent role in the resulting biological activity of the entire EO. In this study, aiming to expand the knowledge on the minor peppermint EO constituents, by minimizing menthol's effect, a novel analytical approach was developed, aiming to remove menthol from the oxygenated fraction. In a second step, the EO, menthol, the entire oxygenated fraction, and the oxygenated collection devoid of menthol were evaluated in terms of biological activity. **Methods:** GC-MS and GC-FID analyses were carried out to evaluate *Mentha piperita* × L. essential oil volatile composition. Multidimensional preparative gas chromatography was exploited to guarantee the isolation of target fractions from the essential oil, while enantioselective multidimensional gas chromatography was used to evaluate the preservation of the enantiomeric ratios from the essential oil to the fraction collected. **Results:** Multidimensional preparative gas chromatography, operated by means of Dean's switch transfer devices, efficiently guaranteed the collection of the oxygenated terpenes, drastically reducing menthol and hydrocarbons' content. Moreover, the chiral GC analyses confirmed the preservation of the enantiomeric excesses of the terpenols of interest from the EO until the collections, representing a consistent step ahead with respect to common practices of standards' addition. Finally, biological tests revealed a synergistic/complementary effect between menthol and the oxygenated fraction devoid of the latter. **Conclusions:** The isolation of distinct sub-fractions of the sample facilitated the successful elucidation of the inhibitory effect attributed to each fraction, circumventing the challenges associated with the conventional use of standard compounds to reconstitute the fraction to be biologically tested. Specifically, while comparable inhibitory activity was observed for both the total essential oil and the entire oxygenated fraction, approximately half of this activity was attributed to pure menthol and the oxygenated fraction devoid of menthol. These findings significantly advance the current understanding of the contribution of individual essential oil components to the overall biological effect.

ACKNOWLEDGMENTS
The project was funded by the Italian Ministry for the University and Research (MUR) with a "PRIN: Progetti di ricerca di rilevante interesse nazionale Bando 2022" Project n. 2022359CZA "Advanced analytical characterization and synergic evaluation of volatile terpenes of selected β-caryophyllene containing spices for diabetes treatment". The authors thank Shimadzu Corp. and Merck KGaA for their support.

REFERENCES
[1] Kamatou, G.P.P., Vermaak, I., Viljoen, A.M., Lawrence, B.M., (2013). Menthol: A simple monoterpene with remarkable biological properties. *Phytochemistry*, 96, 15–25.

Chemical characterization and evaluation of antimicrobial/antibiofilm potential of *Thuja occidentalis* and *Myrtus communis L.* essential oils against diverse multidrug-resistant clinical pathogens

Dănilă, E.[a,*], Marinas, I.C.[b], Gaboreanu, M.D.[b], Marin, M.M.[c], Kaya, D.A.[d], Kaya, M.A.[a]

[a]*INCDTP - Leather and Footwear Research Institute, Collagen Department, Bucharest, Romania*
[b]*The Research Institute of the University of Bucharest, ICUB, Bucharest, Romania*
[c]*Politehnica University of Bucharest, Bucharest, Romania*
[d]*Faculty of Agriculture, Hatay Mustafa Kemal University, Antakya, Turkey*

***Corresponding author:** elena.danila23@yahoo.com*

***Keywords:** Antimicrobial and antibiofilm activity, essential oils (EOs), Myrtus communis L., Thuja occidentalis.*

Objective: Essential oils (EOs) are complex mixtures of volatile compounds, including terpenoids, oxygenated terpenes, sesquiterpenes, and hydrocarbons, which determine their distinct flavors, purity, and therapeutic properties. They not only contribute to the fragrance but also serve antibacterial and preservative roles [1]. An illustrative example is Thuja (*Thuja occidentalis*) essential oil, renowned for its positive effects on the skin in conditions like acne, psoriasis, and eczema. It can contribute to revitalizing tired and congested skin by promoting a healthy microbial balance. Furthermore, specialized literature has demonstrated that the major component of thuja essential oil, thujone, possesses the ability to inhibit metastasis in melanoma [2]. The myrtus (*Myrtus communis L.*) is reported to be used in the treatment of various disorders such as urinary tract infections, digestive problems, bronchitis, sinusitis, dry cough, neurological issues (epilepsy), hemorrhoids, pyorrhea, rheumatic pain, swelling, diarrhea, dysentery, hemorrhoids, and large wounds. Researchers have discussed its antimicrobial, antioxidant, anti-diabetic, analgesic, pesticide, hepatoprotective, and antigenotoxic effects. Additionally, it is used for the treatment of rheumatism, inflammation, bacterial infections, edema, spasms, depression, fungal infections, blood sugar, cough, chest pain, and general pain [3]. This study aimed to evaluate the antimicrobial and antibiofilm activity of two EOs—*Thuja occidentalis* and *Myrtus communis L.*—used alone or in combination, as potential natural alternatives to conventional antibiotics. **Methods:** The EOs were extracted via hydrodistillation and microwave-assisted extraction (for Thuja, from both fresh and dried leaves/cones) and analyzed by GC–MS. The antibacterial and antibiofilm effects of these EOs were tested against clinical strains of *S. epidermidis, S. aureus, E. coli, P. aeruginosa, C. albicans*, and *C. parapsilosis*. **Results:** The primary components of Thuja EOs included α-pinene, β-terpinene, α-thujone, β-thujone, α-phellandrene, terpinyl acetate, and bornyl acetate, while Myrtus EOs predominantly contained α-pinene, limonene, linalool, and 1,8-cineole. Results for antimicrobial activity indicated that it varied depending on chemical composition and the type of microorganism tested. Myrtle EO exhibited broad-spectrum activity, effectively inhibiting cell growth and adhesion in Gram-positive bacteria (*S. aureus, S. epidermidis*), Gram-negative bacteria (*E. coli*), and yeasts (*C. albicans, C. parapsilosis*). Thuja EO showed selective efficacy, with pronounced effects against Gram-positive bacteria and yeasts. **Conclusion**: These findings suggest that both EOs, particularly myrtle, hold promise as natural antimicrobial agents for preventing infections or biofilm formation on tissues and medical devices.

ACKNOWLEDGMENTS
This research was funded by Ministry of Research, Innovation and Digitalization, grant number 26PED from 08/01/2025 (PN-IV-P7-7.1-PED-2024-2326).
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Vella, F. M., Cautela, D., Laratta, B., (2021), Determination of Antioxidant Activity and Sun Protection factor of Commercial Essential Oils, *Biology and Life Sciences Forum*, 6, 96.
[2] Caruntu, S., Ciceu, A., Olah, N. K., Don, I., Hermenean, A., Cotoraci, C., (2020), *Thuja occidentalis L.* (Cupressaceae): Ethnobotany, Phytochemistry and Biological Activity, *Molecules,* 25, 5416.
[3] Sümbül, S., Ahmad, A.M., Asif, M. and Akhtar, M., (2011), *Myrtus communis* Linn. - A review, *Indian Journal of Natural Products and Resources*, 2(4), 395-402.

Chemical Diversity and Bioactivity of *Knema* (Myristicaceae) Essential Oils: New Insights into Enzyme Inhibition

Salihu, A.S.[a,b], Hakimi Wan Salleh, W.M.N.[a,*]

[a]*Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia*
[b]*Department of Pure and Industrial Chemistry, Faculty of Natural and Applied Science, Umaru Musa Yar'adua University, Katsina, Nigeria*

Corresponding author: wmnhakimi@fsmt.upsi.edu.my

Keywords: *Enzyme inhibition, K. hookeriana, K. furfuracea, K. intermedia, K. malayana, molecular docking.*

Objective: This study aimed to evaluate the chemical composition and enzyme inhibitory activities of essential oils derived from four Malaysian Knema species (Myristicaceae): *Knema intermedia* Warb.*, K. furfuracea* Warb.*, K. hookeriana* Warb.*, and K. malayana* Warb*.* The genus *Knema* are known for their extensive use in traditional medicine across Southeast Asia. *K. intermedia* is used as a blood tonic and anticancer agent; *K. hookeriana* leaves are applied as a stomach remedy; *K. furfuracea* bark and twigs are used for treating sores and inflammation; and *K. malayana* leaves are used for fever, jaundice, and spleen issues. These traditional applications provide a pharmacological foundation supporting the investigation of Knema essential oils as potential enzyme inhibitors and therapeutic agents. This research also sought to investigate their potential as natural sources of tyrosinase and acetylcholinesterase inhibitors through in vitro assays and molecular docking analyses. **Methods:** Fresh leaves were collected from Malaysian rainforests, and essential oils were extracted via hydrodistillation using a Clevenger-type apparatus. The oils were subsequently analyzed using GC-MS and GC-FID systems equipped with HP-5MS columns. Chemical constituents were identified by comparing their mass spectra and retention indices with those in the NIST and Wiley libraries. Anti-tyrosinase activity was evaluated using mushroom tyrosinase (Sigma-Aldrich), while acetylcholinesterase (AChE) inhibitory activity was determined using Ellman's method. In silico molecular docking was conducted using AutoDock Vina 1.1.2, targeting tyrosinase (PDB ID: 2Y9X) and AChE (PDB ID: 1C2B). Ligand-enzyme interactions were further visualized using Discovery Studio. **Results:** The essential oil of *K. intermedia* (37 constituents, 97.3%) was dominated by τ-muurolol (20.1%) and α-copaene (14.4%), exhibiting notable tyrosinase inhibitory activity (IC_{50} = 70.2 µg/mL). *K. furfuracea* oil (31 constituents, 96.0%) was characterized by high levels of bicyclogermacrene (23.1%) and δ-cadinene (17.2%), with an IC_{50} of 80.3 µg/mL. The oil of *K. hookeriana*, rich in β-caryophyllene (26.2%) and germacrene D (12.5%), showed AChE inhibition with an IC_{50} value of 70.5 µg/mL. *K. malayana* oil (38 constituents, 98.9%) was dominated by δ-cadinene (20.2%) and α-cadinol (13.7%), yielding an IC_{50} of 85.3 µg/mL. Molecular docking studies revealed binding energies ranging from −6.7 to −8.3 kcal/mol, with active site interactions involving key residues such as His263 and Phe264. **Conclusions:** All four *Knema* species demonstrated distinct essential oil profiles and moderate enzyme inhibitory activities. The integrated in vitro and in silico results highlight their potential as promising candidates for the development of cosmetic depigmenting agents and neuroprotective therapeutics.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

Volatile Clues to Healing: Antimicrobial and Essential Oil Composition of *Tamarix smyrnensis* Bunge (Tamaricaceae)

Karakaş, Ş.[a,*], Göger, G.[b], Demirci, B.[c], Karakaya, S.[d]

[a]*Department of Pharmacognosy in Pharmacy, University of Atatürk-Faculty of Pharmacy, Erzurum, Türkiye*
[b]*Department of Pharmacognosy in Pharmacy, University of Afyonkarahisar Health Sciences-Faculty of Pharmacy, Afyonkarahisar, Türkiye*
[c]*Department of Pharmacognosy in Pharmacy, University of Anadolu-Faculty of Pharmacy, Eskişehir, Türkiye*
[b]*Department of Pharmaceutical Botany in Pharmacy, University of Atatürk-Faculty of Pharmacy, Erzurum, Türkiye*

***Corresponding author:** seydakrks4@gmail.com*

***Keywords:** Antimicrobial activity, essential oil, GC-MS/MS, Tamarix smyrnensis.*

Objective: *Tamarix smyrnensis* (Tamaricaceae) is a traditionally valued medicinal plant, commonly used in the treatment of gastrointestinal disorders, hemorrhages, rheumatism, and fever, and as a natural diuretic [1-3]. The antimicrobial activity of the essential oil (EO) was evaluated by determining its minimum inhibitory concentration (MIC) against selected microbial strains. In parallel, the chemical composition of the essential oil was thoroughly analyzed using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS), allowing for the identification and quantification of its major and minor constituents. **Methods:** EOs and various solvent extracts—including hexane, dichloromethane, ethyl acetate, butanol, methanol, lyophilized, and aqueous extracts—were obtained from the aerial parts and flowers of the plant. Antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC), and the essential oil composition was characterized using GC-MS/MS [4]. **Results:** GC-MS/MS analysis of the EOs obtained from *T. smyrnensis* revealed distinct chemical profiles between pink- and white-flowered specimens, as well as between flower and aerial parts. In the pink-flowered flowers, the major volatile constituents were hexahydrofarnesyl acetone (16.6%), hexadecanoic acid (12.9%), dodecanoic acid (9.6%), and farnesyl acetone (7.1%). In contrast, the aerial parts of the same plant were characterized by a high abundance of nonadecane (34.5%), heneicosane (22.5%), and citronellal (10.7%). For the white-flowered flowers, the EO composition was similarly dominated by hexahydrofarnesyl acetone (22.4%), hexadecanoic acid (13.8%), and dodecanoic acid (9.6%). However, the aerial parts showed a different profile, with hexadecane (15.1%), octadecane (12.2%), nonadecane (12.1%), and eicosane (8.2%) being the primary constituents. These results indicated that both flower color and plant part significantly influence the EO composition of *T. smyrnensis*, particularly in terms of saturated hydrocarbons, fatty acids, and aliphatic ketones. Antimicrobial assays revealed that the extracts of *Tamarix smyrnensis* exhibited notable antibacterial and antifungal activities. The extracts demonstrated antibacterial effects with minimum inhibitory concentration (MIC) values ranging from 156.25 to 1250 µg/mL. Notably, strong anticandidal activity was observed against *Candida tropicalis*, with MIC values ranging from 39.06 to 2500 µg/mL. Additionally, the EO obtained from the pink-flowered flowers exhibited potent antifungal activity with an MIC value of 78.125 µg/mL. EOs derived from the aerial parts and flowers of both pink- and white-flowered specimens showed antifungal activity with MIC values ranging between 625 and 2500 µg/mL. These findings highlighted the potential of *T. smyrnensis*, particularly its flower-derived EOs, as a natural source of antifungal agents, especially effective against *Candida* species. **Conclusions:** The phytochemical profile and antimicrobial activity of *T. smyrnensis* vary according to flower color and the plant part analyzed. Key compounds identified in the EOs, including hexahydrofarnesyl acetone, hexadecanoic acid, and citronellal, exhibited significant biological activity against bacterial and fungal pathogens. Overall, the rich phytochemical composition and antimicrobial potential of *T. smyrnensis* provided a scientific basis for its traditional use in treating infections, inflammation, and gastrointestinal disorders. Furthermore, these findings offer a solid foundation for future research aimed at developing natural antifungal agents, particularly against drug-resistant *Candid*a species.

ACKNOWLEDGMENTS
The authors sincerely acknowledge the support provided by Atatürk University (TDK-2024-13908).
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES
[1] Baytop, T. (1984). *Türkiyede bitkiler ile tedavi (geçmişte ve bugün)* (No. 3255). İstanbul Üniversitesi.
[2] Khan, A., et al. (2016)."Ethnopharmacological review of *Tamarix* species and their medicinal applications." *Journal of Ethnopharmacology*, 182, 202-217.
[3] Sharifi-Rad, M., et al. (2017)."Traditional uses, phytochemistry and pharmacology of Tamarix spp.: A review." *Phytotherapy Research,* 31(3), 300-312.
[4] Karakaya, S., Yuca, H., Yılmaz, G., Aydın, B., Tekman, E., Ekşi, G., Bona, M., Göger, G., Karadayı, M., Gülşahin, Y., Öztürk, G., Demirci, B. & Güvenalp, Z. (2023). Phytochemical screening, biological evaluation, anatomical, and morphological investigation of *Ferula tingitana* L. (Apiaceae). *Protoplasma*, *260*(6), 1581-1601.

Aroma Fingerprinting and Chemometric Classification of 26 Finger Lime *(Citrus australasica)* Cultivars Grown in Japan

Touyama, A.[a,*], Kuraya, E.[b]

[a]*Okinawa Flora Co., Ltd., Room 204, 1-19-24 Onaka, Nago City, Okinawa, Japan*
[b]*National Institute of Technology, Okinawa College, 905 Henoko, Nago City, Okinawa, Japan*

***Corresponding author:** touyama@flora.okinawa*

***Keywords:** Aroma profiling, Citrus australasica, cultivar classification, finger lime.*

Objective: Finger lime (*Citrus australasica*) is a citrus fruit native to Australia that is known for its diversity, with over 250 cultivars being identified to date. It exhibits a wide range of shapes, peel colors, and juice vesicle colors. Notable features include caviar-like vesicles that release a strong aroma only when crushed in the mouth, contributing to the fruit's popularity in high-end cuisine. In recent years, finger limes have been cultivated in various countries worldwide. Although their aromatic characteristics have been studied [1], limited information is available regarding finger limes grown and harvested in Japan. Our group previously investigated the aroma of Okinawa-grown finger limes, which exhibit diverse peel and vesicle colors, along with unique aromatic profiles. This study analyzed the aroma characteristics of 26 finger lime cultivars grown in Japan to identify the distinguishing traits among cultivars and contribute to future cultivar identification using deep learning models trained on aromatic compound data. **Methods:** Twenty-five finger lime varieties were obtained from Finger Lime Japan (Nagano, Japan), a trusted supplier of verified cultivars. The fruits were harvested in October 2024. One additional variety was grown from certified nursery seedlings and harvested after three years at our experimental farm in Okinawa. For aroma analysis, the juice vesicles were removed, and the peel was cut into 5 mm pieces and placed in vials. The samples were then sonicated in *n*-hexane for 10 min to extract the volatile compounds. Aroma components were analyzed using gas chromatography–mass spectrometry (GC-MS). Volatile compounds were identified by comparing their retention indices and mass spectra with the NIST05 library. Linear retention indices were calculated using a homologous series of *n*-alkanes under identical chromatographic conditions. The identified compounds were subjected to two-dimensional hierarchical cluster analysis using Ward's method to create heatmaps and fingerprints. Leave-One-Out Cross-Validation (LOOCV) was performed using a Random Forest classification model. All analyses were conducted using the Python Seaborn and Scikit-learn libraries. **Results**: GC-MS analysis of the aroma components of 26 finger lime cultivars with well-defined cultivars revealed that the main aroma components varied widely among the cultivars. 55 aroma components were subjected to LOOCV using a random forest classification model. The weighted average F1-score obtained a classification accuracy of ~99.3%, indicating that the aroma components discriminate varieties with a high accuracy. The Durham Emerald and Collet samples exhibited similar aromatic characteristics, making it difficult to distinguish between varieties based on sample size. However, fingerprinting analysis using a combination of two-dimensional cluster analysis and heat mapping revealed that *cis*-2-hexen-1-ol and *trans*-dihydrocarvone were key components in determining varietal differences**. Conclusions:** Recent reports on the aroma characteristics of finger lime fruit did not encompass differences in aroma characteristics among domestic cultivars. This study is the first comprehensive classification of the aroma diversity of 26 finger lime (*C. australasica*) cultivars grown in Japan. In this study, aroma characteristics of various varieties were investigated in detail, and deep learning analysis was conducted to identify characteristic aroma components. The performance of the variety model was evaluated using LOOCV on the fragrance components of 26 well-defined finger lime varieties, and it was found that the varieties could be inferred from the fragrance characteristics with high classification accuracy.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Toyomi Nakadaira of Finger Lime Japan for providing finger lime fruit specimens. First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] D'Auria M., Racioppi R., (2022), Volatile organic compounds from *Citrus australasica* growing in Basilicata (Southern Italy), *Nat. Prod. Res.* 25, 1-4. doi: 10.1080/14786419.2022.2063857.

Design of a workflow for the extraction and characterisation of essential oils and bioactive compounds to valorise *Citrus* industry biomass

Irrera, E.[a,*], Russo, M.[a], Giuliano, C.[b], Chirico, F. D.[b], Dugo, P.[a,c], Mondello, L.[a,c]

[a]*Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Viale G. Palatucci snc 98168 – Messina, Italy*
[b]*Agrumaria Reggina S.r.l., Via Nazionale, 167, 89135 – Reggio Calabria, Italy*
[c]*Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Viale G. Palatucci snc 98168 – Messina, Italy*

***Corresponding author:** elisa.irrera@studenti.unime.it

Keywords: *Aroma, bioactive compounds, Citrus by-products, circular economy, essential oils, functional foods.*

Objective: The agro-food industry is responsible for the generation of a consistent amount of waste, the correct disposal of which is of paramount importance in order to mitigate the industry's significant environmental impact. It is imperative to explore the potential for re-evaluating the waste produced, with a view to transforming it into value-added goods, thus moving from a linear approach to a circular model. The objective of this project was to extract the residual aroma and bioactive molecules (phenols) that were still present in *citrus* biomass. These could then be used to fortify the final products, such as *citrus* juices. In particular, four by-products were considered in this study, namely peels and three subsequent pomaces derived from sweet orange (*Citrus sinensis (L.) Osbeck*) juice production. In light of the industry's requirements, a comprehensive evaluation of green extraction methodologies was conducted, encompassing the characterization of both the essential oils and the extracts obtained. **Methods**: The extraction of the essential oil from the biomass was operated using two different techniques, hydro-distillation (HD) and microwave-assisted extraction (MAE). The experimental conditions were optimized by investigating two factors: the ratio of the matrix to the solvent and the power set in MAE. Subsequently, the distilled oils were analyzed by means of gas chromatography coupled with a flame ionization detector (FID) and a single quadrupole mass spectrometer (qMS) to provide a comprehensive qualitative and quantitative characterization of the volatile fraction. In order to assess the composition of the non-volatile fraction, the samples were subjected to solid-liquid extraction, and the extracts obtained were analyzed by liquid chromatography coupled with a photodiode array (PDA/MS) detector. **Results:** Essential oils were successfully extracted from each sample, resulting in a yield ranging from 0.01% to 0.2%. Better results were obtained when microwaves were used. The essential oils were characterized, revealing a profile consistent with that reported in the literature for *cold-pressed* essential oils. Limonene was identified as the predominant compound, accounting for 92-96% of the total composition. This finding suggests the potential for recovering the essential oil to fortify consumer products, as well as the possibility of purifying limonene, which has a variety of potential applications in the food and cosmetic industries, as well as as a solvent, biofuel, or insecticide. Considering the phenol fraction of the obtained extracts, their high flavanone content, particularly hesperidin, suggests the potential to re-evaluate these by-products as sources of antioxidant compounds. **Conclusions:** The unique workflow developed in this study enables the recovery of both the residual aroma and bioactive molecules still present in the biomass. These ingredients can then be used to fortify the final products destined for consumers, thereby enhancing the flavor and the nutraceutical properties.

ACKNOWLEDGMENTS
The authors thank Shimadzu Corp. and Merck KGaA for their support and Agrumaria Reggina S.r.l. for providing the samples.
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

High-throughput analysis of furocoumarins in *Citrus* essential oils by subcritical fluid chromatography with green solvents

Irrera, E.[a,*], Reale, C.[a], Cafeo, G.[a], Russo, M.[a], Donato, P.[a], Mondello, L.[a,b]

[a]*Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Viale G. Palatucci snc, 98168 – Messina, Italy*
[b]*Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Viale G. Palatucci snc, 98168 – Messina, Italy*

***Corresponding author:** elisa.irrera@studenti.unime.it*

***Keywords**: Citrus essential oils, furocoumarins, green chemistry, green solvents, high-throughput, supercritical/ subcritical fluid chromatography.*

Objective: This study aimed to develop a fast and sustainable method for the quantification of furocoumarins (FCs) in the non-volatile fraction of essential oils (EOs) derived from *Citrus* species. These matrices are widely used in the formulation of fragrances and topical cosmetic products. Due to the reported phototoxicity of FCs, concerns regarding their impact on consumer safety are mounting [1, 2]. In light of this evidence, it is crucial for industries to have access to quantitative analytical methods for monitoring that are accurate and reliable while enabling rapid quality control. Traditional approaches consisting of high-performance liquid chromatography (HPLC) are time-consuming and often rely on the use of toxic solvents. This work proposes an alternative approach based on subcritical fluid chromatography (SubFC) with photodiode array detection (PDA) to improve speed and sustainability. **Methods:** Instrumental analyses were carried out on the Nexera-UC system with a PDA detector (Shimadzu, Duisburg, Germany). Apart from the stationary phase selectivity, the effects of numerous analytical parameters (type and concentration of modifier, flow rate, column temperature, and outlet pressure) were studied on the basis of the separation of a standard mixture containing coumarin and 16 FCs (Furocoumarin Mix, CRM from Merck KgaA (Darmstadt, Germany)). From these studies, isocratic conditions were determined to obtain a satisfactory separation on Ascentis® Express 90 Å F5 (2 x 15 cm L., 4.6 mm I.D., 2.7 µm) HPLC columns (Merck KgaA), using bio-methanol (HPLC, Merck KgaA) as the mobile phase modifier into carbon dioxide. The figures of merit were evaluated according to Eurachem guidelines. Afterwards, the content of FCs in cold-pressed *Citrus* EOs was determined, i.e., bergamot (*Citrus × bergamia*), grapefruit (*Citrus paradisi*), lemon (*Citrus limon*), lime (*Citrus × aurantiifolia*), and orange (*Citrus sinensis*). **Results:** Compared to conventional HPLC techniques, the method developed using SubFC resulted in a substantial decrease in solvent usage (around 800 mL per run) and a significant reduction of analysis time (around 3.5 minutes per sample). Additionally, sample dilution and separation were performed using green, bio-based solvents, thus reinforcing the method's adherence to the principles of green analytical chemistry. In the screening of cold-pressed EOs, the content of FC compounds was determined with Limit of Detection (LOD) in the range of 0.035–0.090 mg kg^{-1} (35-90 ppb) and Limit of Quantification (LOQ) in the range of 0.117–0.234 mg kg^{-1} (117-234 ppb). Notably, elution of the target FC peaks was obtained isocratically (column reconditioning not needed) and free from interfering sample components (i.e., coumarins, polymethoxyflavones). **Conclusions:** The developed SubFC method offers a fast, accurate, and sustainable solution for the quantification of coumarin and furocoumarins in *citrus* essential oils. High-throughput capability, allowing for the analysis of up to 17 samples per hour (289 target analytes), and minimal environmental impact (no need for high-temperature operation, absence of toxic solvent and additives) make the method particularly suitable for industrial routine quality control.

ACKNOWLEDGMENTS
The authors gratefully acknowledge the support from Merck KGaA (Darmstad, Germany) and Shimadzu Europa (Duisburg, Germany).

REFERENCES
[1] European regulation (EC) No 1223/2009, Off. J. Eur. Union. 2009.
[2] IFRA Standard, 50th Amendment, 2021.

Endemic *Salvia* (*Salvia pisidica*, *Salvia absconditiflora*, and *Salvia heldreichiana*) Essential Oils as Aromatic Defenders: GC-MS Characterization and Multi - Target Bioactivity Against Diabetes and Neurodegeneration

Ezzulddin, S.H.[a], Civaş, A.[a,e,*], Özer, E.B.[a,f], Aydın, B.[b], Yuca, H.[a], Demirci, B.[c], Karakaya, S.[d]

[a]*Department of Pharmacognosy, University of Ataturk-Faculty of Pharmacy, Erzurum, Türkiye*
[b]*Department of Pharmacognosy, University of Erzincan Binali Yıldırım-Faculty of Pharmacy, Erzincan, Türkiye*
[c]*Department of Pharmacognosy, University of Anadolu -Faculty of Pharmacy, Eskişehir, Türkiye*
[d]*Department of Pharmaceutical Botany, University of Ataturk-Faculty of Pharmacy, Erzurum, Türkiye*
[e]*Department of Pharmacy Services, Iğdır University-Tuzluca Vocational School, Iğdır, Türkiye*
[f]*Department of Pharmacy Professional Sciences, Ondokuz Mayis University, Faculty of Pharmacy, Samsun, Türkiye*

***Corresponding author:** ayse.civas@igdir.edu.tr*

***Keywords:** Antidiabetic, cholinesterase, GC-MS, Salvia pisidica, Salvia absconditiflora, Salvia heldreichiana.*

Objective: The objective of this study was to determine the chemical composition of essential oils obtained by GC-MS from the flowering aerial parts of *Salvia pisidica* Boiss. & Heldr. Ex Benth., *S. absconditiflora* (Montbret & Aucher ex Benth.) Greuter & Burdet, and *S. heldreichiana* Boiss. ex Benth. species, which are endemic to Türkiye. Simultaneously, this study aimed to evaluate the antioxidant, alpha-amylase, alpha-glucosidase, acetylcholinesterase, and butyrylcholinesterase inhibitory activities of these essential oils, as well as their methanol and water extracts obtained by maceration. Additionally, collagenase and tyrosinase inhibitory activities were also targeted to understand their potential in different biological processes such as aging and skin problems. This research thereby aims to establish new sources for conditions such as neurodegenerative diseases and diabetes, which currently lack radical treatments. **Methods:** Essential oils were obtained from the flowering aerial parts of the plants by hydro-distillation using a Clevenger apparatus. The resulting essential oils were analyzed by GC-MS/MS, with components identified through computer matching against commercial (Wiley GC/MS Library, MassFinder Software 4.0) [1, 2] and in-house "Başer Library of Essential Oil Constituents." Alpha-amylase, alpha-glucosidase, acetylcholinesterase, and butyrylcholinesterase inhibition tests were carried out on both the essential oils and their methanol (MeOH) and water extracts obtained by maceration. Additionally, the antioxidant activities of the essential oils were evaluated using ABTS•+ and DPPH• assays. **Results:** In terms of antioxidant activity, the essential oils of *S. pisidica* and *S. heldreichiana* showed low activity, while no activity was detected (N.D.) for *Salvia absconditiflora* essential oil. These activities were significantly lower compared to control substances. For antidiabetic activity, *S. absconditiflora* essential oil exhibited the highest α-glucosidase inhibition (51.98%) among essential oils, though lower than control acarbose (74.72%). *S. heldreichiana* essential oil showed slightly more effective α-amylase inhibition (36.29%). Among extracts, *S. heldreichiana* water extract displayed the highest α-glucosidase inhibition (47.01%) with the lowest IC50 (724 µg/mL). Regarding cholinesterase inhibition, *S. heldreichiana* essential oil demonstrated the highest acetylcholinesterase inhibition (11.55%), while *S. pisidica* essential oil (30.40%) was more effective against butyrylcholinesterase, yet significantly lower than control donepezil (97.96%). Extracts also exhibited varying cholinesterase inhibition, generally lower than the control. In essential oil components analysis, sabinyl acetate (54.2%) was identified as the predominant component in the essential oil of *S. pisidica*. In *S. absconditiflora*, camphor (9.2%) and humulene epoxide II (26.6%) were the major constituents. The essential oil of *S. heldreichiana* was characterized by high levels of *trans*-verbenol (6.4%), α-pinene (7.9%), caryophyllene oxide (8.4%), and spathulenol (9.5%). All three *Salvia* species' essential oils generally contained high proportions of oxygenated monoterpenes. *Salvia* species showed weak/no inhibition against collagenase and tyrosinase enzymes, while positive controls were more effective. **Conclusions:** These results indicate that the investigated *Salvia* species possess antidiabetic and cholinesterase inhibitory potential, although their activity levels are generally lower compared to synthetic control substances. The chemical composition of the essential oils is related to the observed biological activities. The relatively high potential of *S. absconditiflora* in α-glucosidase inhibition is particularly noteworthy, suggesting these species are promising natural sources for further investigation.

ACKNOWLEDGMENTS
The authors sincerely acknowledge the support provided by Atatürk University.
Corresponding author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES
[1] McLafferty F.W., Stauffer D.B., (1989). The Wiley/NBS Registry of Mass Spectral Data, J Wiley and Sons: New York.
[2] Hochmuth D. H., (2008). MassFinder 4.0, Hochmuth Scientific Consulting, Hamburg, Germany.

GC/MS-Based Phytochemical Characterization of *Rhus coriaria* L. Fruit Essential Oil

Ivazović, I.[a], Dedić, A.[c], Alispahić, A.[c], Boškailo, E.[b], Imamović, B.[a], Džudžević-Čančar, H.[c,*]

[a]*Department of Drug analysis, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[b]*Department of Ecology and Environmental Protection, Faculty of Social Sciences Dr. Milenko Brkić, Herzegovina University, Mostar, Bosnia and Herzegovina*
[c]*Department of Chemistry in Pharmacy, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*

***Corresponding author:** hurija.dzudzevic-cancar@ffsa.unsa.ba*

***Keywords:** Chemical characterisation, essential oil, GC/MS, Rhus coriaria L., sumac fruit.*

Objective: *Rhus coriaria* L., commonly known as sumac, is a plant from the Anacardiaceae family, native to subtropical and temperate regions. It is mainly found in the Middle East and the Mediterranean region, where its dried fruits are traditionally used as a spice and flavoring due to their tart and astringent taste. The fruits are harvested in late summer to early autumn, with old and new fruits being on the same plant at the same time. This is valuable because it is a source of nutrients all year round. In folk medicine, sumac is used to treat a variety of ailments, such as liver disease, diarrhea, urinary tract complications, and ulcers. These diverse therapeutic effects are attributed to the pharmacological properties of the plant: antioxidant, anti-inflammatory, hypoglycemic, and hypolipidaemic. Sumac is rich in different classes of phytochemicals, including flavonoids, tannins, polyphenolic compounds, organic acids, and many others [1, 2, 3]. The objective of this work was to evaluate the phytochemical composition of *Rhus coriaria* fruit essential oil (EO) growing in Sarajevo (Bosnia and Herzegovina) as an ornamental plant. **Methods:** Fresh sumac fruits were collected in October 2024 from Sarajevo flower gardens. The fruits were air-dried, and EO was extracted by hydrodistillation for 3 hours using a Clevenger-type apparatus. The phytochemical composition of the EO was analyzed using gas chromatography coupled with mass spectrometry (GC/MS). Samples were introduced into the GC instrument via a split/splitless injector maintained at 250 °C, operating in split mode with a 20:1 ratio. Volatile organic compounds (VOCs) were separated using a 30 m DB-FFAP capillary column (0.25 mm internal diameter, 0.25 µm film thickness). The oven temperature was initially set at 40°C (held for 10 minutes), then increased at a rate of 2°C/min to 250°C, and held at the final temperature for 5 minutes. High-purity helium (99.995%) was used as the carrier gas at a flow rate of 1.0 mL/min. **Results:** Compound identification was carried out by comparing the retention times and/or mass spectra (MS) of the analytes with those of standards, or by matching the obtained mass spectra with those stored in the MS libraries. Quantification of the essential oil components was performed using the normalization method, without the application of correction factors. In total, 78 compounds were separated and identified, with δ-cadinene being the most abundant (10.07%). Other major constituents included γ-muurolene (4.60%), caryophyllene (4.43%), α-cadinol (4.29%), and n-hexadecanoic acid (3.05%). **Conclusion:** To the best of our knowledge, this is the first time that δ-cadinene chemotypes have been recognized in the *Rhus coriaria* L. fruit EO. According to literature data, depending on geographical origin, harvesting time, processing methods, and agricultural practices, different chemotypes of EO of sumac fruit were recorded. Thus, β-caryophyllene was the most abundant component in EO from Iran and Sicily, naphthalene and α-pinene in EO from Jordan and Palestine, β- and o-ocimene and limonene in EO of Egyptian sumac, and limonene/nonanal/(Z)-2-decenal in EO from Turkey [4]. δ-Cadiene, a natural sesquiterpene identified as the dominant compound in this study, has shown cytotoxic and apoptosis-inducing effects against cancer cells, as well as insecticidal and irritant properties [5, 6]. Based on this knowledge, we focused our further research on the evaluation of the anticancer properties as well as the toxicological evaluation of the sumac fruit EO.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Batiha, G. E., Ogunyemi, O. M., Shaheen, H. M., Kutu, F. R., Olaiya, C. O., Sabatier, J. M., & De Waard, M. (2022). *Rhus coriaria* L. (Sumac), a Versatile and Resourceful Food Spice with Cornucopia of Polyphenols. *Molecules, 27*(16), 5179.
[2] Alsamri, H., Athamneh, K., Pintus, G., Eid, A. H., & Iratni, R. (2021). Pharmacological and Antioxidant Activities of *Rhus coriaria* L. (Sumac). *Antioxidants*, 10(1), 73.
[3] 4. Shahrivari, S., Zeebaree, S. M. S., Alizadeh-Salteh, S., Feizy, H. S., & Morshedloo, M. R. (2024). Phytochemical variations antioxidant, and antibacterial activities among zebaria sumac (Rhus coriaria var. zebaria) populations in Iraq. *Scientific reports*, *14*(1), 4818.
[4] 3.Calabrò, A., Ligotti, M. E., Accardi, G., Di Majo, D., Caruso, C., Candore, G., & Aiello, A. (2023). The Nutraceutical Properties of *Rhus coriaria Linn*: Potential Application on Human Health and Aging Biomedicine. *International journal of molecular sciences*, *24*(7), 6206.
[5] Kurucu, S., Koyuncu M., Guvenç A., (Koroglu), Ozek T., (1993). The Essential Oils of Rhus coriaria L. (Sumac). Journal of Essential Oil Research, 5(5):481- 486.
[6] Hui, L. M., Zhao, G. D., & Zhao, J. J. (2015). δ-Cadinene inhibits the growth of ovarian cancer cells via caspase-dependent apoptosis and cell cycle arrest. *International journal of clinical and experimental pathology*, *8*(6), 6046–6056.

Antibacterial and Antifungal Potential of *Origanum vulgare* L. Essential Oil for Food Applications: Bioguided Fractionation and Microencapsulation in Tomato Sauce

Pavarino, M.[a,b,*], Costa, S.D.O.[b], Galdeano, M.C.[b], Rubiolo, P.[a], Tonon, R.V.[b], Lima, J.P.[b], Sgorbini, B. [a], Bizzo, H.R.[b]

[a]*Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125 Turin, Italy*
[b]*Embrapa Food Technology, Avenida das Américas, Rio de Janeiro 29.501, RJ, Brazil.*

***Corresponding author:** m.pavarino@unito.it*

***Keywords**: Bio-guided fractionation, foodborne pathogens, oregano essential oil, microencapsulation, tomato sauce.*

Objective: Foodborne illnesses caused by pathogens such as *Staphylococcus aureus*, *Salmonella enterica*, and various fungi continue to pose a major global health threat, affecting millions of people every year [1]. As consumer demand for natural preservatives shifts away from synthetic additives, the search for safe and effective alternatives is becoming increasingly urgent. Essential oils from aromatic plants are known to contain bioactive compounds—especially phenolic compounds such as carvacrol and thymol—that exhibit strong antimicrobial activity. However, their practical application in food is limited by factors such as high volatility, poor water solubility, instability, and strong flavors that can affect sensory quality [2]. This study aimed to address these challenges by first screening essential oils from selected aromatic plants and spices for their antibacterial and antifungal activity against common foodborne pathogens. A promising essential oil was then selected and subjected to bio-guided fractionation to identify its main antimicrobial constituents. Finally, its potential as a natural food preservative was evaluated in a tomato sauce model, comparing the performance of both the free and microencapsulated forms under simulated storage conditions. **Methods:** The essential oils (EOs) and their fractions were chemically characterized by GC-MS/FID. The antibacterial activity of the free EOs, the EO fractions, and the microencapsulated *Origanum vulgare* L. essential oil (OEO) were tested *in vitro* against *Staphylococcus aureus* and *Salmonella enterica* serovar Typhimurium using the well diffusion method. The antifungal activity against *Penicillium purpurogenum* was evaluated using the same and two additional confirmatory methods. Microencapsulation was performed by emulsifying OEO with gum arabic while maintaining a total solids content of 30% (w/w) with 10% EO. The particle sizes of the emulsion and powder were measured by laser light scattering. For the *in situ* antimicrobial evaluation, commercially available tomato sauce was inoculated with bacteria or fungi and treated with free or microencapsulated EO to a final EO concentration of 0.3% (w/w). The samples were stored at 25°C for 28 days. Antibacterial and antifungal activities were monitored over time by plating on selective media and using the automated TEMPO® fluorescence detection system. The presence of *Salmonella* was confirmed using the mini-VIDAS® Easy Salmonella protocol. **Results**: Among the tested essential oils, oregano essential oil showed the highest antimicrobial activity and was selected for further investigation. Bio-guided fractionation identified oxygenated fractions as the main contributors to the antibacterial and antifungal activity. In tomato sauce, both the free and microencapsulated forms of oregano essential oil effectively inhibited the growth of *Staphylococcus aureus*, *Salmonella enterica*, and *Penicillium purpurogenum* throughout the storage period. The use of microencapsulation technology enabled up to three times lower concentrations and at the same time improved sensory acceptance by reducing strong odors and flavors. **Conclusions:** Microencapsulated *Origanum vulgare* L. essential oil is an effective natural preservative that exhibits strong antimicrobial activity and improved sensory acceptability. Bio-guided fractionation identified oxygenated compounds as key contributors to its efficacy, while its microencapsulated form showed successful application in tomato sauce, indicating its potential for use in various food systems alongside existing preservation strategies.

REFERENCES

[1] Adley C., Ryan M., (2025) The Nature amd Extent of Foodborne Disease, *Antimicrobial Food Packaging*, 2nd edition, 3-14.
2] Maurya A., Prasad J., Das S., Dwivedy A., (2021). Essential Oils and Their Application in Food Safety, *Frontiers in Sustainable Food Systems, 5.*

Digestive Delivery of Microencapsulated *Origanum Onites* Oil to Japanese and Texas White Quails

Çetinkaya, B.[a], Avşar, A.[a], Foysal, MD. S. K.[a], Şahiner, F.[a], Yavuz, M.[a,*]

[a]*Isparta University of Applied Sciences, Agriculture Faculty, Animal Science Department, Isparta, Türkiye*

***Corresponding author:** musayavuz@isparta.edu.tr*

Keywords: *Digestive delivery, Japanese quail, microencapsulation, Origanum onites L., Texas white quail.*

Objective: The first aim of this study was to obtain a product resistant to environmental conditions for use in animal husbandry by applying the microencapsulation technique of *Origanum onites* L. oil, and the second aim was to investigate the digestive delivery of Microencapsulated *Origanum onites* Oil (MOO) to Japanese (*Coturnix coturnix japonica*) and Texas White Quails. **Methods:** Sodium alginate was used to microencapsulate the *Origanum onites* L. oil in the Isparta University of Applied Sciences, Animal Science Department, Feeds and Animal Nutrition Laboratory. The study was approved by the Isparta University of Applied Sciences Animal Experiments Local Ethics Committee, number E-77211729-804.01-18969.336. Laying Japanese (8) and Texas White (8) quails (Coturnix Coturnix) obtained from the Isparta University of Applied Sciences, Agriculture Faculty, Education, Research, and Application Farm's Poultry Unit. At 8:00 am on the day of sampling, all animals were fasted for two hours and then given 2 g of feed with MOO (containing 200 pieces). After finishing the 2 g of feed, all the animals received 100 g of basal feed, and the animals were euthanized after feeding for 1, 2.5, 4.5 and 6.5 hours with MOO. Quails esophagus, crop, proventriculus, ventriculus (gizzard), small intestine, ceca, and large intestine were removed from the carcass. Each part was removed, and inside, the feed particles were washed into a small container. MOO parts that remained intact were counted. **Results**: Japanese and Texas White quails had average 275 g and 314 g live weight, 26 g and 34 g feed intake, respectively. MOO was discovered after 1 hour in proventriculus and ventriculus and after 2.5 hour only in ventriculus of the quails. No MOO was observed in the small intestine, ceca and large intestine at any time. All of the MOO were broken down in the ventriculus. After feeding the white opaque MOO, it increased in size. Texas white quails had 8 MOO in the ventriculus after 1 hour and 7 MOO in the ventriculus after 2.5 hours. Japanese quails had 21 MOO in the ventriculus after 1 hour and 2 MOO in the ventriculus after 2.5 hours. **Conclusions:** This study was conducted considering that there would be differences between Texas white and Japanese quail because their digestion speed and feed consumption were different. However, the area where the microencapsulated product is digested (broken down) in the gizzard area in both quail types, and after 2.5 hours, they completely lost their integrity in both quail types. Increased feed intake also increases MOO digestion. It has been observed that sodium alginate-coated Origanum onites oil successfully reaches the gizzard without disintegrating. This application may lead to the development of various products that can be used in poultry feed.

Antitumor Effect of Juniper, Angelica and Clove Essential Oils and Vir Bak Preparation *In Vitro*

Milovanović, B.[a,*], Žižak, Ž.[b], Petrović, S.[c], Petrović, N.[c], Marković, N.[a], Stanojković, T.[b]

[a]*Institute for Cardiovascular Diseases Dedinje, University of Belgrade, Medical Faculty, Belgrade, Serbia*
[b]*Institute of Oncology and Radiology of Serbia, Belgrade, Serbia*
[c]*Development and Production Center BIOSS - PS and Others, Belgrade, Serbia*

Corresponding author: profdrbranislavmilovanovic@gmail.com

Keywords*: Angelica, antitumor activity, clove, essential oils, juniper, Vir Bak.*

Objective: This study investigated the antiproliferative effects of essential oils derived from juniper (*Juniperus communis* L.), angelica (*Angelica archangelica* L.), clove (*Syzygium aromaticum* (L.) Merr. & Perry), and the Vir Bak preparation on selected human malignant cell lines *in vitro*. Essential oils, composed primarily of monoterpenes and sesquiterpenes, are known bioactive secondary metabolites with potential therapeutic effects**. Methods:** Essential oils were extracted by water and steam distillation using SP-120 and SP-450 distillers. The Vir Bak preparation was developed using acacia honey, which was deep-frozen at –40°C and lyophilized to produce a fine-grained powder used as a capsule filler. Antiproliferative activity was assessed using the 72 h MTT assay on K562 (myelogenous leukemia), LS-174 (colon carcinoma), PC-3 (prostate cancer), and MRC-5 (normal lung fibroblast) cell lines. Oils and Vir Bak were diluted in complete RPMI-1640 medium (without phenol red) supplemented with L-glutamine, antibiotics, 10% fetal bovine serum, and buffered to pH 7.2. **Results:** All tested essential oils exhibited potent inhibitory effects on malignant cells. Clove oil demonstrated the highest cytotoxic activity with IC_{50} values of 0.044 µL/mL, 0.064 µL/mL, and 0.083 µL/mL for K562, LS-174, and PC-3 cells, respectively. Juniper oil showed notable selectivity against K562 cells (IC_{50} = 0.077 µL/mL; Selectivity Index (SI) = 7.5). Clove oil exhibited lower selectivity (SI = 2.7, 1.8, and 1.4 for K562, LS-174, and PC-3, respectively). Angelica oil was less effective across all tested lines. Vir Bak showed moderate antiproliferative activity (IC_{50} = 657 µg/mL, 794 µg/mL, and 396 µg/mL for K562, LS-174, and PC-3 cells, respectively). **Conclusion:** Juniper and clove essential oils demonstrated strong antiproliferative activity with favorable selectivity toward malignant cells. These findings support their potential as natural antitumor agents and justify further *in vivo* studies.

Phenolic acid combinations: synergistic and antagonistic impacts on essential oils bioactivity

Klepo, L.[a,*], Nokto, M.[a]

[a]*Department of Chemistry, University of Sarajevo-Faculty of Science, Zmaja od Bosne 33, Sarajevo, Bosnia and Herzegovina*

***Corresponding author:** lklepo@pmf.unsa.ba*

***Keywords:** Antagonism, antioxidant activity, DMPD method, phenolic acids, synergism.*

Objective: Phenolic acids, such as rosmarinic and caffeic acid, are important constituents of essential oils from *Lamiaceae* species (e.g., rosemary, sage, oregano), where they play a key role in antioxidant activity by scavenging free radicals and enhancing overall oxidative capacity. This effect has been particularly documented in *Salvia officinalis* and *Rosmarinus officinalis*, whose phenolic compounds, including carnosol, rosmarinic and caffeic acid, significantly contribute to the antioxidant properties of their essential oils. Similarly, in the essential oil of *Pittosporum tobria*, phenolic acid such as caffeic, cinnamic, and gallic acid have been identified, showing notable antioxidant activity in assays such as DPPH, as well as protective effects against H_2O_2 – induced hemolysis in erythrocytes. These findings highlight the central role of phenolic acids in determining the bioactivity of plant-derived essential oils and their potential use in functional food and pharmaceutical application. The present study further investigated the synergistic and antagonistic interaction among selected phenolic acid with respect to their antioxidant activity (AA%). **Methods**: For this purpose, four phenolic acids: gallic, rosmarinic, chlorogenic, and caffeic acid were selected to investigated their synergistic and/or antagonistic effects of antioxidant activity. Each compound was prepared and tested individually at four concentrations (0.25 do 1.5 mg/mL), and subsequently in binary, ternary, and quaternary mixtures at specific ratios. Antioxidant activity was measured spectrophotometrically using DMPD (*N,N*-dimethyl-*p*-phenylenediamnine) method, with maximum absorption observed at 515 nm. **Results:** The results revealed a broad spectrum of interaction, ranging from pronounced synergism, where combined activities surpassed the sum of individual effect, to antagonism, where mixtures showed lower activity than expected. Among individual acids, rosmarinic acid exhibited the strongest antioxidant effect (67.28% at 1.5 mg/mL), whereas gallic acid displayed the weakest activity (47% at 1.5 mg/mL). Binary mixtures indicated that higher proportion of rosmarinic or caffeic acid favored synergism, while gallic and chlorogenic acid often led to antagonistic effects. In ternary mixtures, the most pronounced activity (AA% = 72.58%, synergistic effect +25.89%) was observed when rosmarinic and caffeic acid were present at higher rations compared to chlorogenic acid, while other combination of these three acids, tended toward antagonism. A similar trend was confirmed on quaternary mixtures, where the highest antioxidant activity (56.32%) was recorded in blends dominated by rosmarinic and caffeic acid waswe have additive effect (+2.40). Other ratios of these acids in these quaternary mixtures showed antagonism (difference between experimental and theoretical value were ranging from -16.00 to -6.02). **Conclusions:** Overall, these results highlight that the antioxidant activity of essential oils is strongly influenced, among other components, by the presence and interaction of phenolic acid, emphasizing their key role in modulating bioactivity and guiding the design of functional formulation.

Antibacterial Activity of *Origanum majorana* L. var. *tenuifolium* Essential Oil from Cyprus

Ödüncüoğlu, G.[a,*], Güler, E.[b]

[a]*Department of Pharmacognosy, Bahcesehir Cyprus University-Faculty of Pharmacy, Nicosia, Cyprus*
[b]*Department of Molecular Biology and Genetics, European University of Lefke -Faculty of Art and Sciences, Lefke, Cyprus*

***Corresponding author:** goksu.oduncuoglu@baucyprus.edu.tr*

***Keywords**: Antibacterial activity, essential oil, Origanum majorana L. var. tenuifolium.*

Objective: *Origanum majorana* L., known as sweet marjoram, is commonly found in the Mediterranean region. *Origanum majorana* L. var. *tenuifolium* (Cypriot Marjoram) is a variety of marjoram endemic to Cyprus. As previously reported, the essential oil of this plant is mainly rich in carvacrol, *p*-cymene and myrcene [1]. The increasing prevalence of antibiotic resistance emphasizes the importance of developing novel therapeutic compounds. The objective of this study was to evaluate the antibacterial activity of *Origanum majorana* L. var. *tenuifolium* Weston leaf essential oil (WD) from Cyprus against *Escherichia coli* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), and *Enterococcus faecalis* (ATCC 29212). **Methods:** Aerial parts of *Origanum majorana* were collected from the Korucam (Kormakitis) region of Northern Cyprus in March 2025. The essential oil from air-dried aerial parts of the plant was obtained by hydrodistillation for 3 h, using a Clevenger apparatus. The antibacterial activity of the oil against *Escherichia coli* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), and *Enterococcus faecalis* (ATCC 29212) was assessed using the disk diffusion method. **Results:** Essential oil of *O. majorana* demonstrated strong antibacterial activity against all bacterial strains. The inhibition zone diameter on *E. coli* is 24 mm, on *Enterococcus faecalis* is 11 mm, and on *Staphylococcus aureus* is 22 mm (Figure 1). **Conclusions:** The findings indicate that the essential oil of *O. majorana var. tenuifolium* possesses significant antibacterial activity and may serve as a potential source for the development of novel antimicrobial agents.

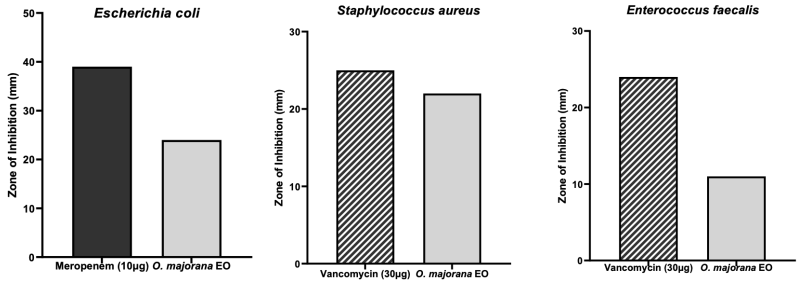


Figure 1: Antibacterial activity of *Origanum majorana* L. var. *tenuifolium* Weston leaf essential oil (EO) and positive controls against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*.

REFERENCES

[1] Güler E, Özbilgin A, Çavuş İ, Baddal B, Etikan İ, Başer KHC, Şanlıdağ T., (2021) In vitro Anti-Leishmanial Activity of Essential Oils Extracted from Plants Growing in Northern Cyprus Against Leishmania tropica, *Turkiye Parazitol Derg*, 45, 101-107.

Cytotoxic activity of *Laurus nobilis* leaf, fruit and seed essential oils

Čamdžić, A.[a], Džudžević-Čančar, H.[b], Dedić, A.[b], Malenica, M.[a], Alispahić, A.[b,*], Stanojković, T.[c]

[a]*Department of Pharmaceutical Biochemistry and Laboratory Diagnostics, University of Sarajevo-Faculty of Pharmacy, Zmaja od Bosne 8, Sarajevo, Bosnia and Herzegovina*
[b]*Department of Chemistry in Pharmacy, University of Sarajevo-Faculty of Pharmacy, Zmaja od Bosne 8, Sarajevo, Bosnia and Herzegovina*
[c]*Institute of Oncology and Radiology of Serbia, Pasterova 14, Belgrade, Serbia*

Coresponding author: amra.alispahic@ffsa.unsa.ba

Keywords: *Cytotoxicity, essential oil, HeLa cells, IC50, Laurus nobilis, MTT, MRC-5 cells.*

Objective: *Laurus nobilis L.* belongs to the family *Lauraceae*, which comprises numerous aromatic and medicinal plants. It is commonly known as bay, sweet bay, or true laurel, and it is widely distributed in the Mediterranean area and Europe. Bay is traditionally used as a carminative, stomachic, and nervine as well as in the treatment of amenorrhea, colic, hysteria, polyps, sclerosis, and spasms. Dried bay leaves are mainly used as a spice and flavoring agent in culinary practices and meals, while the essential oil (EO) is generally used in the flavoring industry. Leaves EO has proven antibacterial and antimicrobial properties. [1, 2]. Herein, we present the evaluation of the activity of bay essential oil from the Herzegovina region on human cervical adenocarcinoma (HeLa) and non-transformed human lung fibroblast (MRC-5) cell lines. **Methods:** In vitro cytotoxic activity of EOs extracted by hydrodistillation in a Clavanger-like apparatus from leaves, fruits, and seeds of *Laurus nobilis* L. was investigated, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cell survival assay. 3000 HeLa cells and 5000 MRC-5 cells were cultured per well in 96-well cell culture microplates. After 24 h incubation in medium, the cells were treated with the three-bay EOs within a concentration range from 0.03125 µL/mL to 0.500 µL/mL for HeLa and from 0.0625 µL/mL to 1.000 µL/mL for MRC-5 cell lines. Wells with non-treated control cells were filled with the same volume of RPMI-1640 nutrient medium. The cell survival was measured after 72 h of treatment with the MTT cell survival assay by following Mosmann and Ohno and Abe's instructions. The absorbance was measured after 24 h at a 570 nm wavelength. A minimum of three independent IC50 concentration measures were performed in triplicate. IC50 represents the concentration of the oil that reduced cell survival by 50% in comparison with the control cell sample. **Results:** All three tested EOs show concentration-dependent cytotoxic effects on both HeLa and normal MRC-5 cells. Both cell lines showed similar sensitivity to the cytotoxic effects of all three essential oil samples. Bay fruit and seed EOs had stronger cytotoxicity against the HeLa cell line than bay leaf EO. In the HeLa cell line, the IC50 value was 3.35 µl/mL of leaf EO, 0.21 µl/mL of flower extracts, and 0.17 µl/mL of seed EO. After the 72 h treatment of MRC-5 with the three EOs, IC50 values were as follows: 2.95 µl/mL (leaf), 0.23 µl/mL (fruit), and 0.15 µl/mL (seed). It appears that the bay seed EOs were most efficient on MRC-5 cell lines too. **Conclusions:** The results of this study indicate that bay leaf, fruit, and seed EOs have great cytotoxic activity against the HeLa cancer cell line, but with no prominent selectivity to MRC-5. This indicates that their genotoxic properties need to be investigated. Also, further studies are needed regarding the anticancer effects of bay essential oil on other cancer cell lines. *L. nobilis* EOs from different regions and countries, as well as different plant parts, have great potential and could be introduced as low-cost and easy-to-access cytotoxic agents in pharmacies as well as for different applications, after rigorously assessing their safety for different applications.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Humaira Rizwana, Noorah Al Kubaisi, Nadeen N. Al-Meghailaith, Nadine MS Moubayed and Gadah Albasher, Evaluation of Chemical Composition, Antibacterial, Antifungal, and Cytotoxic Activity of Laurus nobilis L Grown in Saudi Arabia, *J Pure Appl Microbiol.,* 2019; 13(4):2073-2085.
[2] Berrington D, Lall N. Anticancer Activity of Certain Herbs and Spices on the Cervical Epithelial Carcinoma (HeLa) Cell Line. *Evid Based Complement Alternat Med.* 2012;2012:564927.
[3] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983;65(1-2):55-63.
[4] Ohno M, Abe T. Rapid colorimetric assay for the quantification of leukemia inhibitory factor (LIF) and interleukin-6 (IL-6). *J Immunol Methods.* 1991;145(1-2):199-203.

Phytochemical profile of *Helichrysum italicum* (Roth) G. Don Essential Oils from two areas in Bosnia and Herzegovina

Alispahić, A.[a], Džudžević-Čančar, H.[a,*], Dedić, A.[a], Špánik, I.[b]

[a]*Department of Chemistry in Pharmacy, Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*
[b]*Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, Bratislava, Slovakia*

**Corresponding author:* hurija.dzudzevic-cancar@ffsa.unsa.ba

Keywords: *Essential oils, GC/MS, Helichrysum italicum, , hydrodistillation, phytochemicals.*

Objective: The Asteraceae family includes the genus Helichrysum (Miller), which is a relatively large genus with about 600 species found worldwide. The Mediterranean herb *Helichrysum italicum* (Roth) G. Don, commonly referred to as immortelle, is frequently used in traditional medicine as an antibacterial and anti-inflammatory medication [1]. *H. italicum* grows wild in the Mediterranean, but in many countries [2...] it's mainly cultivated for the production of essential oils used in the cosmetic and perfume industry. The pharmaceutical, cosmetic, and fragrance industries have shown a great deal of interest in essential oil (EO) immortelle in recent years due to the rich composition and advantageous effects of its essential oil on skin [1, 3]. Immortelle essential oil and extracts show antifungicidal, antibacterial, anti-inflammatory, and antioxidant qualities, among other positive properties [2...]. The aim of this study was to (i) conduct an analysis of H. italicum (Roth) G. Don essential oil from plants cultivated in two different biogeographical and agro-ecological zones in Bosnia and Herzegovina (BiH) and (ii) compare their composition with each other and with the composition of essential oils from other countries in the Balkan region. **Methods:** Immortelle EOs were collected from two areas in BiH, continental (Sample 1 - Bijeljina, Janja NS 44°40'39.201''; EW 19°14'39.537''; Altitude 105.5; and submediterranean Sample 2 - Mostar, Livač NS 43°24'42.775''; EW 17°52'32.219''; Altitude 60 m). Essential oils were obtained by hydrodistillation in a Clevenger-type apparatus. **Results:** The GC and MS analyses enabled the identification of 79 constituents in sample 1, which accounted for 97.47% of the total EO, and 123 constituents in sample 2, which accounted for 95.44%. The main compounds in sample 1 were nerol acetate (15.62%), γ-curcumene (13.91%), β-eudesmene (12.78%), and 4,6,9-trimethyldec-8-ene-3,5-dione (Italidione I) (7.36%). For sample 2 the main compounds were 2,4,6,9-trimethyldec-8-ene-3,5-dione (Italidione II) (15.22%), g-curcumene (8.74%), β-Eudesmene (5.78%), and nerol acetate (5.58%). The sum of the amount of both diketones characteristic of immortelle essential oils, in individual samples, ranged from 9.16% in Sample 1 to 19.23%. 19.23% in 2. **Conclusions:** According to the GC/MS data, sesquiterpenes and monoterpenes were abundant in the examined *H. italicum* EOs. The sample from the continental area of BiH contained nerol acetate, a volatile metabolite that contributes to the scent of the plant, as the main compound, followed by γ-curcumen and β-eudesmene, compounds with potential antimicrobial, antioxidant, and anti-inflammatory bioactivities, with almost the same amounts. The EO sample from the BiH Mediterranean region lacks nerol acetate and also has a lower content of γ-curcumin and β-eudesmane than the continental sample. Both EOs contain italidones, compounds known for their anti-inflammatory properties and skin protection against UV rays and pollutants, with sample 1 being richer in italidone I than sample 2, but sample 2 containing italidone II as the main compound. The chemical composition of the tested oils is comparable to those from different countries in the Balkan region, with the continental EO corresponding in content to that of the EO of immortelle from Corsica, which makes it interesting as it could be used for multiple purposes.

REFERENCES

[1] Viegas, D.A., Palmiera-de-Olivera, A., Salgueiro, L.J. Martinez-de-Olivera, R. Palmeirade-Olivera. (2014) Helichrysum italicum: from traditional use to scientific data. *J. Ethnopharm.* 151, 54-56.
[2] Oliva, A., Garzoli, S., Sabatino, M., Tadić, V., Costantini, S., Ragno, R., & Božović, M. (2019). Chemical composition and antimicrobial activity of essential oil of Helichrysum italicum (Roth) G. Don fil. (Asteraceae) from Montenegro. *Natural Product Research, 34*(3), 445–448.
[3] Combes, C.; Legrix, M.; Rouquet, V.; Rivoire, S.; Grasset, S.; Cenizo, V.; Moga, A.; Portes, P. (2017) Helichrysum italicum essential oil prevents skin lipids peroxidation caused by pollution and UV. *J. Investig. Dermatol.* 137, S221.
[4] Bianchini A., Pierre Tomi, J. Costa, A.F. Bernardini. Composition of Helichrysum italicum (Roth) G. Don fil subsp. Italicum essential oils from Corsica (France). *Flavour and Fragrance Journal*, 2001, 16 30-04.
[5] Aćimović, M.; Ljujić, J.; Vulić, J.; Zheljazkov, V.D.; Pezo, L.; Varga, A.; Tumbas Šaponjac, V. Helichrysum italicum (Roth) G. Don Essential Oil from Serbia: Chemical Composition, Classification and Biological Activity—May It Be a Suitable New Crop for Serbia? *Agronomy* 2021, 11, 1282.

Cytotoxicity of *Prunus spinosa* L. fruit, flower, and leaf essential oils

Dedić, A.[a,*], Džudžević-Čančar, H.[a], Alispahić, A.[a], Čančar, I.F.[a], Uzunović, A.[b], Korić, E.[c]

[a]*Department of Chemistry in Pharmacy, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[b]*Agency for Medicinal Products and Medical Devices of Bosnia and Herzegovina*
[c]*Department of Pharmacognosy, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*

***Corresponding author:** alema.dedic@ffsa.unsa.ba*

Keywords: *blackthorn, cytotoxic activity, essential oils, HeLa, MRC-5.*

Objective: *Prunus spinosa L.*, commonly known as blackthorn or blackthorn, is a member of the Rosaceae family. This plant is used for various purposes in traditional Bosnian medicine. All plant organs of the blackthorn are known in folk medicine, but the edible fruit has always been the most important. Due to the numerous therapeutic benefits of the fruit, flower and leaf, there is a growing interest in their study in order to improve and modify pharmaceutical forms containing extracts and essential oils of the plant in the treatment of various diseases. Plant material was collected from three different areas in Bosnia and Herzegovina with the aim of evaluating the in vitro cytotoxic activity of essential oils (EOs) against human cervical adenocarcinoma (HeLa) cells and non-transformed human lung fibroblasts (MRC-5) [1]. **Methods:** Hydrodistillation was performed in a Clevenger apparatus to obtain EOs. The cytotoxic activity of blackthorn essential oils against HeLa and MRC-5 was measured by the MTT cell survival assay. Stock solutions of the tested oils were diluted in complete nutrient medium (RPMI-1640 without phenol red) supplemented with 3 mM L-glutamine, 100 µg/mL streptomycin, 100 IU/mL penicillin, 10% heat-inactivated fetal bovine serum (FBS) and 25 mM Hepes, adjusted to pH 7.2 with bicarbonate solution. Absorbance at 570 nm was measured 24 hours later. The number of viable cells in each well was proportional to the intensity of light absorbance, which was read on an enzyme-linked immunosorbent assay (ELISA) plate reader to determine cell survival. IC50 was defined as the concentration of agent that inhibited cell survival by 50% compared to vehicle-treated control. All experiments were performed in triplicate. RPMI-1640, FBS, Hepes, and L-glutamine were from Sigma Chemical Co., St. Louis, MO [2,3]. **Results:** The produced oils were a pale-yellow liquid with a characteristic pleasant odor. The obtained results indicate that tested EOs show dose-dependent cytotoxic activity against tested cancer cell lines in vitro. The IC50 values ranged from 153.10 to 258.62 µg/mL for HeLa cells and from 142.40 to 346.46 µg/mL for MRC-5, depending on plant parts. The best result of cytotoxic activity has been obtained for leaf EOs. HeLa cells are more sensitive to these EOs than MRC-5. **Conclusion:** As far as we know, there is no available literature data related to the *in vitro* determination of cytotoxic activity against HeLa and MRC-5 cancer cell lines of *Prunus spinosa* L. essential oils, so this research cannot rely on literature values specifically for this plant species. *In vitro* analysis of essential oil gave reliable results about bioactivity to analysed cells In order to verify the overall cytotoxic potential of blackthorn EO as well as the cytotoxicity value, it is necessary to continue the research on other human cancer cell lines. The traditional use of blackthorn could direct this research.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Dedić A., Džudžević-Čančar H., Stanojković T., Roje M., Damjanović A., Alispahić A., Jerković-Mujkić A. **(**2023), *Kem. Ind.*, *72*, 323−330. doi: https://doi.org/10.15255/KUI.2022.077.
[2] Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, J. Immunol. Methods. 65, 55–63 doi: https://doi. org/10.1016/0022-1759(83)90303-4.
[3] Ohno M., Abe T. (1991). Rapid colorimetric assay for the quantification of leukemia inhibitory factor (LIF) and interleukin-6 (IL- 6), J. Immunol. Methods. 145, 199–203 doi: https://doi.org/10.1016/0022-1759(91)90327-c.

Chemical Composition Evaluation of Essential Oil Extracted from Aerial Parts of *Artemisia abrotanum* L. from Bosnia and Herzegovina

Mušinbegović, S.[a], Džudžević-Čančar, H.[a,*], Dedić, A.[a], Boškailo, E.[b], Alispahić, A.[a] Koljančić, N.[c]

[a]*Department of Chemistry in Pharmacy, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[b]*Department of Ecology and Environmental Protection, Faculty of Social Sciences dr. Milenko Brkić, Herzegovina University, Mostar, Bosnia and Herzegovina*
[c]*Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovakia*

Corresponding author: hurija.dzudzevic-cancar@ffsa.unsa.ba

Keywords: *Artemisia abrotanum L., composition essential oil, chemotype, monoterpenes, traditional medicine.*

Objective: The Artemisia genus (consisting of approximately 500 species) is distributed all around the globe, except Antarctica. In traditional medicine they have been used as antimalarial, antioxidant, anticancer, anti-inflammatory, and antiviral agents. *Artemisia abrotanum* L. (southern wormwood) is a widely distributed plant from the Asteraceae family that produces a variety of essential oils (EOs). For most essential oils (EOs) of medicinal and aromatic plants, the diversity of compounds and their composition are strongly influenced by factors such as the origin of the plant, climate, time of harvest, part of the plant used and processing methods. This is also the case with this species, from the Artemisia family. During the past years, a number of studies have been performed concerning the application of EOs and active compounds from *Artemisia abrotanum* L. [1]. This research presents insight into the chemical composition of EO of *A. abrotanum* L. ariel parts, from Bosnia and Herzegovina (BiH) **Methods:** Aerial parts of *A. abrotanum* before flowering were collected in Sarajevo (August, 2024). Voucher specimens were deposited in the herbarium of the University of Sarajevo-Faculty of Pharmacy). The EO was prepared from dried plant by hydrodistillation for 2 hours using a Clevenger-type apparatus, according with recommendation of the European Pharmacopoeia (1997). The extracted VOCs were separated using an Agilent 7890A gas chromatograph connected to an Agilent 5975C MS detector. The sample was introduced into GC via a split/splitless injector heated at 250°C working in split mode (20:1 ratio) autosampler Agilent GC80 for sample *A. abrotanum* EO. Injected VOCs were separated on a 30 m DB-FFAP column, 30 m, 0.25 mm, 0.25 μm (Agilent J & W Column, Agilent Technologies, USA). **Results:** 87 compounds were idenificited in *A. abrotanum* EO with a predominance of monoterpenes up to 74.65% (mainly belonging to oxygenated monoterpenes) and sesquiterpenes up to 13.83%. The major constituents of EO were (+)-2-Bornanone (26.89%), artemisia ketone (16.58%), eucalyptol (15.41%), and camphene (2.28%). Obtained results confirmed by research from other geographic areas, such as Egypt [2], Iraq [3], etc. Accordingly, there are several different chemotypes of *A. abrotanum* different in geographical origin: the ((+)-piperitone chemotype, *trans*-sabinyl acetate/α-terpineol chemotype, 1,8-cineole/α-thujene/α-pinene chemotype, eucalyptol chemotype, and davanol/davanone/hydroxydavanone chemotype) [4]. The *A. abrotanum* EO from Bosnia and Herzegovina was particularly rich in (+)-2-Bornanone, and we could conclude that it is a new (+)-2-Bornanone chemotype that occurred in this *A. abrotanum* EO. **Conclusions:** To our knowledge, no analysis of EO *A. abrotanum* from Bosnia and Herzegovina has been conducted to date. The presented study of EO *A. abrotanum* showed a great diversity of certain compounds as well as their content, which is in line with recent phytochemical studies of EO *A. abrotanum* in the region and wider. The main constituent of EO was (+)-2-bornanone, which reveals a new chemotype when compared to other studies. Due to the potential of *A. abrotanum* for application in the cosmetic and food industries, further research should be conducted regarding the composition during different vegetation phases, while the application of in silico research would contribute to a better understanding of the mechanisms of action of the main components as well as the overall biological activity of EO.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Ekiert, H., Knut, E., Świątkowska, J., Klin, P., Rzepiela, A., Tomczyk, M., & Szopa, A. (2021). Artemisia abrotanum L.(southern wormwood)—history, current knowledge on the chemistry, biological activity, traditional use and possible new pharmaceutical and cosmetological applications. *Molecules*, *26*(9), 2503.
[2] Khalid, K. A., & El-Gohary, A. E. (2020). Productivity of wormwood (Artemisia abrotanum) enhanced by trace elements. *Bulletin of the National Research Centre*, *44*(1), 120.
[3] Aruba, O. S., Jasim, G. A., & Nasser, A. A. (2019). Detection of terpenes of Iraqi Artemisia abrotanum L. by GC/MS in hexane extract. *Al Mustansiriyah Journal of Pharmaceutical Sciences*, *19*(4), 239-248.
[4] Politeo, O., Cajic, I., Simic, A., Ruscic, M., & Bektasevic, M. (2023). Comparative study of chemical composition and cholinesterase inhibition potential of essential oils isolated from artemisia plants from Croatia. *Separations,* 10(10), 546.

Chemical Profile of the Essential Oil of *Helichrysum italicum* (Roth) G. Don subsp. *italicum* from Cultivation in Herzegovina

Talić, S.[a,*], Odak, I.[a]

[a]*Department of Chemistry, Faculty of Science and Education, University of Mostar, Matice hrvatske bb, Mostar, Bosnia and Herzegovina*

***Corresponding author:** stanislava.talic@fpmoz.sum.ba*

***Keywords:** Asteraceae, cultivation, essential oil composition, γ-curcumene, Helichrysum italicum, neryl acetate.*

Objective: Immortelle (Helichrysum italicum (Roth) G. Don subsp. italicum) is a thermophilic aromatic plant that grows in sub-Mediterranean and Mediterranean regions in its natural habitats. The plantation cultivation of immortelle in Herzegovina is of important economic importance. In recent years, numerous plantations have been established in the Herzegovina region. However, it has been observed that essential oils from wild and plantation-grown immortelle differ in the proportion of important chemical components. This paper has investigated the chemical composition of the essential oil of Herzegovinian plantation immortelle. **Methods:** Samples were collected during flowering from a total of eight localities in central, southern, western, and eastern Herzegovina. Essential oil isolation was performed by hydrodistillation using a Clevenger apparatus. The chemical composition of the isolated immortelle essential oil was determined by GC-MS/FID analysis. Samples were analyzed in triplicate on two capillary columns with stationary phases of different polarities. **Results**: The yields of essential oils ranged from 0.19% to 0.29%. A total of 60 components were identified, representing 97.3% to 99.9% of the total essential oil composition. All investigated essential oils had a high content of monoterpene hydrocarbons (37.8% - 50.0%), which is significantly higher than in wild populations [1, 2]. Other components include oxygenated monoterpenes (7.0% - 10.7%), sesquiterpene hydrocarbons (9.6% - 39.6%), oxygenated sesquiterpenes (1.4% - 11.9%), non-terpenic esters (0.9% - 4.6%), and β-diketones (9.7% - 24.1%). The most abundant component in all samples was α-pinene, with an average value of 33.5%, followed by γ-curcumene (8.7%), limonene (5.6%), 4,6,9-trimethyldec-8-en-3,5-dione (4.6%), neryl acetate (4.3%), 2,4,6,9-tetramethyldec-8-en-3,5-dione A (4.2%), β-selinene (3.9%), and trans-caryophyllene (3.4%). **Conclusions:** It was determined that the investigated essential oils from cultivated immortelle have the same volatile components as wild immortelle, but with a significant difference in the relative proportion of the main constituents.

ACKNOWLEDGMENTS

This work was supported by Federal Ministry of Education and Science, Bosnia and Herzegovina [Grant No. 05-39--1/15 and Grant No. 05-39- -1/24].

REFERENCES

[1] Talić S., Odak I., Lukić T., Brkljača M., Martinović Bevanda A., Lasić A., (2021). Chemodiversity of *Helichrysum italicum* (Roth) G. Don subsp. *italicum* Essential Oils from Bosnia and Herzegovina, *Fresenius Environmental Bulletin*, 30 (3): 2492–2502.

[2] Talić S., Odak I., Martinović Bevanda A., Crnjac N., Paštar M., (2019). *Helichrysum italicum* (Roth) G. Don subsp. *italicum* from Herzegovina: Volatile Composition, Variations during Seasons, Total Polyphenols, Acetylcho-linesterase Inhibition and Antioxidant Activity, *Croatica Chemica Acta*, 92(1): 69-77.

Essential Oil of *Teucrium polium* L. from Herzegovina: The Chemical Composition and Acetylcholinesterase inhibition

Džeba, A.[a], Odak, I.[b], Talić, S.[b,*]

[a]*Faculty of Pharmacy, University of Mostar, Matice hrvatske bb, Mostar, Bosnia and Herzegovina*
[b]*Department of Chemistry, Faculty of Science and Education, University of Mostar, Mostar, Bosnia and Herzegovina*

***Corresponding author:** stanislava.talic@fpmoz.sum.ba*

***Keywords:** Acetylcholinesterase, α-cubeben, essential oil composition, Teucrium polium L., trans-caryophyllene.*

Objective: The species from the *Teucrium* genus are part of the aromatic and medicinal flora of Bosnia and Herzegovina. *Teucrium polium* L. is naturally found in dry and rocky areas of central and southern Herzegovina. This paper presents the chemical composition of the essential oil of *Teucrium polium* L. from Herzegovina and its inhibitory effect on the enzyme acetylcholinesterase. **Methods**: The aerial parts of the plant (leaves, flowers, and stem) were collected during flowering in central Herzegovina near Mostar, then dried and subjected to distillation in a Clevenger apparatus. The composition of the essential oil was determined using a Shimadzu GC–MS QP2010 system. The inhibitory effect of the essential oil on acetylcholinesterase was determined *in vitro* according to the Elman method (Microplate reader IRE/96/SFRY). **Results:** A total of 29 volatile components were identified in the essential oil, representing 89.6% of all essential oil components. The most abundant group of compounds was sesquiterpenes (57.8%), followed by oxygenated sesquiterpenes (22.8%), and the rest were monoterpenes. The main components of the essential oil were *trans*-caryophyllene (27.0%), *α*-cubebene (12.5%), and *β*-selinene (4.0%). The maximum inhibitory effect on acetylcholinesterase was achieved by the essential oil at a concentration of 450 μg/mL (58%), while 50% of the enzyme inhibition (IC_{50}) was recorded at 198 μg/mL. **Conclusions:** According to this research, the chemical composition of *Teucrium polium* L. essential oil can be characterized as a *trans*–caryophyllene/*α*-cubebene type with a weak to moderate inhibitory effect on acetylcholinesterase.

ACKNOWLEDGMENTS
This work was supported by Federal Ministry of Education and Science, Bosnia and Herzegovina [Grant No. 05-39- -1/24].

Effect of the vapor phase of patchouli essential oil on cell proliferation of lung carcinoma lineage A549

Kanunfre, C.C.[a,*], Verde, L.L.[a], Silva, M.P.B.[b], Faria, M.F.[c]

[a]*Postgraduate Program in Biomedical Sciences, State University of Ponta Grossa, Ponta Grossa, PR, Brazil.*
[b]*Department of General Biology, State University of Ponta Grossa, Ponta Grossa, PR, Brazil*
[c]*Department of Clinical and Toxicological Analysis, State University of Ponta Grossa, Ponta Grossa, PR, Brazil*

Corresponding author: cckanunfre@uepg.br

Keywords: *A549 cell line, cell viability, lung cancer, Pogostemon cablin Benth, selectivity index.*

Objective: The aim of this study was to evaluate the effect of the vapor phase (VP) of commercial patchouli essential oil (EO) on the viability and proliferation of human lung adenocarcinoma cells (A549 BCRJ Code 0033) and human lung fibroblasts (MRC-5 – Adolfo Lutz Institute CCIAL 023). **Methods:** The patchouli EO, from Indonesia and extracted by steam distillation from the leaves of the species *Pogostemon cablin*, Benth, was purchased from the company Quinári, located in the city of Ponta Grossa, Paraná, Brazil. Based on the calculation of the EO density (900 mg/mL^{-1}), a stock solution at 100 mg/mL^{-1} was prepared. The EO was dissolved in a vehicle solution consisting of 75% PA ethanol and 25% propylene glycol. Subsequently, saline (0.9% NaCl) was used to perform the dilutions/concentrations tested. The chemical composition of OE patchouli obtained by high-resolution gas chromatography showed that the main compounds were patchoulol (31.9%), α-bulnescene (19.5%), seychelene (14.3%), and α-patchoulene (8.6%). As an experimental strategy, the cells were plated in the eight central wells of the 24-well culture plates and treated for 72 hours with the EO vapor at different concentrations (0-1000 µg/mL/well), which was added to the remaining 16 cell-free wells. Cytotoxicity was determined using the MTT reduction and sulforhodamine B (SRB) staining assays, from which the concentration of EO capable of generating enough vapor to reduce cell viability by 50% (ICV50) and the selectivity index (SI), calculated by the ratio of the ICV50 of the non-tumor cell line (MRC-5) to the ICV50 of the tumor cell lines (A549), were calculated. Furthermore, the effect of the VP of patchouli EO on the A549 cell line was also assessed in terms of cell migration capacity and cell distribution in different phases of the cell cycle, using the scratch assay and flow cytometry using propidium iodide (PI), respectively. **Results:** The VP of the patchouli EO reduced A549 cell viability with an ICV50 value obtained for the MTT reduction assay of 169.74 µg/mL and 156.13 µg/mL for the SRB assay. For the control cell line (MRC-5), the EO exhibited a cytotoxic effect with an ICV50 of 333.75 µg/mL (MTT) and 316.30 µg/mL (SRB). The SI values calculated were 1.96 and 2.02, respectively. The results of the scratch assay suggest that patchouli EO significantly reduces migration in the A549 cell line. The VP of patchouli EO decreased cell proliferation at a concentration of 170 µg/mL in a time-dependent manner and induced cell cycle arrest in A549 cells by increasing the percentage of cells in the G0/G1 and decreasing the percentage of cells in the S and G2/M phases. **Conclusions:** These findings indicate that the VP of patchouli EO reduces the cell proliferation of A549 cells. Due to their pharmacological properties [1,2], patchouli OEs emerge as a potential complementary treatment modality for lung cancer. In fact, EOs are composed of volatile components that can be delivered directly to the lung tissue through the inhalation pathway. However, future studies still need to be carried out to elucidate the mechanisms involved.

ACKNOWLEDGMENTS
This research was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES (Finance Code 001) and Fundação Araucária (PBA2022011000208/2022).

REFERENCES
[1] Sun J., Sun P., Kang C, Zhang L., Guo L., Kou Y. (2022). Chemical composition and biological activities of essential oils from six lamiaceae folk medicinal plants. *Frontiers in Plant Science*, v. 13, p. 1-18.
[2] Pandey S. K., Bhandari S., Sarma N., Begun, T., Munda S., Baruah, J. (2022). Essential oil compositions, pharmacological importance and agro technological practices of Patchouli (Pogostemon cablin Benth.): a review. *Journal of Essential Oil Bearing Plants*, v. 24, n. 6 p. 1212-1226.

Volatile constituents in the scent of *Spartium junceum* L.

Stanić, T.*[a], Radonić, A.[a]

[a]*Department of Organic Chemistry, University of Split-Faculty of Chemistry and Technology, Split, Croatia*

***Corresponding author:** tstanic@ktf-split.hr*

***Keywords:** Essential oil, GC-MS analysis, headspace volatiles, hydrolate, Spartium junceum L..*

Objective: *Spartium junceum* L. (Spanish broom) is a wild-growing, thornless shrub with spindly branches and deep golden-yellow flowers that belongs to the legume family (Fabaceae). It is indigenous to the Mediterranean region and is widespread in the Mediterranean part of Croatia. It is believed that the city of Split, Aspalathos in Greek, owes its name to the Spanish broom. *S. junceum* is also commonly known as fragrant broom due to the fragrance of its flowers. Knowing that the floral scent is derived from the release of specific volatile compounds, the aim of this study was to isolate and identify the volatile compounds from the *S. junceum* flowers collected on the Marjan hill just above the city of Split. **Methods:** Two isolation methods were used for this purpose: hydrodistillation and headspace solid-phase microextraction. Freshly plucked, fully opened flowers were subjected to hydrodistillation in order to obtain the essential oil and hydrolate. Solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) was used for the analysis, i.e., isolation and identification of the volatile compounds in the flowers as well as in hydrolate. The essential oil was also analysed by gas chromatography-mass spectrometry (GC-MS). All GC-MS analyses were performed using a non-polar, HP-5MS column. **Results:** The main compounds in the essential oil were saturated fatty acids myristic (14.13%) and palmitic (13.14%) acid, followed by *n*-alkane tricosane (8.22%). Among ten terpene compounds identified in the oil, the most abundant were sesquiterpene hydrocarbon a-farnesene (6.69%) and monoterpene alcohols geraniol (3.34%) and linalool (2.99%). The total content of terpenes was 15.86%. Other compounds present in the essential oil in significant amounts were alcohol oct-1-en-3-ol (6.72%), fatty acid lauric acid (5.50%), and aldehydes phenylacetaldehyde (3.43%) and nonanal (3.42%). The main compounds in headspace volatiles of hydrolate were the monoterpene alcohols geraniol (9.90%) and linalool (8.60%), as well as ester methyl anthranilate (9.01%), oct-1-en-3-ol (6.68%), and phenylacetaldehyde (6.36%). From the composition of the hydrolate headspace volatiles, it is evident that almost all of the identified compounds were also present in *S. junceum* essential oil. As expected, fatty acids and fatty acid esters, compounds that are insoluble in water, were not identified in the hydrolate. The main constituents of the headspace volatiles of *S. junceum* flowers were sesquiterpene hydrocarbon a-farnesene (14.24%) and ester hex-2-enyl butanoate (10.28%), followed by oct-1-en-3-ol (5.08%). The most numerous volatile compounds in headspace volatiles were esters and carbonyl compounds, with a total content of 31.78% and 21.24%, respectively. Except for a-farnesene, only three other terpenes were identified: geraniol, geranial, and bornyl acetate, so that the total content of terpenes was almost the same as in essential oil (15.64%). Same as in the headspace volatiles of hydrolate, fatty acids and their esters were not identified in headspace volatiles of *S. junceum* flowers. **Conclusion:** This approach to the analysis of volatile compounds, i.e., by isolating volatile compounds using various methods, which resulted in different mixtures of volatile compounds, enabled a more comprehensive insight into the chemical composition and content of volatile compounds of this plant.

Combined Antibacterial Effects of Thymol and Terpinen-4-ol against methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Bordás, B.[a,*], Kocsis, B.[b] Horváth, Gy.[a], Balázs, V.L.[a]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs, Pécs, Hungary*
[b]*Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Pécs, Hungary*

***Corresponding author:** bordasbence01@gmail.com*

***Keywords**: Checkerboard titration, terpinen-4-ol, thymol, methicillin-resistant, Pseudomonas aeruginosa, Staphylococcus aureus, synergistic effect.*

Objective: Essential oils are complex mixtures of plant-derived secondary metabolites, predominantly composed of monoterpenes, sesquiterpenes, and phenylpropanoid derivatives [1]. Each essential oil exhibits a characteristic profile of major and minor constituents, the relative abundance of which plays a crucial role in determining their biological activities, including antibacterial effects [2]. In the present study, we aimed to investigate whether the co-application of two isolated major components of essential oils, terpinen-4-ol (T4) and thymol (TH), exhibits synergistic antibacterial activity similar to that observed in the complex mixtures of essential oil constituents (preliminary experiments). It was evaluated the individual and combined antibacterial effects of T4 and TH against methicillin-resistant *Staphylococcus aureus* Rosenbach (MRSA, ATCC 700698) and *Pseudomonas aeruginosa* (Schröter) Migula (ATCC 27853). In addition, the aim of our study was to support the inhibitory effect of T4 and TH on biofilm formation. **Methods**: Using the microdilution method, the minimum inhibitory concentrations (MICs) of the individual compounds were determined. Biofilm inhibition tests were performed using the crystal violet method. In order to investigate the interaction between the components, a checkerboard titration was performed. **Results**: Based on our results, it can be concluded that both T4 and TH have antibacterial effects. Of the two bacteria, *P. aeruginosa* proved to be more resistant (MIC-T4: 0.312 mg/mL; MIC-TH: 0.625 mg/mL) compared to MRSA (MIC-T4: 0.156 mg/mL; MIC-TH: 0.312 mg/mL). In terms of biofilm inhibition, T4 was more effective - inhibitory rates: 80.3% (MRSA); 72.1% (*P. aeruginosa*). The results of the checkerboard titration supported that the combination of T4 and TH is synergistic (FICI: 0.49) for MRSA and additive (FICI: 0.75) for *P. aeruginosa*. **Conclusion**: Based on our results, we can conclude that terpinen-4-ol and thymol have antibacterial and biofilm inhibition effects. Furthermore, we confirmed that when used together, they have a synergistic effect against MRSA, meaning that the two components together show increased antibacterial effect even at lower concentrations.

ACKNOWLEDGMENTS

The research work was supported by Development and Innovation Office NKFIH PD 147156 (Balázs VL) and by 2021-1.2.6-TÉT-IPARI-MA-2022-00015.

REFERENCES

[1] Reichling J. (2020). Anti-biofilm and Virulence Factor-Reducing Activities of Essential Oils and Oil Components as a Possible Option for Bacterial Infection Control. *Planta medica*, 86(8), 520–537.

[2] Tariq, S., Wani, S., Rasool, W., Shafi, K., Bhat, M. A., Prabhakar, A., Shalla, A. H., Rather, M. A. (2019). A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. *Microbial Pathogenesis*, 134, 103580.

Evaluation of the Cytotoxic Potential of Sage Essential Oil

Trajkovska, A.[a*], Zhivikj, Z.[b], Davkova, I.[a], Bajatovska, AM.[b], Kadifkova Panovska, T.[b], Stoilkovska Gjorgievska, V.[a], Cvetkovikj Karanfilova, I.[a], Stefkov, Gj.[a], Karapandzova, M.[a]

[a]*Ss. Cyril and Methodius University, Faculty of Pharmacy, Institute of Pharmacognosy, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia*
[b]*Ss. Cyril and Methodius University, Faculty of Pharmacy, Institute of Applied Biochemistry, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia*

***Corresponding author:** ana.trajkovska@ff.ukim.edu.mk*

***Keywords:** Brine shrimp lethality assay, cytotoxicity, essential oil, Salvia officinalis.*

Objective: *Salvia officinalis* L. (common sage) is a pharmacopoeial species recognized for its production of biologically active metabolites, including terpenes and flavonoids. Traditionally used for its antimicrobial, antioxidant, anti-inflammatory, and metabolic effects, *S. officinalis* has recently gained interest due to its potential cytotoxic activity [1]. The aim of this study was to evaluate the cytotoxic potential of commercially available *S. officinalis* essential oil using the Brine Shrimp Lethality Assay (BSLA), a widely accepted preliminary screening method for bioactive compounds**. Methods:** The cytotoxic potential of sage essential oil was evaluated using the *in vivo* BSLA according to the method of Meyer et al. [2]. After 24 hours of incubation, mortality was recorded, and LC_{50} values were calculated using probit regression analysis**. Results:** Based on the results from the BSLA test, the essential oil demonstrated significant cytotoxicity with a calculated LC_{50} value of 16.68 µg/mL. According to Meyer's toxicity scale [3], this classifies the oil as toxic, while Clarkson's scale [4] ranks it as highly toxic. **Conclusions:** The chemical composition of *S. officinalis* essential oil, which is rich in monoterpenes such as 1,8-cineole, α-thujone, and camphor [1], supports its potent cytotoxic and neurotoxic effects as well as possible antimicrobial activity. This chemical profile aligns with the known bioactivity of similar *Salvia* species and supports further investigation into their pharmacological applications, especially cytotoxic activity, which also underlines the need for its careful dose evaluation in potential therapeutic use.

REFERENCES

[1] Cvetkovikj, I., Stefkov, G., Karapandzova, M., Kulevanova, S., Satović, Z., 2015. Essential Oils and Chemical Diversity of Southeast European Populations of *Salvia officinalis* L.. *Chemistry & Biodiversity,* 12: 1025-1039.
[2] McLaughlin, J.L., Rogers, L.L., Anderson, J.E., 1998. The use of biological assays to evaluate botanicals. *Ther. Innov. Regul.Sci.* 32(2), 513-524.
[3] Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.* 45(5), 31-34.
[4] Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M.G., Bhagwandin, N., Smith, P. J., Folb, P. I., 2004. *In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *J. Ethnopharmacol.* 92(2-3), 177-191.

Cytotoxic Potential of Commercially Available Pine Essential Oil Evaluated by Brine Shrimp Lethality Assay

Davkova, I.[a,*], Zhivikj, Z.[b], Trajkovska, A.[a], Bajatovska, AM.[b], Petreska Ivanovska, T.[b], Stoilkovska Gjorgievska, V.[a], Cvetkovikj Karanfilova, I.[a], Stefkov, Gj.[a], Karapandžova, M.[a]

[a]*Ss. Cyril and Methodius University, Faculty of Pharmacy, Institute of Pharmacognosy, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia*
[b]*Ss. Cyril and Methodius University, Faculty of Pharmacy, Institute of Applied Biochemistry, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia*

***Corresponding author:** iskra.davkova@ff.ukim.edu.mk

Keywords: *Brine shrimp lethality assay, essential oil, Pine.*

Objective: Essential oils from *Pinus* species have long been used for their antimicrobial, anti-inflammatory, and respiratory benefits [1, 2]. These plants are a notable source of bioactive compounds, and their essential oil is increasingly available on the commercial market. However, toxicological data for such commercially sold products remain limited, particularly given the variability in chemical composition depending on the manufacturer, geographical origin, and extraction methods. Generally, the most dominant components in the pine essential oil are monoterpenes, including α-pinene, β-pinene, limonene, β-phellandrene, and bornyl acetate, as well as sesquiterpenes such as *trans*-(E)-caryophyllene and germacrene D [1]. While these compounds have known biological activities, including cytotoxicity, variation among commercial samples from the Macedonian market requires thorough toxicity assessment to ensure their safety and support therapeutic use. Therefore, this study aims to evaluate the cytotoxic activity of commercially available *Pine* essential oil from the Macedonian market using the Brine Shrimp Lethality Assay (BSLA), a rapid, low-cost, and validated preliminary screening method. **Methods:** The cytotoxic activity was evaluated using the Brine Shrimp Lethality Assay (BSLA), following the methodology described by McLaughlin et al. [3] and Meyer et al. [4]. LC_{50} values were calculated using probit regression analysis. Based on the obtained LC_{50} values, the essential oil was categorized according to the toxicity scales proposed by Meyer et al. [4] and Clarkson et al. [5]. **Results:** The LC_{50} value was determined to be 10.95 µg/mL. According to both Meyer's and Clarkson's toxicity scales, the analyzed essential oil is classified as toxic and very toxic, respectively. **Conclusions:** The findings highlight the necessity for caution when using pine essential oils and underscore the importance of thorough toxicological assessment before their therapeutic use. The observed cytotoxicity may be attributed to the high content of bioactive terpenes; however, additional studies are needed to confirm this result through more advanced biological testing.

REFERENCES

[1] Karapandzova, M., Stefkov, G., Cvetkovikj, I., Trajkovska-Dokic, E., Kaftandzieva, A., Kulevanova, S., 2014. Chemical composition and antimicrobial activity of the essential oils of *Pinus peuce* (Pinaceae) growing wild in R. Macedonia, *Natural Product Communications,* 9(11), 1623–1628.
[2] Karapandzova, M., Stefkov, G., Trajkovska-Dokic, E., Kaftandzieva, A., Kulevanova, S., 2011. Antimicrobial activity of needle essential oil of *Pinus peuce* Griseb. (Pinaceae) from Macedonian flora, Macedonian Pharmaceutical Bulletin, 57(1), 2.
[3] McLaughlin, J.L., Rogers, L.L., Anderson, J.E., 1998. The use of biological assays to evaluate botanicals. Ther. Innov. Regul.Sci. 32(2), 513-524.
[4] Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.* 45(5), 31-34.
[5] Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M.G., Bhagwandin, N., Smith, P. J., Folb, P. I., 2004. *In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *J. Ethnopharmacol.* 92(2-3), 177-191.

Chemical characterization and antibacterial evaluation of *Mentha x piperita* L. and *Thymus serpyllum* L. essential oils and their infusion preparations

Demirci, B.[a,*], Özturk, G.[a], Kırcı, D.[b], Demirci, F.[a]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Türkiye*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, İzmir Katip Çelebi University, İzmir, Türkiye*

***Corresponding author:** betuldemirci@gmail.com

Keywords: *Antibacterial activity, essential oil, herbal tea, Mentha x piperita* L.*, Thymus serpyllum* L.*.*

Objective: This study aimed to determine the volatile components of essential oils and their infusion preparations from *Mentha x piperita* L. and *Thymus serpyllum* L. aerial parts to evaluate their antibacterial activities against selected human respiratory tract pathogens. **Methods:** The herbal drugs were obtained from a pharmacy in Munich, Germany. The herbal tea of each herbal drug was freshly prepared as a 5% and 10% infusion, followed by the analyses of the volatiles by HS-SPME-GC/MS [1]. The essential oils were obtained by hydrodistillation, subsequently analyzed by GC-FID and GC/MS systems [1]. The in vitro antibacterial activities of the test samples were evaluated by broth microdilution and vapor diffusion using *Escherichia coli* NRRL B-3008, *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* NRRL B-4378, *Staphylococcus aureus* ATCC 6538, *Streptococcus mutans* ATCC 25175, and *Salmonella typhimurium* ATCC 13311 pathogens [2, 3]. **Results:** The yields of essential oils of *M. piperita* and *T. serpyllum* were 1.25% and 0.31%, respectively. Menthol (33.1, 24.8, 31.8%), menthone (25.8, 32.0, 19.8%), menthyl acetate (10.9, 9.2, 9.5%), and isomenthone (5.7, 10.5, 8.1%) were characterized as the main compounds in the *M. piperita* essential oil, 5% and 10% infusions, respectively. Thymol (27.9, 31.8, 36.2%), linalool (16.5, 18.7, 16.6%), *p*-cymene (15.0, 1.8, 1.7%), and carvacrol (4.7, 8.8, 9.9%) were the main compounds in *T. serpyllum* essential oil, 5% and 10% infusions, respectively. The minimum inhibition concentration (MIC) of the tested samples was in the range of 6.25-0.78 mg/mL, compared with standard antibacterial agents. Inhibition zone diameters (25-60 mm) of essential oils and infusions were determined along with positive and negative controls. When compared with current literature, the volatile constituents were in line, and the antibacterial activities were relatively moderate. The infusions were more susceptible to the tested pathogens compared to the tested oils. **Conclusions:** Both essential oils and infusion combination preparations have the potential for enhanced flavored antimicrobial activity.

ACKNOWLEDGMENTS
This work has been supported by Anadolu University Scientific Research Projects Coordination Unit under grant number #1867.

REFERENCES
[1] Demirci, B., Kırcı, D., Öztürk, G., Demirci, F. (2022). Effect of Extraction Time on *Origanum onites* L. Infusions and Essential Oils– Biological Evaluation, Statistical Principal Component and Hierarchical Cluster Analyses. *Chemistry & Biodiversity*, 19(12), e202200482.
[2] CLSI. (2007). Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement, M100-S17, Clinical and Laboratory Standards Institute 27.
[3] Demirci, F., Karaca, N., Tekin, M., Demirci, B., (2018). Anti-inflammatory and antibacterial evaluation of *Thymus sipyleus* Boiss. subsp *sipyleus* var. *sipyleus* essential oil against rhinosinusitis pathogens. *Microbial Pathogenesis*, 122, 117-121.

Evaluation of *Allium sativum, Syzygium aromaticum,* and *Cymbopogon citratus* Essential Oil Combinations against Upper Respiratory Tract Microbial Pathogens

Kahya, S.E.[a], Al Zalak, S.[a], Karadağ A.E.[a], Demirci, B.[b], Demirci, F.[b,*]

[a]*Department of Pharmacognosy, School of Pharmacy, Istanbul Medipol University, Beykoz, Istanbul, Türkiye*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, Türkiye*

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

***Keywords:** Antibacterial, checkerboard, clove, garlic, disk diffusion, Lemongrass oils, microdilution.*

Objective: Essential oils are well known and used for their antimicrobial properties, especially against resistant microbial pathogens. In this study, the aim is to evaluate the potential of *Syzygium aromaticum, Cymbopogon citratus,* and *Allium sativum* essential oils in combination for their antibacterial activity against selected upper respiratory tract pathogens to formulate an effective oral care formulation**. Methods:** The quality of the commercially available essential oils was verified by GC-MS and GC-FID methods. *In vitro* antibacterial evaluation of the essential oils against *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC BAA44, *Moraxella catarrhalis* ATCC 23245, *Streptococcus mitis* NCIMB 13770, *and Streptococcus mutans* ATCC 25125 was performed using a microdilution assay. The synergistic, additive, and indifferent effects of oil combinations were evaluated using the checkerboard method, where the inhibitory zones of the oral formulations were also evaluated by using a disc diffusion model. **Results:** The main components for the analyzed essential oils were determined as eugenol (78.4%), methyl thiirane (52.3%), geranial (45.4%), diallyl disulfide (29.1%), neral (28.2%), and β-caryophyllene (9.3%). Among the tested oils, *S. aromaticum* showed the strongest inhibitory activity against *M. catarrhalis* (MIC: 125 mg/mL) and *S. aureus* (MIC: 250 mg/mL). The calculated fractional inhibition concentration index (FICI) for *S. aromaticum-C. citratus* was FIC: 0.375, where *S. aromaticum- A. sativum* essential oil combinations against *M. catarrhalis* resulted in an FIC of 0.265, suggesting a synergistic effect. Calculated effective essential oil concentrations were incorporated into the oral formulation, where the *S. aromaticum* and *C. citratus* combination was the most inhibitory against *S. mutans*, as indicated by the largest inhibition zone (23 mm). To the best of our knowledge, this oil combination was characterized by its synergistic effect for the first time. **Conclusions:** The initial combinations findings suggest future potential towards more effective oral care formulations. Further *in-vivo* studies are needed for safety to assess their efficacy in clinical settings.

ACKNOWLEDGMENTS
This work was part of the graduation project of S. Al Zalak. The commercial essential oils were kindly provided by OrlifeGlobal, İstanbul, Türkiye. This work was partially supported by Anadolu Scientific Projects: BAP-YTT-2024-2601.

Chemical composition of essential oil and glucosinolate-derived volatiles in *Raphanus raphanistrum* L. (Brassicaceae)

Avdović, E.[a,*], Gusinac, A.[b], Simijonović, D.[a], Marković, Z.[c], Dekić, M.[c]

[a]*Institute for Information Technologies, University of Kragujevac, Kragujevac, Serbia*
[b]*Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, Niš, Serbia*
[c]*Department of Sciences and Mathematics, State University of Novi Pazar, Novi Pazar, Serbia*

Corresponding author: edina.avdovic@pmf.kg.ac.rs

Keywords: *Brassicaceae, essential oil, GC-MS, glucosinolate breakdown products, Raphanus raphanistrum.*

Objective: *Raphanus raphanistrum* L. (wild radish) is a widely distributed species native to Europe, northern Africa, and parts of Asia and has spread to the Americas, Australia, and other regions [1–3]. Edible in the Mediterranean region and traditionally used for antirheumatic, hypoglycemic, and gastrointestinal purposes, it is also considered an invasive weed in Australia [1, 3]. Phytochemical investigations have revealed the presence of a range of compounds in this species, including polyunsaturated fatty acids, tocopherols, flavonoids, terpenoids, tannins, coumarins, and alkaloids [4–6]. Numerous glucosinolates or their respective breakdown products have been identified in this species, including glucoraphasatin, glucobrassicanapin, neoglucobrassicin, glucoerucin, glucoraphanin, glucoraphenin, glucotropaeolin, glucoiberin, sinigrin, gluconasturtiin, glucosinalbin, glucobassicin, progoitrin, gluconapin, glucoalyssin, gluconapoleiferin, 4-hydroxyglucobrassicin, glucohesperin, 4-methoxyglucobrassicin, and 5-methylsulfopentyl glucosinolate [2–5, 7]. Despite previous studies, the essential oil of *R. raphanistrum* originating from Serbia has not been previously analyzed. **Methods:** Samples of *R. raphanistrum* were collected during the flowering stage in late May 2023 near Niš, Republic of Serbia. Essential oil was extracted by hydrodistillation using a standard Clevenger-type apparatus. The essential oil composition was analyzed by GC–MS using an Agilent 7890B gas chromatograph with an HP-Innowax capillary column and a 240-MS ion trap detector (Agilent Technologies) under previously described conditions [8]. **Results:** A detailed GC-MS analysis of the essential oil revealed that the predominant constituents were (*E*)-4-methylthio-3-butenyl isothiocyanate (*trans*-raphasatin, 32.7%) and (*Z*)-4-methylthio-3-butenyl isothiocyanate (*cis*-raphasatin, 15.9%), degradation products of the glucosinolate glucoraphasatin. Additionally, several other glucosinolate breakdown products were identified in the oil that had not previously been reported in this species. These include 4-methylhexanenitrile, 5-methylhexanenitrile, heptanenitrile, octanenitrile, 4-(methylthio)butanenitrile, and 6-(methylthio)hexanenitrile. **Conclusion:** The essential oil of Serbian *R. raphanistrum* is rich in *trans*- and *cis*-raphasatin, derived from glucoraphasatin. Several previously unreported glucosinolate breakdown products were also identified, indicating a distinct chemical profile.

REFERENCES

[1] U.S. Department of Agriculture-Agricultural Research Services (USDA-ARS). 2025. Germplasm Resources Information Network (GRIN). https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch. Accessed July 6, 2025.
[2] Maldini M., Foddai M., Natella F., Petretto G. L., Rourke J. P., Chessa M., Pintore G., (2016). Identification and quantification of glucosinolates in different tissues of *Raphanus raphanistrum* by liquid chromatography tandem-mass spectrometry, *Journal of Food Composition and Analysis*, 16, 20-27.
[3] Malik M. S., Norsworthy J. K., Culpepper A. S., Riley M. B., Bridges W. Jr., (2008). Use of wild radish (*Raphanus raphanistrum*) and rye cover crops for weed suppression in sweet corn, *Weed Science*, 56, 588–595.
[4] Malik M. S., Riley M. B., Norsworthy J. K., Bridges W. Jr., (2010). Glucosinolate profile variation of growth stages of wild radish (*Raphanus raphanistrum*), *J. Agric. Food Chem.*, 58, 3309–3315.
[5] Iyda J. H., Fernandes Â., Ferreira F. D., Alves M. J., Pires T. C. S. P., Barros L., Amaral J. S., Ferreira I. C.F.R., (2018). Chemical composition and bioactive properties of the wild edible plant *Raphanus raphanistrum* L., *Food Research International,* 121, 714-722.
[6] Muthusamy B., Shanmugam G., (2020). Analysis of flavonoid content, antioxidant, antimicrobial and antibiofilm activity of *in vitro* hairy root extract of radish (*Raphanus sativus* L.), *Plant Cell, Tissue and Organ Culture,* 140, 619-633.
[7] Khalil N., Gad H. A., Musayei N. M. A., Bishr M., Ashour M. L. (2022). Correlation of glucosinolates and volatile constituents of six Brassicaceae seeds with their antioxidant activities based on partial least squares regression, *Plants*, 11, 1116.
[8] Dekić, M. S., Plojović, S. A., Gusinac, A. M., Selimović, E. S., & Radulović, N. S. (2025). Comprehensive mass spectral libraries and gas chromatographic data for esters of regioisomeric furanmethanols: addressing identification challenges in food chemistry. *Food research international*, *209*, 116294.

Chemotype profile and *In vitro*/*In silico* Evaluation of Anticancer Potential of *Mentha spp.* Essential Oils

Bečić, E.[a], Džudžević-Čančar, H.[b,*], Alispahić, A.[b], Dedić, A.[b], Lukšič M.[c], Stanojković, T.[d]

[a]*Department of Pharmaceutical Chemistry, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[b]*Department of Chemistry in Pharmacy, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[c]*Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia*
[d]*Institute for Oncology and Radiology of Serbia, Belgrade, Serbia*

***Corresponding author:** hurija.dzudzevic-cancar@ffsa.unsa.ba*

***Keywords:** Anticancer activity, Mentha pulegium, Mentha carvina, molecular docking.*

Objective: *Mentha* spp., the genus of the Lamiaceae family (formerly Labiatae), includes more than 20 species that are spread all over the world. Some pharmacological effects of essential oils (EOs) from *Mentha* spp., such as their abortifacient effect in rat myometrium, cytotoxic activity against different human cell lines, and antioxidant effect, were confirmed [1, 2]. The objective of this research was to evaluate the phytochemical composition and cytotoxic potential of EOs derived from *Mentha pulegium* (*MP*) and *Mentha cervina* (*MC*), using *in vitro* and *in silico* approaches. **Methods:** The extraction procedure of *MP* and *MC* EOs was performed using hydrodistillation in a Clevenger-type apparatus. The phytochemical composition of the EOs was analyzed using gas chromatography coupled with mass spectrometry (GC-MS). The cytotoxic activity was evaluated using the MTT assay on three cell lines: HeLa, LS174, and A549. Molecular docking was performed using AutoDock 4.2.6 software. Ligands: pinene, 1,8-cineole, menthofuran, pulegone, limonene, and piperitenone were selected based on their relative abundance in the EOs. Selected ligands were prepared by obtaining SDF (3D conformer) files from the PubChem database and converting them to PDB files using PyMol software. Docking was carried out against three cancer-related target proteins: the kinase domain of epidermal growth factor receptor (EGFR, PDB ID: 1M17), the colchicine-binding site of tubulin (PDB ID: 1SA0), and topoisomerase I (PDB ID: 1T8I). The binding energies (ΔG) of the ligand complexes were determined and expressed in kcal/mol. **Results:** The main identified compound in *MP* EO is pulegone with a concentration of 73.8%. Other compounds, including carvone (6.0%), dihydrocarvone (4.6%), menthone (2.1%), and p-mentha-3,8-diene (2.0%), were also detected. Conversely, *Mentha cervina* EO exhibited a distinct phytochemical composition, predominantly comprising piperitenone (40.75%) and pulegone (37.0%), while other compounds were detected in comparatively lower concentrations. MTT assay results demonstrated that *MC* EO exhibited stronger cytotoxic activity against cancer cell lines (IC_{50}HeLa = 1.63±2.33 µL/mL, IC_{50}LS174 = 1.41±0.81 µL/mL, IC_{50}A549 = 1.63±0.72 µL/mL) than *MP* EO (IC_{50}HeLa = 2.35±4.61 µL/mL, IC_{50}LS174 = 1.18±1.33 µL/mL, IC_{50}A549 = 1.18±1.33 µL/mL). On the other hand, *MP* EO showed a higher selectivity index (SI = 20), indicating greater selectivity towards cancer cells compared to control cells, while *MC* had lower selectivity (SI = 15). Docking simulation results supported the experimental findings and provided a mechanistic explanation for the observed stronger cytotoxic activity of *MC* EO. Piperitenone and pulegone exhibited the most favorable predicted binding affinities towards EGFR, tubulin, and topoisomerase I (ΔG ≈ −6 to −7.5 kcal/mol), suggesting effective inhibition of key cancer-related targets. Other compounds, such as menthofuran, showed moderate interactions (ΔG ≈ −5.2 kcal/mol), while monoterpenes like 1,8-cineole, limonene, and β-pinene (ΔG ≈ −3.8, −4.0, and −3.5 kcal/mol) exhibited weaker binding energies. **Conclusions:** *MC* EO exhibited stronger cytotoxic activity than *MP* EO, while *MP* EO showed higher selectivity. While both EOs contained pulegone, *MC* EO is enriched with piperitenone (40.75%), which, together with pulegone, displayed the most favorable binding affinities toward EGFR as a major target for drugs in treating lung carcinoma, tubulin, and topoisomerase I. The absence of piperitenone in *MP* EO likely underlies its comparatively weaker cytotoxic activity. These results demonstrate a concordance between *in vitro* and *in silico* findings, highlighting *MP* and *MC* as potential natural anticancer agents.

ACKNOWLEDGMENTS
Work is result of the Bilateral cooperation between Bosnia and Herzegovina and Slovenia supported by Federal Ministry of Education and Science of BiH and Slovenian Research and Innovation Agency (05-35-2795-1/23:BI-BA/24-25-036).
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES
[1] Stringaro A., Colone M., Angiolella L., (2018). Antioxidant, antifungal, antibiofilm, and cytotoxic activities of *Mentha* spp. essential oils, *Medicines*, 5(4), 112.
[2] Mahboubi M., Haghi G., (2008). Antimicrobial activity and chem. composition of *Mentha pulegium* L. essential oil, *J. of Ethnopharmacology*, 119(2), 325–327.

Aroma characteristics of *Alpinia zerummbet* growing in Bolivia

Kuraya, E.[a,*], Touyama, A.[b], Jahana, K.[c]

[a]*National Institute of Technology, Okinawa College, 905 Henoko, Nago, Japan*
[b]*Okinawa Flora Ltd.,1-19-24 Onaka, Nago, Japan*
[c]*RYUX Inc., 7-22 Suzaki, Uruma, Japan*

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

***Keywords:** Alpinia zerumbet, aroma characteristics, Bolivia.*

Objective: *Alpinia zerumbet* (Pers.) Burtt and Smith is a perennial herb that is widely distributed in tropical and subtropical regions, and is characterized by its fragrant flowers, leaves, and rhizomes. The essential oil extracted from this herb is commonly used as a fragrance and cosmetic ingredient because of its pleasant aroma and strong antioxidant properties [1]. Previous studies have shown that the leaves and essential oils of *A. zerumbet* exhibit diverse aromatic properties. Although *A. zerumbet* was not originally native to Bolivia, the migration of ~3,200 Okinawans between 1954 and 1969 potentially led to *A. zerumbet* being imported into Bolivia due to the importance of its roots and flowers in Okinawan folk medicine. In March 2025, the authors visited Colonia Okinawa, a Japanese immigrant settlement in Santa Cruz Province, Bolivia, to investigate whether *A. zerumbet* could grow there. Additionally, the aromatic characteristics of *A. zerumbet* grown in Bolivia were investigated. **Methods:** One sample was collected from the city of Santa Cruz (B-SA1) and two samples were collected from Colonia Okinawa (B-C1, B-C2) in March 2025. After collection, the leaves and stems were separated, cut, and dried at 45 °C until reaching a moisture content of ≤10%. Each leaf and stem sample were placed in a vial and heated at 60 °C for 10 min. The aroma components were then captured in TENAX TA-filled glass tubes by passing air through which volatile components were removed by activated carbon for 10 minutes. Dynamic headspace–thermal desorption–gas chromatography–mass spectrometry was employed to analyze the collected components. Volatile components were identified by comparing their retention indices and mass fragmentation patterns with those of mass spectrometry libraries. Linear retention indices were determined for all constituents using a homologous series of *n*-alkanes injected under identical chromatographic conditions. The identified aromatic constituents were subjected to a two-dimensional hierarchical cluster analysis using Ward's method to create heat maps and pattern fingerprints by phylogeny for evaluation of the aromatic constituents. Heat maps and two-dimensional cluster analyses were performed using the Python Seaborn Library. **Results:** One sample collected from Santa Cruz (B-SA1) and one sample from Colonia Okinawa (B-C1) contained high concentrations of sabinene, γ-terpinene, and terpinen-4-ol (13.9–19.7, 10.6–11.8, and 9.1–12.4%, respectively). Based on analysis of the aroma characteristics, the *A. zerumbet* var. *excelsa* variant was identified. The other sample (B-C2), collected from Colonia Okinawa, contained trace amounts of these components and was characterized as *A. Zerumbet*. Previous studies showed that *A. Zerumbet* and *A. Zerumbet* var. *excelsa* possess many chemotypes; however, B-SA1 and B-C1 were found to have aromatic characteristics similar to those of individual samples collected from Kita-daito Island and Okinawa Island, respectively. The aroma characteristics of the B-C2 sample were similar to those of samples collected on the Ishigaki, Iriomote, and Okinawa islands, despite their transplantation to Colonia Okinawa ~70 years ago. **Conclusions:** The aromatic characteristics of *A. zerumbet* growing in Bolivia were investigated for the first time. Although only three *A. zerumbet* samples were investigated, the results clearly indicate the importation of *A. zerumbet* to Colonia Okinawa.

ACKNOWLEDGMENTS
The authors would like to thank Mr. Yamazato of the Okinawa Prefectural Government, Mr. Tsukayama of CAICO, and Mr. Isa of the Association de Bolivia en Okinawa for their invaluable assistance in conducting this study.

REFERENCES
[1] Tu. P., Tawata S., (2015). Antioxidant, anti-aging, and anti-melanogenic properties of the essential oils from two varieties of *Alpinia zerumbet*, *Molecules*, 20, 16723-16740.

Orris Butter Authentication: Analytical Approach Using Orthogonal Techniques to Ensure Origin and Purity

Merle, P.[a], Felix, J-A.[a], Neuter, A.[a], Baroux, L.[a,*]

[a]*Dsm-Firmenich, Rue de la Bergere 7, 1242, Satigny, Switzerland*

***Corresponding author:** lucie.baroux@dsm-firmenich.com*

***Keywords:** Analytical techniques, chirality, GC-IRMS, HPTLC, Orris butter, rhizome authenticity.*

Objective: In recent years, the authentication of orris butter has become a priority for both industry stakeholders and regulators, particularly in the context of origin-based quality claims and increasing demand for traceable, sustainably sourced botanical ingredients. Ensuring authenticity requires precise analytical methodologies that can address both compositional integrity and geographical origin, especially given the emergence of blends or substituted products that mimic orris butter's aromatic profile [1]. **Material**: Orris butter, obtained from the aged rhizomes of *Iris germanica, Iris pallida, and Iris florentina*, is a premium natural ingredient with a long-standing tradition in perfumery and flavoring. Characterized by its unique violet-like aroma, underpinned by woody and floral notes, orris butter derives its olfactory complexity from irones and other sesquiterpenes formed during an extensive post-harvest aging process. Due to its labor-intensive production, long maturation (up to five years), and limited global supply, orris butter commands a high price and is highly susceptible to economic adulteration and mislabeling. **Methods:** To address these challenges, this study proposes an integrated analytical workflow that combines

1. Enantioselective Gas Chromatography (Es-GC): Applied to assess the enantiomeric ratios of key chiral compounds (notably ionones and irones), which can serve as chemical markers indicative of botanical origin and processing methods. ES-GC allows for discrimination between authentic orris sources and synthetic analogues or misrepresented batches [1, 2].
2. Gas Chromatography–Isotope Ratio Mass Spectrometry (GC-IRMS): Utilized to measure stable isotope ratios (e.g., $\delta^{13}C$, $\delta^{2}H$) of selected volatile markers, offering insights into the geographical and environmental conditions influencing the plant material. These isotopic fingerprints are particularly useful in distinguishing between production regions such as Italy, Morocco, and France.
3. High-Performance Thin-Layer Chromatography (HPTLC): Employed as a fast, cost-effective method for fingerprinting the entire chemical profile of orris butter. It enables side-by-side comparisons of batches and rapid screening for anomalies, blends, or adulterants.

Results and Conclusion: The complementary use of these techniques provides a multidimensional authentication strategy that enhances both specificity and robustness. **Conclusion:** Beyond routine quality control, this approach supports broader industry goals, including fair trade certification, sustainable sourcing verification, and protection of geographical indications (GIs) and terroir-linked identity.

REFERENCES

[1] Bicchi C., Joulain D. (2025). A comprehensive review on essential oil and extracts from Iris rhizomes, *Phytochem Rev*, 24, 1629-1665.
[2] Marner F-J., Runge.T., König W A. (1990). Separation of enantiomeric irones by gas liquid chromatography on modified cyclodextrins, *Helvetica Chimica Acta*,7.3

Characterization of terpene and terpenoid compounds in *Cannabis Sativa* L. hemp inflorescences by means of different and complementary analytical techniques

Micalizzi, G.[a], Tropea, A.[a], Irrera, E.[a], Salerno, T.M.G. [a], Mondello, L.[a,b,*]

[a]*Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy*
[b]*Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy*

***Corresponding author:** lmondello@unime.it*

***Keywords:** Cannabis sativa L. inflorescences, cannabis essential oil, GC-MS, GC-FTIR.*

Objective: The main aim of this research is the characterization of the volatile fraction in distilled *Cannabis sativa* L. essential oils by using gas chromatography techniques coupled to different detector systems, namely mass spectrometry (MS) and Fourier Transform Infrared Spectroscopy (FTIR). All samples were distilled by using a microwave-assisted hydro distillation (MAHD) system. The univocal characterization of the volatiles in the distilled Cannabis essential oils was performed by using GC-MS and GC-FTIR analytical techniques. This approach allowed the complete and accurate characterization of all the terpene and terpenoid compounds, including the structural isomers characterized by similar MS spectra, which make it difficult to univocally identify in MS detection. **Methods: *Cannabis sativa* L.** hemp inflorescences (c. 100 g) were distilled through the microwave-assisted hydrodistillation (MAHD) system. The identification of the volatile species was carried out by using GC-MS and GC-FTIR systems equipped with an SLB-5ms fused-silica capillary column of 30 m x 0.25 mm ID x 0.25 µm d*f* (Merck, Darmstadt, Germany). Helium was used as a carrier gas at a linear velocity of 30 cm s^{-1}. **Results:** Around 100 different volatile compounds belonging to monoterpene, sesquiterpene, and oxygenated derivatives were identified by comparison of the MS spectra in a commercial spectral database (FFNSC 4.0, Chromaleont S.r.l.) using the Linear Retention Index (LRI) filter and through library search in a solid-phase IR spectral library, thus expanding the knowledge of cannabis essential oil composition. The combination of the MS, FTIR, and LRIs allowed the univocal and confident identification of flavor compounds, an aspect of fundamental importance considering the unique and characteristic aroma of the *Cannabis Sativa L.* plants. **Conclusions:** This approach demonstrates the capability and complementarity of the MS and FTIR instrumentations to increase the confidence in compound identification, offering benefits from each technique. Compound identification was attained by comparison with libraries of GC retention indices, mass spectra, and infrared spectra.

ACKNOWLEDGMENTS
2, INVESTIMENTO 1.1 - BANDO PRIN 2022 - D.D. n. 104 del 02-02-2022 e D.D. 1401 del 18-09-2024: "High revenue-generating molecules from industrial hemp: cost-effective, chemical classification-driven solutions for their production (CHempion)", supported by the Italian Ministry of University and Scientific Research, no. Prot. 2022AJA7H8, CUP: J53C24002510006 J53C24002510006.

Essential oils variability of *Viscum album* subsp. *album*, *Viscum album* subsp. *abietis* and *Viscum album* subsp. *austriacum* from Poland

Kacprzyk, M.[a], Machado, A.M.[b,*], Bonifácio, L.[c,d], Faria, J.M.S.[c,d], Figueiredo, A.C.[b]

[a]*University of Agriculture in Krakow, Department of Forest Ecosystems Protection, Al. 29 Listopada 46, Krakow, Poland*
[b]*Centre for Ecology, Evolution and Environmental Changes (CE3C) & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, Lisboa, Portugal*
[c]*Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P), Quinta do Marquês, Oeiras, Portugal*
[d]*GREEN IT Bioresources for Sustainability, ITQB NOVA, Av. da República, Oeiras, Portugal*

Corresponding author: ampmachado@fc.ul.pt

Keywords: *Essential oil, GC-FID, GC-MS, Viscum, mistletoe, volatiles.*

Objective: *Viscum* L. species (mistletoes) are hemiparasitic epiphytes well-known as traditional medicinal plants in Africa, Asia, and Europe, which are also relevant for nutrient cycling, biodiversity maintenance, and the survival of species that depend on forests [1, 2]. Nevertheless, recent evidence shows that they also contribute to trees decline and mortality, particularly in massively infested host plants in regions with seasonal water deficit [2]. The objective of this work was to expand knowledge on the ecological relevance of volatiles from mistletoe by assessing the chemical variability of essential oils (EOs) isolated from the leaves (L) and wooden (W) parts of *Viscum album* subsp. *abietis* (Va_abi), *Viscum album* subsp. *album* (Va_alb), and *Viscum album* subsp. *austriacum* (Va_aus), collected in Poland. Mistletoe volatile profiles were compared with those from the parasitized portions of two hosts, *Pinus sylvestris* and *Acer rubrum*. **Methods:** EOs were isolated by hydrodistillation and analysed by Gas Chromatography with Flame Ionisation Detection (GC-FID) for quantification and by Gas chromatography-Mass spectrometry (GC-MS) for component identification [3]. *Viscum* volatile profiles were used in the evaluation of the chemical correlation among the samples by cluster analysis. **Results:** The EOs yield was low (< 0.05%, v/d.w), but 186 compounds were identified in all *Viscum* and host EOs. Agglomerative cluster analysis based on the quantitative variation of the analyzed samples evidenced two main clusters poorly correlated, despite the qualitative similarity of the main components. Va_abi and Va_aus leaf EOs were dominated by *n*-nonanal (7-15%), *n*-hexanal (1-14%), and *trans*-β-farnesene (1-13%), whereas 2-*trans*,4-*trans*-decadienal (7-13%), and palmitic acid (9-11%), were the main components of the EOs of the woody parts of the same species. Va_alb leaf EOs were dominated by *trans*-β-farnesene (13-23%), α-pinene (traces-11%) and *n*-tricosane (2-11%). **Conclusions:** The results obtained highlighted the tendency of separation of leaf EOs from those of the woody parts, with most leaf EOs from Va_abi and Va_aus showing a higher correlation than each with Va_alb leaf EOs. The comparison between the present data and an updated survey of the existing literature reinforces the need for further studies to confirm *n*-hexanal, *n*-nonanal, 2,*trans*-4,*trans*-decadienal, *trans*-*β*-farnesene and palmitic acid as representative dominant components of *V. album* volatiles, independently of the subspecies, variety, or host.

ACKNOWLEDGMENTS
Magdalena Kacprzyk was supported by the MSHE (Poland) SUB/040013-D019 and Own Rector's Scholarship Fund for academic teachers at the University of Agriculture in Krakow. Partially funded by FCT/MCTES (Portugal) through CE3C UID/00329/2025, CHANGE, and GREENIT (DOI: 10.54499/UIDB/04551/2020 and DOI: 10.54499/UIDP/04551/2020).

REFERENCES
[1] Song C., Wei X.-Y., Qiu Z.-D., Gong L., Chen Z.-Y., Ma Y., Shen Y., Zhao Y.-J., Wang W.-H., Lai C.-J.-S., Yang B., (2021). Exploring the resources of the genus *Viscum* for potential therapeutic applications, *Journal of Ethnopharmacology*, 277, 114233.
[2] Andrés. E. G., Valeriano C., Camarero J. J., (2024). Long-term effects of mistletoe removal on radial growth of semi-arid Aleppo pine forests, *Forests*, 15, 1113.
[3] Póvoa O., Farinha N., Lopes V., Machado A. M., Figueiredo A. C. (2024). Coriander (*Coriandrum sativum* L.) from Alentejo (South Portugal) - Ethnobotany and Potential Industrial Use, *Foods*, 13, 929.

Volatile compounds from European liverworts

Nowaczyński, F.[a], Asakawa, Y.[b], Ludwiczuk, A.[a,*]

[a]*Department of Pharmacognosy with the Medicinal Plant Garden, Medical University of Lublin, Chodzki Street, 20-093 Lublin, Poland*
[b]*Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan*

***Corresponding author:** agnieszka.ludwiczuk@umlub.pl*

***Keywords**: bibenzyls, chemical markers, liverworts, pinguisanes, 2,3-seco-aromadendranes, sesquiterpene lactones,*

Objective: Liverworts are spore-forming plants that can grow in almost every available habitat, although most often in humid locations. Many liverwort species demonstrate wide geographical distribution, grow under diverse ecological conditions, and often are pioneers in extreme habitats. Liverwort diversity is significantly higher in the Southern Hemisphere compared to Europe. While Europe has around 484 liverwort species, the Southern Hemisphere boasts an estimated 5,971 species [1]. One of the outstanding features of the liverworts is their chemistry. They produce a wide array of secondary metabolites, mainly terpenoids and aromatic compounds. Many of these compounds are characterized by unprecedented structures [2, 3]. The aim of this study was the analysis of the volatiles present in selected European liverworts and comparing their chemical composition with species originating from outside Europe. **Methods:** The subjects of the present study were eight liverwort species collected in Poland, Hungary, and France. These were *Diplophyllum albicans*, *Frullania dilatata*, *Marchantia polymorpha*, *Plagiochila asplenoides*, *Plagiochila porelloides*, *Porella cordaeana*, *Radula complanata,* and *Scapania nemorea*. Plant material was air-dried and extracted by diethyl ether by use of the ultrasound-assisted extraction method. Volatile extract was analyzed by GC-MS. Retention indices for all compounds were calculated based on *n*-alkanes as standards. Compounds were identified using a computer-supported spectral library, mass spectra of reference compounds, and MS data from the literature, along with the library database of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University. Compound identities were confirmed comparing retention indices with reference compounds and published data. **Results:** GC-MS analysis of the European liverworts showed great chemical diversity. The most popular compounds present in the analyzed species were sesquiterpenoids and bibenzyls. Sesquiterpenoids from pinguisane-type were characteristic for *P. cordaeana*. Eudesmane-type sesquiterpene lactones were present in *D. albicans* and *S. nemorea,* belonging to the same family, Scapaniaceae. Sesquiterpene lactones, frullanolide, and dihydrofrullanolide also were found in *F. dilatata*. However, the main compounds in this liverwort species were aromatic compounds from the bibenzyl group. 2-Geranylo-3,5-dihydroxybibenzyl and 3-hydroxy-4,5-methylenedioxybibenzyl were identified. Bibenzyls are also characteristic compounds found in *R. complanata*. This liverwort mainly produces compounds possessing a dihydrooxepin skeleton, e.g., radulanin A. *P. asplenoides* and *P. porelloides* both produce 2,3-*seco*-aromadendranes. The presence of plagiochilide as well as plagiochillins A, B, C, and H was confirmed. The European specimens biosynthesize metabolites similar to those found in the same species growing in other countries and climates. This is further evidence that the metabolites present in liverworts have chemotaxonomic potential. **Conclusions:** European liverworts produce a diverse array of volatile compounds, primarily sesquiterpenoids and bibenzyl derivatives, many of which are chemotaxonomically significant. The chemical profiles of the studied species closely resemble those of the same species found in other regions, suggesting a strong genetic basis for metabolite production. These findings support the use of liverwort volatiles in species classification and ecological studies.

ACKNOWLEDGMENTS
This research was funded by project 101135305—HORIZON-CL6-2023-CircBio-01-4 (Bioprospecting and production of bioactive molecules from European bryophytes 'BRYOMOLECULES').

REFERENCES
[1] Söderström L., Séneca A., (2008). Species richness and range restricted species of liverworts in Europe and Macaronesia, *Folia Cryptog. Estonica, Fasc.*, 44, 143-149.
[2] Asakawa Y., Ludwiczuk A., Nagashima F. (2013) Chemical constituents of bryophytes: Bio- and chemical diversity, biological activity and chemosystematics. In: A. D. Kinghorn, H. Falk, J. Kobayashi (Eds.) *Progress in the Chemistry of Organic Natural Products*, Vol. 95, Springer-Verlag, Wien, pp.1-796.
[3] Asakawa Y., Ludwiczuk A., Novakovic M., Bukvicki D., Anchang K.Y., (2022) Bis-bibenzyls, bibenzyls, and terpenoids in 33 genera of the Marchantiophyta (liverworts): structures, synthesis, and bioactivity. *J. Nat. Prod.*, 85, 729-762.

Composition of the Essential Oil from the Needles and Twigs of Organic Dwarf Pine (*Pinus mugo* Turra) from Tyrol

Bosilcov, A.[a,*], Vergeiner, C.[a], Joas, K.[a], Reith, M.[a]

[a]*Brüder Unterweger GmbH, Austria*

***Corresponding author:** alin.bosilcov@unterweger-oils.com

***Keywords**: Essential oil composition, dwarf pine, Pinaceae, Pinus mugo, Tyrol,*

Objective: The aim of this study was to assess the chemical composition of the essential oil of organic dwarf pine (*Pinus mugo* Turra) from Tyrol, a region encompassing South-West Austria and Northern Italy. *Pinus mugo*, also known as Mountain Pine, is a shrub with a height of up to 3.5 meters that grows spontaneously in the mountainous regions of Central Europe and the Carpathians at altitudes ranging from 1300 to 2200 meters [1, 2]. The essential oil is obtained through steam distillation of the crushed needles and branches and has a very pleasant pine-type odor, with balsamic-sweet, slightly woody, and spicy nuances and an undertone of great tenacity [3]. In this study, 33 industrial batches of *Pinus mugo* essential oil have been analyzed, spanning the years from 2020 to 2024. **Methods:** The batches of *Pinus mugo* essential oil were produced by steam distillation for 6 hours (using a 5 m^3 still) to 10 hours (using a 10 m^3 still). The yield was 0.2 to 0.35% of a colorless to pale yellow essential oil. The composition was determined by GC-MS and dual-channel GC-FID. Additionally, the enantiomeric distribution of selected constituents was evaluated using enantio-GC with a chiral cyclodextrin-based stationary phase. **Results:** The essential oil of *Pinus mugo* mainly comprised monoterpene hydrocarbons, with α-pinene (11–20%), β-pinene (4.5–8.4%), myrcene (5.8–12.8%), δ-3-carene (19–39%), limonene (3.6–9.6%), and β-phellandrene (11–17%) as the major constituents. Chiral analysis indicated that the levorotatory enantiomer predominated in each case. **Conclusions:** The primary constituent of *Pinus mugo* essential oil is δ-3-carene, a compound typically found in minor concentrations or entirely absent in essential oils from other *Pinus* species. This also applies to bornyl acetate (up to 1.9%). Another characteristic constituent is ß-phellandrene, which has been found in similarly high concentrations only in the essential oil from *Pinus cembra*.

REFERENCES.

[1] Gildemeister E., Hoffman Fr. (1956). Die ätherischen Öle, Band 4, Akademie Verlag, Berlin.
[2] Adler, W., Oswald K., Fischer R., (1994). Exkursionsflora von Österreich, Ulmer Verlag, Stuttgart und Wien.
[3] Arctander, S., (1960). Perfume and Flavor Materials of Natural Origin, Elizabeth, New York.

Antibacterial and Anti-biofilm Activity of *Solidago gigantea* Essential Oil Against MDR Pathogens

Balázs, V.L.[a,*], Kocsis, B.[b], Szilágyi-Utcás, M.[c], Ormai, E.[a], Bodás, B.[a], Biró, V.[a], Purger, D.[a], Horváth Gy.[a], Filep, R.[a]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs, Rokus str. 4, Pécs, Hungary*
[b]*Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Szigeti str. 12, Pécs, Hungary*
[c]*Center for Sports Nutrition Science, Hungarian University of Sports Science, Alkotás str. 42-48, Budapest, Hungary*

Corresponding author: viktoria.balazs@gytk.pte.hu

Keywords*: Antibiotic resistance, Escherichia coli, giant goldenrod, nosocomial infection, Pseudomonas aeruginosa, Staphylococcus aureus.*

Objective: The global rise in antibiotic resistance represents a serious public health challenge. One of the major contributors to this issue is bacterial biofilm formation-a process in which microorganisms, including bacteria such as *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Escherichia coli*, form complex, protective communities on both living and non-living surfaces. Biofilms enhance bacterial survival and facilitate resistance by protecting microbes from antibiotics and the host immune system. They are also associated with horizontal gene transfer, persistence of infections, and treatment failures, particularly in immunocompromised patients. Since essential oils (EOs) have shown promising antimicrobial properties, this study investigates the chemical composition and antibacterial as well as biofilm-inhibiting activity of *Solidago gigantea* essential oil (SEO), an underexplored plant-derived oil, against these key pathogens. **Methods**: Aerial parts of *S. gigantea* were collected from three different regions in Hungary: Vejti (SEO1), Homokmégy (SEO2), and Hévíz (SEO3). After drying the plant at room temperature, the SEOs were extracted by water-steam distillation at 175°C for 3 hours. The composition of the SEOs was determined using gas chromatography-mass spectrometry (GC-MS). Standard strains of *P. aeruginosa* (ATCC 27853), MRSA (ATCC 25923), and *E. coli* (ATCC 25922) were cultured in Brain Heart Infusion broth. The minimal inhibitory concentrations (MICs) of the SEOs were determined using a microdilution assay in 96-well plates. To prove the antibiofilm effect of SEOs, a crystal violet assay was used. **Results:** GC-MS analysis revealed Cyclocolorenone as the major compound, with its highest concentration (29.69%) found in the Hévíz sample (SEO3), suggesting that environmental conditions influence the plant's secondary metabolite profile. Other common components included α-Gurjunene, α-Pinene, Spathulenol, Bornyl acetate, Germacrene D, and *p*-Cymene. The SEOs exhibited inhibitory effects against all tested pathogens. The most sensitive strain was *E. coli* (MIC: 0.312–0.625 mg/mL), followed by MRSA, while *P. aeruginosa* showed the highest resistance. Among the samples, SEO3 demonstrated the strongest overall antibacterial activity across all tested bacteria. The SEO3 sample showed the highest biofilm degradation efficiency, with *E. coli* being the most sensitive pathogen (95.7% inhibition), followed by MRSA and *P. aeruginosa*. All SEO samples inhibited biofilm formation in all three pathogens, with *E. coli* consistently showing the greatest susceptibility. **Conclusions:** This study highlights the potential of *S. gigantea* essential oil as an effective natural antibacterial and anti-biofilm agent, particularly against *E. coli*. The biological activity of SEO is influenced by its chemical composition, which varies depending on environmental factors at the collection site. These findings support further research into *S. gigantea* EO as a complementary or alternative therapy for combating biofilm-associated infections and multidrug-resistant pathogens.

ACKNOWLEDGMENTS
The research work was supported by Development and Innovation Office NKFIH PD 147156 (Balázs VL) and by 2021-1.2.6-TÉT-IPARI-MA-2022-00015.

The possibility of imparting antimicrobial activity to shoe insoles by spraying them with essential oil of *Origanum vulgare*

Bielak, E. [a,*], **Syguła-Cholewińska, J.**[b]

[a]*Department of Non-food Product Quality and Safety, Krakow University of Economics, Krakow, Poland*
[b]*Department of Microbiology, Krakow University of Economics, Krakow, Poland*

***Corresponding author:** bielake@uek.krakow.pl*

***Keywords:** Antimicrobial activity, consumer application, oregano oil, spraying, shoe insoles.*

Objective: Among the wide range of shoe insoles available on the market, there are insoles that have antimicrobial properties, but in most cases this effect is achieved through the use of synthetic biocides, about which there is no detailed information on the product packaging. As part of the study, an attempt was made to impart antimicrobial activity to four commercial insoles for which the manufacturers do not claim such activity, i.e., insoles made of: cotton terry knit fabric and coconut fiber (**A**), cotton terry knit fabric and latex foam with active carbon (**B**), cotton fabric and latex foam with active carbon (**C**), and pigskin leather and latex foam with active carbon (**D**). For this purpose, oregano oil originating from Portugal, obtained by steam distillation from the herb *Origanum vulgare,* was sprayed onto the shoe insoles. **Methods**: Two samples (**a** and **b**) in the shape of a disc with a diameter of 25±5 mm were taken from each insole and then sprayed (sample **a**–application on top, i.e., the side that has close/direct contact with the foot; sample **b**–application on the bottom) with oregano oil in two applications, each time in an amount of approximately 1 mL, which was intended to imitate the method of application by the consumer. The antimicrobial activity of the modified insoles was tested 30 days after the application of the oil in accordance with the guidelines of PN-EN ISO 20645:2006 against the strains *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 10231). The samples, conditioned for 24 hrs. at a temperature of approx. 22–25°C, were applied with sterile tweezers to the sprayed side of culture media inoculated with 0.1 mL of microorganism suspensions and then incubated for 18 to 24 hrs. for bacteria at 37 ± 1°C and 48 hrs. for yeasts at 37 ± 1°C. The presence of growth inhibition zones around the samples and the possible growth of microorganisms underneath them were then assessed. **Results**: The results of antimicrobial activity tests of shoe insoles modified with oregano oil were as follows: - INSOLE **A**: For samples sprayed from the top (**a**) and bottom (**b**) in the case of all bacteria and yeasts, no growth inhibition zones were observed, while moderate (bacteria) or slight (yeasts) growth of microorganisms was visible underneath them. - INSOLE **B**: For samples sprayed from the top (**a**), no growth inhibition zones were observed for all bacteria and yeasts, while moderate growth of microorganisms was visible under the samples. The same result was observed for the sample sprayed from the bottom (**b**) and *P. aeruginosa*. For the remaining samples sprayed from the bottom (**b**), growth inhibition zones of 3 mm (*S. aureus*), 4-5 mm (*E. coli*), and 3-4 mm (*C. albicans*) were observed, while no growth of microorganisms was visible under these samples. - INSOLE **C**: For samples sprayed from the top (**a**), the growth inhibition zones of *S. aureus* and *E. coli* were 0-1 mm, and no growth of these bacteria was observed under the discs. No zones were observed for *P. aeruginosa* and *C. albicans* strains, with simultaneous slight/moderate (bacteria) or slight (yeast) growth under the samples. The effect obtained for samples sprayed from the bottom (**b**) was similar, i.e., growth inhibition zones of 0 mm and no growth under the samples characterized the plates with *S. aureus* and *E. coli*; the absence of growth inhibition zones and slight/moderate or slight growth under the samples concerned plates with *P. aeruginosa* and *C. albicans*, respectively.- INSOLE **D**: For samples sprayed from the top (**a**), the growth inhibition zones were 0 mm, 0-1 mm, and 3 mm for *E. coli*, *S. aureus*, and *C. albicans*, respectively, with no growth under the discs. Under the samples sprayed from the bottom (**b**), no growth of these strains was observed either, and the zones were slightly larger: 4 mm (*E. coli*), 3 mm (*S. aureus*) and 4-5 mm (*C. albicans*). Regardless of the side sprayed, the samples showed the weakest effect against *P. aeruginosa* - no zone of growth inhibition and slight growth of the strain under the discs. **Conclusions**: It was found that the application of oregano oil resulted in a good antimicrobial effect of the insole made of pigskin and latex foam with an active carbon (**D**). A similar effect was observed in the case of a cotton terry knit fabric and latex foam with an active carbon insole (**B**) with oil applied to the bottom side and a cotton fabric and latex foam with active carbon insole (**C**) with oil applied to the top side. The tests confirmed the insufficient antimicrobial effect of the cotton terry knit fabric and coconut fiber insole (**A**) sprayed with oregano oil.

ACKNOWLEDGMENTS

The publication presents the results of the Project No. 019/ZJB/2025/DOS financed from the subsidy granted to the Krakow University of Economics.

Essential Oil Analysis of *Swertia* L. *(Gentianaceae)* Species Growing in Türkiye

Tunc, F.[a], Köse, Y.B.[a,*], Yalçın, F.N.[b], Kürkçüoğlu, M.[c]

[a]*Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, Eskişehir, Türkiye*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Türkiye*
[c]*Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, Türkiye*

Corresponding author: ybkose@anadolu.edu.tr

Keywords*: Essential oil, GC/GC-MS, Swertia iberica, Swertia longifolia.*

Objective: The genus *Swertia* L., the third-largest genus in the *Gentianaceae* family, is represented in Türkiye by two species: *S. iberica* and *S. longifolia*. While this genus has a wide global distribution, in Türkiye, it grows in the Northeastern and Eastern Anatolia regions [1, 2]. This study aimed to evaluate the chemical composition of the essential oils obtained from the flowers of *S. iberica* Fischer ex. C. A. Mey. and *S. longifolia* Boiss., species growing in Türkiye. **Methods:** Volatile compounds were obtained from the flowers *of S. iberica* and *S. longifolia* using a microdistillation device at Anadolu University BİBAM (Plant Medicine and Scientific Research Center) [3]. The essential oils obtained through microdistillation were analyzed by GC (Gas Chromatography) and GC/MS (Gas Chromatography/Mass Spectrometry). Gas Chromatography (Agilent 6890N GC) and Gas Chromatography/Mass Spectrometry (Agilent 5975 GC-MSD) systems were used. Components were identified using the BASER Essential Oil Components Library and Wiley and Adams LIBR (TP) Library scanning software. **Results:** As a result of the analysis, 90.1% of *S. iberica* was analyzed and 24 compounds were identified, while 71.5% of *S. longifolia* was examined and 23 compounds were identified. The major compound in both species was determined to be tetracosane. It is present at 31% in *S. iberica* and 25.2% in *S. longifolia*. **Conclusions:** Long-chain hydrocarbons are the most prevalent compound class in both species. The essential oil analysis of these species was performed for the first time.

ACKNOWLEDGMENTS
This study was supported by Anadolu University Scientific Research Projects Coordination Unit under project number TYL-2023-1848. The authors acknowledge the financial support provided by the Scientific Research Projects Commission.

REFERENCES
[1] https://wfoplantlist.org/
[2] https://www.bizimbitkiler.org.tr/v3/demo/details.php?id=7686&t=1.
[3] Baser, K. H. C., Demirci, B., Demirci, F., Kirimer, N., Hedge, I. C. (2001). Microdistillation as a useful tool for the analysis of minute amounts of aromatic plant materials. Chemistry of Natural Compounds, 37, 336-338.

Exploring the Chemical and Antibacterial Characteristics of Essential Oils from Three *Eugenia* P. Micheli ex L. Species

Okida, M.[a], Lima, M.L[a], Pinto, D.L.[b], Kaneko, T.[b], Cordeiro, I[c], Moreno, P.R.H.[a,*]

[a] *Institute of Chemistry, University of São Paulo, Av. Prof. Line Prestes 748, São Paulo-SP, Brazil*
[b] *Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Line Prestes 560, São Paulo-SP, Brazil*
[c] *Institute for Environmental Research, Curatorship of the Herbarium, São Paulo, SP, Brazil*

***Corresponding author:** prmoreno@iq.usp.br*

Keywords: *Antibacterial activity, chemical composition, GC-MS, essential oils, Eugenia species.*

Objective: This study aims to expand the understanding of the chemical composition and antibacterial activity of essential oils extracted from three *Eugenia* P. Micheli ex L. species (*Eugenia cerasiflora* Miq., *Eugenia mosenii* (Kausel) Sobral, and *Eugenia stigmatosa* DC.) found in the Atlantic Forest of São Paulo state, Brazil. The research seeks to fill a gap in knowledge about southeastern *Eugenia* species, as most previous studies have focused on those from the southern region of Brazil [1]. **Methods**: The essential oils were obtained from dried leaves of the three *Eugenia* species through 4-hour hydrodistillation using a Clevenger apparatus. The chemical composition of the oils was analyzed through gas chromatography-mass spectrometry (GC-MS). Antibacterial activity was evaluated against *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), and *Staphylococcus aureus* (ATCC 6538) using the microplate dilution method [2]. **Results:** The major components of *E. cerasiflora* oil were α-pinene (11.9%), β-pinene (17.6%), and bicyclogermacrene (11.9%). For *E. mosenii*, α-pinene was the sole dominant compound (65.3%). *E. stigmatosa* showed a distinctive profile, with humulene epoxide II (41.8%) and tetradecanal (29.7%) as major components. Two of the oils were dominated by monoterpenes, consistent with findings in other *Eugenia* species. No essential oils inhibited the growth of *E. coli*. However, oils from *E. stigmatosa* and *E. cerasiflora* demonstrated activity against *S. aureus* and *P. aeruginosa*, with *E. stigmatosa* oil showing significant inhibition rates (94.8% and 94.0%, respectively). The oil from *E. mosenii* was inactive against all tested bacteria. These differences were attributed to structural variations in bacterial cell walls and the presence of long-chain aldehydes like tetradecanal in *E. stigmatosa* oil, which are known for their antimicrobial properties [3]. **Conclusions:** This study highlights the chemical diversity and antibacterial potential of Eugenia species from southeastern Brazil, with *E. stigmatosa* oil exhibiting the strongest antibacterial activity, likely due to the presence of tetradecanal. The findings support the importance of exploring Brazilian biodiversity for potential pharmacological applications, emphasizing the chemical similarity between southeastern and southern Eugenia species.

ACKNOWLEDGMENTS
USP, FAPESP, CNPq.

REFERENCES

[1] Apel M. A., Volatile Oils of Subtribe Eugeniinae (Myrtaceae): Chemical Composition, Antimicrobial, and Anti-inflammatory Activity, PhD thesis, UFRGS, Porto Alegre, 2001.
[2] Moreno P.R.H., Lima M. E. L., Caruzo M. B. R., Torres D. S. C., Cordeiro I., Young, M. C. M. (2009) Chemical composition and antimicrobial activity of the essential oil from *Croton heterocalyx* Baill.(Euphorbiaceae ss) leaves. *J. Essent. Oil Res.* 21(2), 190 – 192.
[3] Burt S. (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.*, 94(3), 223–253.

Antibacterial activity of *Gaultheria fragrantissima* essential oil as a surface disinfectant on nosocomial pathogens

Csikós, E.[a], Ormai, E.[a], Kocsis, M.[b], Bencsik, T.[a], Bordás, B.[a], Biró, V.[a], Ollmann, T.[c], Kocsis, B.[d], Szilágyi-Utczás, M.[e], Horváth, Gy.[a], Bencsik-Kerekes, E.[f], Balázs, V.L.[b, *]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs, Pécs, Hungary*
[b]*Department of Agricultural Biology, Institute of Biology, University of Pécs, Pécs, Hungary*
[c]*Centre for Neuroscience, University of Pécs, Pécs, Hungary*
[d]*Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Pécs, Hungary*
[e]*Center for Sports Nutrition Science, Hungarian University of Sports Science, Budapest, Hungary*
[f]*Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary*

***Corresponding author:** balazs.viktoria@gytk.pte.hu*

***Keywords:** Antibiotic resistance, essential oil, Gaultheria fragrantissima, methyl salicylate, nosocomial pathogens.*

Objective: Antibiotic resistance is an increasingly pressing issue in healthcare. Nosocomial pathogens are widely present on hospital surfaces, and due to their reduced susceptibility to commonly used disinfectants and antibiotics, their control remains challenging. The presence of bacterial biofilms further exacerbates the situation, as bacteria in this form are significantly more resistant on both biotic and abiotic surfaces. Essential oils are gaining attention as alternative surface disinfectants. The essential oil of creeping wintergreen (*Gaultheria fragrantissima* Wall., commercially obtained from Panarom Naturkozmetika Kft.) is rich in methyl salicylate and has shown promising antibacterial and anti-biofilm properties. **Methods**: Our study focused on the most common nosocomial pathogens: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pneumoniae* (DSM 20566), and vancomycin-resistant *Enterococcus faecalis* (VREF, ATCC 3987). Microbiological analyses included determination of minimum inhibitory concentrations (MIC), evaluation of biofilm inhibition rates, assessment of membrane-degrading activity, and investigation of synergistic effects between *Gaultheria* essential oil and selected antibiotics using the checkerboard titration method with fractional inhibitory concentration indices (FICI) ranging from 0.25 to 0.5. **Results:** The main component of *Gaultheria* essential oil was methyl salicylate (96.35%). The essential oil exhibited antimicrobial activity against all tested pathogens. *E. coli* was the most sensitive pathogen (MIC: 0.154 mg/mL, biofilm inhibition rate: 91.3%). *K. pneumoniae* also demonstrated high sensitivity in terms of biofilm inhibition (inhibition rate: 91.5%). Membrane degradation studies showed that exposure to twice the MIC for 1 hour resulted in substantial damage to *E. coli* (75.7%) and *K. pneumoniae* (80.4%). Partial membrane disruption was observed in all pathogens within 10 minutes. Synergistic interactions were confirmed: between *Gaultheria* essential oil and ceftriaxone (*E. coli*), *Gaultheria* essential oil and gentamicin (*P. aeruginosa*), and *Gaultheria* essential oil and linezolid (VREF). **Conclusions:** Our findings support the antibacterial and anti-biofilm effects of *Gaultheria* essential oil against nosocomial pathogens. The observed synergistic interactions with commonly used antibiotics further highlight its potential as an adjunct in hospital surface disinfection protocols.

ACKNOWLEDGMENTS

The research work was supported by the University Research Scholarship Program, Ministry of Culture and Innovation, National Research, Development and Innovation Fund (EKÖP-2024-4-II-PTE-114, EKÖP-24-4-II-PTE-242), Development and Innovation Office NKFIH PD 147156 (Balázs VL), NKFIH PD 142122 (Kerekes E) and by 2021-1.2.6-TÉT-IPARI-MA-2022-00015.

Essential oils of some aromatic plants in Cameroon: inter- and intraspecific variations in yield and chemical composition and pharmacodynamic properties

Ndengte, E.P.[a], Bella Bella, J. M.[a*].

[a]*University of Douala, Rue Ange Raphael, Douala, Cameroon*

Corresponding author: bellajm99@yahoo.com

Keywords*: Autoxidation, biological effects, chemical composition, Chenopodiaceae, essential oils, Myrtaceae.*

Objective: The work focuses on optimizing the production of essential oils by studying inter- and intraspecific variations in yields and chemical compositions of 215 samples of essential oils from two families (Myrtaceae and Chenopodiaceae), three genera (Eucalyptus, Melaleuca, and Chenopodium), and seven species (*camaldulensis, citriodora, globulus, tereticornis, torelliana, quinquenervia,* and *ambrosioides*), botanically controlled, and on determining the biological effects of 7 of these samples on five microorganisms (*Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, and Candida albicans*). **Methods:** Essential oils were extracted by hydrodistillation from shade-drying plant samples. The chemical composition of the oils was analyzed by gas chromatography–mass spectrometry (GC-MS), and antimicrobial/antifungal activity was assessed using the disk diffusion method. **Results:** Yields of extracted oils depend more on the site than the harvesting period for *E. citriodora*, whereas for *E. camaldulensis*, both factors, place and date of harvesting, need to be taken into account. For *M. quinquenervia*, it was noted that the harvesting period had little influence on yield, that there was no loss of volume after 60 days of storage, and that 6 to 14 days of shade-drying of the plant material seemed to be the time required for optimum yield. As for chemical composition, three chemical types can be distinguished within *E. camaldulensis*, influenced by the period, place of harvest, and age of the leaves: the 1,8-cineole type, the paracymene type, and the y-terpinene type. Essences of E. citriodora have citronellal levels of over 70%. Their chemical composition is influenced more by the site than by the harvesting period. The essential oil of *Eucalyptus globulus* has a 1,8-cineole content of 70% or more. Analysis of the essential oil of *E. tereticornis* from Cameroon indicates the existence of two chemotypes: the 1,8-cineole type and the farnesole type (84%, including all isomers). The study of *M. quinquenervia* reveals the existence of three chemotypes: the 1,8-cineole type, with equal levels of 1,8-cineole and viridiflorol; the viridiflorol type; and a homogeneous chemical composition throughout the year with no seasonal influence. The ascaridol content of the *Chenopodium ambrosioides* essential oils analyzed ranged from 6.4% to 26.45%. Ascaridol oxide, a newly isolated product, is characterized by a percentage ranging from 14.40 to 50.7%. A sample rich in α-terpinene (44.4%) and poor in ascaridol (8.32%) becomes, after aging by autoxidation, very rich in ascaridol (52.28%) and very poor in α-terpinene (0.05%). **Conclusion:** Regarding the biological activity considering the effect of essential oils, *Staphylococcus aureus* (Gram+) proved to be the most sensitive bacterium, and *Klebsiella pneumoniae* (Gram-) the most resistant. Antifungal activity observed for *Candida albicans* varied depending on the series of essential oils tested. *E. citriodora* essential oil was the most active, followed by *M. quinquenervia* oil. *Chenopodium ambrosioides* essential oil, after autooxidation, was the most active.

ACKNOWLEDGEMENTS
Corresponding author is awarded by the IFEAT within the ISEO2025 – YSFA.

Antibiofilm activity of chestnut honey with lavender essential oil against multidrug-resistant otitis media pathogens

Ángyán, V.D.[a], Koloh, R.[a,*], Balázs, V.L.[a], Nagy-Radványi, L.[a], Kocsis, M.[b], Kocsis B.[c], Horváth, Gy.[a], Farkas, Á.[a]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, University of Pécs, 7624 Pécs, Hungary;*
[b]*Department of Agricultural Biology, Institute of Biology, University of Pécs, 7624 Pécs, Hungary*
[c]*Department of Medical Microbiology and Immunology, Medical School, University of Pécs, 7624 Pécs, Hungary*

***Corresponding author:** koloh.regina@pte.hu*

***Keywords**: Antibacterial effect, biofilm eradication, checkerboard titration, chestnut honey; lavender essential oil, otitis media.*

Objective: Acute otitis media is the most common childhood illness. The infection is often caused by biofilm-forming bacteria, which can reduce the success of antibiotic therapy, thereby slowing down the recovery process [1]. Complementary therapy for infectious diseases can rely on natural, antibacterial substances, such as essential oils and honey, which have proven antibacterial and anti-biofilm effects [2]. *Lavandula angustifolia* Mill. is a well-known medicinal plant, with up to 20-60 different bioactive components in lavender essential oil (LEO). The antimicrobial properties of honey are attributed to plant-derived polyphenols, hydrogen peroxide, and bee-derived components, such as the defensin-1 peptide. The aim of the study was to investigate the effects of chestnut honey (CH) (*Castanea sativa* Mill.) from a Hungarian apiary, LEO, and their combinations against the multidrug-resistant otitis media pathogens *Haemophilus influenzae* (DSM 4690), *H. parainfluenzae* (DSM 8978), *Moraxella catarrhalis* (DSM 9143), *Pseudomonas aeruginosa* (ATCC 27853), and *Streptococcus pneumoniae* (DSM 20566). **Methods:** The aerial parts of lavender were collected before the flowering period in Hungary, and LEO was extracted through hydrodistillation. To verify the botanical origin of CH, microscopic pollen analysis was conducted. Minimum inhibitory concentrations (MIC) were established through microdilution assays. The potential synergistic effects of CH-LEO combinations were analyzed using a checkerboard titration method. A crystal violet assay was used to evaluate the elimination of bacterial biofilms. **Results:** LEO displayed more potent antibacterial activity compared to CH (CH-MIC range: 111.1-176.47 mg/mL; LEO-MIC range: 0.31-2.5 mg/mL). CH and LEO also achieved biofilm eradication when used separately, although not to the same extent as when used in combination. CH-LEO combinations showed high inhibition rates for biofilm eradication, with *P. aeruginosa* being the most resistant and *S. pneumoniae* the most sensitive bacterium (inhibitory rate: 82.1% and 91.1%, respectively). The synergistic effect of CH-LEO was proven using the checkerboard titration method (FICI: 0.375). **Conclusions:** Due to its synergistic effect, the CH-LEO combination may be effective as an adjunctive therapy against otitis media pathogens.

ACKNOWLEDGMENTS

This research was supported by the Fund of National Research, Development and Innovation Office NKFIH PD 147156 (Balázs V.L.) and NKFIH K18 128217 (Horvath G.), the Research Grant of the Faculty of Pharmacy University of Pécs, GYTK KA-2024-05 (Farkas Á.), the University Research Scholarship Program of the Ministry for Culture and Innovation from the Source of the National Research, Development and Innovation Fund (Nagy-Radványi L.: EKÖP-24-4-I-PTE-113; Balázs V.L.: EKÖP-24-4-II-PTE-114), and the Kriszbacher Ildikó Talent Management Programme of the University of Pécs: PTE-KITEP-2023-262 and PTE-KITEP-2024-385 (Ángyán V.D.)

REFERENCES

[1] Paramasivan, S.; Bassiouni, A.; Shiffer, A.; Dillon, M.R.; Cope, E.K.; Cooksley, C.; Ramezanpour, M.; Moraitis, S.; Ali, M.J.; Bleier, B.; et al. (2020). The international sinonasal microbiome study: A multicentre, multinational characterization of sinonasal bacterial ecology. *Allergy*, *75*, 2037–2049.
[2] Nagy-Radványi, L., Ormai, E., Koloh, R., Ángyán, V. D., Kocsis, B., Bencsik-Kerekes, E., Szabó, P., Csikós, E., Farkas, Á., Horváth, G., Kocsis, M., & Balázs, V. L. (2024). Biofilm Inhibition Activity of Fennel Honey, Fennel Essential Oil and Their Combination. *Microorganisms*, *12*(11), 2309.

Membrane degradation and anti-biofilm effects of thyme and ceylon cinnamon bark essential oils

Biró, V.[a,*], Balázs, V. L.[a], Horváth, Gy.[a], Filep, R.[a], Kocsis, B.[b] Szilágyi-Utczás, M.[c]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Univ. of Pécs, Rókus St. 4., Pécs, Hungary*
[b]*Department of Medical Microbiology and Immunology, Medical School, Univ. of Pécs, Pécs 7624, Hungary*
[c]*Center for Sports Nutrition Science, Hungarian Univ. of Sport Science, Alkotás St. 42-48, Budapest, Hungary*

Corresponding author: biro.viktoria@pte.hu

Keywords*: Biofilm, essential oils, membrane degradation, Pseudomonas aeruginosa, Staphylococcus aureus.*

Objective: Bacterial biofilms are a major problem in many areas (food industry, agriculture, hospital environment). In the majority of cases, bacterial communities that settle in biofilms show increased resistance to antibiotics and disinfectants, making their containment difficult [1]. Plant essential oils have the potential to be effective against these microorganisms due to their complex composition. In this study, the antibacterial and biofilm inhibitory activity of thyme (*Thymus vulgaris* L.) and Ceylon cinnamon bark (*Cinnamomum zeylanicum* J. Presl.) essential oils (EOs) were tested against *Pseudomonas aeruginosa* (ATCC 27853) and methicillin-resistant *Staphylococcus aureus* (ATCC 700698). **Methods:** The chemical composition of EOs was determined using gas chromatography. The antibacterial effect was determined by the microdilution method in 96-well microtiter plates. The EOs were applied at concentrations of 5, 2.5, 1.25, 0.625, and 0.3125 mg/mL, using pure cell suspension medium. After 24 hours of incubation at 37°C, absorbance was measured at 600 nm, and then the minimum inhibitory concentration (MIC) was determined. The biofilms were developed on polystyrene surfaces (4 hours, 37°C). The EOs were applied at a concentration of MIC/2 (24 hours, 37°C), then washed and dehydrated and stained with crystal violet solution. The bound dye was dissolved with a 33% acetic acid solution, and their absorbance was measured at 595 nm using a plate reader. Scanning electron microscopy (SEM) images of the biofilms were also taken [3]. In the membrane degradation assay, DNA release was measured with samples treated with different concentrations of EOs for 1 hour and with MIC×2 concentrations for different times. The samples were centrifuged, and the absorbance of the supernatant was measured at 260 nm. The results were expressed as a percentage of the control [4]. **Results:** The main component of thyme EO was thymol, and the main component of Ceylon cinnamon bark EO was trans-cinnamaldehyde. The tested EOs had antibacterial effects. The MIC value for *P. aeruginosa* was 1.25 mg/mL, and for MRSA it was 0.625 mg/mL for both EOs. Their biofilm-degrading effect was also outstanding. (inhibitory rates: 72.5-89.3%). This fact was confirmed by SEM. Based on the results of the membrane degradation assay, the EOs were able to destroy the membranes of *P. aeruginosa* and MRSA to different degrees. The effect was strongly concentration-dependent and time-dependent for both bacteria. Thyme EO had a stronger membrane-degrading effect. **Conclusions:** Based on our results, EOs may be effective against the pathogens tested. Thus, they can be used as alternative disinfectants, even for cleaning food industry and hospital environment surfaces. This provides an effective, sustainable, and easily biodegradable alternative for cleaning.

ACKNOWLEDGMENTS
This research was supported by the Fund of National Research Development and Innovation Office (grant number: NKFIH PD 147156; Viktoria Lilla Balazs).

REFERENCES

[1] Rather, M. A., Gupta, K., & Mandal, M. (2021). Microbial biofilm: Formation, architecture, antibiotic resistance, and control strategies. Brazilian Journal of Microbiology, 52(4), 1701–1718. https://doi.org/10.1007/s42770-021-00624-x .
[2] Yang, P., Huo, Y., Yang, Q., Zhao, F., Li, C., & Ju, J. (2025). Synergistic anti-biofilm strategy based on essential oils and its application in the food industry. World Journal of Microbiology and Biotechnology, 41(3), 81. https://doi.org/10.1007/s11274-025-04289-8.
[3] Kerekes, E. B., Vidács, A., Takó, M., Petkovits, T., Vágvölgyi, C., Horváth, G., Balázs, V. L., & Krisch, J. (2019). Anti-Biofilm Effect of Selected Essential Oils and Main Components on Mono- and Polymicrobic Bacterial Cultures. Microorganisms, 7(9), Article 9. https://doi.org/10.3390/microorganisms7090345.
[4] Bennis, S., Chami, F., Chami, N., Bouchikhi, T., & Remmal, A. (2004). Surface alteration of Saccharomyces cerevisiae induced by thymol and eugenol. Letters in Applied Microbiology, 38(6), 454–458. https://doi.org/10.1111/j.1472-765X.2004.01511.x.

Use of nitrogen as alternative carrier gas for GC-FID analysis of Citrus essential oils

Cannizzaro F. [a,*], Micalizzi, G.[a], Vento, F.[a], Alibrando, F.[a], Mondello, L.[a,b]

[a]*Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy*
[b]*Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy*

***Corresponding author:** lmondello@unime.it*

Keywords: *Alternative carrier gas, Citrus essential oils, enantio-GC, GC-FID, nitrogen.*

Objective: Recent global challenges, such as the helium shortage or its slow supply, have led to increasing costs per analysis and pushed the research towards alternative solutions such as hydrogen (H_2) and nitrogen (N_2) gases for GC analyses [1]. Both carrier gases meet the eco-sustainability principles, given that they can be produced in-lab using dedicated generators (ready-to-use) while minimizing environmental, social, and economic impact [2]. N_2 is considered a valid and sustainable alternative due to its inertness, ready availability (can be generated in situ using a generator), low cost, and safety. In particular, the objective of this research work is to optimize a chromatographic method for routine analyses of citrus essential oils (*Citrus bergamia*, *Citrus limon*, *Citrus deliciosa Ten.*, *Citrus sinensis L.*) with the use of gas chromatography (GC) analysis combined with flame ionization detection (FID). In addition to the volatile profile, for the determination of the quality of citrus essential oil, an innovative enantio-GC method based on the utilization of nitrogen carrier gas was developed for the evaluation of several enantiomeric ratios. The enatio-GC method consisted in the employment of two different GC chiral columns and two FID detectors (parallel detection). **Methods:** The separation and quantification of the terpenes and oxygenated derivatives in citrus EOs was carried out on a GC-2030 system (Shimadzu Europa, Germany) equipped with an SLB-5ms fused-silica capillary column of 30 m x 0.25 mm ID x 0.25 µm d*f* (Merck, Darmstadt, Germany). Nitrogen was used as a carrier gas at a linear velocity of 20 cm s^{-1}. A new chromatographic setup has been developed for the enantio-GC analysis: an uncoated segment (1 m × 0.25 mm ID) was installed on the injector side, while the outlet was connected to a "Y" splitting unit that allowed the eluate to be simultaneously split into two chiral columns of different stationary phases named MERCK β-DEX™ 120 of 30 m x 0.25 mm ID x 0.25 µm d*f* and Mega DEX DET-Beta of 25 m x 0.25 mm ID x 0.25 µm d*f*. **Results:** Nitrogen carrier gas was revealed to be a valid and eco-sustainable alternative to helium for the GC-FID profiling of terpene and terpenoid compounds in citrus EOs. Although a higher than optimum (12 cms^{-1}) linear velocity was employed, a satisfactory chromatographic performance was attained for all the EOs. Volatiles were separated in about 50 min, which is comparable to helium-based analysis. Enatio-GC analyses allowed the profiling of the chiral compounds by using two different chiral stationary phases. This GC-column setup ensures the correct evaluation of the enantiomeric ratios in citrus EOs, a fundamental criterion for avoiding eventual fraud or alteration in the essential oil field. **Conclusions:** The present research explored the performance of N_2 as an alternative carrier gas in routine GC-FID and enantio-GC analyses of Citrus EOs. The developed method guarantees reliability in the separation of the components of *Citrus bergamia*, *Citrus limon*, *Citrus deliciosa Ten.*, and *Citrus sinensis L.* EOs, allowing the use of nitrogen as an alternative carrier gas in GC-FID and enantio-GC analysis.

ACKNOWLEDGMENTS
The authors thank Shimadzu Europa and Merck KGaA for their continuous support.

REFERENCES
[1] Margolin Eren K. J., Prest H. F., Amirav A., (2022). Nitrogen and Hydrogen as Carrier and Make-up Gases for GC-MS with Cold EI, *J. Mass Spectrom*, 57, 4830.
[2] (2025). Green Techniques and Materials for Essential Oil Analysis, *IFEAT World, Interantional Federation of Essential Oils & Aroma Trades*, 65, 18-21.

Volatile glucosinolate degradation products and the essential oil of *Barbarea vulgaris* W. T. Aiton (Brassicaceae)

Gusinac, A.[a,*], Dekić, M.[b], Radulović, N.[a]

[a]*Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, Višegradska 33, Niš, Serbia*
[b]*Department of Sciences and Mathematics, State University of Novi Pazar, Vuka Karadžića 9, Novi Pazar, Serbia*

***Corresponding author:** amina.gusinac@pmf.edu.rs*

***Keywords:** Barbarea vulgaris, Brassicaceae, essential oil, glucosinolate breakdown product, GC-MS.*

Objective: The Brassicaceae family comprises over 4000 plant species across 372 genera. It holds significant economic value globally due to its members' diverse applications in agriculture—as vegetables, oilseed crops, condiments, ornamental plants, and fodder [1]. *Barbarea vulgaris* W.T. Aiton, commonly known as winter cress, is a wild crucifer with a broad native distribution across Eurasia and has been introduced to North America, Africa, and Australia, where it is often regarded as a noxious weed. Traditionally, *B. vulgaris* has been used both medicinally and culinarily, particularly in salads and garnishes. Additionally, it shows promise as a potential oilseed crop and represents an important genetic resource for food and agriculture [2]. Due to the limited literature on the volatile chemistry of *B. vulgaris*, particularly from the Serbian region, we undertook an investigation of its volatile profile, including glucosinolate-derived compounds. **Methods**: The plant material of the plant species *B. vulgaris* was collected through the flowering stage, at the beginning of May 2015, in the village of Ribarićе (the municipality of Tutin, Republic of Serbia), on the roadside. The essential oil was obtained by hydrodistillation using the original Clevenger apparatus and extracted with diethyl ether. Hydrolysis of GLSs and isolation of the autolysis volatiles were carried out according to the procedure described in the same paper [3]. The volatiles of the essential oil and autolysates were analyzed using gas chromatography and mass spectrometry (GC-MS). GC-MS analyses were performed on an Agilent Technologies 7890B gas chromatograph equipped with an HP-5MS capillary column and coupled with a 240-MS ion trap detector from the same company under the experimental conditions described previously. The GC analyses were carried out using an Agilent 7890A GC system equipped with a single injector, flame ionization detector (FID), and an HP-5 capillary column under the experimental conditions previously described [4]. **Results:** The essential oil obtained from the entire plant was found to be dominated by 3-phenylpropanenitrile (25.5%), dihydroedulan (isomer II) (17.8%), and benzaldehyde (10.6%). In the autolysates, the underground parts were rich in 2-phenylethyl isothiocyanate (49.3%), while the aerial parts primarily contained 5-phenyl-1,3-oxazolidine-2-thione (63.2%), both indicative of glucosinolate breakdown products. **Conclusions:** This paperwork revealed the volatile degradation products found in essential oil and autolysates of plant species *B. vulgaris*. The glucosinolate-derived product 5-phenyl-1,3-oxazolidine-2-thione was tentatively identified due to lack of data. The identity of the product will be confirmed in the future using the "synthetic approach."

ACKNOWLEDGMENTS

This research was supported by funding through grant numbers 451-03-137/2025-03/200124, 451-03-136/2025-03/200124, and 451-03-137/2025-03/200252 provided by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia.
First author is awarded by the IFEAT within the ISEO2025 – YSFA.

REFERENCES

[1] Tamokou J. D. D., Mbaveng A. T., Kuete V., (2017). Antimicrobial activities of African medicinal spices and vegetables, *Medicinal Spices and Vegetables from Africa*, 207-237.
[2] Agerbirk N., Ørgaardb M., Nielsena J. K., (2003). Glucosinolates, flea beetle resistance, and leaf pubescence as taxonomic characters in the genus *Barbarea* (Brassicaceae), *Phytochemistry*, 63, 69–80.
[3] Dekić M. S., Radulović N. S., Danilović-Luković J. B., Stojanović D. Z., (2017). Volatile glucosinolate breakdown products and the essential oil of *Descurainia sophia* (L.) Webb ex Prantl, *Facta Universitatis, Series: Physics, Chemistry and Technology*, 15, 95-102.
[4] Dekić M. S., Selimović E. S., (2021). New natural products from *Asphodelus albus* Mill. Essential Oil, *Chemistry & Biodiversity*, 18, e2100103.

Chemical Composition and Biological Activities of Essential Oils from *Gomphrena nitida* Rothrock from Nigeria

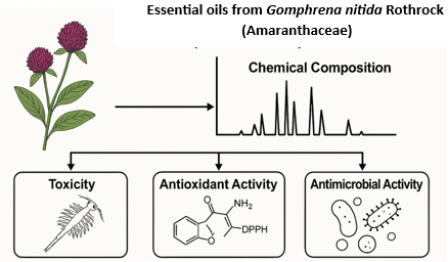
Sotade, A.[a]*, Luchen O.[a], Aiyelaagbe, O.[a]

[a]*Department of Chemistry, Faculty of Science, University of Ibadan, Ibadan. Nigeria.*

***Corresponding author:** drayopo16@gmail.com*

***Keywords:** Antioxidant activity, biological activities, caryophyllene, essential oils, Gomphrena nitida.*

Objective: The objective of this work was to investigate the chemical composition and biological activities of the essential oils obtained from the root, stem, and leaves of *Gomphrena nitida* Rothrock (Amaranthaceae), a medicinal plant native to Nigeria, Angola, and Senegal [1]. Despite the traditional medicinal use of Gomphrena species, there is currently no report on the essential oil composition or bioactivity of *G. nitida*. This study therefore aims to provide the first comprehensive analysis of its volatile constituents and evaluate their antimicrobial, antioxidant, and cytotoxicity properties. **Methods:** Fresh and air-dried plant parts (root, stem, and leaves) of *G. nitida* were collected from Ibadan, Nigeria, and taxonomically identified at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Essential oils were extracted via hydrodistillation using a Clevenger-type apparatus for 2-3 hours. The chemical composition of the oils was analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). The general toxicity of the oils was assessed using the *Artemia salina* (brine shrimp) lethality assay. Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and ferric-reducing antioxidant power (FRAP) assays. Antimicrobial activity was evaluated using the broth microdilution method against standard strains including Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 3366), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 14028, and *Klebsiella pneumoniae* ATCC 700303), and clinical fungal isolates (*Candida albicans* and *Trichophyton rubrum*). The bacteria were obtained from the Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria, and the fungi were obtained as clinical isolates from the University College Hospital (UCH), Ibadan. **Results:** A total of 33, 31, 41, 39, 39, and 37 constituents were identified in the fresh root, dry root, fresh stem, dry stem, fresh leaves, and dry leaves oils, respectively, accounting for 99.44%, 99.98%, 98.53%, 98.47%, 95.20%, and 95.88% of the total oils. The major components included caryophyllene (18.43%, 18.65%) and squalene (13.46%, 12.85%) in the root oils; α-humulene (18.17%, 15.46%) and caryophyllene oxide (15.42%, 17.15%) in the stem oils; and spathulenol (14.89%, 10.97%) and caryophyllene oxide (14.51%, 12.04%) in the leaf oils. The oils exhibited toxicity to *A. salina*, with LC_{50} values of 1.65, 2.65, and 1.95 ppm for the root, stem, and leaf oils, respectively. Antioxidant activity was moderate with IC_{50} values of 402.16 µg/mL (root), 901.83 µg/mL (stem), and 1602.30 µg/mL (leaves), compared to ascorbic acid (IC_{50} = 273.45 µg/mL). Antimicrobial screening revealed that the leaf essential oil was the most active, with minimum inhibitory concentration (MIC) values ranging from 0.781 to 12.50 mg/mL against *S. typhi* and *T. rubrum*.
Conclusions: The findings highlight the potential of *G. nitida*, particularly the root and leaf oils, as potential sources of bioactive compounds with antimicrobial and antioxidant potential, and lay the groundwork for further pharmacological exploration and development of natural therapeutic agents.



Potential sources of bioactive compounds

ACKNOWLEDGEMENTS
First author is awarded by the IFEAT within the ISEO2025 – YSFA.

REFERENCES

[1] Hogan, T. (2019). A Floristic Survey of the Boulder Mountain Park. *J. Bot. Res. Inst. Texas*. 13(1): 279–314.

Headspace SPME-GC/MS Analysis of *Laurus nobilis L.* Volatiles

Helać, H.[a], Džudžević-Čančar, H.[b], Bečić, E.[a], Alispahić, A.[b], Šahinović M.[c], Jerković, I.[d], Dedić, A.[b*]

[a]*Department of Drug Analysis, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[b]*Department of Chemistry in Pharmacy, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[c]*Department of Pharmaceutical Technology, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[d]*Mediterranean Institute for Life Sciences, University of Split, Meštrovićevo šetalište 45, 21000, Split, Croatia*

***Corresponding author:** alema.dedic@ffsa.unsa.ba*

***Keywords:** Chemical composition, essential oil, HS-SPME-GC/MS, Laurus nobilis L..*

Objective: *Laurus nobilis L.* is classified as part of the *Lauraceae* family, which includes several aromatic and therapeutic plants. Bay laurel, sweet bay, and true bay, as it is commonly called, are widespread throughout Europe and the Mediterranean region, as well as in North and South America. In addition to treating amenorrhea, colic, hysteria, polyps, sclerosis, and spasms, bay has long been used as a nervine, stomachic, and carminative. While dried bay leaves are mostly used as a spice and flavoring agent in culinary, essential oil (EO) is applied in aromatherapy, cosmetic, and the food industries for its analgesic, anti-inflammatory, antiseptic, antioxidant, and antimicrobial properties. Common applications include topical use for pain and skin conditions, diffusion for respiratory support, inclusion in baths for aches, and oral use in mouthwashes [1, 2, 3]. The regions of Počitelj and Mostar in Herzegovina have favorable Mediterranean microclimates, long-standing traditions of *Laurus nobilis* cultivation, and specific ecological conditions that support optimal growth and phytochemical development of the species. The aim of this research was to analyze the volatiles [4] composition of domestic laurel. **Methods**: *Laurus nobilis L.* was collected from Mostar and Počitelj, Bosnia and Herzegovina. Essential oils from leaves, fruits, and seeds were extracted by hydrodistillation using a Clevenger-type apparatus and stored in sealed vials after drying with anhydrous Na_2SO_4. To enhance the characterization of the volatile profile, headspace solid-phase microextraction (HS-SPME) [4] was employed. Volatile compounds were identified in samples from both locations using two fibre types: Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) and Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) by (GC-MS). **Results:** A total of 76 volatile compounds were identified across leaf, fruit, and seed samples. The predominant volatile compound across all *Laurus nobilis L.* samples was 1,8-cineole, reaching up to 18.92% in the Mostar leaf and consistently present in all tissues. α-Terpinyl acetate also showed high abundance, particularly in the Počitelj leaf (25.33%) and fruit (23.52%). Linalool was abundant in leaf tissues, especially from Mostar (14.07%), but significantly lower in fruit and seed samples. In the Mostar leaf methyl eugenol was notable, while β-elemene and *trans*-caryophyllene were dominant in the seed and fruit, respectively. Other notable monoterpenes included α-pinene, sabinene, and limonene, particularly in fruit and seed samples. Sesquiterpenes such as β-selinene, germacrene A, and δ-cadinene were more prevalent in seeds. Overall, the identified volatiles primarily belonged to monoterpenes, oxygenated monoterpenes, and sesquiterpenes, reflecting both organ-specific and geographic variability. HS-SPME-GC-MS proved effective for profiling bay leaf aroma compounds with minimal sample preparation. Variation reflects the differing affinities of the fibre coatings for volatile compounds of varying polarity. **Conclusions:** The volatile profile of *Laurus nobilis* varied across tissues and locations, with 1,8-cineole, α-terpinyl acetate, and linalool as major constituents. Monoterpenes and sesquiterpenes dominated the composition. HS-SPME-GC-MS proved effective for profiling, highlighting organ-specific and geographic influences on essential oil composition. Advantages of this method include minimal sample requirements and shorter extraction times, making it efficient for comprehensive volatile compound analysis.

ACKNOWLEDGEMENTS
First author is awarded by the IFEAT within the ISEO2025 – YSFA.

REFERENCES

[1] Alejo-Armijo A., Altarejos J., Salido S., (2017). Phytochemicals and biological activities of laurel tree (Laurus nobilis), *Natural Product Communications*, 12, 743–757.
[2] Özcan, M., Erkmen, O. (2001). Antimicrobial activity of the essential oils of Turkish plant spices. *European Food Research and Technology*, 212, 658–660.
[3] Marques A, Teixeira B., NunesL.N.(2016), Bay Laurel (*Laurus nobilis*) Oils, Ch26 in *Essential Oils in Food Preservation, Flavor and Safety,* AcademicPress.
[4] Yalçin, H., Anik, M., Sanda, M. A., Cakir, A. (2007). Gas chromatography/mass spectrometry analysis of *Laurus nobilis* essential oil composition of Northern Cyprus. *Journal of Medicinal Food*, 10, 715-719.

Effect of *Cryptomeria japonica* foliage age in the essential oil yield and chemical composition

Arruda, F.[a,b,*], Lima, A.[a,c], Wortham, T.[d], Janeiro, A.[a,b], Rodrigues, T.[a,b], Baptista, J.[a,c], Lima, E.[a,c]

[a]*Institute of Agricultural and Environmental Research and Technology, University of the Azores, Angra do Heroísmo, Portugal*
[b]*Department of Biology, Faculty of Science and Technology, University of the Azores, Ponta Delgada, Portugal*
[c]*Department of Physics, Chemistry and Engineering, University of the Azores, Ponta Delgada, Portugal*
[d]*The Perfumery, 621 Park East Blvd. New Albany, IN, USA*

***Corresponding author:** filipe.mp.arruda@uac.pt*

***Keywords:** Cryptomeria japonica, chemical composition, leaf age, waste valorisation, yield.*

Objective: *Cryptomeria japonica* (Thunb. ex L.f.) D. Don (Cupressaceae) is the most economically important tree species in the Azores archipelago, Portugal. The foliage of Azorean *C. japonica* (Az–CJF) is particularly rich in essential oil (EO), which exhibits multiple bioactivities. It is well established that the developmental stage of plant organs can significantly influence the chemical profile of EOs. However, the impact of leaf age on the EO composition of CJF has not yet been investigated. Therefore, the present study aimed to explore the dynamics of EO composition in Az–CJF at different maturation stages. **Methods:** The Az–CJF was collected in May 2024 and separated into two subsamples: mature leaves (CJL) and shoots (CJS). Essential oils (EOs) were extracted from fresh CJL and CJS by hydrodistillation using a Clevenger-type apparatus for 3 hours. The chemical composition of the resulting EO samples was analyzed by gas chromatography–mass spectrometry (GC–MS), following the methodology described in [1]. The yields of the EOs were calculated based on the dry weight of the plant material. **Results:** The EO yields of CJS and CJL were 1.92% and 1.07% (v/w, dry weight basis), respectively, indicating that the maturation stage has a substantial impact on EO production. GC-MS analysis also revealed marked differences in the chemical profiles between the CJS and CJL EO samples. Notably, variations were observed in monoterpene hydrocarbons, including α-pinene (17.8% vs. 15.4%), sabinene (4.3% vs. 7.9%), limonene (5.6% vs. 3.7%), and γ-terpinene (3.1% vs. 1.1%). The content of the oxygenated monoterpene terpinen-4-ol was also higher in CJS (9.8% vs. 3.3%). Among oxygenated sesquiterpenes, elemol (3.6% vs. 11.3%) and eudesmol isomers (23.0% vs. 10.4%) showed notable differences. Additionally, the diterpene hydrocarbon phyllocladene (9.8% vs. 15.9%) and the oxygenated diterpene nezukol (0.1% vs. 2.7%) also varied considerably between the two developmental stages. It is well established that, within the biosynthetic pathway of EO components, different compounds may be derived from common precursors. For example, elemol and eudesmol isomers (α-, β-, and γ-eudesmol) share the same precursor, namely hedycaryol [2]. However, the biosynthesis of eudesmol isomers appears to be more prominent in young shoots, whereas elemol production tends to increase in mature foliage. Previous research reported that, within the *Myrtaceae*, the eudesmols could be derived from the hydrolysis of hedycaryol during leaf aging [2], which can explain the significant decrease of eudesmol isomer content in CJL EO. **Conclusions:** This study demonstrates that the developmental stage of Az–CJF significantly influences both the yield and chemical composition of its EO. These compositional variations may also impact the biological activity of the EOs, underlining the importance of selecting specific leaf maturity stages for targeted applications.

ACKNOWLEDGEMENTS
Filipe Arruda acknowledges the PhD scholarship (M3.1.a/F/008/2021) awarded by FRCT – Fundo Regional para a Ciência e Tecnologia. This work was supported by DRCT - Direção Regional da Ciência e Tecnologia M1.1.C/PROJ.EXPLORATÓRIOS/003/2022, by FCT – Fundação para a Ciência e a Tecnologia, I.P., under the Scientific Employment Stimulus – Individual Call – 6th Edition (2023.06172.CEECIND) and by IITAA – Institute of Agricultural and Environmental Research and Technology (UID/00153/2023).

REFERENCES
[1] Arruda, F., Lima, A., Wortham, T., Janeiro, A., Rodrigues, T., Baptista, J., Rosa, J. S., & Lima, E. (2024). Sequential Separation of Essential Oil Components during Hydrodistillation of Fresh Foliage from Azorean *Cryptomeria japonica* (Cupressaceae): Effects on Antibacterial, Antifungal, and Free Radical Scavenging Activities. *Plants*, *13*, 1729.
[2] Cornwell, C.P., Reddy, N., Leach, D.N. and Wyllie, S.G. (2000). Hydrolysis of hedycaryol: the origin of the eudesmols in the Myrtaceae. *Flavour Fragr. J.*, 15: 421-431.

Antibacterial activity of the essential oil from Azorean *Cryptomeria japonica* foliage harvested in two different seasons

Rodrigues, T.[a,b], Lima, A.[a,c], Wortham, T.[d], Arruda, F.[a, b], Janeiro, A.[a,b,*], Baptista, J.[a,c], Lima, E.[a,c]

[a]*Institute of Agricultural and Environmental Research and Technology, University of the Azores, Angra do Heroísmo, Portugal*
[b]*Department of Biology, Faculty of Science and Technology, University of the Azores, Ponta Delgada, Portugal*
[c]*Department of Physics, Chemistry and Engineering, University of the Azores, Ponta Delgada, Portugal*
[d]*The Perfumery, 621 Park East Blvd. New Albany, IN, USA*

Corresponding author: alex-19961917@hotmail.com

Keywords: *Antibacterial, bioeconomy, Cryptomeria japonica, essential oils, seasonal variation, sustainable circular.*

Objective: The timber production industry and associated forest operations produce significant amounts of Azorean *Cryptomeria japonica* (Thunb. ex L.f.) D. Don (Cupressaceae) biomass residues, particularly foliage (Az–CJF), which is used for local essential oil (EO) production, constituting, thus, economically and environmentally sustainable return materials with zero competition for land areas. On the other hand, the growth opportunity for the EO industry is on the rise across the globe, which requires quality assurance for consistent product performance. As a result, this study evaluated, for the first time, the effect of seasonal variation on the Az–CJF EO's antibacterial efficacy, measured via minimum inhibitory concentration (MIC) determination assay. **Methods:** The Az–CJF was harvested from a tree population located in Vila Franca do Campo (latitude 37° 44′38.347" N, longitude 25° 21′56.263" W, altitude 400 m) in São Miguel Island. The plant material was collected on two different occasions, namely, November 2022 (autumn) and April 2023 (spring). The EO from each seasonal Az–CJF sample (referred to as Aut–EO and Spr–EO samples, respectively) was obtained via hydrodistillation, using a Clevenger-type apparatus, according to Rodrigues et al. [1]. The antibacterial activity (MIC values) of the EOs, α-pinene (a key component of Az–CJF EO), and kanamycin (an antibacterial drug) was evaluated via the broth microdilution method, according to the CLSI guidelines [2]. The bacterial strains tested include four Gram-positive strains, namely, *Bacillus licheniformis* (Weigmann) Chester (DSM13), *Bacillus subtilis* (Ehrenberg) Cohn (DSM10), *Staphylococcus aureus* Rosenbach (DSM1104), and *Micrococcus luteus* (Schroeter) Cohn (DSM20030), and three Gram-negative strains, namely, *Serratia marcescens* Bizio (DSM 48), *Enterobacter cloacae* (Jordan) Hormaeche & Edwards (DSM 30054), and *Escherichia coli* (Migula) Castellani & Chalmers (DSM 498). **Results:** The EOs' MIC values revealed that, among the selected bacteria, *M. luteus* was shown to be the most susceptible strain to both EOs (MIC = 5.0 mg/mL for *M. luteus vs.* MIC > 5.0 mg/mL for the other tested bacteria), which fit well with the activity of α-pinene (MIC = 2.5 mg/mL for *M. luteus vs.* MIC ≥ 5.0 mg/mL for the other tested bacteria). These results are interesting, since *M. luteus*, a generally underestimated bacterium, has been recently reported as a clinically potential opportunistic pathogen [3]. Overall, this research demonstrated that, although quantitative differences were found in the chemical composition of Aut–EO and Spr–EO, as reported in Rodrigues et al. [1], these variations were not significant enough to alter, i.e., increase or decrease, the antibacterial properties of the studied Az–CJF EOs. However, the results for either the antibacterial property of Az–CJF EOs or α-pinene suggest that, under the experimental conditions used, they are weaker antibacterial agents against the selected bacterial strains when compared to the utilized positive control. **Conclusions**: This research demonstrates that the antibacterial efficacy of the Az–CJF EO was unaffected by seasonal period, specifically autumn and spring. The results also revealed that both EOs, albeit having mild effects, can inhibit the growth of a broad range of bacteria, warranting further investigation concerning their effect against other bacterium types.

REFERENCES

[1] Rodrigues T., Lima A., Wortham T., Arruda F., Janeiro A., Baptista J., Lima E. (2024). Essential oil composition and anti-cholinesterase properties of *Cryptomeria japonica* foliage harvested in São Miguel Island (Azores) in two different seasons. *Plants*, 13, 3277.
[2] Weinstein M. P., Patel J. B., Burnhman C. A., Zimmer, B. L. (2018) Clinical and laboratory standards institute methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically standard, approval CDM-A. In: *M07 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria, That Grow Aerobically*, 112.
[3] Zhu M., Zhu Q., Yang Z., Liang Z. (2021) Clinical characteristics of patients with *Micrococcus luteus* bloodstream infection in a Chinese tertiary-care hospital. *Pol. J. Microbiol.,* 70, 321–326.

Evaluation of chemical composition and antibacterial activity of *Pinus sylvestris* L. essential oil

Öztürk, G.[a,*], Demirci, F.[a], Demirci, B.[a]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkiye.*

***Corresponding author:** g.ozturkau@gmail.com*

***Keywords:** Antibacterial activity, essential oil, Pinus sylvestris L., skin pathogens.*

Objective: The skin is the largest organ in the human body. It serves as a barrier between the internal organs and the external environment. The skin is a complex and dynamic ecosystem inhabited by bacteria, archaea, fungi, and viruses. These microorganisms are collectively known as the skin microbiota. Tissue integrity is compromised by trauma, cuts, wounds, and UV exposure to the skin. Opportunistic microorganisms, particularly *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains, proliferate in these areas and cause infections [1]. *Pinus sylvestris* L. is an important forest tree species and belongs to the Pinaceae family within the conifer class of Gymnospermae. *P. sylvestris* has a wide range of medical applications due to its antiparasitic, antiviral, antispasmodic, antihyperglycemic, anti-inflammatory, and expectorant properties. Furthermore, the essential oil of *P. sylvestris* has been used in the pharmaceutical, chemical, cosmetic, and perfume industries as a food additive and preservative [2, 3]. This study aimed to determine the volatile components of *P. sylvestris* essential oil and to evaluate their antibacterial activities against selected skin pathogens. **Methods:** The *P. sylvestris* essential oil was obtained from a commercial source in Türkiye. The essential oil was analyzed by GC-FID and GC/MS systems [1]. The antibacterial activity of the sample was determined by broth microdilution against *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 27853 [4-6]. **Results:** *a*-Pinene (55.2%), *trans*-verbenol (7.8%), verbenone (4.0%), and *trans*-pinocarveol (3.2%) were found as the main compounds in *P. sylvestris* essential oil. The minimum inhibitory concentration (MIC) of samples was in the range of 6.25-0.78 mg/mL, compared with standard antibacterial agents. **Conclusions:** When compared with current literature, the volatile constituents were in line, and the antibacterial activities were relatively moderate.

ACKNOWLEDGMENTS

This work has been supported by Anadolu University Scientific Research Projects Coordination Unit under grant number #2958.

REFERENCES

[1] Proksch, E., Brandner, J.M., Jensen, J.M. (2008) The skin: an indispensable barrier, *Experimental Dermatology*, 17 (12), 1063–1072.

[2] Sezik, E., Üstün, O., Demirci, B., Başer, K.H.C. (2010). Composition of the essential oils of *Pinus nigra* Arnold from Turkey. *Turkish Journal of Chemistry*, 34(2), 313-325.

[3] Silori, G.K., Kushwaha, N., Kumar, V. (2019) Essential Oils from Pines: Chemistry and Applications. In: Malik S (ed) Essential Oil Research, Trends in Biosynthesis, Analytics, Industrial Applications and Biotechnological Production. Springer, Cham.

[4] Demirci, B., Kırcı, D., Öztürk, G., Demirci, F. (2022). Effect of Extraction Time on *Origanum onites* L. Infusions and Essential Oils– Biological Evaluation, Statistical Principal Component and Hierarchical Cluster Analyses. Chemistry & Biodiversity, 19(12), e202200482.

[5] CLSI. (2007). Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement, M100-S17, Clinical and Laboratory Standards Institute 27.

[6] Demirci, F., Karaca, N., Tekin, M., Demirci, B., (2018). Anti-inflammatory and antibacterial evaluation of *Thymus sipyleus* Boiss. subsp *sipyleus* var. *sipyleus* essential oil against rhinosinusitis pathogens. Microbial Pathogenesis, 122, 117-121.

Integrated Chemical, Biological, Morphological, and Anatomical Characterization of *Tripleurospermum monticolum* Bornm. (Asteraceae): Insights into a Promising Medicinal Plant

Sönmez, H.R.[a], Karakaş, Ş.[a], Civaş, A.[a], Kararenk, A.C.[a], Özer, E.B.[a,j], Tekman, E.[b], Yuca, H.[a], Bona, M.[c], Çoban, F.[d], Acar Şahin, A.[e], Pınar, N.M.[e], Nobarirezaeyeh, M.[a], Ekşi Bona, G.[f], Demirci, B.[g], Göger, G.[h], Karakaya, S.[b,*]

[a]*Department of Pharmacognosy in Pharmacy, University of Atatürk-Faculty of Pharmacy, Erzurum, Türkiye*
[b]*Department of Pharmaceutical Botany in Pharmacy, University of Atatürk-Faculty of Pharmacy, Erzurum, Türkiye*
[c]*Department of Botany in Science, University of Istanbul-Faculty of Science, Istanbul, Türkiye*
[d]*Department of Field Crops in Agriculture, University of Atatürk-Faculty of Agriculture, Erzurum, Türkiye*
[e]*Department of Biology in Science, University of Ankara-Faculty of Science, Ankara, Türkiye*
[f]*Department of Pharmaceutical Botany in Pharmacy, University of Istanbul-Cerrahpaşa-Faculty of Pharmacy, Istanbul, Türkiye*
[g]*Department of Pharmacognosy in Pharmacy, University of Anadolu-Faculty of Pharmacy, Eskişehir, Türkiye*
[h]*Department of Pharmacognosy in Pharmacy, University of Afyonkarahisar Health Sciences-Faculty of Pharmacy, Afyonkarahisar, Türkiye*
[i]*Department of Pharmacognosy in Pharmacy, University of Ağrı İbrahim Çeçen-Faculty of Pharmacy, Ağrı, Türkiye*
[j]*Department of Pharmacy Professional Sciences, Ondokuz Mayis University, Faculty of Pharmacy, Samsun, Türkiye*

***Corresponding author:** snglkarakaya@yahoo.com*

***Keywords:** Anticholinesterase, antidiabetic, antioxidant, antimicrobia, Tripleurospermum monticolum.*

Objective: Alzheimer's disease (AD), driven largely by aging, impairs memory and cognition, with U.S. cases expected to reach 16 million by 2050. Oxidative stress plays a key role in AD, and natural antioxidants are under preclinical investigation as potential therapies. Diabetes mellitus (DM) is a chronic metabolic disorder marked by hyperglycemia due to insulin deficiency or resistance. Type 2 DM increases dementia risk, as AD involves disrupted insulin signaling and neurodegeneration, leading to its description as "type 3 diabetes" [1, 4]. This study aims to comprehensively evaluate the biological potential of Tripleurospermum monticolum Bornm. (Asteraceae), a species traditionally used to treat fever, cough, and gastrointestinal disorders, with a focus on its relevance to the management of these chronic diseases. **Methods:** Methanol and aqueous extracts were prepared from the capitulum, root, and aerial parts of the plant. These extracts were evaluated for their inhibitory effects on key enzymes associated with diabetes and neurodegeneration, namely acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-amylase, and α-glucosidase [3]. Antioxidant activities were assessed using DPPH• and ABTS•$^{+}$ assays, while total phenolic, flavonoid, and tannin contents were quantified using the Folin–Ciocalteu, $AlCl_3$, and vanillin–H_2SO_4 methods, respectively. The essential oil composition of the capitulum, root, and aerial part was analyzed using GC-MS/MS. Additionally, the plant underwent morphological, anatomical, and qualitative phytochemical analyses to identify secondary metabolites. **Results:** GC-MS/MS analysis revealed that the dominant compound in the capitulum essential oil was (2Z,8Z)-matricaria ester (64.1%), whereas the aerial parts were rich in pentacosane (22.2%) and caryophyllene oxide (13.5%). The root essential oil contained high amounts of geranyl isovalerate (30.7%). Among the extracts, the methanol extract of the aerial parts exhibited the highest contents of phenolics (74.686 µg GAE/mg), flavonoids (259.083 µg RE/mg), and tannins (83.000 µg TAE/mg). In antioxidant assays, the methanol extract of the root showed the strongest DPPH• radical scavenging activity (20.855%), while the capitulum methanol extract was most effective in the ABTS•$^{+}$ assay (9.362%). Antimicrobial assays revealed antibacterial activity with MIC values of 1250–2500 µg/mL and particularly strong anticandidal activity against Candida tropicalis (MIC: 625–2500 µg/mL). Essential oils from the root and flower exhibited antifungal activity (MIC: 625 and 1250–2500 µg/mL, respectively). Phytochemical screening detected alkaloids, flavonoids, and tannins in all extracts, with lipids selectively present in the methanol extracts of capitulum, aerial parts, and root, indicating metabolic diversity. **Conclusions:** Tripleurospermum monticolum displays significant antioxidant, enzyme inhibitory, antimicrobial, and phytochemical properties. These findings highlight the species as a multifunctional medicinal plant with potential applications in the supportive treatment of complex chronic diseases such as diabetes and neurodegenerative disorders. This study enriches the pharmacological profiling of T. monticolum and provides a foundation for future clinical and pharmacodynamic research.

ACKNOWLEDGMENT
Hatice Rümeysa SÖNMEZ and Enes TEKMAN expresses heartfelt gratitude for the scholarship awarded by the Turkish Scientific and Technical Research Council (TÜBİTAK) and appreciates the support received throughout his postgraduate studies.
First author is awarded by the IFEAT within the ISEO2025 – YSFA.

REFERENCES
[1] Chen, X., Drew, J., Berney, W., & Lei, W. (2021). Neuroprotective natural products for Alzheimer's disease. *Cells,* 10(6), 1309.
[2] Collins, A. E., Saleh, T. M., & Kalisch, B. E. (2022). Naturally occurring antioxidant therapy in Alzheimer's disease. *Antioxidants,* 11(2), 213.
[3] Nasri, H., Shirzad, H., Baradaran, A., & Rafieian-Kopaei, M. (2015). Antioxidant plants and diabetes mellitus. *J. of Res. in Medical Sciences,* 20(5), 491-502.
[4] Gamzé, R., elangre, E., Tolu, S., Moreau, M., Janel, N., Bailbé, D., & Movassat, J. (2022). Type 2 diabetes mellitus and Alzheimer's disease: shared molecular mechanisms and potential common therapeutic targets. International Journal of Molecular Sciences, 23(23), 15287.

Essential oil of *Satureja montana L.*: antibacterial properties

Pašić, I.[a,*], Preljevic, K.[b], Stanojkovic, T.[a], Matić, I.Z.[a]

[a]*Institute for Oncology and Radiology of Serbia, Belgrade, Serbia*
[b]*University of Montenegro, Faculty of Natural Sciences and Mathematics, Department of Biology, Podgorica, Montenegro*

Corresponding author: ivannapasic@gmail.com

Keywords: *Antioxidants, antibacterial properties, essential oil, Satureja montana L..*

Objective: This research is focused on investigation of the antibacterial properties of essential oil derived from the aromatic plant species *Satureja montana*, collected in Montenegro at the locality of Garač, Danilovgrad, under voucher number 2078661 at an altitude of 1130 m. The EO was stored at +4°C until subsequent analyses. The essential oil was extracted from the plant's aerial parts using an industrial-scale extractor, employing the conventional method of steam distillation. **Materials and Methods:** The research was conducted using two complementary techniques: the microdilution method to determine the minimum inhibitory concentration (MIC) and the disk diffusion method to measure the diameter of the inhibition zone. The EO was evaluated against a selection of clinically significant Gram-positive and Gram-negative bacterial strains. **Results:** The MIC assay demonstrated significant antimicrobial effectiveness of the essential oil. The lowest MIC value was recorded for Enterococcus faecalis at 0.78 µL/mL, whereas Escherichia coli, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Bacillus subtilis* were inhibited at a concentration of 3.125 µL/mL. *Pseudomonas aeruginosa* exhibited the greatest resistance, with an MIC of 12.5 µL/mL. The disk diffusion method also confirmed strong antibacterial activity, especially against *S. aureus* (37 mm), *Proteus mirabilis* (34 mm), and *E. coli* (31 mm) when pure EO was used. In many instances, the inhibition zones produced by the EO were similar to or even surpassed those of standard antibiotics. When compared to antibiotics from various pharmacological classes, such as β-lactams, aminoglycosides, fluoroquinolones, tetracyclines, and sulfonamides, the essential oil showed remarkable efficacy. This effect is likely due to a synergistic interaction among the multiple bioactive compounds in the essential oil, which collectively contribute to its broad and potent antibacterial activity. In EO, the most prevalent components were oxygenated monoterpenes and monoterpane hydrocarbons, with p-cymene (28.65%), thymol (22.1%), linalool (4.86%), trans-caryophyllene (4.52%), and carvacrol (3.28%) being the primary compounds. **Conclusions**: The antimicrobial properties observed could be linked to the unique chemical composition of the essential oil Satureja montana from Montenegro, which is abundant in phenolic and terpene compounds.

Exploring Green Alternatives to Manage Pine Wilt Disease

Bacińska, Z.[a,*], Faria, J.M.S.[b,c], Figueiredo, A.C.[d], Strub, D.J.[a,e]

[a]*Department of Chemical Biology and Bioimaging, Faculty of Chemistry, Wrocław Tech, Wyb. Wyspiańskiego 27, 50-370 Wrocław, Poland*
[b]*Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P), Quinta do Marquês, 2780-159 Oeiras, Portugal*
[c]*GREEN-IT Bioresources for Sustainability, ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal*
[d]*CE3C & CHANGE, Faculdade de Ciências da Universidade de Lisboa, Biotecnologia Vegetal, DBV, Campo Grande, 1749-016 Lisboa, Portugal*
[e]*International Federation of Essential Oils and Aroma Trades (IFEAT) Panel of Experts, 6th Floor, King's House, 9-10, Haymarket, London SW1Y 4BP, United Kingdom*

***Corresponding author:** zuzanna.bacinska@pwr.edu.pl*

***Keywords:** Bursaphelenchus xylophilus, essential oils, nematicidal activity, pinewood nematode.*

Objective: The Pinewood nematode (PWN), *Bursaphelenchus xylophilus,* is responsible for pine wilt disease by infecting the stem of conifer trees, mainly *Pinus* species. After infection of internal tissue, there is a progressive blockage of water transport leading to reduced transpiration and photosynthesis [1], and ultimately to the death of infected trees. This disease contributes to serious economic problems and negative environmental impacts; therefore, it is imperative to identify biocidal natural alternatives that can serve as environmentally safe biopesticides, effectively mitigating this global problem. Essential oils (EOs) are notable for their complex composition of natural products, offering potent bioactivity, environmental degradability, and a wide spectrum of effects, which together reduce the likelihood of resistance development in pathogenic organisms [2]. **Methods:** This study aimed to screen the nematicidal activity of eight commercially available EOs, *namely*, Birch white – *Betula alba* Roth. (Poland, Hermitage); Buddhawood – *Eremophila mitchellii* Benth. (Australia, Ultra International); Coriander leaf – *Coriandrum sativum* L. (Egypt, A. Fakhry & Co.); Dittany – *Origanum dictamnus* L. (Greece, Vessel Essential Oils); Parsley leaf - *Petroselinum crispum* L. (Egypt, A. Fakhry & Co.). The bioassays were conducted on a PWN reference isolate kept at the Plant Nematology Lab of the INIAV, I.P., at Oeiras, Portugal, for research purposes. Direct contact assays were carried out in flat-bottom 96-well microtiter plates, where 95 µl of an aqueous suspension of mixed-life-stage PWNs (~80 PWNs) and 5 µl of EO stock solution were placed in each well, with a concentration range of 1 - 0.125 µl/ml. The dead and alive nematodes were counted after 0.5, 1, 3, and 24 h. **Results:** Corrected mortality (Cm) was calculated for the tested EOs. The most active EOs, which inhibited PWNs activity at a concentration of 0.125 µl/ml, were coriander leaf EO (Cm = 100% ± 0.00), geranium EO (Cm = 97.62% ± 0.29), and lemon-verbena basil CT citral EO (Cm = 93.11% ± 1.67). **Conclusions:** Of the 27 EOs tested, seven of them showed activity at the lowest tested concentration of 0.125 µl/ml. The study has shown that EOs show high potential for inhibiting the activity of PWNs, making them strong candidates for the development of environmentally friendly biopesticides.

ACKNOWLEDGMENTS
Z. Bacińska thanks Prof. Ana Cristina da Silva Figueiredo and Dr Jorge M. S. Faria for the opportunity to undertake a research internship as part of the Erasmus+ programme, as well as for their support and scientific cooperation. Z. Bacińska thanks IFEAT for awarding the Young Scientist Fellowship at the 55th ISEO. We would like to thank representatives of the EOs industry and members of IFEAT: A. Fakhry & Co., Lluch Essence, and Ultra International for the donation of the samples of EOs for this study. This work was supported by the PhD Training Programme within Erasmus+ (PLWROCLAW02) (Wrocław, Poland). Thanks to FCT (Portugal) through national funds, under UID/00329/2025 CE3C & CHANGE.
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES
[1] Cui, H., Jin, H., Liu, Q., Yan, Z., Ding, L., Qin, B. (2014) Nematicidal metabolites from roots of *Stellera chamaejasme* against *Bursaphelenchus xylophilus* and *Bursaphelenchus mucronatus*. *Pest Manag. Sci*. 70, 5, 827-835.
[2] Figueiredo A.C., Barroso J.G., Pedro L.G., Scheffer J.J.C. (2008) Factors affecting secondary metabolite production in plants: Volatile components and essential oils. *Flavour Fragr. J*. 23, 213–226.

A recent review of the impact of lavender essential oil on male and female sex hormones

Bogevski, K. [a,*], **Maksimova, V.**[a]

[a]*Department of Pharmacy, Faculty of Medical Sciences, Goce Delcev University, Stip, North Macedonia*

***Corresponding author:** krume.153976@student.ugd.edu.mk*

Keywords: *Anti-androgenic activity, endocrine disruption, estrogenic activity, lavender essential oil, linalool, linalyl acetate.*

Objective: This review critically evaluates the evidence regarding the potential endocrine-disrupting effects of lavender (*Lavandula angustifolia* Mill., Lamiaceae) essential oil and its main components, linalool (Lin) and linalyl acetate (LinAc). Case reports have suggested that continuous dermal exposure to lavender-containing products may be linked to prepubertal gynecomastia and premature thelarche in children [1]. Early *in vitro* findings indicated weak estrogenic and anti-androgenic activities of lavender oil constituents, raising concerns about their possible role as endocrine disruptors [2]. This review aims to determine whether these concerns are supported by studies and regulatory data generated in recent years. **Methods:** A structured literature review was conducted, covering case reports, mechanistic *in vitro* assays, *in silico* approaches, and follow-up *in vivo* studies in rodents. Eligible studies included those examining lavender essential oil or its isolated constituents, provided that experimental details and models were adequately described. **Results:** Although early investigations reported weak Endrogen Receptor (ER) activation and anti-androgenic effects in cell-based systems, these findings were not reproduced in recent guideline ER or Androgen Receptor (AR) reporter assays. Regulatory *in vitro* studies showed no consistent estrogenic or anti-androgenic activity for Lin or LinAc, with AUC values of 0 or close to 0 in EPA ToxCast analyses [3]. *In vivo* reproductive and developmental toxicity screening tests in rats revealed no significant changes in sex steroid hormone–sensitive endpoints, such as nipple retention, timing of puberty, or fertility parameters, apart from nonspecific systemic toxicity at high oral doses. Epidemiological support remains weak, as case reports are limited by methodological issues, potential confounders, and lack of plausible dose–response relationships. **Conclusions:** The current body of evidence does not support lavender essential oil or its main constituents as clinically relevant endocrine disruptors. Concerns raised by case reports and isolated *in vitro* findings are not substantiated by validated *in vitro* assays, computational models, or *in vivo* studies. These results emphasize the importance of integrating multiple lines of evidence before proposing causal links between exposure to natural products and endocrine-related outcomes. Further research should focus on potential confounding components in cosmetic formulations and employ standardized protocols to resolve remaining uncertainties.

ACKNOWLEDGMENTS

First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Henley D. V., Lipson N., Korach K. S., Bloch C. A., (2007). Prepubertal gynecomastia linked to lavender and tea tree oils, *New England Journal of Medicine*, 356, 479–485.

[2] Linklater A., Hewitt J. K., (2015). Premature thelarche in the setting of high lavender oil exposure, *Journal of Paediatrics and Child Health*, 51, 235.

[3] Hareng L., Kolle S. N., Gomes C., Schneider S., Wahl M., (2024). Critical assessment of the endocrine potential of Linalool and Linalyl acetate: proactive testing strategy assessing estrogenic and androgenic activity of Lavender oil main components, *Archives of Toxicology*, 98, 347–361.

Comprehensive Analysis of Mandarin Essential Oils by means of Enantioselective Multidimensional Gas Chromatography coupled to Isotopic Ratio Mass Spectrometry

Cannizzaro, F.[a,*], Cucinotta, L.[a], Sciarrone, D.[a], Mondello, L.[a,b]

[a]*Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Viale G. Palatucci snc 98168 – Messina, Italy*
[b]*Chromaleont s.r.l., c/o Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Viale G. Palatucci snc 98168 – Messina, Italy*

***Corresponding author:** francesca.cannizzaro@studenti.unime.it*

Keywords: *Citrus, chiral gas chromatography, isotopic ratio mass spectrometry, mandarin essential oil, multidimensional gas chromatography, quantitative analysis.*

Objective: The authenticity of essential oils (EOs) is a growing concern due to the increasing global demand and the widespread risk of adulteration [1]. This study aimed to develop a comprehensive analytical approach for authenticity assessment through the simultaneous characterization of the qualitative, quantitative, chiral, and isotopic profiles of mandarin EO samples. For this purpose, a novel analytical approach was implemented, based on a heart-cut enantioselective multidimensional gas chromatographic system coupled in parallel to an isotopic ratio mass spectrometer and a single quadrupole mass spectrometer (Es-MDGC-C-IRMS/qMS). **Methods:** Sixty-three cold-pressed mandarin EO samples from Sicily and Calabria, collected over two consecutive harvest seasons (2021/2022 and 2022/2023), were analyzed. The Es-MDGC-C-IRMS/qMS system was employed to perform simultaneous qualitative, quantitative, chiral, and isotopic profiling. The first dimension enabled standby analysis for quali-quantitative data, while the second dimension, equipped with a chiral column, allowed the simultaneous determination of enantiomeric and isotopic ratios. Chemometric analysis, including PCA, was utilized to assess seasonal and geographical variability. **Results:** The multidimensional method exhibited comparable results between the standby analysis and conventional GC-FID methods. Seasonal variations were evident, with increasing limonene and decreasing γ-terpinene levels over the harvest period. Chiral analysis revealed the dominance of dextrorotatory enantiomers (β-pinene, sabinene, limonene, linalool) and levorotatory enantiomers (α-thujene, α-terpineol). Isotopic analysis provided for the first time $\delta^{13}C$ values of specific enantiomers, distinguishing samples from Reggio Calabria and Palermo. **Conclusions:** The proposed Es-MDGC-C-IRMS/qMS approach demonstrated its efficacy in achieving a reliable characterization of mandarin EOs. This integrated approach, compared to using multiple instruments, enhanced the accuracy of the analytical data by mitigating common issues such as co-elution and peak overlap, which are often encountered in monodimensional systems. Furthermore, it led to a significant reduction in total analysis time, electricity and solvent consumption, and waste generation. Ongoing efforts will focus on expanding the sample set to further validate and strengthen the method's applicability across broader contexts.

ACKNOWLEDGMENTS
The authors thank Shimadzu Corp. and Merck KGaA for their support.
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES
[1] Do T. K. T., Hadji-Minaglou F., Antoniotti S., Fernandez X. (2015). Authenticity of essential oils, *TrAC Trends Anal. Chem.*, 66. 146-157.

Aromatic Allies in Herbal Medicine: Essential Oil Composition and Broad-Spectrum Enzyme Inhibition of *Salvia aucheri* subsp. *canescens, S. quezelii,* and *S. candidissima* subsp. *occidentalis*

Apdullah Fadhıl, A.[a], Akyüz, B,[a,*], Özer, E.B.[a,e], Bilge A.[b], Yuca, H.[a], Demirci, B.[c], Karakaya, S.[d]

[a]*Department of Pharmacognosy, University of Ataturk-Faculty of Pharmacy, Erzurum, Türkiye*
[b]*Department of Pharmacognosy, University of Erzincan Binali Yıldırım-Faculty of Pharmacy, Erzincan, Türkiye*
[c]*Department of Pharmacognosy, University of Anadolu -Faculty of Pharmacy, Eskişehir, Türkiye*
[d]*Department of Pharmaceutical Botany, University of Ataturk-Faculty of Pharmacy, Erzurum, Türkiye*
[e]*Department of Pharmacy Professional Sciences, Ondokuz Mayis University, Faculty of Pharmacy, Samsun, Türkiye*

***Corresponding author:** byznrakyz@outlook.com*

***Keywords:** Anticholinesterase, antioxidant, enzyme inhibition, essential oil, GC–MS, α-glucosidase, Salvia.*

Objective: Salvia species are known for their essential oils (EOs), rich in monoterpenes and sesquiterpenes, and for their biological effects, including antioxidant, antidiabetic, and neuroprotective properties [1, 2]. This study aimed to investigate and compare the phytochemical compositions and biological activities of essential oils, methanolic, and aqueous extracts obtained from aerial parts of three Anatolian Salvia taxa: Salvia aucheri subsp. canescens (SAC, endemic species from Karaman, Ermenek, 1256 m, 13 June 2022; KMUB 7377), S. quezelii (SQ, endemic species from Mersin, Anamur, Boğuntu, 700 m, 09 May 2020; KMUB 6000), and S. candidissima subsp. occidentalis (SCO, Karaman, Kazancı, 950 m, 20 June 2020; KMUB 5452). **Methods:** Essential oils (EO) were obtained from the dried aerial parts by hydrodistillation using a Clevenger-type apparatus. Methanolic extracts were prepared via maceration (3 days, 8 h/day), and aqueous extracts were prepared by boiling plant material in distilled water for 2 h followed by filtration. GC–MS/FID was used for EO analysis. Biological activities were tested using standard spectrophotometric assays for ABTS•+, DPPH•, collagenase, tyrosinase, α-amylase, α-glucosidase, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) inhibition [3-6]. **Results:** GC–MS analyses revealed distinctive chemotypes: SAC EO was dominated by 1,8-cineole (25.6%) and camphor (24.1%), while SQ EO was rich in pinocamphone (33.7%) and α-pinene (11.2%). All essential oils exhibited very low radical scavenging activities when compared to the standards Trolox and α-tocopherol. In particular, the essential oil of SAC demonstrated minimal activity in both ABTS•+ and DPPH• assays, with scavenging effects of 1.797 ± 0.0122% and 1.994 ± 0.0068%, respectively. Among EOs, only SQ exhibited moderate collagenase inhibition (4.56 ± 0.48%), while epigallocatechin gallate (EGCG) inhibited 54.90 ± 0.30%. The inhibitory activity of the essential oils against α-amylase was observed at low levels, ranging from 26.9% to 29.8%, while α-glucosidase inhibition was found to be even more limited. Specifically, the essential oil of SAC exhibited the highest α-glucosidase activity at 4.55% ± 2.79, whereas SCO showed the highest α-amylase inhibition at 29.82% ± 9.23. However, the inhibitory effects of both essential oils were considerably lower compared to the positive control acarbose, which demonstrated α-glucosidase and α-amylase inhibition of 74.72% ± 1.41 and 67.87% ± 2.74, respectively. The essential oil of SCO exhibited the highest inhibitory activities against AChE and BChE, with values of 4.96% ± 3.68 and 19.43% ± 2.62, respectively. Notably, the aqueous extract of SAC showed moderate α-glucosidase inhibition (42.22 ± 3.77%, IC_{50} = 867 µg/mL). Methanolic extract of SQ exhibited strong AChE inhibition (88.11 ± 7.42% at 100 µg/mL; 77.0 ± 0.3% at 5 µg/mL), comparable to donepezil, but weak BChE inhibition (<9%). **Conclusions:** Each Salvia taxon exhibited unique phytochemical and biological profiles. The methanolic extract of S. quezelii presented strong potential as an anti-Alzheimer agent, while the aqueous extract of S. aucheri appears promising for antidiabetic purposes. Although the EOs demonstrated limited antioxidant and enzymatic activities, their specific chemical fingerprints (e.g., cineole, pinocamphone) suggest their utility in cosmetic applications and as chemotaxonomic markers.

ACKNOWLEDGMENTS

The authors express their gratitude to Atatürk University for the support and resources provided throughout this study.
Coresponding author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES

[1] Wu Y-B, Ni Z-Y, Shi Q-W, et al. Constituents from Salvia species and their biological activities. Chemical reviews. 2012;112(11):5967-6026.
[2] Kamatou GP, Makunga N, Ramogola W, Viljoen AM. South African Salvia species: a review of biological activities and phytochemistry. Journal of ethnopharmacology. 2008;119(3):664-672.
[3] Ozkan A, Erdogan A, Sokmen M, Tugrulay S, Unal O. Antitumoral and antioxidant effect of essential oils and in vitro antioxidant properties of essential oils and aqueous extracts from Salvia pisidica. Biologia. 2010;65: 990-996.
[4] Ozkan G, Sagdic O, Gokturk RS, Unal O, Albayrak S. Study on chemical composition and biological activities of essential oil and extract from Salvia pisidica. LWT-Food Science and Technology. 2010;43(1):186-190.
[5] Maral H. Chemical and antioxidant diversity of essential oils of some Salvia species from Turkey. Biochemical Systematics and Ecology. 2023;106:104575.
[6] Yilmaz G, Eruygur N, Bona GE, et al. Phytochemical analysis, antioxidant, and enzyme inhibition activity of five Salvia taxa from Turkey. South African Journal of Botany. 2023; 152:212-221.

Evaluation of Tyrosinase Inhibitory Effect of *Cupressus sempervirens* L. Essential Oil and *Cucumis melo* L. Fixed Oil

Kahya, S.E.[a,*], Karadağ, A.E.[a], Demirci, B.[b], Demirci, F.[b]

[a]*Department of Pharmacognosy, School of Pharmacy, Istanbul Medipol University, Beykoz, Istanbul, Türkiye*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, Türkiye*

***Corresponding author:** sumeyye.kahya@medipol.edu.tr*

Keywords: *Cucumis melo, Cupressus sempervirens, essential oil, tyrosinase inhibitory.*

Objective: Tyrosinase is a copper-containing enzyme known to play a key role in the synthesis of melanins. These pigments are responsible for human skin color and enzymatic browning in nature. Essential oils, derived from aromatic plants, contain bioactive compounds with biological activities such as anti-inflammatory, antioxidant, and anti-aging. In this study, the inhibitory effect of *Cupressus sempervirens* L. essential oil and *Cucumis melo* L. fixed oil on the tyrosinase enzyme was evaluated individually. **Methods:** The quality of commercially available essential oil and fixed oil was analyzed by GC-MS and GC-FID. The tyrosinase inhibition assay was tested by modifying the method of Yang et al. [1]. The inhibitory activities of *C. sempervirens* and *C. melo* were evaluated against mushroom tyrosinase. Initial concentrations were at 1140 mg/mL and were applied in a two-fold serial dilution. Kojic acid was used as a standard compound, and the initial concentration was 250 mg/mL under the same conditions. **Results:** *C. sempervirens's* and *C. melo's* main components were determined as α-pinene (65.0%), d-3-carene (13.0%), linoleic acid (39.4%), and oleic acid (27.4%), respectively. The results indicated that essential oil and fixed oil exhibited anti-tyrosinase activities with IC50 values of 1676.70 ± 0.006 mg/mL and 641.47 ± 0.014 mg/mL, respectively. The positive control, kojic acid, was determined to have an IC50 value of 6.74±0.03 µg/mL. **Conclusions:** To the best of our knowledge, the tyrosinase inhibition of the selected oils was determined by IC50 values for the first time. Based on the comparison of IC_{50} values, *C. melo* fixed oil was demonstrated to be as effective as kojic acid. These results indicate that fixed oil has potent anti-tyrosinase activities. It may be a beneficial source for skin-whitening agents. It may be evaluated in combination with various essential oils for potential synergistic effects.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the ISEO 2025 – YSFA.

REFERENCES
[1] Yang Z., Wang Y., Wang Y., Zhang Y. (2012). Bioassay-guided screening and isolation of α-glucosidase and tyrosinase inhibitors from leaves of Morus alba, *Food chemistry*, 131(2), 617-625.

.

Cytotoxic Activity of *Rhus coriaria* L. Essential Oil Against Human Cancer Cell Lines

Meseldžić, N.[a], Dedić, A.[b], Džudžević-Čančar, H.[b,*], Bego, T.[a], Alispahić A.[a], Žižek, Ž.[c]

[a]*Department of Pharmaceutical Biochemistry and Laboratory Diagnostics, University of Sarajevo-Faculty of Pharmacy, Zmaja od Bosne 8, Sarajevo, Bosnia and Herzegovina*
[b]*Department of Chemistry in Pharmacy, University of Sarajevo-Faculty of Pharmacy, Zmaja od Bosne 8, Sarajevo, Bosnia and Herzegovina*
[c] *Institute of Oncology and Radiology of Serbia, Pasterova 14, Belgrade, Serbia*

Corresponding author: hurija.dzudzevic-cancar@ffsa.unsa.ba

Keywords: *Cytotoxicity, essential oils, IC5, K562/PC-3/S174cell line, MTT assay, Rhus coriaria L..*

Objective: Natural products, particularly essential oils derived from medicinal plants, have gained attention for their biological activities and potential anticancer properties. *Rhus coriaria* L. (sumac), traditionally used in many countries for its antimicrobial and antioxidant effects, has also shown promising results in cancer research [1]. Although *Rus coriaria* is widely distributed throughout the world, there are no examples of sumac being used in traditional medicine or culinary arts in Bosnia and Herzegovina or the region. It is grown for ornament and has only recently been shyly appearing as a spice, under the influence of Mediterranean cuisine. There is also no literature data on any sumac research in BiH or neighboring countries. The aim of this research was to fill that gap and to evaluate the bioactivity of Rhus coriaria fruit essential oil (EO), focusing on the cytotoxic effect on selected human cancer cell lines. **Methods:** Air-dried *Russ c.* fruit is hydrodistillated for 3 hours in a Clevenger-type apparatus to obtain essential oil (EO) samples for constituent determination and cytotoxicity analysis. Stock solutions of essential oil were prepared by dilution in complete RPMI-1640 medium (without phenol red), supplemented with 3 mM L-glutamine, 100 μg/mL streptomycin, 100 IU/mL penicillin, 10% heat-inactivated fetal bovine serum (FBS), and 25 mM HEPES, adjusted to pH 7.2. Cytotoxic activity was assessed using the MTT assay after 72 hours of incubation on chronic myelogenous leukemia (K562), colon carcinoma (LS-174), and prostate cancer (PC-3) cell lines. IC50 values were calculated based on cell viability reduction, and all tests were conducted in triplicate. **Results:** The EO of *Rhus coriaria* exhibited cytotoxic activity against the K562 cell line with an IC50 value of 10.65 ± 0.5 μL/mL. No significant activity was observed against LS-174 and PC-3 cell lines, with IC50 values exceeding 20 μL/mL. Results are presented as average ± standard deviation from three independent experiments. **Conclusions**: Cytotoxic effects are highly dependent on the concentration of the essential oil, so the activity is dose-dependent. *Rhus coriaria* L. essential oil demonstrates selective cytotoxicity against K562 cells, suggesting its potential as a candidate for further investigation in leukemia-related research. However, its limited activity on solid tumor lines (LS-174, PC-3) indicates specificity that warrants additional mechanistic studies.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the YSFA at ISEO 2025.

REFERENCES
[1] (Eds) Atta-ur-Rahman, M. Iqbal Choudhary, Sammer Yousuf (2021). Science of Spices and Culinary Herbs - Latest Laboratory, Pre-clinical, and Clinical Studies. Publisher: Bentham Science.

Characterization and Antiproliferative Activity of Wild Growing *Salvia officinalis* L. Essential Oil

Boškailo, E.[a,*], Džudžević-Čančar, H.[b], Alispahić, A.[a], Sevim, B.[c], Dedić, A.[b], Ramić, E.[a], Stanojković, T.[d]

[a]*Department of Ecology and Environmental Protection, Faculty of Social Sciences dr. Milenko Brkić, Mostar, Bosnia and Herzegovina*
[b]*Department of Chemistry in Pharmacy, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[c]*Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsü Müdürlüğü, Konya-Türkiye*
[d]*Institute of Oncology and Radiology of Serbia, Pasterova 14, Belgrade, Serbia*

***Corresponding author:** emina.boskailo@hercegovina.edu.ba*

***Keywords:** antiproliferative properties, chemical composition, essential oil, Salvia officinalis, terpenes,*

Objective: *Salvia officinalis* L. has long been used in medicine for its essential oil (EO), which has therapeutic effects on respiratory, digestive, cardiovascular, metabolic, and endocrine disorders. Its leaves also offer antiseptic, anti-inflammatory, and anticancer benefits [1]. Previous studies showed α-thujone, eucalyptol, β-pinene, borneol, and camphor as the major phytochemical molecules of *S. officinalis* EO. *S. officinalis* showed cytotoxic activity on normal and immortalized fibroblasts (HF77FA and HDF-Tert), immortalized lung line (BEAS-2B), and breast adenocarcinoma (MDA-MB-231) [2]. The focus of this research was on the chemical composition profiling of *S. officinalis* EO extracted from wild-grown plants and assessing the ability of the EO to inhibit the growth of K562 lymphoblast cell lines from a leukemic patient. **Methods:** Aerial parts of *S. officinalis* were collected in the post-flowering period (October, 2023) in Mostar (hill Fortica: 43°21′04″N 17°49′45″E). Voucher specimens were deposited in the herbarium of the University of Sarajevo-Faculty of Pharmacy (Bosnia and Herzegovina). Following the recommendation of the European Pharmacopoeia (1997), EO is obtained during 2 hours of hydrodistillation in Clevenger-type apparatus. Extracted VOCs were separated by an Agilent 7890A gas chromatograph connected to an Agilent 5975C MS detector. The sample was introduced into GC via a split/splitless injector, heated at 250°C, working in split mode (20:1 ratio), autosampler Agilent GC80. Injected VOCs were separated on DB-FFAP column, 30 m, 0.25 mm, 0.25 µm (Agilent J & W Column, Agilent Technologies, USA). The EO antiproliferative properties were evaluated by MTT assay. The percentage of reduction of yellow tetrazolium dye was determined, and the IC50 of EO was calculated**. Results:** Using GC/MS analysis, 93 compounds in total were identified in *S. officinalis* EO, which represented 97.53% of oil. The chemical compounds found in a high amount in EO were (+)-camphor (19.15%), thujone (15.57%), eucalyptol (10.58%), humulene (7.10%), and camphene (6.77%). The cytotoxic activity of *S. officinalis* EO was evaluated using the MTT assay on K562 lymphoblast cell lines. IC50 (µL/mL) values for K562 cell lines were 1.08±0.16, which represent high cytotoxic activity (IC50<20 mg/L) that might be linked to determined major compounds. Synergy between EO compounds may produce an effect greater than the sum of their individual effects. The *S. officinalis* EOs contain compounds that have been proven to reduce the viability of cancer cells. Research conducted with human hepatoma (Hep3B) cell lines showed that camphor has a cytotoxic effect [3], and eucalyptol showed cytotoxicity on human gingival fibroblasts [4]. **Conclusions:** The chemical analysis of *S. officinalis* EO showed high percentages of (+)-camphor, thujone, eucalyptol, humulene, and camphene. The antiproliferative activity of *S. officinalis* EO on cell viability was tested on K562 cancerous cells, and the results showed low IC50 values. The EO's chemical composition and its cytotoxic effects on cancer cells support the plant's therapeutic potential. Further research into the interactions between its compounds is needed to better understand its pharmacological properties.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the YSFA at ISEO 2025.

REFERENCES

[1] Kačániová, M., Galovičová, L., Valková, V., Ďuranová, H., Borotová, P., Štefániková, J., Vuković, N.L., Vukičć, M., Kunová, S., Felsöciová, S., Miklášová, K., Savitskaya, T., Grinshpan, D. (2021). Chemical composition and biological activity of *Salvia officinalis* essential oil. *Acta Hortic. Regiotect,* 24, 81-88.
[2] Jažo, Z., Glumac, M., Paštar, V., Bektic, S., Radan, M., Carev, I. (2023). Chemical Composition and Biological Activity of *Salvia officinalis* L. Essential Oil. *Plants* 2023, 12, 1794.
[3] Celen Yuceturk, S., Aydogan Turkoglu, S., Kockar, F., Kucukbay, F. Z., Azaz, A. D. (2021). Essential oil chemical composition, antimicrobial, anticancer, and antioxidant effects of *Thymus convolutus* Klokov in Turkey. Zeitschrift für Naturforschung C, 76(5-6), 193-203.
[4] Ivanov, M., Kannan, A., Stojković, D. S., Glamočlija, J., Calhelha, R. C., Ferreira, I. C., Sanglard, D., Soković, M. (2021). Camphor and eucalyptol—Anticandidal spectrum, antivirulence effect, efflux pumps interference and cytotoxicity. *Int. J.of Molecular Sciences,* 22(2), 483.

Investigating the anxiolytic activity of selected essential oils in zebrafish larvae and identification of active constituents using biochemometrics

Maphanga, V.[a], Sandasi, M.[a,b], Enslin, G.[a], Skalicka-Woźniak, K.[c], Salifu, E.[d], Ramharack, P.[d], Viljoen, A.[a,b,*]

[a]*Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa*
[b]*SAMRC Herbal Drugs Research Unit, Faculty of Science, Tshwane University of Technology, Pretoria 0001, South Africa*
[c]*Department of Natural Products Chemistry, Medical University of Lublin, 1 Chodzki Street, 20-093, Lublin, Poland*
[d]*Biomedical Research and Innovation Platform, South African Medical Research Council, Cape Town 7505, South Africa*

***Corresponding author:** viljoenam@tut.ac.za*

***Keywords**: Anxiety, biochemometrics, essential oils, light-dark transitions, molecular docking, zebrafish larvae.*

Objective: The objective of this study was to investigate essential oils as natural alternatives for the management of anxiety, as they have a long history of traditional use for various central nervous system (CNS) disorders. **Methods:** Commercially available essential oils (n = 62) reported to exhibit possible anxiolytic activity were sourced from Pranarôm International® (Belgium) and Scatters Oils® (South Africa). The oils were chemically profiled on an Agilent gas chromatograph coupled to a quadruple mass spectrometer (MS) and a flame ionization detector (FID) (GC-MS/FID). Anxiety-like behavior in five-day post-fertilization (5-dpf) zebrafish larvae was induced by light/dark transitions in a DanioVision observational chamber (Noldus), and locomotor activity was monitored. The anxiolytic activity of the oils was assessed as reverse thigmotaxis behavior (percentage distance travelled (%DT) and time spent (%TS)) in the dark by the pretreated larvae. A biochemometrics approach was used to correlate the oil profiles to anxiolytic activity and subsequent identification of bioactive compounds. *In silico* target prediction was used to identify potential biological targets for all the compounds that exhibited favorable ADMET properties. **Results:** Thirty one out of the 62 oils alleviated anxiety in the larvae to some extent, with *Boswellia carterii* (%DT = 40.6 ± 2.7, %TS = 39.0 ± 3.3), *Pogostemon cablin* (%DT = 38.8 ± 2.5, %TS = 37.9 ± 2.3), and *Lavandula burnatii* (%DT = 37.0 ± 2.0, %TS = 38.6 ± 3.4), as well as the positive control, diazepam (%DT = 45.1 ± 2.7, %TS = 41.7 ± 2.9), displaying the best anxiolytic activity recorded as a significant ($p < 0.05$) increase in the distance travelled and time spent in the central arena during the dark phase (reverse-thigmotaxis behavior) compared to the negative control (%DT = 26.5 ± 2.1, %TS = 26.6 ± 2.3). α-Pinene, *p*-cymene, camphene, eucalyptol, linalyl acetate, linalool, and *trans*-anethole were predicted as potential bioactive molecules, and subsequent experimental validation confirmed significant anxiolytic activity ($p < 0.05$) of the compounds. Target prediction identified seven biological targets, and of those, cannabinoid (CB2), serotonin 7 (5-HT7), and serotonin 2C (5-HT2C) receptors bound favorably to the compounds after molecular docking. **Conclusion:** The study provides scientific evidence to support the use of some essential oils to alleviate anxiety. The active essential oil compounds can be explored further as possible leads for anxiolytic drug development. Biochemometrics is herein demonstrated as a useful tool for identifying possible bioactive compounds, as supported by the experimental validation results and *in silico* molecular docking.

ACKNOWLEDGMENTS
We would like to acknowledge the National Research Foundation (Grant number 86923), the South African Medical Research Council (EMU grant number 23015), and Tshwane University of Technology for funding.
First author is awarded by the IFEAT within the YSFA at ISEO 2025.

Tracing the Flavor Origin in Cosmetics Using Enantiomeric Profiles of Selected Chiral Compounds

Khvalbota, L.[a,*], Rušinová S.[a], Špánik, I.[a]

[a]*Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovakia*

***Corresponding author:** liudmyla.khvalbota@stuba.sk*

***Keywords:** Cosmetics, chiral separations, enantiomers, essential oils, heart-cutting gas chromatography.*

Objective: This work aims to determine the origin of flavor in essential oil-based cosmetic products by evaluating the enantiomeric composition of selected chiral markers through advanced chromatographic techniques. Some essential oils can be beneficial in skin care by helping to treat acne, slowing skin aging, improving skin tone, or providing protection against the sun. They can also serve as natural preservatives, either alone or in combination with other agents, by preventing the growth of bacteria and mold in products [1]. Modern cosmetic consumers are increasingly concerned about the quality of the products they use. However, due to strong market competition, manufacturers are sometimes tempted to adulterate cosmetic products by replacing essential oils with lower-quality substitutes. Therefore, the development of methods for detecting such adulterations is of great interest. **Methods:** In this work, 20 essential oils originating from Brazil, Bhutan, Barbados, Bolivia, Guatemala, and Uganda were analyzed. The essential oils were derived from lemongrass, citrus, eucalyptus, rosemary, and artemisia. In addition, eight commercially available cosmetic products containing essential oils or their ingredients were also included in the study. For VOC analysis, essential oil samples were diluted with hexane in a ratio of 1:3, except for the sample originating from Barbados, which was analyzed undiluted. VOCs from cosmetic products were extracted by use of SPME with DVB/PDMS fiber. The extracted VOCs were analyzed by GC-MS using a DB-FFAP column. Enantiomer separations were performed by a heart-cut two-dimensional GC system with two independent GC ovens connected via Dean's microfluidic switching system. For chiral separation Chirasil-β-Dex or MEGA-DEX DMT-Beta columns were used. **Results:** Using GC-MS analysis, several volatile organic compounds, including chiral compounds, were identified in cosmetic products and essential oil samples from diverse botanical and geographical origins. 10 terpenoic compounds present in essential oils and cosmetics were selected for analysis of their enantiomeric profiles (camphene, linalool oxide, limonene, linalool, 4-terpineol, α-terpineol, citronellol, limonene oxide, nerolidol, and β-pinene). Depending on the botanical or geographical origin, the identified chiral compounds were present either as racemic mixtures or with one dominant enantiomer. By combining VOC profiling with enantiomer distribution analysis, the presence or absence of essential oils in the studied products was determined. **Conclusions:** The chemical complexity of essential oils and cosmetic products presents significant challenges for the detection of falsifications. Complex analytical approaches, such as volatile profiling combined with comprehensive chiral analysis of present chiral compounds, can serve as effective tools for the identification of fraud. This study demonstrated the potential of such an approach.

ACKNOWLEDGMENTS

The project has received funding from Slovak Research and Development Agency under Contract No. APVV-21-0211. First author is awarded by the IFEAT within the YSFA at ISEO 2025.

REFERENCES

[1] Guzman E., Lucia A. (2021) Essential oils and their individual components in cosmetic products, *Cosmetics*, 8(4), 114.

Phytochemical profile of *Prunus spinosa* L. flower and leaf essential oils

Džudžević-Čančar, H.[a,*], Dedić, A.[a], Alispahić, A.[a], Begić, S.[b], Koljančić, N.[c]

[a]*Department of Chemistry in Pharmacy, University of Sarajevo-Faculty of Pharmacy, Sarajevo, Bosnia and Herzegovina*
[b]*Department of Chemistry, University of Sarajevo-Faculty of Science, Bosnia and Herzegovina*
[c]*Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, Bratislava, Slovakia*

Corresponding author: hurija.dzudzevic-cancar@ffsa.unsa.ba

Keywords: blackthorn, essential oils, GC-MS, long-chain alkanes and alcohols, phytol, *Prunus spinosa* L.

Objective: Numerous beneficial properties have been attributed to EOs, such as strong antibacterial, antiproliferative, and antifungal activities; insecticidal and repellent effects; antispasmodic effects; purgative and diuretic properties; and other bioeffects in different medical fields. This research presents the investigation of the phytochemical composition of *Prunus spinosa* L. flower and leaf essential oils because their bioactivity is attributed to certain compounds. Blackthorn (sloe) is known in traditional medicine of Bosnia and Herzegovina (BiH) for different purposes. Usually raw fruit and extracts of fruit, leaf, and flower are used for their astringent, depurative, diaphoretic, diuretic, febrifuge, laxative, and stomachic properties [1, 2] But use of the EOs and assessment of their therapeutic potential for human health, to the best of our knowledge, is at the beginning**. Methods:** EOs of fresh flowers and leaves of *Prunus spinosa* L. from BiH were obtained by hydrodistillation in a Clevenger-type apparatus. EOs were dried over anhydrous Na_2SO_4 and stored in sealed dark vials until analysis. Phytochemical composition of EO volatile compounds was analyzed by gas chromatography coupled with mass spectrometry (GC/MS) on a GC by the Agilent 7820A model (Palo Alto, CA, USA). The identification of compounds was performed using GC peaks by comparing their retention indices (RI) relative to C9-C25 *n*-alkanes for HP-5MS with RI data from El-Sayed and by comparing their mass spectra with those from the Wiley 275 (Wiley, NY, USA) and NIST02 (Gaithersburg, MD, USA) libraries. The quantification of volatile components was calculated from the GC peak areas (average of duplicate analyses) by applying the normalization method with no correction factors. **Results:** The GC/MS analyses contained 23 constituents, which accounted for 94.9% of the total leaf EO from Borije, 4 constituents from leaf EO from Trnovo (accounted for 95.00%), and 2 constituents from leaf EO from Vareš (accounted for 94.50%). Long-chain alkanes and alcohols [3] such as phytol were found to be the major compounds of leaf EOs extracted from blackthorn, including docosane and tricosane as main constituents. The analysis indicated that the flower collected from Borije was the richest in VOs components. In three fresh samples of *P. spinosa*, a total of 38 compounds were identified in flowers with EOs. It extracted 13 to 38 compounds, making up 94.6% to 98.7% of the total chromatogram area. The predominant compounds were tricosane and hexadecanoic acid. **Conclusion:** Due to the high presence of phytol in leaf EO, based on literature, antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activity can be expected, and it can be used as an anti-cancer agent and in cosmetics for skin health. The presence of long-chain alkanes such as heneicosane in flower EO assumes good antimicrobial activity. Also, the presence of a large number of components of low content is not without significance. The possibility of a synergistic effect of all components on the overall activity of the analyzed EOs should not be ignored. In order to obtain a complete picture of the biological activity of the essential oils of *Prunus spinosa* L. from the different regions of BiH, further research is necessary, which is the goal of our future research.

REFERENCES

[1] Džudžević-Čančar H., Dedić A., Jerković I., Marijanović Z., Mandal Š., Čančar I.F., Alispahić A. (2019). Chemical Characterization of *Prunus spinosa* L. fruit Volatiles, 6th World Congress on Medicinal and Aromatic Plants for Human and Animal Welfare (WOCMAP VI), 13-17 November 2019 u Famagusta, N. Cyprus, Book of abstracts, pp 174.
[2] Spadaccino G., Frabboni L., Petruzzi F., Disciglio G., Mentana A., Nardiello D., Quinto M. (2021). Essential oil characterization of *Prunus spinosa* L., *Salvia officinalis* L., *Eucalyptus globulus* L., *Melissa officinalis* L. and *Mentha x piperita* L. by a volatolomic approach a volatolomic approach, *Journal of Pharmaceutical and Biomedical Analysis*, 202.
[3] Islam M.T, et. al (2021). Phytol: A review of biomedical activities. *Food and Chemical Toxicology*, Vol 121, pp 82.94.

Biofilm and efflux pump inhibition of tea tree and thyme essential oils against two nosocomial bacterial species

Ormai, E.[a], Balázs, V. L.[a], Szilágyi-Utczás, M. [b], Ollmann, T.[c], Szemerédi, N. [d], Spengler, G.[d], Horváth, Gy.[a,*]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Univ. of Pécs, Rókus St. 4., Pécs, Hungary*
[b]*Center for Sports Nutrition Science, Hungarian Univ. of Sport Science, Alkotás St. 42-48, Budapest, Hungary*
[c]*Institute of Physiology, Medical School, Univ. of Pécs, Szigeti St. 12., Pécs, Hungary*
[d]*Department of Medical Microbiology, Albert Szent-Györgyi Medical School, Univ. of Szeged, Semmelweis St. 6., Szeged, Hungary*

***Corresponding author:** horvath.gyorgyi@gytk.pte.hu*

***Keywords:** Antibiofilm effect, efflux pump inhibition, essential oils, nosocomial infections, thyme. tea tree.*

Objective: Nosocomial infections are acquired in the healthcare system and typically develop 48 hours after hospital admission [1]. These infections are often caused by multidrug-resistant bacteria, which pose significant challenges in their treatment. Nosocomial bacteria frequently form biofilms, which protect them from antimicrobial agents [2]. Additional resistance mechanisms include efflux pumps, which actively remove antibiotics from the cells. These concerns highlight the need to explore alternative approaches, such as essential oils (EOs), which have demonstrated antimicrobial activity [3]. In this study, the effects of tea tree (TTEO; *Melaleuca alternifolia* (Maiden & Betche) Cheel) and thyme EOs (TEO; *Thymus vulgaris* L.) were investigated against *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 13883) reference strains. This study aimed to assess whether these EOs can both inhibit biofilm formation and efflux pump activity *in vitro*. **Methods**: The EO samples were purchased commercially (Panarom Ltd, Hungary). The chemical composition of the EOs was analyzed with gas chromatography coupled to mass spectrometry (GC-MS). Minimum inhibitory concentrations (MICs) of the oils and the positive controls (ceftriaxone and polymyxin B) were determined with the microdilution method [4]. The crystal violet assay was used to evaluate the antibiofilm effect of the EOs, and inhibitory rate values were calculated [4]. To investigate the efflux pump inhibition ability, fluorescence activity was monitored at excitation and emission wavelengths of 525 and 615 nm every minute for one hour on a real-time basis. Relative fluorescence index (RFI) was calculated and compared to the positive control (carbonyl cyanide 3-chlorophenylhydrazone, CCCP) [5]. Higher RFI means more effective inhibition of the efflux pump compared to the control. **Results**: The main component of TTEO was terpinen-4-ol (38.4%), and the major constituent of TEO was thymol (55.9%). The MIC of TTEO was 0.43 mg/mL against both *E. coli* and *K. pneumoniae*. For TEO, the MIC was 0.63 mg/mL for *E. coli* and 0.22 mg/mL for *K. pneumoniae*. The biofilm inhibition assay showed that *E. coli* biofilm formation was reduced by 75% in the presence of TTEO and by 71% with TEO. In the case of *K. pneumoniae*, biofilm formation was inhibited by 81% with TTEO and by 73% with TEO. The efflux pump inhibition assay showed relative fluorescence index (RFI) values of 0.51 (TTEO) and 1.32 (TEO) for *E. coli*. For *K. pneumoniae*, the RFI values were 0.50 with TTEO and 0.31 with TEO. **Conclusion**: Our findings demonstrate that TTEO and TEO exhibit notable anti-biofilm and potential efflux pump-inhibitory activities against nosocomial pathogens such as *K. pneumoniae* and *E. coli*. These results can support the development of surface disinfectant formulations intended to help control healthcare-associated infections in clinical settings. However, further studies are required to determine whether TTEO and TEO may be safely and effectively incorporated into routine medical practice.

ACKNOWLEDGMENTS
This work was supported by the Albert Szent-Györgyi Medical School, University of Szeged, Hungary, (grant number: SZTESZAOK-KKASZGYA2023/5S772; Gabriella Spengler). This research was supported by the Fund of National Research Development and Innovation Office (grant number: NKFIH PD 147156; Viktoria Lilla Balazs). And the János László Doctoral Research Scholarship of University of Pécs (Edit Ormai).
First author is awarded by the IFEAT within the YSFA at ISEO 2025.

REFERENCES

[1] Lemiech-Mirowska E., Kiersnowska Z. M., Michałkiewicz M., Depta A., Marczak M., (2021). Nosocomial infections as one of the most important problems of healthcare system, *Annals of Agricultural and Environmental Medicine*, 28(3):361-366.
[2] Sauer K., Stoodle P., Goeres D. M., Hall-Stoodley L., Burmølle M., Stewart P. S., Bjarnsholt T., (2022). The biofilm life cycle: expanding the conceptual model of biofilm formation, *Nature Reviews Microbiology*, 20(10), 608–620.
[3] Puvača N., Milenković J., Galonja Coghill T., Bursić V., Petrović A., Tanasković S., Miljković T., (2021). Antimicrobial activity of selected essential oils against selected pathogenic bacteria: In vitro study, *Antibiotics*, 10(5), 546.
[4] Nagy-Radványi L., Ormai E., Koloh R., Ángyán V. D., Kocsis B., Bencsik-Kerekes E., Balázs V. L., (2024). Biofilm inhibition activity of fennel honey, fennel essential oil and their combination, *Microorganisms*, 12(11), 2309.
[5] Ghazal T. S. A., Schelz Z., Vidács L., Szemerédi N., Veres K., Spengler G., Hohmann J., (2022). Antimicrobial, multidrug resistance reversal and biofilm formation inhibitory effect of *Origanum majorana* extracts, essential oil and monoterpenes, *Plants*, 11(11), 1432.

Chemical profiles, antioxidant, and antimicrobial activities of *Juniperus indica* found in Indian Himalayan Region

Gupta A.[a,b,*], Baleshwar M.[b], Dwivedy A. K.[c]., Rana T. S.[d]

[a]*CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow-226001, India*
[b]*CSIR-Traditional Knowledge Digital Library, New Delhi-110 067, India*
[c]*Department of Botany, Banaras Hindu University-221005, India*
[d]*CSIR-Human Resource Development Centre, Sector -19, Central Govt. Enclave, Kamla Nehru Nagar, Ghaziabad 201002, Uttar Pradesh, India*

***Corresponding author:** abhigupta962@gmail.com*

***Keywords:** Antioxidant, antimicrobial, essential oil, Juniper, terpenes.*

Objective: *Juniperus indica* Bertol. (Cupressaceae) is a dioecious, evergreen aromatic shrub widely distributed across the Indian Himalayan Region (IHR) at elevations between 2500 and 4700 m [1]. Traditionally, it is used in food, perfumery, medicine, and religious practices [2]. However, limited research exists on its population-level chemical variability and bioactivity. The present study aimed to investigate the essential oil (EO) yield, chemical composition, antioxidant potential, and antimicrobial activity of six wild populations (J1–J6) of *J. indica* from three Himalayan states of India: Himachal Pradesh, Uttarakhand, and Sikkim. **Methods:** Mature needle samples (500 g dry weight per population) were collected between October 2019 and October 2022. Plant identity was confirmed by morphological and microscopic evaluation, with voucher specimens deposited at LWG Herbarium, CSIR-NBRI, Lucknow. EOs were obtained by hydrodistillation using a Clevenger-type apparatus at 70–80°C for 8 hours. Chemical composition was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) on a Shimadzu GC/MS-QP2010 system. Identification of compounds was performed by comparing mass spectra with NIST and Wiley libraries. Antioxidant activity was evaluated using the DPPH radical scavenging assay, with IC50 values determined and compared across populations. Gallic acid served as the reference standard. Antimicrobial activity was assessed using the disc diffusion method against five human pathogenic bacterial strains (*Acinetobacter baumannii*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) and three fungal strains (*Aspergillus niger*, *Candida albicans*, *Fusarium oxysporum*). Ampicillin and fluconazole served as positive controls, with DMSO as a negative control. **Results:** EO yield varied significantly among populations, ranging from 2.40% (J6) to 5.80% (J3) (v/w dry weight). GC-MS profiling revealed a total of 75 distinct compounds across all populations, with 26 common across all six. Population-specific chemical signatures were observed, with J6 having 11 unique constituents. The major chemical classes included monoterpene hydrocarbons (26.05–47.04%), oxygenated sesquiterpenes (27.03–44.66%), oxygenated monoterpenes (3.14–22.96%), sesquiterpene hydrocarbons (2.15–21.94%), and non-terpenoids (0.32–1.72%). α-Pinene and sabinene were the predominant monoterpenes, while terpinen-4-ol and α-elemol were notable oxygenated sesquiterpenes. Multivariate analyses (PCA and HCA) based on 15 major volatile compounds classified the six populations into two chemotypic clusters: J1, J4, and J5 grouped together, while J2, J3, and J6 formed the second group. Antioxidant activities (IC50 values) ranged from 25.75 µg/mL (J3) to 42.5 µg/mL (J2), indicating moderate to good free radical scavenging potential. J3 exhibited the highest antioxidant activity, close to the standard gallic acid (9.64 µg/mL). Antimicrobial testing revealed inhibition zones between 8.3 mm and 15.7 mm across tested bacterial and fungal strains. EO from the J1 population exhibited the strongest antibacterial and antifungal effects, especially against *Bacillus subtilis* and *Candida albicans*. **Conclusions:** This study highlights substantial population-level variation in EO yield, composition, and bioactivity of *J. indica* from the IHR. The dominance of monoterpenes and oxygenated sesquiterpenes with notable bioactivity suggests significant potential for pharmaceutical, food, and industrial applications. Populations J3 and J1 showed superior antioxidant and antimicrobial activities, respectively. The findings also emphasize the need for conservation and sustainable utilization strategies for this valuable Himalayan species.

ACKNOWLEDGMENTS
First author is awarded by the IFEAT within the YSFA at ISEO 2025.

REFERENCES
[1] Adams, R.P. (2014). Junipers of the World: The Genus *Juniperus*, 4th ed. Trafford Publishing, Victoria, BC.
[2] Maurya, A.K., Devi, R., Kumar, A., Koundal, R., Thakur, S., Sharma, A., Kumar, R., Padwad, Y.S., Chand, G., Singh, B., & Agnihotri, V.K. (2018). Chemical composition, cytotoxic and antibacterial activities of essential oils of cultivated clones of *Juniperus communis* and wild *Juniperus* species. *Chem. Biodiversity* 15: e1800183.

Optimising microwave-assisted hydrodistillation for enhanced essential oil extraction from *Cinnamomum tamala* using response surface methodology: A comparative analysis with conventional hydrodistillation

Debajani, M.[a], Sudipta J.[a], Asit R,[a,*]

[a]*Centre for Biotechnology, Siksha O Anusandhan University, Kalinga Nagar, Bhubaneswar, India*

***Corresponding author:** asitray@soa.ac.in*

Keywords: **Keywords:** *Central composite design, Cinnamomum tamala, essential oil, hydrodistillation, Indian bay leaf, microwave-assisted, response surface methodology*

Objectives: *Cinnamomum tamala* (Buch.-Ham.) Nees & Eberm., commonly known as Indian bay leaf, has been traditionally used in Ayurveda for its therapeutic properties. Its dried leaves are widely used as a spice and flavoring agent in the preparation of various food items. The leaf essential oil (CTLEO) has significant application in food, perfumery, and pharmaceuticals owing to its aromatic and medicinal value. The conventional hydrodistillation (HD) process used for essential oil extraction is time-consuming, energy intensive, yields less oil, and releases environmental waste. Therefore, there is a need to explore an efficient, environmentally friendly, and cost-effective extraction method for recovering essential oil from *C. tamala* leaves. Microwave-assisted hydrodistillation (MAHD) is a promising greener approach complying with the idea of environmentally sustainable engineering practices. The present study aimed at optimizing the extraction parameters of the MAHD process to maximize CTLEO yield and assessing its performance in comparison with conventional HD to determine its overall effectiveness. **Methods:** The process optimization to extract essential oil from *C. tamala* leaf by MAHD was performed using central composite design-based response surface methodology (RSM). The effectiveness of MAHD was assessed with conventional HD by comparing oil yield, kinetics, physical attributes, chemical composition, energy usage, and environmental impact. The micromorphological alterations in *C. tamala* leaf following HD and MAHD processes were examined using scanning electron microscopic (SEM) analysis. The polyphenolic content in residual water following essential oil extraction was assessed using high-performance liquid chromatography (HPLC). **Result:** The optimized parameters of MAHD were identified as microwave power of 800 W, liquid/plant ratio of 3:1, and an extraction time of 60 min. A regression model was obtained, suggesting a quadratic relationship with an R^2 value of 0.9896, revealing its high level of precision in predicting essential oil yield. MAHD resulted in a higher yield of essential oil (0.24% v/w) compared to HD (0.19% v/w). Moreover, MAHD exhibited better efficiency by significantly reducing the extraction time and lowering energy usage (60 min, compared to 240 min in HD). GC-MS analysis revealed that the use of microwave energy did not adversely influence the composition of the essential oils. Fourier transform infrared spectroscopy (FTIR) analysis showed close resemblance in the spectra of oils obtained from HD and MAHD. Moreover, there was no significant difference in the physical properties of essential oils extracted using HD and MAHD. SEM analysis revealed that the higher yield of essential oil from *C. tamala* leaves using MAHD is attributed to the complete destruction of secretory oil glands. HPLC analysis revealed that MAHD produces a higher proportion of bioactive polyphenols than conventional HD wastewater by-product of *C. tamala* leaf. **Conclusion:** MAHD demonstrated an environmentally friendly and sustainable alternative technique for a rapid and green extraction of essential oil from *Cinnamomum tamala*.

ACKNOWLEDGEMENTS
Fitst author is awarded by the IFEAT within the ISEO 2025 – YSFA.

Participants /Listeners

Name and Surname	Country	Institution
Adam Feyaerts	Belgium	UCLL, Baal
Adin Pašić	Bosnia and Herzegovina	University of Sarajevo - Faculty of Pharmacy, Sarajevo
Ajna Avdić	Bosnia and Herzegovina	University of Sarajevo - Faculty of Pharmacy, Sarajevo
Anita Milovanović	Serbia	Clinical hospital center Bežanijska kosa, Belgrade
José Fernando Silva	Portugal	Lisboa
Asim Halač	Bosnia and Herzegovina	University of Sarajevo - Faculty of Pharmacy, Sarajevo
Carlos Cavaleiro	Portugal	University of Coimbra - Faculty of Pharmacy, Coimbra
Carolina Barroso	Portugal	University of Porto,
Çağdaş Özgünay	Türkiye	ORLIFE Global International Essential Oils trade Company, Istanbul
Chris Bowerbank	USA	Young Living Essential Oils, Utah
Christian Reichle	Germany	Primavera Life GmbH, Mittelberg
Cigdem Başokutan	Türkiye	Temas Tarım ve Sanat Ltd Şti, Istanbul
Clemens Vergeiner	Austria	Brüder Unterweger GmbH, Assling
Hussein Fakhry	Egypt	A. Fakhry & Co., Cairo
Ivana Jovančević	Serbia	General Hospital Dr Dragiša Mišović, Čačak
Lena Cholewińska	Poland	University of Agriculture in Krakow, Faculty of Animal Science, Krakow
Medin Omerspahić	Bosnia and Herzegovina	University of Sarajevo - Faculty of Pharmacy, Sarajevo
Mohamed Alajmi	Saudi Arabia	King Saud University, Riyad
Natalija Vajdić	Croatia	Aromara d.o.o., Harmica
Nikunj Harlalka	India	Mumbai University, Mumbai
Paula Cavaleiro	Portugal	University of Coimbra - Faculty of Pharmacy, Coimbra
Robert Piorunski	Poland	Life Solutions, Sp.z_O.o. Warszawa
Rosemarie Ruža Ilić	USA/BiH	
Sanja Sučić	Croatia	Aromara d.o.o., Harmica
Sercan Çelebi	Türkiye	Atelier Aromatherapy, Istanbul
Vesna Mišković	Slovenia	Arxfarm d. o. o. Šentjernej
Zafer Özgünay	Türkiye	ORLIFE Global International Essential Oils trade Company, Istanbul

Main sponsors

Sponsor of Young Scientist Fellowship Award (YSFA)

International Federation of Essential Oils and Aroma Trades" (IFEAT)

Sponsor of Medal of Honor

www.merckgroup.com

Workshop Sponsor

Atelier Aromaterapi

ISEO 2025 Bronze Sponsors

eko aromatik

HERBAL GROUP

[eko aromatik]

[herbalgroup.ba]

HALILOVIC SINCE 1983 Liliya essential oil

[biohalilovic.ba]

[farmaceutskakomora.ba]

BOSNALIJEK Prvo zdravlje!

[bosnalijek.ba]

Exhibitor

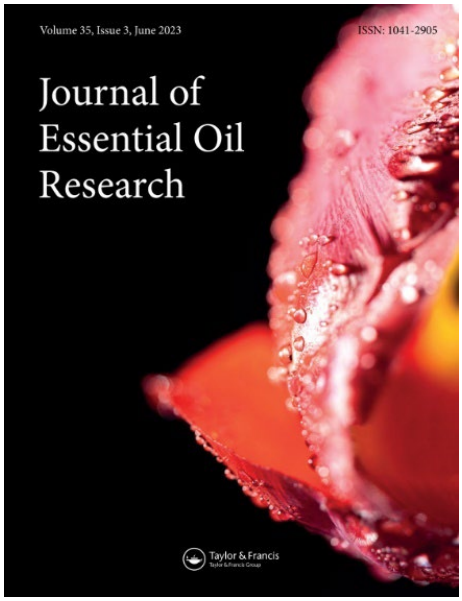
BIOSS www.bioss.rs

[www.bioss.rs]

Publishing Sponsors: Abstract and Full Papers, Special Issue editions

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

[Bulletin of the Chemists and Technologists of Bosnia and Herzegovina]



[Journal of Essential Oil Research]

Supporter

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA

[The Society of Chemists and Technologists of Canton Sarajevo]

INDEX

Participants /Listeners

Name and Surname	Country	Institution
Adam Feyaerts	Belgium	UCLL, Baal
Adin Pašić	Bosnia and Herzegovina	University of Sarajevo - Faculty of Pharmacy, Sarajevo
Ajna Avdić	Bosnia and Herzegovina	University of Sarajevo - Faculty of Pharmacy, Sarajevo
Anita Milovanović	Serbia	Clinical hospital center Bežanijska kosa, Belgrade
José Fernando Silva	Portugal	Lisboa
Asim Halač	Bosnia and Herzegovina	University of Sarajevo - Faculty of Pharmacy, Sarajevo
Carlos Cavaleiro	Portugal	University of Coimbra - Faculty of Pharmacy, Coimbra
Carolina Barroso	Portugal	University of Porto - School of Medicine and Biomedical Sciences
Çağdaş Özgünay	Türkiye	ORLIFE Global International Essential Oils trade Company, Istanbul
Chris Bowerbank	USA	Young Living Essential Oils, Utah
Christian Reichle	Germany	Primavera Life GmbH, Mittelberg
Cigdem Başokutan	Türkiye	Temas Tarım ve Sanat Ltd Şti, Istanbul
Clemens Vergeiner	Austria	Brüder Unterweger GmbH, Assling
Hussein Fakhry	Egypt	A. Fakhry & Co., Cairo
Ivana Jovančević	Serbia	General Hospital Dr Dragiša Mišović, Čačak
Lena Cholewińska	Poland	University of Agriculture in Krakow, Faculty of Animal Science, Krakow
Medin Omerspahić	Bosnia and Herzegovina	University of Sarajevo - Faculty of Pharmacy, Sarajevo
Mohamed Alajmi	Saudi Arabia	King Saud University, Riyad
Natalija Vajdić	Croatia	Aromara d.o.o., Harmica
Nikunj Harlalka	India	Mumbai University, Mumbai
Paula Cavaleiro	Portugal	University of Coimbra - Faculty of Pharmacy, Coimbra
Robert Piorunski	Poland	Life Solutions, Sp.z_O.o. Warszawa
Rosemarie Ruža Ilić	USA/BiH	
Sanja Sučić	Croatia	Aromara d.o.o., Harmica
Sercan Çelebi	Türkiye	Atelier Aromatherapy, Istanbul
Vesna Mišković	Slovenia	Arxfarm d. o. o. Šentjernej
Zafer Özgünay	Türkiye	ORLIFE Global International Essential Oils trade Company, Istanbul

55th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

7-10. September 2025

SARAJEVO, BOSNIA AND HERZEGOVINA

ISEO 2025

55th INTERNATIONAL SYMPOSIUM ON ESSENTIAL OILS

7-10. September 2025

SARAJEVO, BOSNIA AND HERZEGOVINA

ISEO 2025

iseo2025@ffsa.unsa.ba

UNIVERSITY OF SARAJEVO
FACULTY OF PHARMACY