ISEO 2022

52nd International Symposium on Essential Oils

Wrocław, 4-7 September

PROGRAMME AND BOOK OF ABSTRACTS

September 4-7, 2022, Wroclaw, Poland

Organizers:

Wroclaw University of Science and Technology

Wrocław Centre for Technology Transfer

Wroclaw University of Environmental and Life Sciences

College of Physiotherapy in Wroclaw

The Eugeniusz Geppert Academy of Art and Design

Liquid Technologies Sp. z o.o.

Enterprise Europe Network

Editors:

Stanisław Lochyński	Wroclaw University of Science and Technology College of Physiotherapy in Wroclaw
Daniel Jan Strub	Wroclaw University of Science and Technology
Alicja Karolina Surowiak	Wroclaw University of Science and Technology
Lucyna Balcerzak	Wroclaw University of Science and Technology

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Welcome

For the third time, the International Symposium on Essential Oils is held in Wroclaw. In 2001 the Symposium was organized by Wroclaw University of Science and Technology and Wroclaw University of Environmental and Life Sciences. In 2010 Wroclaw College of Physiotherapy joined the Organizing Committee. This year also the Eugeniusz Geppert Academy of Art and Design in Wroclaw empowers the organizers. We also appreciate the support of Liquid Technologies, Wrocław Center for Technology transfer, Enterprise Europe Network in this endeavor.

We expect that this year 100-150 participants from all over the world will attend the Symposium.The scientific program of the Symposium, as you can read in the Book of Abstracts, is a reflection of very broad interests of participants. One can find the papers concerned with all aspects of essential oils and related natural products, ranging from breeding, cultivation, analysis, biogenesis, and chemistry to biological activity and utilization.

We intend to continue the trend regarding aromatherapy and related research aspects. We hope that such a broad area of scientific activity of participants will be a good platform for exchanging scientific information and encouraging partnership between laboratories working with essential oils.

For the first time, we have been able to organize, together with the Eugeniusz Geppert Academy of Art and Design in Wroclaw, the sensory exhibition.

On behalf of the Organizing Committee of ISEO 2022, we would like to wish all participants valuable inspiration during the lectures and poster sessions stimulating scientific discussions, and a nice stay in the beautiful and friendly city of Wroclaw.

Organizing Committee of the 52nd ISEO

ISEO Records 1969 – 2022

1st	1969	Leiden, Netherlands	25th	1994	Grasse, France
2nd	1971	Freiburg i. Br., Germany	26th	1995	Hamburg, Germany
3rd	1972	Helsinki, Finland	27th	1996	Vienna, Austria
4th	1973	Freiburg i. Br., Germany	28th	1997	Eskisehir, Turkey
5th	1974	Freiburg i. Br., Germany	29th	1998	Frankfurt, Germany
6th	1975	Leiden, Netherlands	30th	1999	Leipzig, Germany
7th	1976	Würzburg, Germany	31st	2000	Hamburg, Germany
8th	1977	Freiburg i. Br., Germany	32nd	2001	Wrocław, Poland
9th	1978	Münster, Germany	33rd	2002	Lisbon, Portugal
10th	1979	Würzburg, Germany	34th	2003	Würzburg, Germany
11th	1980	Groningen, Netherlands	35th	2004	Giardini Naxos, Italy
			36th	2005	Budapest, Hungary
12th	1981	Marburg, Germany	37th	2006	Grasse, France
13th	1982	Würzburg, Germany	38th	2007	Graz, Austria
14th	1983	Freising – Weihenstephan, Germany	39th	2008	Quedlinburg, Germany
			40th	2009	Savigliano, Italy
15th	1984	Leiden, Netherlands	41st	2010	Wrocław, Poland
16th	1985	Holminden/Neuhaus, Germany	42nd	2011	Antalya, Turkey
			43rd	2012	Lisbon, Portugal
17th	1986	Bevensen, Germany	44th	2013	Budapest, Hungary
18th	1987	Hordwijkerhout, Netherlands	45th	2014	Istanbul, Turkey
			46th	2015	Lublin, Poland
19th	1988	Zürich – Greifensee, Switzerland	47th	2016	Nice, France
			48th	2017	Pécs, Hungary
20th	1989	Würzburg, Germany	49th	2018	Niš, Serbia
21st	1990	Lahti, Finland	50th	2019	Vienna, Austria
22nd	1991	St. Vincent, Italy	51st	2021	Turkey (Online)
23rd	1992	Auchincruive, Scotland	52nd	2022	Wrocław, Poland
24th	1993	Berlin, Germany			

ISEO 2022

52nd International Symposium on Essential Oils

Wrocław, 4-7 September

GENERAL INFORMATIONS

Symposium venue

The venue of the 52nd International Symposium on Essential Oils will be Concordia Design Wrocław, wyspa Słodowa 7, 50-266 Wrocław

Organizing committee

Chairpersons

Stanisław Lochyński	Wrocław University of Science and Technology College of Physiotherapy in Wroclaw
Daniel Jan Strub	Wrocław University of Science and Technology

Members

Lucyna Balcerzak	Wrocław University of Science and Technology
Alicja Karolina Surowiak	Wrocław University of Science and Technology
Marta Płonka	Eugeniusz Geppert Academy of Art and Design
Anna Pytel	Wrocław Centre for Technology Transfer at Wrocław University of Science and Technology
Agnieszka Turyńska	Wrocław Centre for Technology Transfer at Wrocław University of Science and Technology
Anna Gliszczyńska	Department of Food Chemistry and Biocatalysis, Wrocław University of Environmental and Life Sciences
Daria Kaczmarczyk	College of Physiotherapy in Wroclaw
Beata Szmigiel-Merena	College of Physiotherapy in Wroclaw
Agnieszka Bronowicka-Szydełko	Wrocław Medical University
Arkadiusz Szydełko	Wrocław University of Science and Technology
Bartosz Urbanek	Liquid Technologies
Maria Strub	Liquid Technologies

Local Honorary Committee

Cezary Przybylski	Marshal of Lower Silesia Voivodeship
Jacek Sutryk	President of Wrocław
Prof. Arkadiusz Wójs	Rector of Wrocław University of Science and Technology
Prof. Jarosław Bosy	Rector of Wrocław University of Environmental and Life Sciences
Prof. Andrzej Czamara	Rector of Wrocław College of Physiotherapy
Prof. Wojciech Pukocz	Rector of Eugeniusz Geppert Academy of Art and Design
Prof. Piotr Młynarz	Dean of Faculty of Chemistry of of Wrocław University of Science and Technology

Permanent scientific committee

Yoshinori Asakawa	Tokushima Bunri University, Japan
Nicolas Baldovini	University Côte d'Azur, France
K. Hüsnü Can Baser	Near East University, North Cyprus
Carlo Bicchi	University of Turin, Italy

Humberto Bizzo	Embrapa Food Technology, Brazil
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Fatih Demirci	Anadolu University, Turkey
Jan Demyttenaere	European Flavour & Fragrancee Assoc., Belgium
Adam F. Feyaerts	KU Leuven, Belgium
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Toshio Hasegawa	Saitama University, Japan
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Luigi Mondello	University of Messina, Italy
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

Registration

Conference materials and name badges can be obtained at the registration desk which will be opened in **Concordia Design (4th Floor)** as follows:

Sunday, 4th September	15:00 – 17:00
Monday, 5th September	8:00 – 9:00
Tuesday, 6th September	8:00 – 9:00

Assistants and conference secretariat

If you need any help during the conference you can find the staff at the registration desk. Conference assistants and organizing committee will be recognizable by their green badge. They will help you in all practical aspects of conference participation.

Name badges

Participants and accompanying persons are obliged to wear the conference name badges on all conference events.

Sensory exhibition opening

Opening lecture will be held in the front of the "Old" (renovated part of the historical building) part of the Concordia Design, wyspa Słodowa 7, 50-266 Wrocław on Sunday, 4th September 2022, from 16:00 to 18:00.

Opening ceremony

Opening Ceremony will take place in lecture hall (Sala Widok, 4th floor) in symposium venue (Concordia Design) on Monday, 6th September from 8:30 to 9:30.

Speaker's instructions

PowerPoint, PDF presentations are accepted on USB sticks. The presentation has to be copied to the laptop before the session. Speakers are requested to copy their presentation the day prior their speech **before** the session starts. It is crucial that speakers are on time and that they keep their presentation within the allotted time scheduled. The plenary lectures are foreseen in **40 minutes** including discussion. The time for oral presentations is **15 minutes** including discussion. An audiovisual technician will be available for assistance in the conference hall.

Poster sessions

Poster sessions will take place in poster exhibit area in symposium venue (Taras Widokowy,5th floor) The posters should be placed on certain day in the morning and removed just after the end of the session. Maximum size of a poster is **841 x 1189 [mm]** (Ao format, orientation: portrait).

Poster Session A	Monday, 5th September 16:30 - 18:00
Poster Session B	Tuesday, 6th September 16:00 - 17:30

Language

The conference language is English.

Lunches and refreshments

Lunches are included in the participant's registration fee and will be served in symposium venue (5th floor, Taras Widokowy) as follows:

Monday, 5th September	12:25 – 14:00
Tuesday, 6th September	12:25 – 14:00
Wednesday, 7th September	12:45 – 14:00

Social events

Get-together party (Concordia design, 5th floor Taras Widokowy)

Date	Sunday, 4th September 2022
Time	19:00 – 21:00

Symposium dinner

Date	Tuesday, 6th September 2022
Time	19:00 – 00:00
Place	Restaurant "Z Nurtem" on Wratislavia ship, Bulwar Xawerego Dunikowskiego

City excursion

Date Wednesday, 7th September 2022
Time 14:30-18:00
Place Meeting point: in the front of the Concordia Design building at 13:45 or Niedźwiadek (Main City Square) at 14:30.

Insurance and liability

The organizers do not take any responsibility for injury or damage involving persons or property during the conference. Participants are advised to make their own arrangements with respect to health, travel and property insurance before they depart from their country.

Conference website

www.iseo2022.pl

Exhibitors

Exhibition site will be located in Concordia design (4th floor)

Emergency phone numbers

Mobile phones:

112 - police, fire department, emergency

Stationary phones:

999 – Emergency
998 – Fire Department
997 – Police
981 – Road Assistance
986 – Municipal Police

Scientific Programme

Sunday September 4, 2022

15:00 – 17:00 **Registration**

16:00 – 18:00 **Sensory exhibition opening**

Opening lecture (Concordia Design yard)

(OL-1) Natural perfumery: an olfactive journey in time and space- public own smelling experience

Clio Vidal

WALA Heilmittel GmbH

19:00 – 21:00 **Get- together party** (Concordia design, 5th floor Taras Widokowy)

Monday September 5, 2022

8:30 – 9:00 **Opening Ceremony**

9:00-12:25 Session dedicated to prof. Stanisław Lochyński to honor his 70th birthday and 45th anniversary of scientific activity

SESSION 1

Chairs: Czesław Wawrzeńczyk, Daniel Jan Strub

9:30 – 10:10 **Plenary lecture**
(PL-1) Chemistry of fragrant woods: Agarwood and Sandalwood
Nicolas Baldovini

Côte d'Azur University, France

10:10 – 10:55 **Oral presentations**

(OP-1) Review of volatile marine polyhalogenated monoterpenes from Southern African red marine algae

Michael Knott

University of Namibia, Namibia

(OP-2) An essential oil component as a building block for pheromone synthesis

Anat Zada Byers

Agricultural Research Organization, Israel

(OP-3) Inducing volatile production by plant hormones in peppermint and marjoram

Eva Zamborine Nemeth

Hungarian University of Agriculture and Life Sciences, Hungary

10:55 – 11:25 **Coffee Break**

Session dedicated to prof. Stanisław Lochyński to honor his 70th birthday and 45th anniversary of scientific activity -continued

SESSION 1 - continued

Chairs: Agnieszka Ludwiczuk**,** Györgyi Horváth

11:25 – 12:25 **Oral presentations**

(OP-4) The essential oil composition in medicinally relevant *Salvia* species: A global review

Rattray Ryan

University of Johannesburg, South Africa

(OP-5) Evaluation on Chemical Composition and Bioactivity Potential of the *Thymus transcaucasicus* Ronniger Extracts and Essential Oil

Burcu Sen

Istanbul University, Turkey

(OP-6) Antimicrobial and antibiofilm activities of thyme essential oil with emphasis on the development of oral hygiene compositions

Maryna Kryvtsova

Uzhhorod National University, Ukraine

(OP-7) Eugenol as a promising natural antifungal component

Anna Biernasiuk

University of Lublin, Poland

12:25 – 14:00 **Lunch**

SESSION 2

Chairs: Stanisław Lochyński, Nicolas Baldovini

14:00 – 14:40 **Plenary lecture**

(PL-2) The concept of 'buchu' and the ancient origins of aromatherapy

Ben-Erik Van Wyk

University of Johannesburg, South Africa

14:40 – 15:25 **Oral presentations**

(OP-8) Medicinal plants of Cyprus
K. Husnu Can Baser

Near East University, North Cyprus

(OP-9) Investigations on the sex-specific effect of swiss stone pine essential oil in human well-being. A pilot study

Iris Stappen

University of Vienna, Austria

(OP-10) Essential oils from Côa Valley *Lamiaceae* species: cytotoxicity on glioblastoma cells

Celia Cabral

University of Coimbra, Portugal

15:25 – 16:05 **Medal lecture**

Carlo Bicchi

University of Turin, Italy

16:05 – 16:30 **Coffee Break**

16:30 – 18:00 **Poster Presentation - Session A** (Taras Widokowy,5th floor)

17:30 – 19:00 **ISEO PSC Meeting**

Tuesday September 6, 2022

SESSION 3

Chairs: Jonathan Bonello, Carlo Bicchi

8:30 – 9:10 **Plenary lecture**
(PL-3) Why do liverworts produce so many structurally different volatiles?

Agnieszka Ludwiczuk

Medical University of Lublin

9:10 – 9:40 **Keynote lecture**
(KL-1) F&F Ingredients: World Overview on Feedstocks & Renewability

Alain Frix

Allchemix, Belgium / IFEAT, UK

9:40 – 10:40 **Oral presentations**
(OP-11) Chemical Composition of Industrially Produced Egyptian Jasmine (*Jasminum grandiflorum* L.) Essential Oil Obtained by Steam Distillation

Hussein Fakhry

A. Fakhry & Co., Egypt

(OP-12) Smart solutions for improving performance of analysis of volatile compounds by gas chromatography mass spectrometry

Maciej Kochanowski

Shimpol A.M. Borzymowski, Poland

(OP-13) Influence of organic nutrients on growth, yield and essential oil composition of Patchouli

Tara Mehra

Central Agricultural University, India

(OP-14) Traits specific breeding and varietal registration in *Cymbopogon* genus at CSIR-NEIST, Jorhat, India

Mohan Lal

CSIR- North East Institute of Science & Technology, India

10:40– 11:10 **Coffee Break**

SESSION 4

Chairs: Niko Radulović, Hussein Fakhry

11:10 – 12:25 **Oral presentations**

(OP-15) Organic vs Conventional Farming of Lavender: Effect on Yield, Phytochemicals and Essential Oil Composition

Mima Todorova

Trakia University, Bulgaria

(OP-16) Chamomile essential oils exert anti-inflammatory effects involving human and murine macrophages: evidence to support a therapeutic action

Carmen Formisano

University of Federico II, Italy

(OP-17) Chemical and biological investigations of essential oils from *Juniperus* genus

Laura De Martino

University of Salerno, Italy

(OP-18) Relation between dill apiole content and insecticidal activity of *Piper aduncum* L. essential oil

Humberto Bizzo

Embrapa Agroindústria de Alimentos, Brazil

(OP-19) Essential oils from pineapple processing by-products through ohmic heating

Mohsen Gavahian

National Pingtung University of Science and Technology, Taiwan

12:25 – 14:00 **Lunch**

SESSION 5

Chairs: Humberto Bizzo, Patrizia Rubiolo

14:00 – 14:40 **Plenary lecture**
(PL-4) Analytical strategies for the characterization of non-volatile fraction of *Citrus* essential oil: applications in food and cosmetic.

Paola Dugo

University of Messina, Italy

14:40 – 15:45 **Oral presentations**
(OP-20) CNV and Gene Expression of Monoterpene Synthases Explain Chemotypes of Three Sage Species

Johannes Novak

University of Veterinary Medicine, Austria

(OP-21) Discrimination of the essential oils from three wild goldenrod species growing together in Quebec

Alexis St-Gelais

Laboratoire PhytoChemia, Canada

(OP-22) Essential oils analysis in light of sensory quality. A reliable tool or not?

Jacek Łyczko

Wrocław University of Environmental and Life Sciences, Poland

(OP-23) Lady Pinecone – back to nature

Krzysztof Czapski

Lady Pinecone / Pani Szyszka, Poland

15:40 – 16:00 **Coffee Break**

16:00 – 17:30 **Poster Presentation - Session B** (Taras Widokowy,5th floor)

19:00– 00:00 **Symposium dinner** *(Restaurant "Z Nurtem" on Wratislavia Ship, Bulwar Xawerego Dunikowskiego)*

Wednesday September 7, 2022

SESSION 6

Chairs: Johannes Novak, Éva Zámboriné-Németh

9:00 – 09:40 **Plenary lecture**

(PL-5) Biological activity of essential oils – not a single golden bullet loaded to that revolver?

Niko Radulović

University of Niš, Serbia

10:00 – 11:00 **Young Scientist Session**

(YSP-1) Microwave-Assisted Hydrodistillation of Hop (*Humulus lupulus* L.) Terpenes: A Pilot-Scale Study

Lorenzo Lamberti

University of Turin, Italy

(YSP-2) Involvement of serotonergic neurotransmission in the anxiolytic potential of *Melissa officinalis* essential oil and citronellal

Nikola M. Stojanović

University of Niš, Serbia

(YSP-3) Exploring the mechanisms of action of essential oils: how to undermine the pathogenic biofilm life cycle

Francesca Maggio

University of Teramo, Italy

(YSP-4) Chemical diversity and antifungal activity of *Zataria multiflora* essential oils and influence of abiotic stresses on the compositions

Ali Karimi

Julius Kühn Institute, Germany

11:00 – 11:30 **Coffee Break**

SESSION 7

Chairs: Iris Stappen, Hüsnü Can Baser

11:30 – 12:30 **Young Scientist Session**

(YSP-5) Essential Oils Diversity from the Malayan Flora: Recent Findings and Challenges

Wan Mohd Nuzul Hakimi Wan Salleh

Sultan Idris Education University, Malaysia

(YSP-6) Reuse of Food Waste: Chemical Compositions and Health Properties of Essential Oils from Several Cultivars of Sicilian *Citrus aurantium* L. and *Citrus maxima* (Burm.) Merr.

Natale Badalamenti

University of Palermo, Italy

(YSP-7) Anti-inflammatory effect and possible mechanism of action of bark essential oil of *Neocinnamomum caudatum* in lipopolysaccharide (LPS) stimulated murine macrophage RAW 264.7 cells

Sudipta Jena

Siksha O Anusandhan, India

(YSP-8) *Cymbopogon nardus* essential oil nanoemulsion against fungal infestation and aflatoxin B1 contamination of food system

Abhishek Kumar Dwivedy

Banaras Hindu University, India

12:30 – 12:45 **Closing remarks**

12:45 – 14:00 **Lunch**

14:30 – 18:00 **City guided tour** *(meeting point: the bear, Main Town Square)*

Abbreviations

OL	Opening Lecture
PL	Plenary Lecture
KL	Keynote Lecture
ML	Medal Lecture
OP	Oral Presentation
YSP	Young Scientist Presentation
PP	Poster Presentation

ISEO MEDAL OF HONOUR

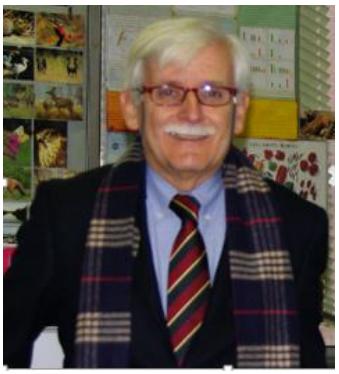
The first promoters of the ISEO Medal were Giovanni Dugo and Luigi Mondello, Chairmens of the 35th International Symposium on Essential Oils organized in Giardini Naxos (Italy) in 2004. The idea of the ISEO Medal came up again in 2017 when Humberto Bizzo proposed to implement the award/medal for lifetime achievements in essential oil research during the Permanent Scientific Committee meeting on the occasion of the 48th ISEO symposium in Pecs (Hungary).
At the ISEO in Niš (Serbia), we decided that the 50th anniversary of the ISEO symposia would be the best time to present the first medal. With the help of the members of the Permanent Scientific Committee and an artist from Messina, as well as the Merck company, the official sponsor of the medal, the ISEO Medal of Honour was created.

Previous ISEO Medal of Honour Recipients

2019 Jan Karlsen (50th ISEO, Vienna, Austria)
2021 Karl-Heinz Kubeczka (51st ISEO, Turkey, online)

ISEO 2022 Medal of Honour Recipient

We are delighted to announce that, by the decision of the ISEO Medal Committee, Prof. Carlo Bicchi from the University of Turin was elected to be the recipient of the ISEO 2022 Medal of Honour. The outline of Prof. Bicchi's achievements has been presented in the "Medal Lecture" section on page 42.



On behalf of the ISEO Permanent Scientific Committee

Agnieszka Ludwiczuk

Sponsor of the ISEO Medal of Honour

MERCK

YOUNG SCIENTISTS FELLOWSHIP

The ISEO 2022 Organizing Committee is very grateful to the Executive Committee of the International Federation of Essential Oils and Aroma Trades (IFEAT), which supported financially the participation of 20 selected young scientists for the participation in the 52nd International Symposium on Essential Oils. Due to a high number of high quality contributions, the ISEO 2022 Organizing Committee decided to support additional 3 young scientists from own resources. IFEAT Young Scientists Fellowship comprises the reimbursement of the registration fee.

Therefore, twenty-three contributions were accepted after a thorough evaluation and selection procedure, eight of which were chosen for an oral presentation, and fifteen for a poster presentation.

This year's awardees are:

Oral Presentations:

Natale Badalamenti	University of Palermo, Italy
Abhishek Kumar Dwivedy	Banaras Hindu University, India
Jena Sudipta	Siksha O Anusandhan, India
Ali Karimi	Julius Kühn Institute, Germany
Lorenzo Lamberti	University of Turin, Italy
Francesca Maggio	University of Teramo, Italy
Wan Mohd Nuzul Hakimi Wan Salleh	Sultan Idris Education University, Malaysia
Nikola M. Stojanović	University of Niš, Serbia

Poster presentations:

Filipe Arruda	University of the Azores, Portugal
Folajimi T. Avoseh	Vaal University of Technology, South Africa
Piyush Bhalla	Forest Research Institute, India
Veronika Chaloupková	CEDER-CIEMAT, Technical University of Madrid, Spain
Furkan Coban	Atatürk University, Turkey
Ayşe Esra Karadağ	Istanbul Medipol University, Turkey
Andreja Komnenić	University of Montenegro, Montenegro
Fotini Plati	Aristotle University of Thessaloniki, Greece
Giulia Mastellone	University of Turin, Italy
Ayoub Abdelkader Mekkaoui	Sultan Moulay Slimane University, Cadi Ayyad University, Morocco
Davy Moussango	University of Douala, Cameroon
Vidak Raičević	University of Novi Sad, Serbia
Chiara Rossi	University of Teramo, Italy
Juliana Huei Zago Wang	University of Southern Santa Catarina, Brazil
Natalia Wolan	Medical University of Lublin, Poland

ISEO 2022

52nd International Symposium on Essential Oils

Wrocław, 4-7 September

ON THE HONOR OF 70TH BIRTHDAY AND 45TH ANNIVERSARY OF SCIENTIFIC ACTIVITY

Prof. Stanisław Lochyński DSc, Ph.d., Eng.

Wroclaw University of Science and Technology
College of Physiotherapy in Wroclaw

Prof. Stanisław Lochyński (born 1952) is a recognized specialist in the field of bioorganic chemistry, in particular chemistry of natural products, in the synthesis of chemical compounds of application importance obtained from a renewable resource base, mainly from plants.

He graduated from the Faculty of Chemistry of the Wroclaw University of Science and Technology (WUST) in 1976, where he also gained further scientific degrees: doctor in 1980 and habilitated doctor in 2004. In 1988-90, he completed a two-year post-doctoral position at Texas Tech University in Lubbock (USA) and in 1991 a nine-month post-doctoral position at the Technische Universitat Berlin (Germany) under the supervision of a renowned specialist in the field of terpenoid chemistry, prof. Peter Weyerstahl. In 2007, he completed a short-term research stay at Anadolu University in Eskisehir (Turkey). In 2012, he obtained the title of full professor of chemical sciences.

The scientific interests of Prof. Lochynski focuses on the use of natural, readily available monoterpene raw materials that constitute a chiral substrate base, hydrocarbons and oxygen derivatives, which can be used in stereocontrolled syntheses and biosynthesis of chiral terpenoid derivatives with potential biological activity, in particular pharmacological, insecticide, and fragrance. In the 1990s of the last century, he became deeply involved in the mainstream of international cooperation with world centers dealing with the subject of the chemistry of essential oils. He established fruitful cooperation with such centers and specialists as: Prof. Yoshinori Asakawa from Tokusima University Japan, Prof. Fatih Demirci, Anadolu University, Turkey, Prof. Hüsnü Can Başer, Near East University, Cyprus, Prof. Nicolas Baldovini, University of Nice, France, Prof. Györgyi Horváth, University of Pecs, Hungary, Prof. Niko Radulović, University of Niš, Serbia, Prof. Luigi Mondello, University of Messina, Italy. In 2011, he was the first Polish member of the Permanent Scientific Committee of International Symposium on Essential Oils. He has been participating in ISEO symposia since 1993, where he co-authored two plenary lectures (Budapest-2013 and Niš-2018). He twice chaired the organization of the International Symposium of Essential Oils (ISEO), which took place in Wroclaw (2001, 2010). The next event, distinguishing Poland as the organizer, is currently taking place in Wroclaw on 4-7.09. 2022, where he chairs the Scientific and Organizational Committees.

Author and coauthor of 231 scientific works, including 213 publications and 18 unpublished works. Particularly noteworthy is the enormous number of patents: 101 granted patents and five patent applications. Member of the scientific committee of 17 international and 27 national conferences.

So far, he has promoted 10 doctors (Bożena Frąckowiak-2005, Kamila Gajcy-2010, Ewelina Wincza-2011, Renata Kuriata-Adamusiak-2013, Jolanta Pękala-Wagner-2014, Daniel Strub-2014, Daria Kaczmarczyk-2016, Lucyna Balcerzak-2016, Agata Kozioł-2018, Agnieszka Stryjewska-2021) and is currently the promoter of 1 open doctoral dissertation (Alicja Surowiak).

From 2005, he was a voluntary vice-chairman, and from 2012 he is the chairman of the WUST Alumni Association. In the activities of the association, he popularizes the knowledge and successes of outstanding graduates from the socio-economic environment among academic youth. Since 2010, he has been the Director of the Institute of Cosmetology at the Colleage of Physiotherapy in Wroclaw. From 01/01/2020 he is the vice-head of the Department of Biological Chemistry and Bioimaging (K-15) of the Wroclaw University of Science and Technology.

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Wrocław, 4-7 September

OPENING LECTURE

ISEO 2022 52nd International Symposium on Essential Oils Wrocław, 4-7 September

Natural perfumery: an olfactive journey in time and space- public own smelling experience

Clio Vidal[1,*]

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Keywords: natural perfumery, multicomponent mixtures, fragrance creation, effect of scent

Overview

This lecture aims to introduce the community to the topic of natural fragrances, the properties of its raw materials as well the creation process behind it. Natural perfumery (which can be seen as aromatherapy with aesthetical exigences) and phytotherapy have a common craddle in Nature's vegetal world, hence the healing properties of natural fragrant raw materials (typical multicomponent mixtures) and its products. Various extraction and distillation techniques offer a great number of natural ingredients to the perfumer as the necessary resources to perform the art of creation. On the other hand, the actual emphasis of scientific research to study isolated molecules to predict behaviours of muti compound mixtures in human metabolism (identification of toxic compounds within natural raw materials, e.g. methyl eugenol.) and its resulting legislation (that can lead up to a ban on some ingredients) heavily influence the art of perfumery creation. This can even force perfumers to modify their existing fragrance compositions. Both often result in a loss of olfactive and healing diversity.

The experience of the effect of smell is an extremely important aspect and a starting point for the creation of natural fragrances since olfactory perception is much more than the simple detection of individual molecules by the human nose. Hereby, it is directly related to essential oils as a multi compound mixtures.

Conclusions

Natural Perfumery today is an exciting and challenging creative journey, that aims to express connection through emotions and healing in an actual segmentated vision of the Living. The reduction of the olfactory palette due to undirectional focused research is one of its biggest challenge. The audience will have the opportunity to get a deeper insight into the art natural perfumery, the effect of scent and how it affects many aspects of human life.

ACKNOWLEDGMENTS

I would like to thank Marianne Martin, the actual president of BSP (British Society of Perfumers), for remembering my passion and offering me the opportunity to share it in this very special context, my current employer WALA Heilmittel GmbH, for allowing me to spend some time on this project and supporting a global vision of human health, my colleague Hannes Bitterling for his inspiring PhD research on furocoumarins and very supportive enthusiasm, and Daniel Strub and the whole ISEO 2022 Organizing Committee for this great idea and organization.

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PLENARY LECTURES

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Chemistry of fragrant woods: Agarwood and Sandalwood

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Keywords: Agarwood, Sandalwood, Key odorants, GC-O

Overview

Several species of fragrant woods are used to diffuse their pleasant smell on burning, as incenses directly available from natural sources. In Asia, two precious woods are employed for such applications : sandalwood (in joss sticks or "agarbattis") and agarwood (oud), and these materials are also used for essential oil production.

The main sandalwood species (*Santalum album*, *S. spicatum* and *S. austrocaledonicum*) are hemiparasitic trees exploited commercially in India, Australia and several islands in the Pacific. The essential oil of the heartwood of these trees is mostly composed of sesquiterpene alcohols in which (*Z*)-α-santalol and (*Z*)-β-santalol (often the major compounds) bring a significant contribution to the specific warm-woody odor of the oil [1]. This communication will give an overview of the composition of several *Santalum* species obtained from samples of secured origin. It will also focus on the characterization of an atypical constituent present in large amounts in sandalwood samples from East Timor.

Agarwood is the resinous infected heartwood of Aquilaria trees (*Aquilaria* sp.), which grow in tropical forests of Southeast Asia. The very specific odor of agarwood has been described by Roman Kaiser, a renowned fragrance specialist from Givaudan Company, as « one of the most fascinating scent source of this world (…) a pervasive, mysterious, wonderful scent, bringing to mind the perfumes of all imaginable precious woods, balsams, and resins, as well as those of amber, musk, and castoreum and, somewhat hidden, even tender floral notes » [2]. Nowadays, it is recognized unanimously as the most expensive wood, as well as the most prestigious natural raw material used in perfumery. In this presentation, the chemical compositions of agarwood essential oils will be discussed, as well as those of the smoke produced from heated wood samples submitted to Purge & Trap sampling followed by GC-MS, GC-FID and GC-Olfactometry analyses.

Conclusions

After fractionation of a sample of essential oil of sandalwood from East Timor involving Girard T reagent for the specific extraction of carbonyl compounds, we could identify several sesquiterpenic aldehydes, including spirosantalal. This compound was never reported so far as a constituent of sandalwood, but the corresponding alcohol have been described previously by Brunke [3]. Additionally, the analysis of the composition of agarwood smoke showed that for our samples, the odor emitted on burning mostly comes from the pyrolysis of non-volatile constituents remaining in the wood after hydrodistillation.

ACKNOWLEDGMENTS

A part of the work on sandalwood was made in collaboration with Cecilia Cagliero, Carlo Bicchi (University of Torino, Italy), and Norbert Braun (Symrise Ltd, Singapore), who are warmly acknowledged for their key contribution.

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The concept of 'buchu' and the ancient origins of aromatherapy

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Keywords: aromatic plants, traditional uses, Khoi**,** San, cosmetics, deodorants, ritual use, spiritual use

Overview

According to genetic evidence, the !Xam (San hunter-gatherers; Sonqua, "bushmen") of the Karoo in South Africa are the most ancient lineage of modern humans, with medicinal and cosmetic practices that potentially date back to the emergence of modern humans, some 160 000 years ago. The habitual massage of the skin with buchu powder and fat resulted in the people being called Sonqua (*son* = buchu; *qua* = people), directly translating to "buchu people" or "aromatic bush people" and less accurately to *bossiesmans*, *boesmans* or *bushmen*. Kolbe (1719), an under-estimated source of information on San and Khoi ethnography [1], provided a rather comprehensive account of the traditional uses of the original buchu of the Western Cape, which was *Diosma hirsuta* L. In 1719, Thunberg [2] reported the use of this species near Mamre on the Cape West coast, where the plant is still used medicinally to this day. A study of the plant use practices of San and Khoi descendants in the Cape region provided profound new insights into the origins of medicine and the cosmetic, medicinal, ritual and spiritual uses of *Diosma* and ca. 50 other species of aromatic plants, known collectively as "buchu" or "son". Apart from the two well-known commercialised species [*Agathosma betulina* (P.J.Bergius) Pillans and *A. crenulata* (L.) Pillans] [3], few of the other buchu plants have yet been subjected to rigorous essential oil analyses.

Conclusions

Despite the profound historical significance of *Diosma* species and other buchu plants from the families Rutaceae, Asteraceae and Lamiaceae, the essential oil chemical compositions of these species have hitherto been incompletely studied and represent an obvious knowledge gap. The way in which aromatic plants have been used by San and Khoi people provides important new clues about how the use of aromatic plants should be interpreted.

ACKNOWLEDGMENTS

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PL-3

Why do liverworts produce so many structurally different volatiles?

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Keywords: spore-forming plants, terpenoids, chemical diversity, biological function

Overview

Liverworts are a small group of plants with 5 to 8 thousand species in the world. In general, liverworts exhibit low morphological complexity, but a high degree of chemical diversification. This group of spore-forming plants produces a wide array of specialized metabolites, and among them, terpenoids are most abundant and structurally diverse. These compounds are accumulated in the oil bodies, which are a prominent and highly distinctive organelle found in liverworts. In the last 40 years, more than 3000 compounds have been reported from this group of plants, and among them, about 1600 compounds are terpenoids [1-3]. The aim of this lecture will be to answer into two questions:

- What kind of volatiles are present in liverworts? and
- Why do such small and simple plants produce such a large variety of compounds?

Liverwort-derived volatile organic compounds in the terpenoid family are mono-, sesqui- and some diterpenoids, while in the aromatics are bibenzyls. Among liverwort terpenoids, the most diverse and largest group are sesquiterpenoids. These belong to more than 60 different skeletal groups, among which the eudesmane and aromadendrane skeletons are the most prevalent. Other, like cuparane, pinguisane and barbatane are also quite common. Many sesquiterpenoids are species specific, and some such as dumortanes and pinguisanes are found only in this group of plants [1-4].

Liverworts are considered to be the oldest terrestrial plants. As such, they are the first land plants to synthesize volatiles and other metabolites. As the first inhabitants of terrestrial habitats they were frequently exposed to adverse environmental conditions. The mentioned high degree of chemical diversification in liverworts suggests that secondary metabolites, and especially terpenoids, may play an important role in bryophyte-environment interactions [2, 3]. The volatiles present in liverworts were shown to be phytotoxic, inhibiting germination and growth of vascular plants in standard laboratory tests. This toxicity inspired the search for other valuable compounds with antibacterial, antifungal, anti-inflammatory, cytotoxic, insect repellent activities, among others [1-4].

Conclusions

The enormous chemical diversity of liverworts is associated with evolution and environmental changes. Plants start to synthesize new compounds as a result of changes in their biotic partners and enemies, when the 'old' plant compounds were no longer effective. There is also the hypothesis that the diversity of compounds in one species provides benefits that a single compound cannot. Unique metabolite blends observed in liverwort species are interesting from a chemistry point of view but also help in the identification process and taxonomic work. The biologically active compounds present in liverworts are also important for human health.

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Analytical strategies for the characterization of non-volatile fraction of *Citrus* essential oil: applications in food and cosmetic.

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Keywords: *Citrus* essential oil, furocoumarins, LC-MS/MS, food, cosmetic

Overview

Citrus essential oils are widely employed in perfumery, as well as food and cosmetic fields. The non-volatile fraction of cold-pressed *Citrus* EOs (2-15% of their composition) includes coumarins (C), furocoumarins (FC), and polymethoxyflavones (PMF), known as oxygen heterocyclic compounds (OHCs).

Several beneficial effects have been demonstrated to be related to the presence of OHCs; however, negative effects have been reported for coumarin, that can cause liver damage, and FCs, that can cause phototoxic reactions to skin.

Coumarin content is regulated in food and cosmetic[1,2] whilst FCs content is regulated only in cosmetic [3,4]; even thoug recent studies suggest adverse effects after their dietary intake, an official limit about their maximum content in food is still missing.

For these reasons, identification and quantification of OHCs fraction in *Citrus* oils, have been attained by several research group. The latter, to develop reliable, sensitive, fast, and accurate analytical methods appliable in complex matrices and at trace level, to cope with the current regulation limits. Several analytical approaches were proposed, inluding liquid chromatography coupled to photodiode array detection and mass spectrometry.

This presentation will review the different approaches developed in our laboratories and will focus on a recently developed liquid chromatography tandem mass spectrometry method (HPLC-MS/MS) used in combination with the linear retention index (LRI) system, for the detemination of 35 OHCs compounds. Method validation was attained by creating calibration curves in mutiple reaction monitoring (MRM) mode both in pure solvent and in distilled essential oil as blank matrices, to overcome matrix interferences. LOQ values were in the ppb levels, making it suitable to detect analytes at trace level.

The method was furthermore tested by participating at a proficiency testing showing excellent results in terms of z-score. Applicability to cosmetic, such as body cream and hand sanitizing gel and in food such as alcoholic and non-alcoholic beverages will be shown.

Conclusions

The aim of this research was to demonstrate the validity and applicability of the method employing Linear Retention Index (LRI) in liquid chromatography to make the analysis of C, FC, PMF automatic and reproducible even at inter-laboratory level.

It represents a very sensitive analytical strategy for the characterization of OHCs in food and cosmetic products, where it could be useful for quality control operation.

ACKNOWLEDGMENTS

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PL-5

Biological activity of essential oils – not a single golden bullet loaded to that revolver?

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Keywords: essential oil, biological activity, mechanism of action, synergy, antagonistic interaction

Overview

Initially, much attention has been devoted to essential-oil research primarily due to their olfactory properties and ease of access. It is still today that the structural and compositional diversity and complexity of essential-oil constituents drive this field forward again owing to their biological properties. It has been long believed that single compounds could be responsible for the observed vast biological activities, and major principles have been recognized in certain cases. Yet, a substantial amount of evidence has accumulated that points to the fact that the major modus operandi of essential oils is coming from the cooperation (synergy) of several if not many constituents simultaneously. Such synergy is now readily quantified in simpler models and even cases of antagonistic interactions are known. Essential oils have been demonstrated to alter the effect of already biologically active compounds again through unspecific pathways usually termed synergistic or antagonistic interactions, although on their own the essential oil showed no similar activity. Although it is quite difficult to pinpoint specific biological interactions in such complex matrices of frequently hundreds of compounds, these deserve intense study as such a mechanism of action is promising in combating drug resistance.

Conclusion

Both the experimental setups and (statistical) treatment of the obtained data, which provide insight into the possible interactions between essential-oil constituents, need development and standardization. Examples that illustrate such an approach to deciphering different biological activities of essential oils will be put forward in this lecture, aiming to provoke future work and caution when reaching conclusions.

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KEYNOTE LECTURE

KL

F&F Ingredients: World Overview on Feedstocks & Renewability

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Keywords: F&F ingredients, Sustainability, Safety, Regulations, Essential Oils, Biotechnology, Petrochemicals, Biomass

Overview

Our Flavour and Fragrance market is a very small industry that transforms over 4000 ingredients into the finest flavours and fragrances, and provides work for more than 10 million people worldwide.

For the past 50 years, and until recently, the ingredients market had a relatively quiet life. There have been a few crises, but in general, natural ingredients for F&F have been fairly available and large industries have continued to supply well most raw materials to produce natural and synthetic F&F ingredients.

However, new factors will affect this availability:

- Global demand for renewable products across all industries, leading to increased competition for renewable feedstocks; waste materials have increasing value and large industries are finding new life for them.
- The acquisition of F&F players by larger groups, mainly from other industries, changing the access to the usual raw materials and adding new ways of using them
- An excessive regulatory hype that seems to have lost its purpose

In this session, Alain Frix shares his view on the overall low renewability of F&F ingredients, quantifying each of the major raw materials such as petrochemicals, forest products, essential oils, biotechnology and others.

He outlines difficulties and the efforts that must be made to preserve F&F access to raw materials, which also includes the cultural need to better understand large and rapidly evolving industries.

Alain Frix highlights the pressing challenge facing the essential oil industry over the next decade, elaborate why biotechnology is not yet ready to meet global demand, and why the essential oil industry will urgently need to accelerate progress in adapting crops to climate change, improving extraction technology and yields, adapting crop protection protocols and composition, and, most importantly, increasing farmer education.

Finally, he explains why there is no perfect sustainability solution, with petrochemical and natural ingredients each having a different, but never complete, sustainability profile.

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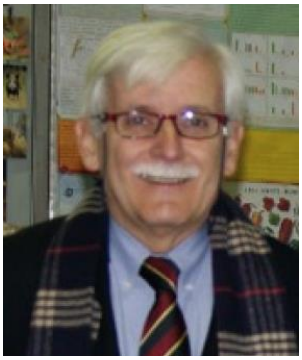
MEDAL LECTURE

Prof. Dr. CARLO BICCI
University of Turin



Carlo Bicchi has been Full Professor of Pharmaceutical Biology at the Faculty of Pharmacy of University of Turin since 1990.
In the same University, he was Director of the Department of Scienza e Tecnologia del Farmaco from 1992 to 1998 and Dean of the Faculty of Pharmacy from 2001 to 2007. His main field of research is development of analytical technologies for biologically active specialized metabolites in vegetable matrices (essential oils, terpenoids, phenolic compounds and alkaloids) and aroma profiling and fingerprinting of important industrial food crops (coffee, cocoa, hazelnuts, olive oil and tea). His main topics include Sample preparation; Gas chromatography (GC, GC-MS, Fast-GC, Multidimensional GC and new stationary phases), Enantioselective GC, High pressure liquid chromatography (HPLC, HPLC-MS), Supercritical fluid extraction and chromatography (SFE and SFC), Chemometric methods, fingerprinting and profiling.
In particular, his recent research work in the natural product field focused on the chemical composition of the volatile fraction taken as a diagnostic marker of specific characteristics/properties of several important aromatic plants (recently including sandalwood and frankincense) and food crops (coffee, cocoa, hazelnuts, olive oil and tea).

Carlo Bicchi's scientific activity is documented by 325 publications in international Journals, and more than 350 plenary lectures, keynote lectures and communications and seminars.He has been Associate Editor of Flavour and Fragrance Journal, member of the advisory board of international journals and member of permanent scientific board of several International Scientific foundations.

Carlo Bicchi has also been invited several times to be member of international juries for professorship (12) and doctorate thesis (53) and Visiting Professor (7) all over the world.

He has also been referee for several International Organization in evaluating international research projects

Since 2016, he is effective member of the Working Group for the Compendium of Botanicals for the European Food Safety Authority (EFSA)

In Mai 2018, **Carlo Bicchi** was awarded from the Interdisciplinary Group of Separation Science of the Italian Chemical Society with the Giovanni Dugo's medal for his contribution to the separation science in food analysis. In September 2019, he was awarded with the Arnaldo Liberti's Medal from the Division of Analytical Chemistry of the Italian Chemical Society for his scientific contribution to the development of separation sciences and of microtechniques in sample preparation. In October 2021, he was awarded with the COLACRO Medal from the Latino-American Association of Chromatography and Related Techniques for his scientific contribution to the development of gas chromatography.

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Essential oils: a great future behind them

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Keywords: Essential oils, State of the art, Future perspectives, Applications, Conclusions

Overview

Essential oils are products obtained from a plant or a specific part of a plant with a rather homogeneous composition in terms of molecular mass and polarity of their components, i.e. the latter is a distinctive trait often underestimated and is mainly due to the isolation technique based on steam distillation or hydrodistillation (with the exception of citrus EO). This characteristic places essential oils and their chemistry at a nodal crossroads between agronomy, biology, botany and biochemistry and their applications in innumerable fields, particularly food, cosmetics and pharmaceuticals. Modern research in the field of essential oils is a clear example of the ever-increasing integration of chemistry and biology and thus involves the synergistic contribution of the expertise of industry, regulatory organisations and universities with their technologists, lawyers, biologists and toxicologists, pharmacologists and medical doctors, agronomists and botanists, analytical and synthetic chemists. The misuse of the magic word 'essential oils', sometimes (indeed often) without knowledge, therefore hides a multidisciplinary entity that 'marries' research subjects and skills of a very different nature. The above concepts will be illustrated with some examples from the author's day-to-day experience.

Conclusions

What role for ISEO in the future? At a time when essential oils are in high fashion, ISEO plays a key role in maintaining/enhancing the culture that underlies this community and, in particular, in passing on innovative research strategies to new generations, acting as a 'sentinel' against the current trend of declining knowledge.

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ORAL PRESENTATIONS

A review of the isolation and biological activity of volatile marine polyhalogenated monoterpenes from Southern African *Plocamium* and *Portieria* marine algal species

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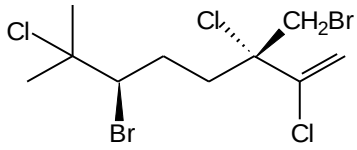
Keywords: *Plocamium*, *Portieria*, marine algae, bioactivity

Overview

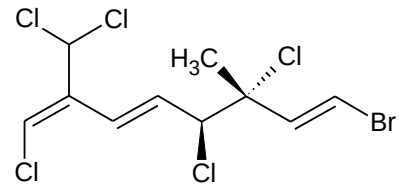
Numerous voltaile polyhalogenated monoterpenes have been isolated from various marine algae such as *Plocamium* and *Portieria* species. The isolatıon, structural characterısatıon and *in vitro* biological actıvıty of these natural products from Southern Afrıca are reviewed with the purpose of finding future pharmaceutical or agricultural applications.

Conclusions

These novel compounds are biologically active, often selective and represent a potentially untapped resource of natural products which are 'just waiting' for commercial application.



Halomon [**1**]



[**2**]

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An essential oil component as a building block for pheromone synthesis

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Keywords: Pheromones, Plant protection, Spanish lavender *Lavandula luisieri* essential oil, mealybug pest

Objective

The spherical mealybug *Nipaecoccus viridis* (Newstead) is an insect pest of several major crops including soybeans, grapes and citrus varieties. Virgin females of *N. viridis* release two volatiles, 2,2,3,4–tetramethyl–3–cyclopentene–1–methanol (γ–necrodol) and γ–necrodyl isobutyrate - a monocyclic terpenoid alcohol and a related carboxylic ester [1].

In the course of the pheromone identification, trans–3,4,5,5–Tetramethyl–2–cyclopentene–1–methanol (trans–α–necrodol) and trans–α–necrodyl acetate, found in the commercially available essential oil of Spanish lavender, *Lavandula luisieri*, were rearranged to γ–necrodol that was then used to synthesize γ–necrodyl isobutyrate. GC–MS and NMR data confirmed the identifications. However, the chirality of trans-α-necrodol obtained from essential oil of Spanish lavender, *Lavandula luisieri*, which is crucial for pheromone application in pest control, was unknown. Our objective was to determine the chirality of *trans*-α-necrodol in the essential oil of Spanish lavender that can serve as a precursor in the synthesis of the spherical mealybug pheromone to control the pest.

Methods

To determine the chirality of the pheromone, we synthesized γ-necrodyl acetate enriched in (+)-(*S*)-enantiomer and separated the enantiomers using a lipase enzyme. Then we compared the retention times and mass-spectra of the synthetic and natural components with chiral GCMS analysis.

Results

We confirmed that the natural components, both in the mealybug and in the lavender essential oil, consist of the (−)-(*R*)-enantiomer [2]. Bioassays conducted in the lab and field show that males are attracted to (−)-(*R*)-γ-necrodyl isobutyrate pheromone alone.

Conclusion

Pheromones of mealybug species usually require multi-step syntheses that are complicated, which has limited their being used widely in pest management. However, since mealybug pheromones are usually built of terpenic structures, we believe that syntheses of these pheromones can be facilitated by using appropriate essential oil components as preformed synthetic precursors.

ACKNOWLEDGMENTS

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Inducing volatile production by plant hormones in peppermint and marjoram

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Keywords: essential oil, elicitation, methyl-jasmonate, salycilic acid

Objective

In the last decades sevaral strategies were studied to enhance the production of secondary metabolites (SM), among other elicitation. Jasmonates, among others methyl-jasmonate (MeJa) are well known plant hormones influencing numerous physiological processes and stress reactions [1, 2]. Their effects on SM production are reported frequently, but reliable data on *in vivo* application are still missing [3]. The goal of our studies was to get information about potential effects of these hormones on volatile accumulation of some Lamiaceae species.

Methods

Peppermint (*Mentha x piperita* L. variety 'Mexian') and marjoram (*Majorana hortensis* Mönch. var. 'Magyar') were grown in open field plots in Budapest 2019 ad 2020. Besides, phytotron experiments were installed in 2020 and 2021 where the plants were grown in pots in controlled environment simulating average summer conditions. Two weeks before harvest aboveground parts of the plants were sprayed with 2.0 mM MeJa dissolved in water and this was repeated after one week. Samples were cut at full flowering stage, air dried and distilled (WD) according to the 7th Hungarian Pharmacopoeia. Essential oil (EO) content was calculated to dry mass. he main compounds of the oil were determined by GC-MS method as described formerly [3].

Results

Our results demonstrated that in most cases the treatments resulted in an enhanced production of essential oil in both species: in peppermint up to 34% and in marjoram up to 30%. In open field '19 in peppermint however, no change could be demonstrated and in the pot trial '21 of marjoram the EO content decreased. Changes in the ratios of EO compound were registered only in some cases, among others increases in the proportion of terpinene-4-ol in marjoram and in l-menthol in peppermint. We did not observed any significant loss in biomass in either of the experiments.

Conclusions

The results ascertained that MeJa may be an effective tool in stimulation of volatile accumulation without deteriorating the quality of the oil and reducing biomass yield. However, the growing conditions have a very large influence on the efficacy of the treatments, thus, development of a standard technology for each species should be a prerequisite for a reliable use of this elicitor.

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The essential oil composition in medicinally relevant *Salvia* species: A global review

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Keywords: Lamiaceae, *Salvia*, regular expression, GC-MS, essential oil

Overview

The genus *Salvia* L. comprises ca. 1,010 species [1] and is one of the largest genera within the Lamiaceae. To date, there have been no comprehensive reviews on the entire genus due to the vast amount of data available and the time required to review such data. As an example, a simple search on Google Scholar using the term "*Salvia*" returns ca. 508,000 results. To further narrow the search, changing the parameters to only include the search term in paper titles, returns ca. 27,000 hits. As a case study, this paper will focus on a method created using a carefully developed regular expression analysis utilizing publication data gathered from SciFinder® with search terms such as "*Salvia*" AND "medicinal" AND "essential oil" OR "volatile compound" in either the paper title, abstract or keywords. Publication data was analysed in R-Studio using the *pdftools*, *stringi*, *stringr* and *tm* packages. Our data shows that from the list of 839 papers returned, a total of ca. 181 *Salvia* species have been investigated for their essential oil chemistry. There are 405 publications for *Salvia officinalis* L., 73 for *S. miltiorrhiza* Bunge, 58 for *S. hispanica* L., 65 for *S. sclarea* L. and 50 for *S. fruticosa* Mill. The ten most reported main chemical constituents identified in the genus *Salvia* are camphor, 1,8-cineole, caryophyllene, borneol, camphene, linalool, caryophyllene oxide, germacrene D and viridiflorol.

Conclusions

A comprehensive review of the genus requires a targeted and refined method 1) with carefully selected search phrases when searching for literature and 2) to scrape data from the immense number of sources available in order to produce accurate data to report. Here we highlight a method used to generate data as a first attempt at a comprehensive and global review of this medicinally and chemically important genus.

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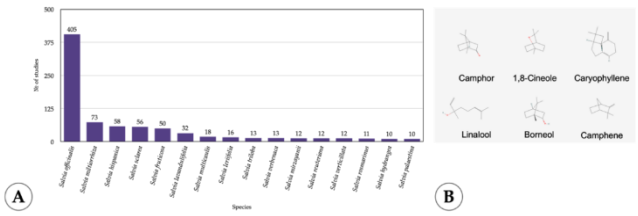

Figure 1: A – Numbers of essential oil studies for medicinally relevant *Salvia* species (only those with > 10 publications are shown). B – Most frequently reported major compounds for medicinally relevant *Salvia* species. Image source: : https://pubchem.ncbi.nlm.nih.gov/

Evaluation on Chemical Composition and Bioactivity Potential of the *Thymus transcaucasicus* Ronniger Extracts and Essential Oil

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Keywords: *Thymus transcaucasicus*, antimicrobial, anti-biofilm, antioxidant, chemical composition

Objective

Nosocomial pathogens can produce a biofilm surrounds them, and survive for a long period in this habitat, protected from immune mechanisms and antimicrobials [1]. Reactive oxygen species are produced as a defense mechanism, but prolonged exposure to their high levels may lead to disease. At that point, antioxidants and antimicrobials are beneficial [2]. In this study, it is aimed to determine the composition and bioactivities of extracts and essential oil (EO) of *Thymus transcaucasicus*, and evaluate its therapeutical potential against infectious diseases.

Methods

The aerial parts of *T. transcaucasicus* from Turkey were successively macerated with n-hexane, $CHCl_3$ (CE), EtOAc, EtOH and n-BuOH, and individually with ethanol, by stirring for 24h. Its infusion and decoction were prepared. The EO was obtained by hydrodistillation for 3 h. All samples (9.76-5000 µg/mL) were tested against *Staphylococcus aureus* Rosenbach (ATCC 29213), *S. epidermidis* (Winslow and Winslow) Evans (ATCC 12228) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC 29212), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC 25922), *Klebsiella pneumoniae* subsp. *pneumoniae* (Schroeter) Trevisan (ATCC 4352), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC 27853), and yeast *Candida albicans* (Robin) Berkhout (ATCC 10231). In anti-biofilm assays, they were applied at 10-fold and 20-fold concentrations of the MIC values. In antioxidant assays, DPPH and CUPRAC methods were performed. Their chemical compositions were analyzed by GC-FID and GC-MS.

Results

While the EO was the most active sample with its antimicrobial and antibiofilm effect, the CE exhibited the highest antimicrobial activity with 78-1250 µg/mL MIC values and mostly ≥3 log10 decrease in biofilm mass, among all extracts. Especially *S. aureus* was found more sensitive microorganism for the extracts. In antioxidant tests, the EO elicited also higher activity (<0.008 mg/mL; 3.571±1.21 mM trolox/mg extract). As major compound in the EO was found to be thymol (58.7%), and in the CE p-cymene (30.5%).

Conclusions

In light of all data, it can be estimated that, *T. transcaucasicus* has a good potential to treat wounds and infectious diseases such as nosocomial infections.

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Antimicrobial and antibiofilm activities of thyme essential oil with emphasis on the development of oral hygiene compositions

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Keywords: antimicrobial effect; antibiofilm formation; essential oil; garden thyme

Objective

The rapid development of antibiotic resistance of microorganisms is a difficult problem for contemporary biology and medical science. Besides, there is a possibility for bacterial existence in the form of biofilm – an elaborate association available in the environment and in the human organism. Medicinal plants containing a broad spectrum of biologically active substances with total additive antimicrobial activity are a mighty source of antimicrobial substances. Thyme, *Thymus vulgaris* L., belongs to a genus encompassing over 215 species of hardy perennial herbaceous plants and sub-shrubs, which is native to Europe, particularly around the Mediterranean. Thyme is widely used in pharmaceutical industry. The objective of this study was to identify the biochemical and antimicrobial characteristics of thyme essential oil against clinical isolates of opportunistic microorganisms.

Methods

Thyme (*Thymus vulgaris* L.) herbs were picked from the Uzhhorod Botanical Garden, where we had several plant populations in 2020/2021. The plant materials were cleaned, dried, packed, labelled and stored in a clean and dry place for extraction of essential oils. Thyme essential oil was prepared by hydro-distillation (2 hours) in Clevenger-type apparatus according to the European Pharmacopoeia. The qualitative and quantitative characteristics were determines by GC/MS analysis – the equipment: Varian 450-GC connected with a Varian 220-MS, NIST 02 MS Library). Test cultures. The following were used for the purpose of the study: reference museum cultures ATCC (American Type Culture Collection, USA) *C. albicans* ATCC 885-653; *S. aureus* ATCC 25923; *E. coli* ATCC 25922, *E.faecalis* ATCC 29212, *S. pyogenes* ATCC 19615, and clinical cultures isolated from the oral cavity of patients suffering from inflammatory periodontal diseases. The antibiofilm activity was tested in 96-well micro titration plates spectrophotometrically (Greiner-BioOne, Austria) according to O'Toole, 2011.

Results

The GC/MS results confirm the earlier reports that major volatile constituents obtained from the aerial parts of thyme species were thymol, γ-terpinene, p-cymene, 3-carene and carvacrol. The selected cultivars, environmental conditions, agronomic management practice and effective steam distillation were obtained of substantial contents of phenolic monoterpenes – thymol (67.68 %) and γ-terpene (8.21 %). Garden thyme essential oil has been established to show a high antimicrobial activity against antibiotic-resistant microorganism strains. The obtained results proved the wide spectrum of antimicrobial activity of thyme essential oil. The highest antimicrobial activity was registered against the typical and clinic strains of *S. aureus* up to (50.0 ± 0.25 mm) and microscopic *Candida* genus fungi. Thyme essential oil was ascertained to show high antibiofilm-forming activity against *S. aureus*. Thus, the oil concentration of 0.1% reduced the intensity of biofilm formation by 81 %; when applying 0.05 % concentration, a tendency to reduce the biofilm to 75.8 % was observed.

Conclusions

The antimicrobial and antibiofilm activities of thyme essential oil and its ability to reveal the antimicrobial activity on both bacterial pathogens of opportunistic infections and microscopic fungi have proven good prospects for development of a broad-spectrum agent against opportunistic microbial associations based on this oil [2].

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Eugenol as a promising natural antifungal component

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Keywords: antifungal activity, mechanism of action, chlorhexidine, cetylpyridinium chloride, interaction, synergism

Objective

In recent years, a worldwide increase in the incidence of superficial fungal infections, especially caused by yeasts belonging to *Candida* spp., has been observed. Moreover, due to the limited range of antifungal agents available for treating of these infections and the emergence of resistant isolates, attention has focused on the antifungal power of natural compounds with promising biological properties, e.g. essential oils and their components. In the present work we verified the antifungal activity of eugenol, its mechanism of antifungal action and the effect in combination with the selected antimycotics.

Methods

The antifungal activity of eugenol (Sigma-Aldrich Chemicals, USA) and the selected antimycotics (chlorhexidine, cetylpyridinium chloride, chlorquinaldolum, silver nitrate, triclosan and nystatin) against the strains of *Candida* spp.: *C. albicans, C. glabrata, C. parapsilosis* and *C. krusei* (from American Type Culture Collection – ATCC) was studied. The broth microdilution method was used according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [1] and Clinical and Laboratory Standards Institute (CLSI) guidelines [2]. The MIC (Minimal Inhibitory Concentration) of the eugenol and antimycotics was examined using their two-fold dilutions in a liquid medium in 96-well polystyrene plates. The MFC (Minimal Fungicidal Concentration) was determined by transferring the culture samples from the wells onto Sabouraud agar after MIC reading. To verify the interactions of eugenol with antimycotics, the fractional inhibitory concentration (FIC and ΣFIC values) was assessed by checkerboard technique. Moreover, the effect of eugenol on the cell wall and cytoplasmic membrane of yeasts was tested using the sorbitol and ergosterol assay, respectively [3].

Results

Based on our results, eugenol showed antifungal activity against the reference *Candida* spp. strains with MIC = 0.25 – 2 mg/mL and MFC = 1 – 4 mg/mL. The values of MFC were 2-4-fold higher than MIC indicating its fungicidal effect (MFC/MIC = 2 – 4). Additionally, a synergistic activity of eugenol in combination with chlorhexidine and cetylpyridinium chloride was found (ΣFIC = 0.375). Moreover, eugenol with silver nitrate and triclosan showed additive effect (ΣFIC = 0.625). In the case of combining of eugenol with nystatin and chlorquinaldolum, indifference (ΣFIC = 1.12 and ΣFIC = 1.5) was evaluated. Antagonistic activity was not observed. It was found also that eugenol bind to ergosterol in the yeast cell membrane. As a result, it disturbs its structure and has a beneficial antifungal effect.

Conclusions

These results showed fungicidal activity of eugenol towards the reference *Candida* spp. strains. Additionally, a stronger antifungal action of eugenol can be obtained by combining it with chlorhexidine and cetylpyridinium chloride due to the favorable synergistic interaction. On the basis of the obtained data, it is concluded that eugenol can be used as component of antifungal preparations used in the prevention and treatment of topical candidiasis.

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Essential oils of Cyprus

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Keywords: Cyprus, essential oils, aromatic plants, distillation, GC/MS

Overview

Cyprus is the 3rd largest island in the Mediterranean sea with a land cover of 9.251 sq.km. Being in eastern Mediterranean, it is under the influence of the floras of Asia, Africa and Europe. Flora of Cyprus is well documented with 1.610 species and altogether 1.738 taxa. 108 species (143 taxa) are endemic plants comprising 6.7% (8.2%) of the flora. The families Asteraceae (66), Lamiaceae (39) and Apiaceae (29) are important for the aromatic flora.

Conclusions

In this review, I shall present our results on the essential oils of the following species: *Anthemis tricolor, Asphodelus aestivus, Chenopodium murale, Eucalyptus spp., Helichrysum conglobatum, Helichrysum italicum, Lagoecia cuminoides, Lathyrus spp., Origanum cordifolium, Origanum dubium, Origanum majorana, Phlomis brevibracteata, Phlomis cypria var. cypria, Pimpinella cypria, Sideritis cypria, Teucrium cyprium, Teucrium kyrenia, Teucrium micropodioides, Teucrium salaminium, Thymus capitatus, Zosima absinthifolia.*.

OP-9

Investigations on the sex-specific effect of swiss stone pine essential oil in human well-being. A pilot study

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Keywords: *Pinus cembra*, aromatherapy, hedonic valence, depression

Objective

Pinus cembra L. (Pinaceae) can be found in the Alps and Carpathian Mountains in altitudes up to 2500 and 1700 m a.s.l., respectively [1]. Besides its insecticidal and antimicrobial activity, swiss stone pine essential oil (SSPEO) is also known for its sleep-enhancing and calming impact [2]. Due to the current COVID-19 pandemic, depression and related ailments have increased within the world population [3]. Odors in general as well as aromatherapy in particular can have a positive influence on mood [4]. The aim of this work was to evaluate the effect of SSPEO on human subjective well-being in the context of aromatherapy. Since sex differences in odor-perception are well known [4b], the study further was created to address this issue.

Methods

90 healthy non-smoking (45 women) volunteeres were devided into three groups consisting of 30 participants (15 men), respectively: group 1 (SSPEO), group 2 (pos. control, orange absolute), group 3 (neg. control, no odor). During one session, subjects inhaled the fragrances for 31 minutes, continously rating odor-intensity. Before and after inhalation subjective well-being was determined. Odors were rated for hedonic valence, familiarity and effect (calming or activating). Data were analysed according to time, odor and sex using an ANOVA and post hoc t-tests.

Results

The analysis of the three groups regarding ratings of well-being showed significant differences for the parameter of calmness and alertness. Both, men and women, felt calmer after the inhalation of SSPEO compared to no odor ($p \leq 0.000$). When exposed to SSPEO, males ($p < 0.025$) and females ($p \leq 0.000$) became more awake while participants of the no-odor group became tired. Under the influence of orange absolute, men's alertness increased, while women fatigued. A trend towards significance was found for the parameter of mood ($p < 0.085$). Men ($p < 0.015$) and women ($p < 0.085$) perceived the fragrance of orange absolute more pleasant than that of SSPEO.

Conclusions

According to the findings of this pilot study, aromatherapy using SSPEO could help increase human well-being. However, sex differences between the fruity odor of orange and the woody odor of SSPEO were also detected and have to be considered when aromatherapy is being applied.

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Essential oils from Côa Valley Lamiaceae species: cytotoxicity on glioblastoma cells

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Keywords: Essential oils, Lamiaceae, Côa Valley, Cytotoxicity, Glioblastoma

Objective

Lavandula pedunculata (Mill.) Cav., *Mentha cervina* L. and *Thymus mastichina* (L) L. subsp. *mastichina* are widely used and fairly representative species of the flora and traditional uses of the Côa Valley, a Portuguese UNESCO World Heritage Site. *L. pedunculata* and *T. mastichina* are used by local populations to preserve olives, as a condiment in traditional cuisine and to aromatize bonfires on Saint John's Eve, while *M. cervina* is mainly used as a spice for river fish dishes. However, despite their popularity among the population and prevalence in the territory of Côa Valley, these aromatic plants are still undervalued and scientific literature on their potential as anti-cancer agents is scarce. Therefore, in this work we aim to study the therapeutic potential of the essential oils (EO) obtained from these three species, by assessing their chemical composition, secretory structures morphology and cytotoxic effect against glioblastoma cell lines.

Methods

Wild-growing *L. pedunculata*, *M. cervina* and *T. mastichina* plants were harvested in the Côa Valley region during the flowering stage. Glandular trichomes morphology was assessed by by scanning electron microscopy (SEM) on fresh leaves. EOs were obtained by hydrodistillation, using a Clevenger-type apparatus during 3 hours, according to the European Pharmacopoeia, preserved at 4 °C and the yield determined. Their effect (0,6 - 1 µL/mL) on cell viability was assessed on five glioma cell lines (A172, H4, U118, U373, U87) and a non-tumor cell line (Hek-293), for 24, 48 and 72 hours, through the Alamar blue assay.

Results

SEM analysis of the secretory structures showed an abundance of glandular trichomes namely peltate and capitate-types, thus explaining the high yields obtained for each EO. Moreover, *L. pedunculata* EO presented the most pronounced cytotoxic/antiproliferative activity against tumor cells, with moderate cytotoxicity against non-tumor cells. In turn, *M. cervina* EO exhibited a slightly lower cytotoxic effect against tumor cells and did not affect the viability of non-tumor cells. On the other hand, the EO obtained from *T. mastichina* was the less cytotoxic among the three EOs tested against glioma cells.

Conclusions

The results herein presented suggest that the EOs from *L. pedunculata* and *M. cervina* have a strong and promising antiproliferative potential to be further studied as efficient antitumor agents, whereasthe EO from *T. mastichina* revealed a weak to moderate cytotoxic activity against glioma cells.

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Chemical Composition of Industrially Produced Egyptian Jasmine (*Jasminum grandiflorum* L.) Essential Oil Obtained by Steam Distillation

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Keywords: Jasmine essential oil, chemical composition, GC-MS, *Jasminum grandiflorum*, steam distillation.

Objective

Jasminum grandiflorum L., a plant species belonging to the *Oleaceae* family possibly originating from the foothills of the Himalayas [1] has been introduced to ancient Egypt at least as far back as the pharaonic 18th dynasty (period 1550-1292 BC) as it has been identified in Tutankhamun's tomb [2] (reign 1332-1323 BC). It was later arguably reintroduced to Egypt and the Mediterranean by the Arabs possibly as early as the 8th century AD [3] during the conquest of Egypt. In modern Egyptian times, it was eventually exploited as early as the 1920s [4] by the modern techniques of solvent extraction to produce one of the most important aromatic raw material in fine fragrance perfumery: jasmine *grandiflorum* absolute.

This study aims to overview the chemical composition of *J. grandiflorum* essential oil (EO) obtained by steam distillation as a novel and desirably sought after raw material for fine fragrance perfumery and flavoring applications, long considered impossible to obtain as it was non-yielding industrially and therefore commercially unviable. To this day, the bulk of Egyptian jasmine blossoms is dedicated quasi-exclusively to the production of concrete (ca. 6-7 tons/yr) and absolute derived from it, obtained by hexane extraction and further ethanol displacement. Other solvent extraction options have been developed over time [4], some have been banned (benzene), others continue to exist singularly or in mixtures (*e.g.* ethyl acetate, SC CO_2, etc.), but never was an oil viably produced industrially by steam distillation — until today with outputs of over 200 kg of Egyptian jasmine oil per year since 2019.

Methods

J. grandiflorum EO was produced in industrial quantities (>200 kg/year) by steam distillation of the fresh, early morning, daily hand picked blooming blossoms from end of May to November, sourced from the area around the village of Shoubra Beloula As-Sakhaweya in the center of the Nile delta (Gharbeya province) Egypt. The chemical composition of the jasmine EO was evaluated by GC-MS/FID on a GC-QP2020 system (Shimadzu, Kyoto, Japan).

Results

EO yield was on average 0.025%. The major typical chemical consituents are: benzyl acetate (14.22%), linalool (12%), benzyl benzoate (10.21%), (*E*,*E*)-α-farnesene (10.12%), (Z)-jasmone (10.03%), isophytol (7.97%), (*Z*)-3-hexenyl benzoate (5.73%), phytol (4.48%), eugenol (1.18%), phytone (0.72%), methyl jasmonate (0.11%), squalene (<0.1%). We identified 98.78% of the jasmine essential oil composition, representing 129 compounds.

Conclusions

Jasmine *grandiflorum* EO presents the advantages of being 100% self-composed (*i.e.* additives-free, no collateral support macerate material, etc.), obtained by a millenia old established technique (steam distillation), without the use of petroleum based solvents (no risk of solvent residues), standardizable under ISO/TC-54 standards, offering great potential towards sustainable, lower carbon impact, green chemistry forms of industrial productions and with innovative applications in fine fragrances, cosmetics, and aromatherapy — while remaining socio-economically sensible. Further study is needed to prove its activity.

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Smart solutions for improving performance of analysis of volatile compounds by gas chromatography mass spectrometry

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Keywords: volatile compounds, gas chromatography, GCMS, flavour, fragrances, Aroma Database

Overview

In the presentation several solutions for improving the performance and throughput of analyses of volatile compounds by GCMS technique are presented.

The most important feature of modern chromatograph is versality and speed. That is why development of automatic solutions is being held. For analyses of volatiles eg flavour and fragrances, solid phase microextraction (SPME) in classical and arrow mode is widely used.

By coupling this technique with automated samplers, efficiency of the system is dramatically improved. From the detector side, there can be an option for splitting the analysis for both mass spectrometer and classical detectors (FID, ECD, BID, SCD) or an olphactometry port.

Big area for improvement lays also in the software. Modern software platforms give opportunity for automatic optimization, and later integration and quantification of compounds, and giving fast and reliable results.

Therefore lots of new databases are introduced like Smart Aroma Database consisting of more than 500 compounds with their retention times, mass chromatograms and mass spectra. Unique is additional odor information and semi-quantitative function.

Conclusions

Presentation of solutions for improving performance of analysis of volatile compounds by gas chromatography mass spectrometry.

Influence of organic nutrients on growth, yield and essential oil composition of Patchouli (*Pogostemon cablin* Benth.) under foothill conditions of Arunachal Pradesh, India.

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Keywords: Essential oil; farmyard manure; neem cake; vermicompost; *Pogostemon cablin* Benth.

Objective

Pogostemon cablin Benth., an aromatic plant species yields essential oil with immense industrial as well as pharmaceutical applications. In the backdrop of promoting organic cultivation of crops yielding essential oil and related products, the present investigation was designed to evaluate the influence of organic nutrients on growth, yield and essential oil composition of *Pogostemon cablin* under foothill conditions of Arunachal Pradesh.

Methods

Different organic nutrients such as FYM (Farmyard manure)@10t/ha; FYM@15t/ha; Vermicompost@5t/ha; Vermicopost@7.5t/ha; Neem cake@1.5t/ha; Neem cake@2.5t/ha; were applied and compared with the check NPK@150:50:50 kg/ha under Randomized Block Design in open field conditions and their influence was observed on plant height, number of branches/plant, collar diameter, herbage yield (fresh and dry), essential oil yield and essential oil composition.

Results

It was observed that Plant height was highest in FYM @ 15t/ha with mean value 73.53 cm while Neem cake @ 1.5t/ha have shown the lowest with mean value of 54.73 cm, but no significant variation was observed among the treatments. The Maximum number of branches/plant and collar diameter was recorded in NPK @ 150:50:50 kg/ha with mean value of 7.8 and 1.74 respectively however, in Neem cake @ 1.5 t/ha those were observed lowest with mean value of 4.93 and 0.86 respectively. Number of branches/plant shows no significant variation among the treatments while collar diameter shows significant variation. Herbage yield for fresh was observed superior in NPK @ 150:50:50 kg/ha with mean value of 15926.15 kg/ha followed by FYM @ 10 t/ha with mean value of 15751.79 kg/ha however, Neem cake @ 1.5t/ha it was observed lowest with mean value of 11563.08 kg/ha. Dried herbage yield was recorded maximum in FYM @ 10 t/ha with mean value of 5649.23 kg/ha and lowest in Neem cake @ 1.5 t/ha with mean value of 2332.31 kg/ha. Essential oil yield l/kg was extracted highest in Vermicompost @ 7.5 t/ha with mean value of 65.62 kg/ha followed by NPK @ 150:50:50 kg/ha with mean value of 62.90 kg/ha and lowest in Neem cake @ 1.5 t/ha with mean value of 22.74 kg/ha.

Conclusions

It can be concluded that depending upon the requirements of *P. cablin* essential oil or fresh/dried herbage, as well as essential oil composition the conditions and composition of organic nutrients varies. Present investigation recommends application of vermicompost@5t/ha for high essential oil yield, FYM@10t/ha for dried and NPK@150:50:50 kg/ha for fresh herbage yield respectively, while maximum patchouli alcohol content can be obtained in the essential obtained from the plants where no input is given.

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Traits specific breeding and varietal registration in *Cymbopogon* genus at CSIR-NEIST, Jorhat, India

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Keywords: Lemongrass, essential oil, elemicin, varietal development, trait specific germplasm

Objective

Genus *Cymbopogon* is a commercially important crop belonging to Poaceae family and used in perfumery, aromatic and pharmaceutical industries. An approximate of 140 species of *Cymbopogon* have been reported so far in tropical and subtropical regions of the world. Many bioactive compounds have been reported in the aromatic essential oil of this genus having high pharmaceutical and biological importance. This crop must be conserved and preserved for utilisation in the breeding programme. Therefore, the plant breeders of CSIR-North East Institute of science and Technology, Jorhat, Assam, India has attempted breeding techniques like selection, mutation and molecular breeding techniques to develop/ identify high yielding varieties of lemongrass. The success was highly striking and unique.

Methods

A total of 634 accessions of *Cymbopogon* has been collected from different regions of Northeast India which were planted and characterized for different morphological, molecular, qualitative and quantitative traits. High yielding superior lines were selected which were followed by multilocation trial in different regions of India. This led to the identification and registration of five novel varieties viz., Jor Lab L-8, Jor Lab L-9, Jor Lab L-10, Jor Lab L-11 and Jor Lab L-15. In contrast, other two novel varieties, Jor Lab C-5 and Jor Lab L-14 were developed through mutation breeding of *Cymbopogon winterianus* and *Cymbopogon flexuosus* respectively using EMS and gamma treatment at different doses.

Results

Jor Lab C-5 (citronellal content >35%) and Jor Lab L-14 (citral content >79.5%) was developed through mutation breeding which yielded essential oil of >1%. Citronellal is an aldehyde which gives lemon like fragrance and has anti-microbial, antinociceptive, anti-inflammatory, anticancer and mosquito repellentactivities. While, citral is the principal source of β-ionone extensively used for the synthesis of vitamin A. Additionally, Jor Lab L-8 (essential oil 0.80%; citral content 74%) and Jor Lab L-11 (essential oil 0.58%; methyl isoeugenol content 39.11% and myrcene content 48.02%) were identified through selection breeding of *Cymbopogon flexuosus*. Myrcene is an earthy, spicy, clove fragrance while methyl iso-eugenol has a typical sweet-warm, spicy- clove, woody, floral odour mostly used in perfume industries. Similarly, Jor Lab L-9, Jor Lab L-10 and Jor Lab L-15 were identified through selection breeding of *Cymbopogon khasianus* followed by multilocation trial. Jor Lab L-9 yielded essential oil of 0.80% and methyl eugenol content 74.56% whereas Jor Lab L-10 and Jor Lab L-15 yielded essential oil of 0.40% (elemicin content 70%) and 0.54% (geraniol content 46.8%) respectively. Methyleugenol has an important characteristic to synthesize methyl dopa, an important hypertensive medication. It is also used to control pests through Male Annihilation Techniques (MAT) when mixed with cue-lure at different concentrations. Similarly, an important alkaloid, mescaline can be synthesized from elemicin. Geraniolhas a rose-like fragrance and is effective at preventing certain types of cancer, and possesses antimicrobial, anti-inflammatory, antiseptic and insect repellent activities. All these germplasm are registered and commercially cultivated by the pharmaceutical and aromatic industries.

Conclusions

All these superior varieties were released for commercial cultivation after rigorous screening in the field trial and already published in high impact Journals. The varieties were registered by Plant Germplasm Registration Committee of Indian Council of Agricultural Research, New Delhi, India. Cultivation of these superior varieties is very beneficial to the cultivators as well as pharmaceutical industries and net return will be too higher as compared to other lemon grass variants.

Organic vs Conventional Farming of Lavender: Effect on Yield, Phytochemicals and Essential Oil Composition

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Keywords: *Lavandula angustifolia* Mill., agriculture systems; plant pigments; quality; volatiles

Objective

The choice for organic cultivation of essential oil crops, such as lavender, is still a challenge for agriculture in some countries, incl. Bulgaria. The essential oil composition is determined by the genotype of the plant, but abiotic and growing conditions can also affect biomass production and oil quality. Soil chemicals, fertilization, pesticides/herbicides and their relationship to volatile chemicals have been the subject of a number of studies. On the other hand, the plant pigments chlorophyll, carotenoids and anthocyanins are key for the absorption of light energy and are involved in various biological processes in the plant. They could provide important information on the condition of plants and may be suitable for assessing the response of different agricultural practices. The aim of our study was to compare phytochemicals, yield and essential oil composition of lavender under organic and conventional agricultural systems in order to find their the impact on productivity and quality of essential oil.

Methods

For 2019/2020 the field study was conducted on six private farms, located in Kazanlak valley, Bulgaria. Three of them are certified as organic farming (OF) and the rest - as conventional one (CF).. The essential oil was obtained by steam distillation, and analyzed using GC via Agilent 7820A GC System, coupled with FID and 5977B MS detector. The determination of chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophylls and total carotenoids was performed by the method of Lichtenthaler and Wellburn. Total anthocyanins content was determined according to the pH differential method, as absorbance was measured at 520 and 700 nm. Experimental data were subjected to analyses of variance (One-way ANOVA).

Results

The average values of natural pigments in CF were statistically proven higher than OF, 500.8 and 433.1 $\mu g.g^{-1}$, respectively for total chlorophyll, 63.6 and 55.8 $\mu g.g^{-1}$ for total carotenoids content, respectively. With regard to anthocyanins the difference is insignificant. During the first year, essential oil yields ranged from 1.60% to 2.39%. The OF lavender was limited in 1.60 – 2.29%, while for CF the interval was 1.80 – 2.39%. There is no statistically proven difference, but the CF levels are slightly higher the OF ones. In the second year, the values of OF essential oil were between 1.43 – 2.75% and for CF one, between 1.75 – 2.78%. The average rates were 2.04% and 2.32% respectively, so the conventional agricultural system had with 14% greater yield. The chemical profile is typical for the essential oil of *L. angustifolia* , and more precisely - for the Bulgarian one. Sixteen compounds were identified and monitored: for CF the main one was linalyl acetate (28.40 – 38.23 %), followed by linalool (20.01 – 31.04 %), β-caryophyllene (7.92 – 14.97%), cis-β- ocimene (1.91 – 12.46%), lavandulyl acetate (2.89 – 4.55%), terpinen-4-ol (1.62 – 4.46%) and trans-β oci-mene (2.74 – 3.72%). For OF the order was: linalyl acetate (28.40 – 34.90%), linalool (23.16 – 26.83%) and β-caryophyllene (7.92 – 11.71%)

Conclusions

For a two-year observation period, a slightly higher oil yield was reported with the CF lavender and insignificant changes in its quality. Regarding pigments, the levels of chlorophylls and carotenoids were higher in CF than organic one, while the anthocyanins level was slightly less.

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Chamomile essential oils exert anti-inflammatory effects involving human and murine macrophages: evidence to support a therapeutic action

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Keywords: chamomile; *Matricaria*; macrophages; inflammation; essential oils

Objective

Matricaria chamomilla L. is a well-known medicinal plant distributed worldwide. It has been used as herbal remedies for thousands of years to treat several diseases, including infections, neuropsychiatric, respiratory, gastrointestinal, and liver disorders. In the present study, we have investigated the potential anti-inflammatory effects of three essential oils (EOs) (GC1, GC2 and GC3) extracted from different chamomile samples cultivated in South-central Italy (Molise), in vitro [1].

Methods

The essential oils from dried samples were isolated by hydrodistillation in a Clevenger apparatus and analysed by gas chromatography–mass spectrometry (GC–MS). Three essential oils (GC1, GC2 and GC3 were tested for their ability to modulate pro-inflammatory murine macrophages and human peripheral blood mononuclear cells (PBMCs) functions.

Results

GC1, GC2 and GC3 essential oils significantly attenuated LPS/IFN-γ-induced inflammation by reducing M1 polarization. In details, they showed significant anti-inflammatory property by inhibiting NO, TNF-α and IL-6 production. These effects were correlated to a suppression of LPS-mediated p65 activation, the critical transactivation subunit for NF-κB transcription factor. Oxidative stress may trigger macrophages activation and elicit strong immune responses. Our study demonstrated that GC1, GC2 and GC3 were highly effective at increasing GCL and HMOX-1 anti-oxidant enzymes expression leading to the rapid scavenging of ROS. Next, we demonstrated that EOs were able to reduce CD4+ T cell activation which are also involved in inflammatory processes [2].

Conclusions

Our data describe for the first time that chamomile EOs exerted their anti-inflammatory and antioxidant activity by modulating macrophages and $CD4^+$ T cells- mediate immune response.

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OP-17

Chemical and biological investigations of essential oils from *Juniperus* genus

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Keywords: *Juniperus* essential oils, GC-MS, phytophatogenic activity, antibacterial activity, antioxidant activity

Objective

In the context of problem solving of environmental damages caused by synthetic pesticides, the objectives of this work were 1) to determine the chemical composition of essential oils from fresh leaves and branches of common juniper (*Juniperus communis* L.), rocky mountain juniper (*Juniperus scopulorum* Sarg.), and creeping juniper (*Juniperus horizontalis* Moench.) from Bighorn Mountains (Wyoming); 2) to evaluate thier antimicrobial activity against four phytopathogenic fungi (*Monilinia fructicola* [G. Winter] Honey, *Aspergillus niger* van Tieghem, *Penicillium expansum* Link and *Botrytis cinerea* Pers.), against two Gram-positive (G+ve), *Bacillus megaterium* de Bary (ITM100) and *Clavibacter michiganensis* Smith and three Gram-negative (G-ve) bacterial strains, *Pseudomonas fluorescens* Flügge (Migula), *P. syringae* pv. *phaseolicola* Van Hall and *Xanthomonas campestris* Pammel; 3) to assay their antioxidant capability.

Methods

The essential oils were obtained by steam distillation from leaves and branches of the three species of *Juniperus* and were characterized by GC-MS. The possible fungicidal activity of the EOs was evaluated at two different doses and fungitoxicity was expressed as percentage of mycelium growth compared to the negative control. The antibacterial test was carried out following the disc diffusion method by measuring the diameter of inhibition hyaline zone (mm) around each treated disc compared to the positive control one. Also cell membrane permeability (CMP) was determined by measuring the potential of electrical current transport through water as molar conductivity or electrolytic conductivity (EC) and the results were expressed as increasing percantage of EC value. The antioxidant activity was also determined using the stable 1,1-diphenyl-2-picrylhydrazyl radical method (DPPH) and EC50 value was defined as the dose of sample which reduced the initial DPPH of 50%.

Results

The assays used for the evaluation of some biological activities of the tested EOs showed variable results. The major component of *J. communis* was α-pinene; sabinene was the main constituent of *J. scopulorum* and *J. horizontalis*. The three tested EOs have antifungal activity against *M. fructicola* and *P. expansum* and effective antibacterial activity against *P. syringae* pv. *phaseolicola* and *B. megaterium*. The results of antioxidant activity showed comparable EC_{50} value between all three evaluated juniper EOs: after 45 minutes of experimental time the EC50 values are 487.1 μg/ml for *J. communis*, 527.4 μg/ml for *J. scopulorum*, 533.3 μg/ml for *J. scopulorum*.

Conclusions

The outfindings of this research showed promising antimicrobial effects against the majority of the tested phytopathogens: these data, corroborated also from the antioxidant capability shown by the three essential oils, highlighted potential for a their possible utilize them as natural alternatives to synthetic drugs, cause of global environmental problems, pathogen resistance and difficulty to control many post-harvest plant diseases.

Relation between dill apiole content and insecticidal activity of *Piper aduncum* L. essential oil

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Keywords: *Piper aduncum*, insecticides, rectification, dillapiole,

Objective

Piper aduncum L. (Piperaceae) can be easily found in the western region of the Brazilian Amazon, more specifically in the state of Acre. Its essential oil (EOPA) has an insecticidal activity particularly related to one of its compounds, dillapiolle, involved in the enzymatic detoxification in arthropods. However, there are no data relating the dillapiole content to the insecticidal activity of EOPA, which is necessary to establish reliable dose-activity parameters for formulated products. Thus, the objective of this study was to establish the relation between the dillapiole content and the insecticidal activity of EOPA.

Methods

The EOPA was obtained by steam distillation of the leaves and then rectified by fractional distillation. Thirteen fractions of EOPA with varying concentrations (from 12.3% to 99.8%) of dillapiole were analyzed. The chemical analysis of EOPA compounds was made by GC-MS and GC-FID. The identifications were made by the injection of authentic standards, calculation of linear retation indices and comparison of the mass spectra with those from different databases [1, 2]. The insecticidal effect by topical and residual contact, using *Spodoptera frugiperda* (J. E. Smith, 1797) *larvae* as target insect, was analysed *in vitro* to determine the lethal dose (LD_{50}) and the lethal concentration (LC_{50}) [3].

Results

The major compounds found in the distilled oil were dillapiole (73.5 - 78.0%), (*E*)-caryophyllene (8.0 - 9.1%) and myristicin (2.0 - 2.6%). The LC_{50} values for the fractions fitted the corresponding concentrations of dillapiole with a high coefficient of determination ($R^2 = 0.94$), and concentrations between 68% and 88% were estimated to promote the highest residual contact toxicity against *S. frugiperda*. Furthermore, the LD_{50} values for the fractions fitted the corresponding concentrations of dillapiole with a high coefficient of determination ($R^2 = 0.96$) and concentrations of dillapiole between 82% and 100% were estimated to promote the highest topical contact toxicity against *S. frugiperda*.

Conclusions

The EOPA with dillapiole content ranging between 68% and 100% showed greater insecticidal toxicity by residual and topical contact against *S. frugiperda larvae*.

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OP-19

Essential oil from pineapple processing by-products through ohmic heating

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Keywords: pineapple essential oil, ohmic heating, extraction, energy consumption, ohmic-assisted hydrodistillation (OAHD)

Objective

Ohmic-assisted hydrodistillation (OAHD) is an innovative distillation method, first proposed in about a decade ago [1] and has been used in several regions of the world [2]. At the same time, pineapple processing has a huge amount of processing by-products [3], which can be valorized to increase profit for the agrofood industry. This study aims to valorize pineapple processing waste through the application of ohmic heating. Also, it aims to evaluate the processing parameters, including heating rate, come up time in comparision with conventional method. Also, it aims to analyze the physichochemical proerties of the essential oil.

Methods

The processing by-products of gold diamond pineapple (*Ananas comosus*) Tainung 17 prepared for the essential oil isolation process. A new system based on novel OAHD was designed and developed in the emerging food processing technology laboratory of National Pingtung University of Science and Technology, Taiwan. The samples were processed by OAHD using water as the solvent. The results were compared with those of the conventional hydrodistillation system. The processing parameters were assessed for both techniques. The products were then analyzed for physical and chemical properties.

Results

The results showed that the heating rate in the OAHD system was higher than that of hydrodistillation. OAHD saved a substantial amounts of processing time. In addition, this emerging technology reduced energy consumption. This were in line with those reported on the literature on energy-saving aspect of ohmic heating which is due to efficient volumetric heating and high rates of energy conversion from electrical to thermal [4]. Furthermore, information on the physical and chemical properties of gold diamond pineapple essential oil was reported for the first time as a result of OAHD implementation.

Conclusions

This study provided a valorization platform for pineapple processing waste. Essential oil can be considered as a product development based on a waste through the application of ohmic heating technology. This system is scalable and can be implemented in the farm and in the pineapple processing factory to produce value-added products. Finally, promising data highlighted the possibility of using OAHD to achieve the Sustainable Development Goals (SDGs).

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Copy Number Variation and Gene Expression of Monoterpene Synthases Explain Chemotypes of Three Sage Species

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Keywords: *Salvia officinalis* L., *S. fruticosa* Mill., *S. pomifera* L., copy number variation, digital PCR, gene expression

Objective

Biosynthesis of plant monoterpenes results from the activity of terpene synthases (TPSs) producing either the final product or a precursor that is further modified to the final monoterpene. Copy number variation (CNV) of TPS genes together with their level of expression may shape intra- and interspecies phenotypic diversity.The objective of this study was to compare the monoterpene composition of three closely related sage species and different plant organs (leaf, calyx, corolla) with CNV and gene expression of TPSs in order to explain the chemotypic variation between the three species.

Methods

This study used whole-genome sequencing by next generation sequencing (NGS) and digital PCR (dPCR) of DNA/RNA in different plant organs of three sage species, Dalmatian sage (*Salvia officinalis*), apple sage (*Salvia pomifera*) and Greek sage (*Salvia fruticosa*). Monoterpene composition stratifying the phenotypes was determined by GC/MS on a non-polar column.

Results

According to the NGS data, the three sage genomes contained 12, 13 and 15 TPS-encoding open-reading frames (ORFs), respectively. In *S. officinalis*, one ORF was disrupted indicating a pseudogene. Genes were present at a single copy per haploid genome with a few exceptions of gene duplication or triplication. Expectedly, CNV was more pronounced across species compared to variation within *S. officinalis*. Transcript expression of TPS genes measured in leaf, calyx, and corolla was found to differ considerably across leaf types and sage species, but occasionally also between the three genotypes within *S. officinalis*. In some cases, gene duplication increased gene expression. For example, the very high content of thujones measured for *S. pomifera* came along with duplication of the gene that encodes the thujone-producing synthase, i.e. (+)-sabinene synthase.

Conclusions

Overall, copy numbers and transcript expressions of terpene synthases correlated well with the composition of monoterpenes.

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Discrimination of the essential oils from three wild goldenrod species growing together in Quebec

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Keywords: *Solidago canadensis*, *Solidago rugosa*, *Euthamia graminifolia*, essential oil, quality control

Objective

In the province of Quebec, Canada, goldenrods are a typical component of the boreal landscapes. *Solidago canadensis* L. (Canada goldenrod, SC) is particularly prominent. Its long, scented panicles are collected from the wild and steam distilled by several local facilities to yield a commercially relevant essential oil (EO). Despite past reports from accross the globe [e.g., 1] on the EOs of this now globally invasive species, surprisingly, no study was apparently conducted on the native North American populations of the plant. In Quebec, two other species of goldenrods often share their habitat with SC, often growing in fully mixed spontaneous populations. *Euthamia graminifolia* (L.) Nutt. (EG), formerly known as *S. graminifolia* (L.) Salisb., flowers in the same period as SC but is morphologically distinct; its EO was studied in Poland [e.g., 2]. Another commonly associated goldenrod is *S. rugosa* Mill. (SR), which flowers slightly later than SC, although overlaps are frequent. The only available study on its EO was obtained from samples grown in a German arboretum [3]. The objective of this study hence was to produce several batches of EO from SC, SR and EG, collected as they are found in their typical native habitat in Quebec, and to find chemical markers for cross-contamination monitoring, in a context of commercial distillation of wild-collected plants.

Methods

Four accessions of SC, and three accessions of each SR and EG fresh inflorescences at full flowering were collected in various locations in Saguenay, Quebec, Canada, between August 16 and August 20, 2020. Batches of 0.5-1.5 kg were steam distilled using a Nano distillation apparatus (AlChemia Solutions, Canada) for 2.5 h. Collected EOs were analyzed by GC-FID (internal normalization) and GC-MS, using previously described parameters [4], and identifying compounds via mass spectra and retention indices from DB-5/DB-Wax columns.

Results

All samples shared bornyl acetate and germacrene D as salient constituents. The EOs from EG differed by their notable proportions of (*E*)-β-ocimene (4.2–11.1%), cosmene (3.3–7.4%) and an important unidentified sesquiterpenol ($RI_{DB\text{-}5}$ = 1791, 2.8–4.5%). Three out of four SC EOs were richer in sesquiterpenes compounds, with α-gurjunene (4.0–10%), γ-gurjunene (1.4–3.2%) and cyclocolorenone (1.6–4.5%) as exclusive constituents, but the fourth batch entirely lacked those markers suggesting potential underlying chemotypes in the species. The overall simpler SR EOs could be differentiated from either SC or EG using combinations of monoterpenes.

Conclusions

Some species-specific markers can help ruling out cross-contamination of Canadian goldenrod EOs obtained from SC, SR or EG. Our results further suggest that chemotypical patterns could exist in SC and that EG features notable amounts of uncommon constituents among EOs.

ACKNOWLEDGMENTS

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Essential oils analysis in light of sensory quality. A reliable tool or not?

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Keywords: GC-MS; herbs; spices; essential oils bearing plants; headspace solid-phase microextraction; glandular trichomes

Objective

The concept of this research design was focused on exploring the relationship amongst actual EOs composition (EOs liquid injection) and the patterns of unlimited (from pure EOs) or limited (by presence of plant matrix) VOCs emission to headspace. This need was established in terms of the malfunction of currently used protocols for sensory quality determination of MAPs in light of the chemical composition of volatiles. For this reason, three different pairs of *Mentha* chemotypes, namely the menthol pathway, carvone pathway and linalool pathway, were subjected to various analytical approaches, to determine if some trends in VOCs emission to plant headspace in relation to EOs composition will occur.

Methods

The hydrodistillation of EOs was carried out by Deryng apparatus, while the headspace VOCs extraction was performed by HS-SPME Arrow technique. Thereafter, isolated VOCs were analysed by GC-MS technique. For statistical analysis principal components analysis and hierarchical clustering analysis were used.

Results

It was proven, that the differences in essential oils analysis by liquid injection and the headspace analysis of source plant material, do not refer only to particular medicinal and aromatic plants, but do represent a tendency to consist despite the differences in chemotype and morphological features of plants.

Conclusions

Clearly, differences in the distribution of the volatile compounds in essential oils and the patterns of their emission from pure essential oils or source plant material should be the reason for avoiding the estimation of the sensory quality of medicinal and aromatic plants just on the basis of total essential oil analysis.

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OP-23

Lady Pinecone back to nature

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Keywords: aromatherapy, drivers, automotive aromatherapy

Overview

Pani Szyszka™ (polish market) and Lady Pinecone™ (international market).

These products were created as a result of his many years of experience in the field of aromatherapy and perfumery. Krzysztof Czapski is a member of the Polish Aromatherapy Society.

A milestone in the project was the development of a fragrance carrier, which was supposed to implement the assumptions of aromatherapy in the car and was to be an ecological product based on natural components. The inspiration was a 9 year-old child who, having fun, came up with the concept of universal product fastening, which was later patented.

Experience and contacts with the most outstanding aromatherapy experts in the world resulted in consultations on the composition of mixtures for drivers.

Conclusions

After market launch, customers confirmed the positive effect on well-being. The blends were created on the basis of the available research on the effects of essential oils.

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Microwave-Assisted Hydrodistillation of Hop (*Humulus lupulus* L.) Terpenes: A Pilot-Scale Study

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Keywords: terpenes, green extraction, GC-MS characterisation, microwaved assisted hydrodistillation, Humus lupulus L.

Objective

Interest in essential oils has consistently increased in recent years. Essential oils have a large variety of applications in multiple fields, including in the food, cosmetics and pharmaceutical industries. The volatile fraction (VF) in hops (Humulus lupulus L.) fits within this domain as it is primarily used in the brewery industry for the aromatization of beer, and is responsible for the floral and fruity tones. This work aims to design an optimized extraction protocol of the VF from hops, using microwaves. Microwave-assisted hydrodistillation (MAHD) has been developed to reduce energy and time consumption in lab-scale reactors up to industrial-scale systems. Hops are principally available in three forms, according to a brewery's applications: i) fresh (FH); ii) dried (DH); iii) pelletized (PH). In this work, all three forms have therefore been studied and the recovered volatiles characterized by means of GC-MS.

Methods

The extractions and the purification process have been carried out using a lab scale reactor (MILESTONE ETHOS X) and the industrial scale prototype (MILESTONE ETHOS XL) for all the different biomasses. While the characterization has been done by using a GC-MS system.

Results

The optimized lab-scale MAHD protocol gave the best extraction yield of 20.5 mLVF/kgdry matrix for FH. This value underwent a slight contraction when working at the highest matrix amount (3 kg), with 17.3 mLVF/kgdry matrix being achieved. Further tests were then performed in a pilot reactor that is able to process 30 kg of material. In this case, huge yield increases were observed for PH and DH; quadruple and double the lab-scale yields respectively.

Conclusions

The MAHD has proven to maintain the high extraction yields even in a larger scale reactor without compromising the quality of the volatile fraction recover nor burning the biomass. In addition, this industrial-scale system also provided dramatic energy savings, practically halving the absorbed kJ/mLVF.

Involvement of serotonergic neurotransmission in the anxiolytic potential of *Melissa officinalis* essential oil and citronellal

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Keywords: *Melissa officinalis*, citronellal, anxiety, serotonin, essential oil

Objective

Numerous scientific and ethnomedicinal data are pointing to *Melissa officinalis* L. (Lamiaceae) as a valuable natural source of phytochemicals for the treatment of various mental health disorders [1]. Anxiety disorders could be considered a group of the most prevalent mental disorders, with limited treatment options. It has been suggested that serotoninergic neurotransmission plays an important role, especially in the septo-hippocampal system and the amygdala [2]. Thus, the aim of the current study was set to investigate whether *M. officinalis* essential oil and/or citronellal, the main oil constituent, act through a specific type of serotoninergic receptors using two animal models mimicking anxiety.

Methods

The essential oil of *M. officinalis* used in this study was isolated using a Clevenger-type apparatus, while citronellal was acquired from commercial sources. The oil was analyzed in detail by GC-MS and the composition corresponded to that usually encountered [3]. As the positive control, a standard serotonin receptor-acting drug, buspirone (10 mg/kg) was used. In order to investigate the role of 5-HT_{1A} receptors in the anxiolytic activity of the oil, a specific antagonist, WAY-100635, was applied 15 min prior to either the oil or citronellal. *In vivo* experiments were conducted on male BALB/c mice, the behavior of which, after appropriate treatment, was traced in an open field and light/dark tests. The obtained data were statistically compared using One Way ANOVA, followed by Tukey's post hoc test, with a level of significance set at 0.05.

Results

The pretreatment with WAY-100635 reduced some of the investigated parameters in mice treated with *M. officinalis* essential oil (at 12.5 mg/kg); however, some parameters (e.g. the number of transitions and the time of the first transition) remained unaltered. On the other hand, the action of citronellal (12.5 mg/kg) was statistically significantly modulated, in all of the investigated parameters in the mentioned tests, by the pretreatment with WAY-100635.

Conclusions

The results of the present study indicate that the anxiolytic activity of *M. officinalis* essential oil is at least partially mediated through 5-HT_{1A} receptors, while the anxiolytic action of citronellal in the two applied tests was predominantly mediated through 5-HT_{1A} receptors.

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Exploring the mechanisms of action of essential oils: how to undermine the pathogenic biofilm life cycle

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Keywords: essential oils, mechanism of action, pathogens**,** biofilm, multidrug resistances

Overview

Centuries-old selective environmental pressures have led to multiple survival strategies in bacteria, allowing the formation of global habitats. Biofilm is the most common survival strategy in bacterial life. It represents an evolutionary form of life, which translates from free-living cells to community lifestyle. What drives bacteria to establish a hierarchical lifestyle condition, is a multi-factorial and complex process based on *Quorum sensing* (Qs). The exchange of small organic molecules in Qs, lead to social cooperation between cells in biofilm and induce the coordinated expression of specific genes. These genes either encode an arsenal of virulence factors, or contribute to resistance to stressors, or even upregulate EPS production and efflux pumps [1]. Tolerance, which is a non-heritable phenotype, can arise when EPS matrix quenches the activity of antimicrobials by using diffusion–reaction inhibition, which enables tolerance against the numerous antimicrobial drugs [2]. For all these reasons, biofilm represents a global challenge, in particular for the clinical, environmental and food sectors. Medical device-related infections occur due to surface contamination by biofilm-forming bacteria during the time of implantation, causing life-threatening diseases, as the pathogenesis related to *P. aerginosa* [1]. On the other hand, biofilms on food processing surfaces can contaminate the food products, with a relevant health impact if the biofilm-associated bacteria are pathogens, causing outbreaks [3]. Among these, *Salmonella*, enterohemorrhagic *E. coli* and *L. monocytogenes* were responsible of about 59.000 confirmed human cases of infection in Europe during the year 2020 [4]. Several strategies have been developed to control the presence of biofilms, which have established multidrug bacterial resistances. Essential oils (EOs), due to their strong antimicrobial activities, can represent a novel biocontrol strategy. For bacteria, it is stated that it is more difficult to develop resistance to the multicomponent EOs, than to the antibiotics often composed of only single molecular entity [5]. The EOs mechanisms may involve the damage of the cytoplasmic membrane, alternating the cell membrane fatty acid profile and disintegrating the cell structures [3]. In particular, EOs showed very high efficiency in controlling biofilm formation and eradication from surfaces [3]. In this overview, genetic, transcriptomic and proteomic approaches have been applied to investigate the mechanisms by which EOs inhibit biofilm formation in *L. monocytogenes, S. aureus, P. aeruginosa, E. coli.*

Conclusions

The EOs strike the pillar targets of physiological pathways in biofilm, sapping of community life advantages. In *L. monocytogenes*, *S. aureus*, *P. aeruginosa* and *E. coli.*, the mechanisms of action affect cellular targets in a completely new way: interfering with the metabolic pathways, or softening virulence factors through the down-regulation of the relates genes. Therefore, the EOs could overcome bacterial resistances, but also restore susceptibility to drugs exposure, suggesting their use as the first strategy to control biofilms.

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Chemical diversity and antifungal activity of *Zataria multiflora* essential oils and influence of abiotic stresses on the compositions

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Keywords: Phenolic compounds, Pathogenic fungi, Chemical diversity, Heat stress, Drought stress

Objective

When searching for natural compounds for sustainable crop disease management it is eligible to be able to predict plant material as a suitable source of these compounds on the basis of environmental factors. The distribution of plants, their major metabolic processes and as a consequence the presence of a certain chemotype are limited by environmental conditions which are shaping the production and accumulation processes of secondary metabolites in plants. Essential oils (EOs), which are naturally produced by aromatic plants, contain a wide range of volatile molecules, including mostly secondary metabolites that have several biological activities. *Zataria multiflora* is an aromatic, perennial plant with interesting pharmacological and biological properties which belongs to the Lamiaceae family. This study aimed to (i) evaluate the chemical composition and antifungal activity of *Z. multiflora* populations; furthermore, (ii) examine the volatile compound profile responses of *Z. multiflora* chemotypes to the heat and drought stresses.

Methods

We collected fourteen populations of *Z. multiflora* from different natural habitats in Iran. The EO of each sampled plant (10 g of leaves) was isolated by hydro-distillation utilizing a clevenger-type system. To determine the phytochemical variation of *Z. multiflora* populations, the EOs were analyzed by GC-FID and GC-MS [1]. Antifungal assays were performed with *Colletotrichum lindemuthianum*, *Fusarium sambucinum*, *Fusarium culmorum*, *Alternaria dauci*, and *Botrytis cinerea* using the filter paper disc diffusion method. In order to investigate the effect of heat (33 °C vs. 20 °C) and drought (50 % reduced irrigation) stresses on the production of main volatile compounds, a greenhouse experiment was performed [2].

Results

The EO content ranged from 2.75 to 5.89 % in dry matter. Fifty-six volatile compounds were detected with carvacrol, thymol, and linalool as major constituents, each representing a distinct chemotype of the studied populations [1]. Meanwhile, carvacrol and thymol chemotypes had a significant inhibitory effect on the studied fungi at low concentrations whilst linalool chemotype exhibited a lower degree of inhibition. Abiotic stresses, particularly heat and the interaction of drought and heat, induced plants from the linalool chemotype to produce higher amounts of carvacrol, while plants from the carvacrol and thymol chemotypes did not alter EO production and composition [2].

Conclusions

There was a significant difference in EOs content and their compositions and antifungal activities among *Z. multiflora* populations. Hence, understanding the effect of environmental conditions on aromatic plant populations and chemotype development helps agriculture and food industry fully exploiting the potential of aromatic plants as a source of natural sustainable fungicides or insecticides.

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Essential Oils Diversity from the Malayan Flora: Recent Findings and Challenges

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Keywords: essential oil, hydrodistillation, GC-MS, Lauraceae, Annonoceae,

Overview

Malaysia is one of the seventeen megadiverse countries in the world. Its flora is home to an estimated 15,000 higher plant species and it is widely known that plants are efficient manufacturers of compounds including essential oils with highly diverse molecular structures and an unending array of biological activities. Therefore, it is logical to assume that a multitude of molecules or essential oils and their activities are yet to be discovered from this beautiful and immense flora. Our laboratory has been involved in the search of essential oils composition from the forest plants notably from species of Annonaceae (*Polyalthia*, *Goniothalamus*, *Xylopia*, *Anaxagorea*, *Pseuduvaria*, *Cyathocalyx sp.*), Lauraceae (*Alseodaphne*, *Actinodaphne*, *Beilschmiedia*, *Cryptocarya*, *Litsea*, *Cinnamomum sp.*), Rubiaceae (*Pavetta*, *Chassalia*, *Rothmannia sp.*), Lamiaceae (*Vitex*, *Premna*, *Plectranthus sp.*), Meliaceae (*Dysoxylum*, *Reinwardtiodendron sp.*), Myristicaceae (*Knema*, *Horsfieldia*, *Myristica sp.*), Myrtaceae (*Syzygium*, *Rhodamnia sp.*), Anisophyllea (*Anisophyllea sp.*), Apocynaceae (*Alstonia sp.*), Aristolochiaceae (*Thottea sp.*), Clusiaceae (*Garcinia sp.*), Dipterocarpaceae (*Dipterocarpus sp.*), Ebenaceae (*Diospyros sp.*), Euphorbiaceae (*Croton sp.*), Irvingiaceae (*Irvingia sp.*), Loganiaceae (*Strychnos sp.*), Magnoliaceae (*Magnolia sp.*), Oxalidaceae (*Sarcotheca sp.*), Piperaceae (*Piper sp.*), and Rutaceae (*Paramignya sp.*) family. All species have been collected from Peninsular Malaysia including Borneo. Most of the essentials were isolated from the leaves, bark, flowers, and fruits part by using the hydrodistillation method. Studies showed that they are rich source of monoterpenoids (α,β-pinene, linalool, 1,8-cineole, myrcene, borneol), sesquiterpenoids (β,δ-elemene, β-caryophyllene, germacrene D, δ-cadinene, bicyclogermacrene, caryophyllene oxide, nerolidol, eudesmol, α-cadinol, elemol), phenylpropanoids (safrole, eugenol, methyl eugenol, asarone, dillapiole) and some of them could be considered as chemotaxonomic markers of the genus. Pharmacological studies indicated that the essential oils exhibited cytotoxicity, antioxidant, antimicrobial, anticholinesterase, antityrosinase, anti-inflammatory, antidiabetic, and anticancer activities [1-4]. Hence, these results are mainly meant to provide relevant information on the phytochemical features of all species, with emphasis on the essential oils, providing guidance for the selection of accessions or species with the best chemical profiles. The outcome of these studies will further support the therapeutic potential of the species and provide convincing evidence for its future clinical applications in modern medicine.

Conclusions

As natural products and drug discovery is an important research area in Malaysia, the challenges and possible directions in this field shall be discussed briefly. For instance, how can we, the essential oil scientists relate our findings and knowledge to the society and then further contribute to the enhancement of its well-being?

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Reuse of Food Waste: Chemical Compositions and Health Properties of Essential Oils from Several Cultivars of Sicilian *Citrus aurantium* L. and *Citrus maxima* (Burm.) Merr.

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Keywords: *Citrus aurantium* L., *Citrus maxima* (Burm.) Merr., Gas Chromatography-Mass Spectrometry, Limonene, PCA and HCA analyses, Carbohydrate Hydrolyzing Enzymes, Lipase.

Objective

Citrus aurantium L. and *Citrus maxima* (Burm.) Merr., belong to the Rutaceae family, are native to a wide area covering Asia (from India to northern China) and Oceania (Queensland, Australia) [1]. The wastes deriving from *Citrus* processing (peels and seeds in primis) are an important part, and various papers have shown that by recycling and using these parts, bioactive compounds such as flavonoids, limonoids, terpenoids, and minerals can be obtained [2]. The aim of the present study was to investigate the chemical profile, antioxidant activity, carbohydrate hydrolising enzymes inhibition and hypolipidemic effect of essential oils (EOs) extracted from Sicilian *Citrus maxima* (pomelo) and *Citrus aurantium* flavedo by-product.

Methods

All EOs were obtained by hydrodistillation for 3 h using Clevenger's apparatus. We analysed by gas-chromatography-mass spectrometry (GC-MS) analysis EOs of five cultivars of *C. maxima*, and seven cultivars of *C. aurantium*. EOs were also combined in 1:1 (v/v) ratio to identify possible synergism or antagonism of actions. The antioxidant activity was performed by using a multi-target approach using 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic acid (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing ability power (FRAP) and β-carotene bleaching tests. The α-amylase, α-glucosidase and lipase inhibitory activities were also assessed.

Results

GC-MS analyses revealed D-limonene as the main monoterpene hydrocarbon in all cultivars, albeit with different percentages in the range 21.72-95.57%. All EOs are able to exert antioxidant activity via different mechanisms of action, as revealed by different applied tests. Among them, the EO of *C. aurantium* 'Fasciata' cultivar showed the highest antioxidant potential, whereas among combinations, a promising antioxidant activity was observed with the sample obtained by mixing, in equal volumes, cultivar 'Canaliculata'+'Bizzaria'. Regarding the inhibitory activity against enzyme involved in metabolic syndrome, EOs of *C. maxima* 'Terracciani' and 'Todarii' cultivars exhibited the highest lipase inhibitory activity.

Conclusions

Our results support the importance of the involvement of minor compounds in the antioxidant activity of the samples: the founded bioactivity is not linked to the main abundant compound D-limonene. However, the comparison between EOs combinations' inhibitory concentration (IC_{50}) and FRAP values' theoretical calculation and real data did not clearly highlight phenomena of synergism or antagonism of action valid in all biological tests. This study evidenced for the first time the chemistry and biological properties of Sicilian pomelo and orange EOs. Further studies are necessary to identify pumelo and orange oils possible application as functional ingredients for the development of functional food or nutraceuticals products alone or as blend.

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Anti-inflammatory effect and possible mechanism of action of bark essential oil of *Neocinnamomum caudatum* in lipopolysaccharide (LPS) stimulated murine macrophage RAW 264.7 cells

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Keywords: Essential oil, inflammation, lipopolysaccharide, murine macrophages, *Neocinnamomum caudatum*

Objective

The present study aimed at investigating the chemical constituents of *N. caudatum* bark essential oil (NCBEO) and elucidate the mechanism of its anti-inflammatory activity in lipopolysaccharide (LPS) stimulated murine macrophage RAW264.7 cells.

Methods

Essential oil was extracted by hydro-distillation and its chemical composition was characterized by gas chromatography mass spectrometry (GC-MS). Lipopolysaccharide (LPS)-stimulated RAW 264.7 cells were used as the inflammatory model. Cytotoxicity of NCBEO in RAW 264.7 cells was evaluated through MTT assay. The production of nitric oxide (NO) was assessed by Griess assay. Cytokine and mRNA levels of inflammatory mediators were measured by the enzyme-linked immunosorbent assay (ELISA) and quantitative real-time polymerase chain reaction (qRT-PCR), respectively. The levels of reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) were determined using 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA) and JC-1 assays, respectively. Immunofluorescence assay was also performed for the localization of nuclear factor-kappa B (NF-κB).

Results

The major constituents found in the *N. caudatum* bark essential oil were epi-*α*-cadinol (11.18%), (Z)-caryophyllene (9.30%), *α*-pinene (10.99%) and *α*-terpineol (6.79%). The results of *in vitro* experiments showed that NCBEO (12.5 and 25 μg/mL) significantly reduced LPS-triggered production of nitric oxide (NO) levels. NCBEO decreased the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β levels. Pre-treatment with NCBEO also reversed the increased mRNA expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 caused by LPS in RAW 264.7 cells. Besides, NCBEO inhibited the production of intracellular ROS and alleviated the depletion of mitochondrial membrane potential *in vitro*. Treatment of NCBEO decreased the nuclear factor-kappa B (NF-κB)/p65 nuclear translocation in LPS-induced RAW 264.7 cells. Further, NCBEO also elevated the expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione (GSH), and glutathione peroxidase (GPx) in LPS-induced RAW 264.7 cells.

Conclusions

The present study indicated that *N. caudatum* bark essential oil possesses significant anti-inflammatory effect, suggesting its application as a potential agent for the treatment of inflammatory diseases.

Cymbopogon nardus essential oil nanoemulsion against fungal infestation and aflatoxin B_1 contamination of food system

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Keywords: essential oils, nanoencapsulation, antifungal, antiaflatoxigenic, preservative, mode of action

Objective

The present investigation aimed to synthesize *Cymbopogon nardus* (L.) Rendle leaf essential oil (CNEO impregnated chitosan nanoemulsion (Ne-CNEO) and its practical application as novel green delivery system for protection of *Syzygium cumini* seeds (model food system) against range of storage fungi, aflatoxin B1 (AFB1) secretion and lipid peroxidation.

Methods

Essential oil was obtained using Clevenger hydrodistillation apparatus followed by chemical standardization through GC-MS. The nanoemulsion was prepared using ionic gelation technique. The Ne-CNEO was characterized using SEM, zeta potential, atomic force microscopy and FTIR analysis. Aflatoxin B1 inhibitory study was performed against most toxigenic isolate of *Aspergillus flavus* (AFLHPSc-1) isolated from *Syzygium cumini* seeds.The antifungal and antiflatoxigenic assay was performed by poisoned food method followed by *in situ* study using HPLC. In silico study was performed using UCSF Chimera software follwed by Sfety assessment through acute oral toxicity assay on male mice.

Results

Chemical characterization of CNEO showed citral (62.73%) as major component. Successful loading of CNEO inside chitosan nanoemulsion was confirmed through SEM, AFM and FTIR analyses. *In vitro* release study showed biphasic release profile with initial burst followed by sustained release of CNEO from chitosan nanomatrix. Ne-CNEO exhibited enhanced *in vitro* antifungal, antiaflatoxigenic (0.16 µL/mL) and antioxidant activity over CNEO. The antifungal and antiaflatoxigenic mechanism of action of Ne-CNEO was associated with increased leakage of cellular contents, inhibition of ergosterol biosynthesis, and impairment in cellular methylglyoxal biosynthesis. *In silico* modeling validated interaction of citral with Ver-1 and Omt-A proteins, confirming the molecular action for inhibition of AFB1 production. *In situ* studies showed signficant protection of *S. cumini* seeds (model food system) against fungal inhabitation, AFB1 production and lipid peroxidation without changing organoleptic properties. The acute oral toxicity assay of Ne-CNEO showed its non-mammalian toxicity.

Conclusions

Potential antifungal, antiaflatoxigenic, antioxidant activity, and higher mammalian non-toxicity strengthens the use of Ne-CNEO as safe nano-green and smart preservative in place of synthetic preservatives in modern food, and agriculture industries.

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POSTER SESSION - A

ISEO 2022 52nd International Symposium on Essential Oils Wrocław, 4-7 September

Analysis of essential oils of new Ukrainian *Lavandula angustifolia* and *L. x intermedia* cultivars

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Keywords: *Lavandula*, lavandin; lavender; essential oil; linalool; GC–MS; caryophyllene oxide

Objective

After the annexation of Crimea, the place where aromatic plants were cropped in Ukraine, there was a need to develop new varieties adapted to the dry steppe conditions in the remaining parts of the country. The Rice Institute of the Ukrainian Academy of Agricultural Sciences conducted works on the breeding and selection of new varieties of *Lavandula angustifolia* Miller and *Lavandula x intermedia* Emeric ex Loiseleur, which show high economic value in addition to being adapted to periodic droughts and significant temperature fluctuations. This research project aimed to analyze the chemical composition of the essential oils of seven new *L. angustifolia* and eight *L. x intermedia* cultivars. This work was part of the characterization process of newly developed cultivars.

Methods

The oils were produced from flowering parts by a hydrodistillation. The oils were analyzed using gas chromatography with a mass spectrometer. All identifications were confirmed by retention data (linear retention indices database).

Results

Over 70 different compounds have been identified in the analyzed oils. The main components of the studied lavender and lavandin oils were linalool and linalool acetate (26.14–57.07% and 9.08–24.45%, respectively). Other significant components of *L. angustifolia* oils were terpinen-4-ol (2.16–22.44%), lavandulol acetate (2.12–10.23%), lavandulol (1.30–3.14%), and in the case of *L. x intermedia* oils: camphor (10.11–12.55%), borneol (5.49–8.71%), and eucalyptol. The oils were characterized by a high linalool content and the substantial presence of lavandulol and its ester – the features that are beneficial and sought for lavender oils. Nonetheless, the studied essential oils did not fulfil the industry standards, largely because of too high terpinene-4-ol content.

A comparative analysis of oils from both species grown under the same conditions showed that lavender oils are characterized by a much higher content of camphor, borneol and eucalyptol than lavender oils. This is consistent with the literature. Furthermore, the performed statistical analyses (PCA and HCA) indicated that caryophyllene oxide may be a potential marker differentiating the studied species. The obtained results enabled characterization of the newly developed cultivars and selection of those that have not only appropriate morphological and physiological features but also a specific chemical composition.

Conclusions

The chemical composition of the new Ukrainian cultivars was evaluated and discussed against the. The Ukrainian lavender oils characterize with high terpinene-4-ol content and therefore do not fit the industry standards for lavender oil. The differences between both studied species are typical. Statistical analysis highlights caryophyllene oxide as potential another marker for distinguishing lavandin and lavender oils.

Could cinnamon essential oil and its nanoemulsion be useful in the *Acinetobacter baumannii* biofilm treatment?

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Keywords: *Acinetobacter baumannii*, biofilm, *Cinnamomum zeylanicum* essential oil, nanoemulsion

Objective:
Nanoemulsion (NA) synthesis is a popular way to increase essential oil (EO) solubility and therefore, to enhance biological activities. The strong antimicrobial effect of cinnamon EO *Cinnamomum zeylanicum,* is already proven, however with its encapsulation is expected to be more effective. *Acinetobacter baumannii* is widely known as a threat to health care system due to its high resistance to antibiotics. Eradicating infections caused by this species is very difficult due to complexly structured biofilms with polysaccharides being one of the most abundant components. Data on the effect of EO and NA on biofilm eradication is very scarce, so the aim of this study was to examine their activity on the already formed biofilm of *A. baumannii*.

Methods:
Bacterial strains used in this study were *A. baumannii* ATCC19606 and clinical isolates originating from blood, swab wound and wound. To determine the minimal inhibitory concentration (MIC) of commercially obtained EO (P0125285, Frey + Lau GmbH Henstedt-Ulsburg, Germany) and NA that prevents visible growth of microorganisms microdilution assay was preformed. The effect of EO and NA on biofilm eradication was done with crystal violet staining, using a range of concentrations between 1/2MIC - 4MIC. Phenol sulphuric method was applied to determine the concentrations of egzopolysaccharides from biofilm matrix.

Results:
For EO MIC values were 0.5mg/mL and 1mg/mL, but for NA concentrations were lower, ranging from 0.125 mg/mL for clinical isolates to 0.25 mg/mL for ATCC strain. EO and NA exhibited remarkable antibiofilm activity with percent of bifilm eradication of 68% and 63%, respectively. However, treatments of biofilm with EO and NA showed an increase of concentrations of exopolysaccharides in the biofilm matrix.

Conclusion:
Results obtained in this study pointed out that EO and NA exhibited strong antibacterial and antibiofilm potential, and therefore could be good candidates for clinical trials with a goal to threat infections caused by *A. baumannii*.

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Cinnamon essential oil and nanoemulsion: antibiofilm activity on *Acinetobacter baumannii* clinical isolates

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Keywords: cinnamon essential oil, *Acinetobacter baumannii*, nanoemulsion, GC/MS, biofilm

Objective

Acinetobacter baumannii is a pathogenic species which presents a danger to healthcare facilities due to the rapid spread of antibiotic resistance. Plants are currently being explored as a potential source of bioactive compounds that could be used to combat infectious diseases. Cinnamon is used as a food spice, but essential oil has been proven for antimicrobial activity. Due to reduced stability and solubility of essential oil, nanoemulsion (NE) synthesis could provide stronger antimicrobial effects. Investigation and comparison of antimicrobial activity of cinnamon (*Cinnamomum zeylanicum* L.) bark essential oil (EO) and NE on the *A. baumannii* ATCC19606 and clinical isolates. Effect of EO and NE on biofilm formation and biofilm eradication.

Methods

GC/MS was performed in order to determine chemical composition of commercially purchased EO (P0125285, Frey + Lau, GmbH, Henstedt-Ulzburg, Germany). Droplet/particle size and polydispersity index of NE was determined by photon correlation spectroscopy (PCS). Minimal inhibitory concentration of EO and NE were defined using MIC assay. Effects of EO and NE were also examined on biofilm formation and eradication. Crystal violet staining was used for biofilm biomass quantification.

Results

GC/MS analysis determined that the most common compound was trans-Cinnamaldehyde (61.9%). NE droplet/particles had multimodal distribution, shown by PCS. MIC values for EO were in range 0.25mg/mL – 0.5mg/mL, and for NE 0.125mg/mL – 0.25mg/mL. Both tested substances showed good effect on biofilm eradication, and destroyed biofilm biomass up to 64%, whilst the inhibition of biofilm formation was up to 70%.

Conclusion

Taking all the results into account, it is a good start for further investigations of both EO and NE as a potential antimicrobial agent. Deeper knowledge about the mechanisms of actions of both of these tested substances could help us to understand the best and the most effective way to use them, in order to combat against *Acinetobacter baumannii*.

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Helichrysum italicum oil degraded bacterial biofilm but aggravated inflammatory parameters

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Keywords: immortelle, essential oil, biofilm, skin, inflammation

Objective

Helichrysum italicum (Roth) G. Don, also known as immortelle, belongs to the Asteraceae family. The *H. italicum* essential oil has been shown to possess anti-inflammatory, antioxidant, antimicrobial, antiviral and biopesticidal properties [1,2]. However, its biofilm degradation activity has not been proved yet. *Pseudomonas aeruginosa* as one of the most prominent nosocomial bacterium can form bacterial biofilm on the surface of catheters or in wounds, and in most cases these are associated with inflammation. The aim of our study was to explore the biofilm degradation and anti-inflammatory effect of *H. italicum* essential oil.

Methods

The composition of essential oil was investigated by GC-MS. The microbiological assay included the determination of MIC value (minimum inhibitory concentration) with broth macrodilution against *P. aeruginosa* ATCC27853. The bacterial biofilm was created in 96-well microtiter plates. After incubation, the essential oil solution was added to the biofilm in MIC/2 concentration. The fixed bacterial cells were stained with 0.1% crystal violet dye. The absorbance was measured at 595 nm with plate reader. For the *in vivo* examination, the different concentrations of *H. italicum* essential oil were formulated in gels, then the inflammatory parameters (ear oedema, myeloperoxidase enzyme activity, and cytokines) were measured in an oxazolone-evoked contact dermatitis mouse model.

Results

The main component of *H. italicum* essential oil was neryl acetate (21.1%). The MIC value was 0.625 mg/ml, the measured inhibitory rate of biofilm degradation study was 59%. However, *in vivo* the 5 and 10% of *H. italicum* oil containing gels aggravated the inflammatory parameters such as some histopathological alterations, myeloperoxidase activity and the level of IL-1β, TNF-α and IL-10.

Conclusions

We conclude that although the *H. italicum* essential oil may be a successful treatment in case of *P. aeruginosa* infection associated with biofilm formation, its use on inflamed skin is not recommended as it may further deteriorate the skin inflammation.

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Volatile compounds from fruits of low commercial value

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Keywords: Peaches, plums, volatile compounds, gas chromatography, shelf-life

Objective

Peaches and plums are very perishable climacteric fruits whose firmness decays rapidly on post-harvest. Fruits without appropriate firmness for marketing in nature, can however be raw material for other food products [1], avoiding waste. The extraction of compounds from these residues with technological and/or functional interest is considered a viable option [2]. Since the aroma is one of the main quality attributes, the goal of this study was to characterize the volatile profile of under-valued peach ('Royal Summer' cultivar) and plum ('Black Splender' cultivar) fruits and their storage stability at room temperature.

Methods

Peach and plum fruits were collected from Granfer orchards and kept at room temperature to mimic shelf-life storage conditions. Fruits volatiles were isolated by Solid Phase Microextraction (SPME) and Hydrodistillation (H), and the chemical composition was analyzed by Gas Chromatography (GC) and Gas Chromatography coupled to Mass Spectrometry (GC-MS) as in [3]. An SPME time-course study was performed while the fruits presented quality characteristics (T0-T7 for peaches and T0-T21 for plums) and when they showed signs of deterioration (T11 for peaches and T24 for plums). Fruits H was performed at two time points, T0, after harvesting, and when the first evidence of perishability was visible (T11 for peaches and T24 for plums).

Results

Ethyl octanoate (9-73 %) and γ-decalactone (8-28 %) were T0-T7 peaches main SPME volatiles, while *n*-heptadecane was the major compound at T11. Peaches H showed *n*-octane (14 %) and 2-furfural (14 %) as dominant compounds at T0, while ethanol (52 %) was the main one at T11, evidencing some fermentation stage. Plums SPME analyses showed that hexanoic acid butyl ester (16-39 %), hexyl acetate (2-28 %) and hexyl butanoate (9-25 %) were T0-T21 major compounds and butyl butanoate (19 %) at T24. *n*-Nonanal (15 %) at T0 and ethyl hexadecanoate (22 %) at T24, were plums H main compounds.

Conclusions

Despite being fruits of low commercial value both peaches and plums showed a typical volatile profile. During studied storage time at room temperature, while fruits retained an acceptable quality, there were only slight variations in volatiles composition.

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The effect of natural fragrances on the inhibition of the growth of bacteria of *Staphylococcus*

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Keywords: Gram-positive bacteria, *Staphylococcus*, natural fragrance raw materials, growth inhibition, MIC

Objective

The aim of this study was to inhibit the growth of *Staphylococcus aureus* (ATCC 6538, Sterbios), and *Staphylococcus epidermidis* (ATCC 12228, Sterbios) with the use of natural fragrance raw materials.

Methods

In the experiment, 96-well plates were used and the effect of 100 natural fragrance raw materials was examined. The viability and proliferation capacity of the cell cultures were assessed using the alamarBlue™ dye. The natural frangrance raw materials that inhibited the growth of bacteria in the screening test were qualified for the next part of the research: the proper test. In this test, the minimum inhibitory concentration (MIC) was determined.

Results

In the screening test, the growth of bacteria of the species *S. aureus* was inhibited by 31 natural fragrance raw materials. In the case of *S. epidermidis*, growth was inhibited by 22 compounds. In proper test, the lowest MIC values for *S. aureus* were shown by Black seed India Nigella sativa and Artichoke abs Eg (MIC = 22,1342 µg/ml) and Blue cypress Australia (MIC = 40,2435 µg/ml). Juniper Berry India showed the lowest MIC value (MIC = 25 µg/ml) for *S. epidermidis*.

Conclusions

The results show that the natural fragrance raw materials have an inhibitory effect on the growth of bacteria. *S. epidermidis* is more resistant to the natural fragrance raw materials than *S. aureus*. The researched natural fragrance raw materials can be used in the future as preservatives in cosmetics due to their ability to inhibit the development of pathogens living in them.

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Natural fragrance raw materials as growth inhibitors of the species *Burkholderia* and *Pseudomonas*

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Keywords: natural fragrance raw materials, Gram – negative bacteria, antibiotics, MDR resistance, antibacterial activity

Objective

The aim of the study was to determine the inhibitory effect of selected natural fragrance raw materials on microorganisms such as *Burkholderia cepacia* (ATCC 25416), and *Pseudomonas fluorescens* (ATCC 13525) from the company Sterbios. The microorganisms used in the experiment are known to be multi-drug resistant (MDR), which means antimicrobial resistance to at least one antimicrobial drug. Bacteria use several mechanisms to achieve MDR such as efflux system or enzymatic deactivation. In addition, multi-drug resistance in Gram – negative bacteria is enhanced by their cell wall structure containing LPS, which causes a lot of difficulties in combating such pathogens.

Methods

The first step of this study was to carry out the screening tests on each of the species, which allowed the marking of 100 natural fragrance raw materials that inhibit bacterial growth. Then, the proper test allowed to define the minimum inhibitory concentration (MIC) of the natural fragrance raw materials, which were selected in the screening test. For this experiment, 96-well cell culture plates and alamarBlue® were used.

Results

In the screening test, the *Burkholderia cepacia* genus was inhibited by 26 natural fragrances raw materials, whereas *Pseudomonas fluorescens* was inhibited by 36 natural raw fragrance materials. In the proper test, the lowest MIC values for *Burkholderia cepacia* showed Blue cypress Australia (MIC = 25 µg/ml), Fir balsam absolute Canada, Coriander herb Russia, and Juniper Berry India (MIC = 50 µg/ml). In the case of *Pseudomonas fluorescens*, the lowest MIC values showed Fir balsam absolute Canada and Juniper Berry India (MIC = 50 µg/ml).

Conclusions

The purpose of the work was achieved, as compounds showing an inhibitory effect on the chosen bacteria were selected and the MIC values of the substances were determined. As a result of the widespread use of natural fragrance raw materials in the food, agricultural, cosmetic, or pharmaceutical industries, it is possible to apply selected inhibitory compounds in the treatment of infections caused by pathogenic bacteria or as preservatives in cosmetic products, and as ingredients in the plant health products.

Synthesis of new derivatives of indole musk and study of the inhibition of the growth on bacteria from *Klebsiella* and *Pseudomonas* species

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Keywords: traseolide, synthetic musks, ether oxime, MIC

Objective

Musk is considered one of the most important fragrances. Because of the high cost of obtaining natural musk, synthetic derivatives are used much more frequently. Traseolid® is an indan derivative and was used in the following work to synthesize oxime and ether oximes by an *O*-alkylation reaction using the appropriate halides [1]. Fragrances and their derivatives that have not yet been introduced into the industry should be tested that will prove their usefulness or indicate possible side effects. The following tests were performed microbiologically with the use of microorganisms found in cosmetics: *Klebsiella aerogenes* (ATCC 13048, WDCM 00175), *Klebsiella pneumoniae* (ATCC 13883, WDCM 00097), *Pseudomonas fluorescens* (ATCC 13525, WDCM 00115), *Pseudomonas putida* (ATCC 49128), *Pseudomonas aeruginosa* (ATCC 15442, NCTC 13359) (all microorganisms were purchased from Sterbios). The minimum inhibitory concentration of the compounds obtained has been evaluated.

Methods

The traseolid oxime was synthesized by reaction with hydroxylammonium chloride in pyridine. Then, the four oxime ethers were obtained using an *O*-alkylation reaction with methyl iodide, ethyl iodide, n-propyl iodide, and n-butyl bromide. All compounds obtained were tested to determine antibacterial activity using alamarBlue® reagent, allowing the MIC value to be dermined.

Results

The yield of the synthesis reaction of traseoild oxime was 16% while the efficiency of the synthesis of traseolide oxime ethers ranged from 47 to 58%. The traseolide oxime most strongly inhibited the growth of the tested bacteria – in the case of two of them, the MIC value was the lowest among the rest of the compounds tested, in the case of *K. aerogenes* this value was 4.4 μg/ml, while the species *P. putida* did not show sensitivity to the presence of this compound. The traseoilde oxime *O*-methyl ether turned out to be the second most inhibitory growth of microorganisms with a chemical compound from among the tested.

Conclusions

In conclusion, in this work new derivatives on indan musk were synthesized and their influence on the growth of selected species of *Klebsiella* and *Pseudomonas* bacteria was tested. The results can be useful to identify opportunities for the subsequent use of these compounds in various industries.

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The main components of essential oils as potential preservatives in cosmetic emulsions

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Keywords: olfactory compounds, antimicrobial activity, cosmetics

Objective

The aim of this study was to assess the antibacterial activity of volatile components of essential oils (EO). As well as assessing their potential as preservatives of cosmetic emulsions. The research was conducted on five pathogens: *Enterococcus hirae* (ATCC 10541) (MediMark), *Bacillus cereus* (ATCC 10876) (TCS), *Staphylococcus aureus* (ATCC 6538) (MediMark), *Escherichia coli*, (ATCC 10536) (MediMark), *Pseudomonas aeruginosa* (ATCC 15442) (TCS). The focus was on the main components of essential oils with proven antimicrobial activity. It was (−)-menthol, linalool, *trans*-cinnamaldehyde, (+)-carvone, (+)-pulegone, piperitone, eucalyptol, (+)-limonene and (+)-α-pinene main constituents of various essential oils [1-5]. The preservation potential of selected volatile organic compounds was analyzed for cosmetic O/W emulsions and for selected finished products.

Methods

Initial activity studies were performed by serial microdilution in 96-well plates with the alamarBlue® reagent to determine MIC (Minimal Inhibitory Concentration). The research on the preservative potential of finished cosmetics was carried out using the USP 51 antimicrobial effectiveness test.

Results

In preliminary studies, thymol (MIC = 300-600 mg/L) and *trans*-cinnamaldehyde (MIC = 300-1200 mg/L) showed the best antimicrobial activity against the strains tested. In studies on the preservative potential in cosmetic emulsion O/W, it was shown that both thymol (MIC = 150 - 18.75 mg/L) and *trans*-cinnamaldehyde (MIC = 150 - 18.75 mg/L) inhibit the growth of the tested microorganisms better than methyl paraben (MIC = 32-2 g/L). Moreover, in USP 51 tests on the finished products: beard shampoo and hair conditioner, it was shown that the current preservatives do not sufficiently protect the product against *S. aureus* and that the added *trans*-cinnamaldehyde and thymol in concentration 1200 mg/L and 600 mg/L, respectively, inhibit the growth of the microorganism.

Conclusions

The main components of essential oils, thymol and *trans*-cinnamaldehyde, can inhibit the growth of microorganisms in cosmetic emulsions. Moreover, both compounds in the tested concentrations support the preservatives present in the finished products. However, the concentrations used are quite high and the allergenic properties at such concentration should be revised. It can therefore be concluded that the addition of a fragrance has an impact on its protection against microorganisms that may contaminate the product.

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Essential oil of Azorean *Cryptomeria japonica* seed cones: chemical composition, antibacterial activity and *Artemia salina* toxicity

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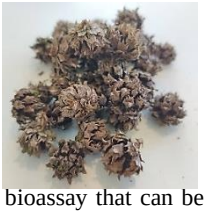
Keywords: Azores, *Cryptomeria japonica*, antimicrobial, forest wastes valorisation, seed cones, essential oil

Objective

The interest in essential oil (EO) from *Cryptomeria japonica* (Thunb. ex L.f.) D. Don (Cupressaceae) wastes, by the scientific community and EO markets, is rapidly increasing. However, some of these biomass wastes, such as seed cones, have been less exploited. Thus, this study aimed to determine the chemical composition of the EO of dried seed cones (DSC) from Azorean *C. japonica*, and to evaluate its antibacterial activity and its toxicity against *Artemia salina*.

Methods



The plant material was collected in São Miguel Island (Azores, Portugal) in October 2020. The OE-DSC was obtained by hydrodistillation for 3 h, using a Clevenger-type apparatus. The chemical composition of the OE-DSC was analyzed by GC-FID and GC-MS. The antibacterial activity was evaluated by the agar disk diffusion method against *Bacilus subtilis* (DSM 10), *Micrococus luteus* (DSM 20030), *Enterobacter cloacae* (DSM 30054), and *Escherichia coli* (DSM 498). The toxic properties were assayed against *Artemia salina* (L.) (Anostraca: Artemiidae), an easy and inexpensive bioassay that can be considered as a preliminary assessment of bioactive compounds.

Results

C. japonica OE-DSC presented a high yield (1.33%, v/w), and showed forty-two components that represented 97.9% of the total EO, being α-pinene (32.6%), sabinene (23.7%), terpinen-4-ol (9.8%) and β-myrcene (3.7%) the major components; it should be noted that all these compounds exhibited important multi-bioactivities, including antimicrobial, antioxidant, anti-inflammatory and anti-acetylcholinesterase [1]. Thus, OE-DSC may be a candidate as a natural therapy for some chronic diseases.

Antibacterial activity of OE-DSC was observed in all target species, except for *E. coli*, presenting growth inhibition zones of 10.0 mm (*E. cloacae*), 12.3 mm (*M. luteus*) and 14.7 mm (*B. subtilis*), which revealed a moderate activity. The OE-DSC also displayed high toxicity against *A. salina* being the LD_{50} and LD_{90} values of 81.1 ppm and 120.2 ppm, respectively, that again indicate potential bioactivities for pharmaceutical applications.

Conclusions

C. japonica OE-DSC is regarded as an abundant source that possess high toxicity against *A. salina* and significant inhibition against common food-borne microbes. Thus, this EO could be a good candidate for the development of food preservatives. Moreover, the study of its bioactivities warrants further investigation in order to accumulate knowledge that will help to find new uses for this biomass waste and thus to give them a surplus value.

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The effect of storage time of dry leaves on yield, chemical profile and antibacterial activity of *Cymbopogon citratus* essential oil

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Keywords: *Cymbopogon citratus*, lemongrass, essential oil, plant material storage time, yield, antibacterial

Objective

Antibiotic resistance has become a major problem worldwide, leading to an increased interest in plant-based antimicrobials. Essential oils (EOs) from leaves of *Cymbopogon citrates* (D.C.) Stapf. (Poaceae), commonly known as lemongrass, are widely used in traditional medicine, perfumery and food technology, due to its high citral (a mixture of isomers geranial and neral) content, which is responsible for the strong lemony aroma and the antimicrobial activity. However, as is well known, the yield, chemical profile, and bioactivity potency of plant-derived EOs depend on many factors, including the plant material storage time. Therefore, and due to the importance of *C. citratus* EO, the objective of this comparative study was to evaluate the yield, chemical composition, and antibacterial activity of Azorean *C. citratus* EO obtained from two different dried leaf samples, one without storage and the other stored during three years.

Methods

Fresh leaves of Azorean *C. citratus* were collected in 2022, air-dried and submitted to hydrodistillation for 6 h, using a Clevenger-type apparatus. A dried sample, stored since 2019, was also submitted to hydrodistillation for comparison purposes. The chemical profile of the obtained EOs was performed by gas chromatography (GC) with flame ionization and mass selective detectors, and the *in vitro* antibacterial activity was evaluated by the agar disk diffusion method. The target species included Gram positive (*Bacillus subtilis* DSM10, *Bacillus licheniformis* DSM13, and *Micrococcus luteus* DSM20030) and Gram negative (*Serratia marcescens* DSM48, *Enterobacter cloacae* DSM30054, and *Escherichia coli* DSM498) bacteria.

Results

The yield of OE-2019 and OE-2022 samples was 0.43% and 0.86%, v/w (dry basis), respectively, showing that aged plant material and consequently aged EO resulted in large losses of EO content. GC analyses of OE-2019 *vs.* OE-2022 samples revealed a high-content of geranial (24% vs. 42%) and neral (15% *vs.* 28%), followed by myrcene (2% *vs.* 5%). Regarding the antibacterial activity (Table 1), OE-2019 sample exhibited moderate-strong growth inhibition zones (> 13 mm) against common food-borne Gram positive bacteria (*B. licheniformis*, *B. subtilis* and *M. luteus*) and weak activity (≤ 8 mm) against the negative ones, except *E. coli* (0 mm). On the other hand, all target bacteria were sensitive to OE-2022.

Table 1. *In vitro* antibacterial activity of Azorean *Cymbopogon citratus* essential oil from leaves collected in 2019 and 2022.

EO samples	Growth inhibition zones in mm					
	B. subtilis	*B. licheniformis*	*M. luteus*	*S. marcescens*	*E. cloacae*	*E. coli*
EO-2019	21 ± 4.5	21.75 ± 2.6	13.3 ± 2.1	7.75 ± 0.96	8 ± 0.82	0
EO-2022	50.5 ± 4.4	39 ± 11	27 ± 5.6	10 ± 0.82	11.75 ± 1.26	10.5 ± 1.29

Values are presented as mean ± SD.

Conclusions

Plant material storage time had significant effect on *C. citratus* leaf EO yield and quantitative chemical profile. *C. citratus* leaf EO had greater activity against Gram positive bacteria than Gram negatives, even obtained 3 years after plant material storage. This novelty represent an important contribute in this field.

Antimicrobial activity of encapsulated *Coridothymus capitatus* essential oil incorporated into chitosan-based film against *L. monocytogenes* in smoked salmon

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Keywords: essential oil, encapsulation, *Listeria monocytogenes*, chitosan, smoked salmon

Objective

Essential oils (EOs) are increasingly used for their antimicrobial activity despite their application in food preservation is hindered by side effects like their odour and limited water solubility. Encapsulation could improve EOs stability and mask their flavour while promoting a controlled release and increasing the antimicrobial activity [1]. The incorporation of essential oils into edible coatings and films represents an innovative and promising strategy to enhance food safety of packed and minimally processed products, like cold-smoked salmon where *L. monocytogenes* represents a main concern [2]. Objective of this study was to evaluate the antimicrobial activity of encapsulated *Coridothymus capitatus* essential oil (eCEO) and of chitosan films incorporating the eCEO against *L. monocytogenes* to improve the safety and preservation of cold-smoked salmon.

Methods

Commercial, food grade *Coridothymus capitatus* L. flowering tops essential oil (CEO) from Italy, obtained by steam distillation and characterized by carvacrol as main component, was used. The CEO was encapsulated by ball-milling of the mix of trehalose and the EO at different concentrations (3, 7, and 10% w). A chitosan film added with 3.75 mg/mL eCEO was prepared according to Kadam et al. [3], with some modifications. *In vitro* antimicrobial and antibiofilm activity of CEO against three *L. monocytogenes* strains isolated from smoked salmon and type strain ATCC 19114 was preliminarily assessed in microtitre plates. Then the antimicrobial efficiency of CEO against *L. monocytogenes* was evaluated as microencapsulated powder both after dispersion in a salmon-based model system at two different concentrations (5 and 3.75 mg/mL) and included in a chitosan film *in vitro* and *in situ* in cold-smoked salmon during storage (4°C).

Results

Results of the *in vitro* test indicated that eCEO loaded at 7 and 10% showed the highest antimicrobial activity with MIC/MBC of 0.63 mg/mL. In addition, sub-MIC of eCEO (0.3 mg/mL) inhibited of ca. 60-80% the biofilm formation of *L. monocytogenes* strains. The antimicrobial activity of eCEO was also confirmed in salmon-based model system where reductions of *L. monocytogenes* between 6.5-7.3 Log and below the detection limit (2 Log CFU/g) were observed for CEO at 3.75 and 5 mg/mL, respectively. Chitosan-based coatings and films inhibited the pathogen growth and high efficacy was observed for the chitosan film (1%) incorporating eCEO (3.75 mg/mL). Furthermore, the *in situ* study revealed a good anti-*Listeria* activity particularly for the eCEO loaded-chitosan film, showing the lowest counts (below the detection limit) during all the storage period of the salmon.

Conclusions

These findings highlight the potential of the CEO encapsulation for the development of biodegradable and edible "active" packaging to control *L. monocytogenes* growth in smoked salmon, offering a sustainable alternative to conventional packaging.

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In vitro Anti-inflammatory and anticancer activities of *Mentha spicata* and *Matricaria recutita* essential oils

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Keywords: Lamiaceae, Astaraceae, anti-inflammatory, anticancer, caspase

Objective

Mentha spicata L. and *Matricaria recutita* (L.) Rausch. are known and used for inflammatory conditions [1,2]. This present study aimed to evaluate *M. spicata* and *M. recutita* essential oils for their *in vitro* anti-inflammatory and anticancer activities *via* COX-1 and COX-2 enzyme inhibitions, as well as their apoptozis potential through the caspase pathway.

Methods

Before activity evaluation, pharma grade essential oils were analysed and confirmed by GC/FID and GC/MS, respectively. MTT was used for *in vitro* cytotoxic/anticancer effects using the HEK293/A549, MCF7, PC3 cell lines. To evaluate caspase, COX-1 and COX-2 inhibition assays commercial test kits were used.

Results

Major components of *M. spicata* essential oil were characterized as 72.8% carvone, 12.6% limonene, 2.2% 1,8-cineole, 1.3% myrcene, and 1% *trans*-dihydrocarvone, respectively. The major components of *M. recutita* essential oil were characterized as 47.9% α-bisabolol oxide A, 16.8% α-bisabolol, 13.8% (*Z*)-β-farnesene, 5.8% α-bisabolol oxide, and 4.7% α-bisabolene oxide A, respectively. The IC_{50} values for *M. recutita* essential oil on A549, MCF7, PC3, HEK293 cells were 208.54±1.39, 315.44±1.17, 197.52±0.98, 638.79±1.15, respectively. The IC_{50} values of *M. spicata* essential oil on A549, MCF7, PC3 cells were 672.13±2.57, 708.27±2.05, 206.49±1.48 respectively. No toxic effect on healthy HEK293 cells were observed. However, the tested essential oils increased apoptosis activity and all results were statistically significant. According to the anti-inflammatory activity results, it was determined that the essential oils of *M. chamomilla* and *M. spicata* essential oils were selective COX2 enzyme inhibitors and their SI values were 0.30 and 0.67, respectively.
Overall, both *M. spicata* and *M. recutitata* essential oils showed selective potential for COX-2 enzyme inhibition and apoptosis against selected cancer cell lines for the first time to the best of our knowledge with this specific mode of action.

Conclusions

In vitro methods are an important stage of biological activity evaluation, standard pharma grade essential oils may help for the repeatability of the results. However, further mechanistical in vivo tests are needed to confirm the anti-inflammatory and anticancer potentials of essential oils, also combination studies are also worthwhile to screen.

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Essential Oil Composition of Aerial Parts of *Prangos platychlaena* subsp. *platychloena* and Its Antimicrobial Activity against Foodborne Microorganisms

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Keywords: *Prangos platychlaena*, essential oil, antimicrobial activity, α-bisabolol

Objective

Prangos platychlaena subsp. *platychloena* is found in the East and North East Anatolian Regions of Turkey. In traditional medicine, the dried and crushed roots are used externally for wound healing in animals. Here we report on the antimicrobial activity of the essential oils from *Prangos platychlaena* subsp. *platychloena* because very little information is available on these endemic perennial herbaceous plants.

Methods

The aerial parts of *Prangos platychlaena* subsp. *platychloena* were collected at the flowering stage in August 2021 in the vicinity of Erzurum (altitude 2100 m, 40°20'21.6"N, 40°48'51.4"E), Turkey. The aerial parts of air-dried plant material (200 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 3 hours. The obtained essential oil was stored at +4 ºC until tested and analyzed. The essential oil was tested individually against a range of 42 microorganisms, including 29 bacteria, 10 mold, and 3 yeast species. Antimicrobial tests were carried out by the disk diffusion method [1, 2] using 100 µL of suspension containing 10^8 colony forming units (CFU)/mL of bacteria, 10^6 CFU/mL of yeast, and 10^4 spores/mL of mold spread on Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA), and potato dextrose agar (PDA) medium, respectively. The MIC values were determined for the bacterial and yeast strains that were sensitive to the essential oil in the microwell dilution assay. The inoculation of the strains was prepared from 12-hour broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity (BioSan DEN-1 McFarland Densitometer). The MIC values of prepared serial the essential oil dilutions between 7,8 to 500 µg/mL were determined on the basis of a microwell dilution method. The agar dilution method, as described previously by Gul et al., (2002), was used to determine the MIC values of the essential oil. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. Each test was repeated at least triplicate.

Results

Essential oil yield (1.92 %) (v/w) of *Prangos platychlaena* subsp. *platychloena* was calculated on the basis of dry weight of the plant material. The main components of the oil were α-bisabolol (21.51 %). It was determined that this essential oil showed an antagonistic effect on all strains. *Bacillus subtilis, Listeria monocytogenes,* and *S. aureus* were the most affected bacterial strains. It has also been observed that the development of pathogenic, spoiling and toxic strains such as *Escherichia coli, Salmonella* Typhimurium, *Proteus* spp., *Pseudomonas* spp., *Candida albicans,* and *Aspergillus flavus* can be inhibited. Especially, the antifungal effect, which shows this essential oil on mildew, is remarkable in terms of mildew development and prevention of mycotoxin formation.

Conclusions

All the above results demonstrated that *Prangos platychlaena* subsp. *platychloena* oil had the potential to control foodborne microorganisms and therefore could be used as a preservative for food products. However, care should be taken in the use in foods containing probiotic bacteria such as kefir and prebiotic yogurt, because of the possible adverse effects of *Prangos platychlaena* subsp. *platychloena* oil on probiotics.

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A closer look at trace volatiles of banana fruit brandy

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Keywords: banana, fruit brandy, trace volatiles, dichloromethane extract, gas chromatography-mass spectrometry

Objective

Banana (*Musa acuminata* Colla) is one of the most widely grown fruit. The aroma profile and odor-active compounds of banana were the subject of several studies [1, 2]. Like other high sugar-content fruit, it can be processed into brandy. The composition of this uncommon alcoholic beverage was previously briefly investigated, with sixteen minor constituents identified in either Brazilian commercial distillates or a product produced exclusively from banana waste [3]. In an effort to identify as many potential aroma contributors as possible, a detailed analysis of the trace volatiles of conventionally prepared banana fruit brandy was made the objective of this work.

Methods

The banana fruit distillate was prepared for this purpose by controlled fermentation of homogenized peeled ripe Colombia-sourced fruit followed by a traditional two-step distillation process. Trace volatiles were isolated by extraction with dichloromethane; the contents of the prepared extract were analyzed by gas chromatography coupled with mass spectrometry (GC-MS).

Results

GC-MS analysis of the dichloromethane extract of the banana brandy revealed the presence of 109 different minor constituents, 93 of which were successfully identified.

Conclusions

Most of the identified trace volatiles were esters (50 in total). A number of other compounds (acetals, (un)saturated alcohols, carboxylic acids, terpenes etc.) were found to be present as well. Isoamyl acetate, frequently referred to as the defining constituent of banana aroma, was present in only a minute quantity. Contributions of individual compounds to the overall sensory properties of the beverage remain to be studied.

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Analysis of mycolic acids fraction obtained from *Mycobacterium tuberculosis* exposed to garlic essential oil

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Keywords: MAME, lipids, TLC, natural products

Objective

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* having specific waxy outer envelope, which is impermeable to drugs and other compounds [1]. According to WHO report TB was the 13th leading cause of death worldwide and the top cause from a single infectious agent in 2019. The COVID-19 pandemic has reversed years of progress in providing essential TB services and reducing the disease burden. Additionally, the emergance of highly resistant strains complicates the treatment, which often fails to successfully eradicate bacteria. For this reason new therapeutic schemes are needed to increase the treatment succes rate. Such schemes inclede adjuvant compounds originating from plants known for its antibacteial actoin [2]. Since garlic (*Allium sativum* L.) posseses antimicrobial activity, we checked the influence of its essential oil on the growth of *Mycobacterium tuberculosis* H37Ra and determined the profile of mycolic acids fraction obtained from bacteria stressed with this inhibiting agent.

Methods

The chemical composition of essential oil was determined through GC-MS analysis [3]. The antimycobacterial activity was checked in two-fold dilution assay with resazurin [3]. Later, mycolic acids fraction was obtained through hydrolysis of bacterial cell wall and subsequent methylation of liberated fatty acyls. Resulted mycolic acids methyl esters (MAME) were separated on TLC [4].

Results

The main components of studied essential oil were diallyl sulfides and methylated diallyl sulfides. The profile of MAME obtained from bacteria stressed with garlic essential oil was compared with MAME from control culture. The visual inspection of TLC plate revealed that MAME fraction contained three typical mycolic acids sub-fractions: alpha, keto and metoxy. The bands for these acids were less abundant for the sample of bacteria stressed with garlic essential oil than the bands for the control sample.

Conclusions

Garlic essential oil affects mycolic acids fraction of mycobacterial cell wall. It indicates a new promising application for this natural product which is usually used in the kitchen.

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Does sweet marjoram essential oil have the same antimicrobial potential as tea tree oil?

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Keywords: Tea tree oil, *Origanum majorana* L., antibacterial activity, antifungal activity, GC-MS

Objective

Tea tree oil (TTO) has been employed as a strong antimicrobial, antifungal, and low toxic agent to treat many dermatological disorders. Terpinen-4-ol, γ-terpinene, α-terpinene, α-terpineol, *p*-cymene, and α-pinene are the major constituents of TTO, with terpinen-4-ol being the main one [1]. The composition of *Origanum majorana* L. (sweet marjoram) essential oil (SMO) is similar, and among the constituents, terpinen-4-ol also predominated [2]. However, the therapeutic potential of SMO lags behind that of TTO. The present study aims to compare the antimicrobial potency of TTO and SMO against 14 bacterial and 3 fungal strains.

Methods

SMO was obtained from the leaves of *O. majorana* by hydrodistillation, and commercial TTO was applied in the experiment. The compositions of the essential oils were determined by GC-MS and GC-FID. The tested microorganisms were *Cutibacterium acnes* ATCC 11827, *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *S. epidermidis* ATCC 12228, *Escherichia coli* ATCC 35218, *E. coli* AG-100, *Klebsiella pneumoniae* ATCC 700603, *Salmonella typhi* SE-01, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Moraxella catarrhalis* ATCC 25238, *Streptococcus agalactiae* ATCC 13813, *S. pyogenes* ATCC 19615, *Candida albicans* ATCC 14053, *C. krusei* ATCC 14243 and *C. auris* CDC B11903. The antimicrobial activity was determined first by the agar disc diffusion method, and then the minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were measured with the microdilution method in a 96-well plate according to the Clinical and Laboratory Standard Institute guidelines.

Results

GC-MS and GC investigations displayed similarities of TTO and SMO in terpinen-4-ol, γ-terpinene, α-terpinene, α-terpineol, and *p*-cymene-content. The difference was in the sabinene-type compounds. The antibacterial activities were in the range of MIC = 0.25–0.5%, except for *P. aeruginosa* and *E. faecalis*, on which both EOs were only slightly effective. SMO against *E. coli* and TTO against *K. pneumoniae* and *S. typhi* displayed higher activity. Antifungal activity expressed as MIC and MFC values were significantly higher for SMO or the same for both essential oils against all tested *Candida* strains.

Conclusions

Our antimicrobial evaluation indicated that, as expected based on composition of TTO and SMO, both oils had equal activity against Gram-positive and Gram-negative bacteria. While against fungal strains, SMO was more potent. These preliminary results suggest that *O. majorana* oil's therapeutic value is underestimated; it may have a broader therapeutic application and worth further studies against various dermatological infections.

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Evaluation of the anti-SARS-CoV-2 properties of essential oils and aromatic extracts

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Keywords: SARS-CoV-2, essential oils, M^{pro}, PL^{pro}, cysteine protease, inhibition, antiviral

Objective

In the present study, we wanted to address the question of whether essential oils (EOs) and aromatic extracts (AEs) are inhibitors of the SARS-CoV-2 main and papain-like proteases, and if these inhibitors exhibit *in vitro* antiviral properties. To the best of our knowledge, our work was the first experimental study [1] regarding the potential of EOs and AEs as a part of the search for the anti-coronaviral strategies.

Methods

538 samples of essential oils and aromatic extracts were screened for the inhibitory activity on SARS-CoV-2 M^{pro} and PL^{pro} using the established microplate assay [1]. The most active protease inhibitors were also tested for their antiviral activity using a fully replication-competent SARS-CoV-2 strain in VeroE6-GFP cell culture.

Results

The best M^{pro} inhibitor benzoin Sumatra resinoid showed selective inhibition (EC_{50}) = 31.5±2.4 μg/mL with limited toxicity (CC_{50}) = 85.5±1.9 μg/mL. The best PL^{pro} inhibitor petitgrain mandarin essential oil showed lower antiviral activity (>100 μg/mL).

Conclusions

Results of this study give the scientific community the selection criteria for further *in vitro* and *in vivo* testing of the most promising EOs, AEs, and isolated natural compounds.

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Composition and Phytotoxic Activity of Essential oil of Invasive *Heracleum* spp. in Slovakia and Poland

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Keywords: hogeweed, hydrodistillation, GCMS, phytotoxicity, biodiversity

Objective

Heracleum mantegazzianum Sommier& Levier (Giant hogweed) and *Heracleum sosnowskyi* Manden (large hogweed or Sosnowsky's hogweed) are invasive plant species widespread in central Europe [1]. Both species belong to the Apiaceae family and negatively affect local plant diversity. Their chemical residues also alter soils' chemical and biological characteristics [2]. This study aimed to compare the essential oil of two populations of *Heracleum* spp. – one from Slovakia and one from Poland, their composition and phytotoxic activity.

Methods

The seeds of plants were collected at Lekárovce in Slovakia (SK) and Krakow-Glinik in Poland (PL), dried, and hydrodistilled by Clevenger-type apparatus for 3 h. The EOs were analyzed by GC-MS-FID on a Trace GC Ultra coupled with DSQII mass spectrometer and an MS-FID splitter. The compounds' identification was based on comparing their mass spectra and linear retention indices.
Phytotoxic activity assay, EOs (doses of 100-0.5 μg/mL) were applied on seeds of two dicots (*Sinapis alba* L. and *Lepidium sativum* L.) and two monocots (*Hordeum vulgare* L. and *Triticum aestivum* L.). Ten seeds in a Petri dish were treated with EO solution and stored in the growth chamber in 14/10 h light/dark periods for 5 days. The number of germinated seeds was counted, and the root lengths were measured and compared to the control (cultivated without EO).

Results

There were 55 components in SK EO sample, and 52 in PL EO sample identified. The dominant compound was octyl acetate in a similar amount in both samples (58.65±0.95% and 54.08±1.07%, resp.). The second dominant compound was hexyl 2-methylbutyrate (10.61±0.57% and 15.52±0.09%, resp.). No significant distinctions in the germination activity of model plants were observed in comparison to control and between particular concentrations of EO. Statistically signifficant phytotoxic effect on root length was noted only at the application of 5 μg/mL and 10 μg/mL of PL EO on *S.alba*.

Conclusions

The phytotoxict activity of the *Heracleum* spp. EO on seed germination and root elongation of monocot and dicot species was very weak.

ACKNOWLEDGMENTS

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Chemical Composition and Antimicrobial activity of Essential Oil from Carrot Seeds 'Tushon' Variety

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Keywords: carrot seeds, GC/MS analysis, essential oils, antimicrobial activity

Objective

Carrot seeds essential oil is a fragrance component in perfumery and cosmetics, as well as mosquito repellent [1] Therefore, the composition and activity of this oil present challenge, especially from the origin of carrot seeds. The aim of the current study was to investigate the chemical composition and antimicrobial activity of essential oil from cultivated carrot (*Daucus carota* L.) seeds 'Tushon' variety grown in Bulgaria.

Methods

Cultivated carrot (Daucus carota L.) seeds 'Tushon' variety was harvested and finely ground in a laboratory homogenizer. The essential oil from this sample was obtained by steam distillation procedure using the Clevenger apparatus. GC-MS analysis was performed on a gas chromatograph Agilent Technology, Hewlett Packard 7890 A, coupled with MS detector. Antimicrobial activity of carrot seeds essential oil was tested against ten microorganisms.

Results

Fifty-two compounds were identified in essential oils of the cultivated carrot (*Daucus carota* L.) seeds 'Tushon' variety by GC-MS analysis, as the major ones were carotol (26.09%), sabinene (16.16%), α-pinene (4.83%), β-caryophyllene (3.27%), β-bisabolene (3.25%), and daucol (1,66%). Sesquiterpenes dominated in carrot seeds essential oils. This essential oil demonstrated the highest inhibitory activity against *Proteus vulgaris* ATCC 6380 (minimal inhibitory activity MIC 0.625 mg/ml). The moderate activity was observed against *Candida albicans* NBIMCC74. *Staphylococcus aureus* ATCC25923 (MIC 2.5 mg/ml). Antimicrobial activity was found against *Escherichia coli* ATCC8739, *Salmonella enteritidis* ATCC 13076, while these seed oil was inactive only against *Pseudomonas aerugenosa* ATCC9027.

Conclusions

The chemical composition of cultivated carrot seeds essential oil was revealed and its antimicrobial activity was evaluated. The presented results demonstrated not only the anti-Candia activity, but also the antimicrobial potential against Gram-negative and Gram-positive pathogenic bacteria. Due to its antimicrobial potential this oil could find possible application in the pharmaceutical industry and also in the natural cosmetic products.

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Diversity of Content and Composition of *Juniperus communis* L. Essential Oil Collected in Different Vegetation Seasons from Locality Spišský hrad (Slovakia)

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Keywords: α-pinene, essential oil, monoterpenes, sesquiterpene, juniper berries

Objective

Juniperus communis L. is an evergreen dioecious gymnosperm shrub, with a broad ecological amplitude adapted to various climatic conditions. It can be found up to 3000 m above sea level [1]. *J. communis* has been used in traditional medicine [2]. The berry cones are also used as a flavour in gin, liquors, bitters, Swedish beer and borovička, a Slovak national alcoholic beverage „Spišská borovička"like gin [3]. The present study evaluated the quantity and quality of essential oil (EO) in ripe berry cones from locality Spišský hrad, Slovakia, during the years 2013 – 2015.

Methods

The juniper berries were manually collected from the wild growing *J. communis* shrubs close to the historical monument and UNESCO site Spišský hrad in eastern part of Slovakia. Collections was provided in three vegetation seasons 2013, 2014 and 2015. Weather conditions, air temperature and precipitation during the years 2013–2015, were obtained from meteorological stations of the Slovak Hydro Meteorological Institute. Berry cones were dried and then crushed and hydrodistilled by the Clevenger apparatus for two hours. The composition of the EO were carried out on a Varian 450-GC connected with a Varian 220-MS. Components were identified by comparison of their mass spectra with those stored in NIST 02 (software library). Correlations between content of EO and its compositions and environmental conditions were statistically evaluated.

Results

The EO yield ranged from 1.2 to 1.4%, depending on the year. In the EO, eight monoterpenes (α-pinene, β-pinene, β-myrcene, sabinene, limonene, terpinene-4-ol, borneol, bornyl acetate) and one sesquiterpene (β-caryophyllene) were identified. The dominant component was monoterpene α-pinene, ranging from 27.0 to 39.0%. In 2013, a significantly lower content of sabinene (1.5%), β-myrcene (5.5%) and limonene (2.5%) was found. In 2014 and 2015, the amount of these components was significantly higher (sabinene 16.5% and 19.5%, β-myrcene 11.5% and 9.3% and limonene 5.5% and 4.3%). In contrast, the amount of α-pinene was higher in the berries from the year 2013 (14.5%) compared to the years 2014 and 2015 (1.5% and 2.0%). The remaining monoterpenes were found in EO in low amounts (0-2.5%). Content of sesquiterpene β-caryophyllene was found in the amount 14.0% and 13.5% (years 2013 and 2014). In the year 2015, the content of β-caryophyllene was found to be only 1.5%.

Conclusions

The results presented in the study showed the impact of the climatic conditions which was changing in different years on the EO yield and its composition in ripped *J. communis* berry cones.

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Headspace GC/MS profiling of aroma compounds in different *Cannabis* cultivars

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Keywords: aroma compounds, terpenes, cannabis, gas chromatography/mass spectrometry, headspace

Objective

Beside the usage in medicinal purposes, industrial hemp (*Cannabis sativa* L. cultivars) is cultivated for fiber and seed production, but also has an incredible number of possible applications as an ingredient in the cosmetics and food industry. Hemp flowers and upper leaves contain an essential oil which is used as a scent in perfumes, soaps, creams, candles and as flavouring agent in food and beverages production. This essential oil is found to be a complex mixture of many volatile compounds, mainly monoterpenes, sesquiterpenes, and other terpenoid-like substances. The main chemical components are myrcene, *β*-caryophyllene, limonene, *α*-pinene, *β*-pinene, terpinolene, and *α*-humulene. The general properties of these substances include antidepressant, relaxant, anxiolytic, sedative, antimicrobial, and antioxidant activity [1,2]. According to literature data, several studies have been carried out on the cannabinoid content and seed oil of *Cannabis sativa* L. cultivars, but few studies have focused on the chemical composition and pharmacology of the essential oil extracted from inflorescences and even fewer studies are concerned with its possible uses. Furthermore, there is no avaliable data about *Cannabis* cultivars aroma compounds, thus the main object of this study was investigation of aroma components in eight different *Cannabis* strains: CBD *Charlotte's Angel*, *Glueberry* ORG, *Passion*, *Elleta Campana*, *Orange Hill Special*, *Gorilla Glue*, *Amnesia Kush* and *Bruse Banner*.

Methods

Headspace GC/MS analysis was carried out using Agilent 7890A Gas Chromatography system equipped with FID detector as well as Agilent 5975C Mass Quadripole detector. For that purpose, HP-5 ms capillary column (30 m × 0.25 mm, film thick-ness 0.25 μm) was used. Only the gas phase (1 mL of highly volatile compounds) was injected and then investigated on an Agilent GC/FID/MS system.

Results

Headspace GC/MS analyses of volatile aroma compounds in dried flowers collected from the grown *Cannabis* plants resulted in identification of 32 components representing 97.54-99.27% of the total amount. In the class of terpenoids, monoterpenoid fraction was dominated by *α*-pinene (14.21% in *Amnesia Kush* and 62.60% in *Passion*), myrcene (11.17% in *Orange Hill Special* and 57.79% in *Elleta Campana*), limonene (3.16% in *Glueberry* ORG and 23.49% in *Gorilla Glue*), *β*-pinene (2.89% in *Elleta Campana* and 13.52% in *Orange Hill Special*), terpinolene (0.08% in *Passion* and 19.05% in *Orange Hill Special*) and *β*-Ocimene (1.00% in *Elleta Campana* and 9.53% in *Orange Hill Special*). Monoterpenoid fraction was followed by sesquiterpenes where caryophyllene was declared as the most abounded component present in different amount in the analysed cultivars (0.87% in *Amnesia Kush* and 7.75% in CBD *Charlotte's Angel*).

Conclusions

As high volatile aroma compounds, these identified components are probably important and responsible for the *Cannabis sativa* cultivars specific scent. Futhermore, terpenes analysis can be benifical for breeders and patients in order to selectively modulate the terpene rations of their cultivars and to identify the right strain for their symptoms.

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Bioautographic evaluation of selected Apiaceae essential oils

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Keywords: Apiaceae, bioautography, antimicrobial, antioxidant, essential oil

Objective

This study aimed to evaluate the antimicrobial and antioxidant bioauthographic activity of commercial *Anethum graveolens* L., *Foeniculum vulgare* Mill., *Apium graveolens* L., *Pimpinella anisum* L., *Coriandrum sativum* L., *Cuminum cyminum* L., *Petroselinum crispum* L., and *Illicium* verum L. essential oils, for possbile combinations.

Methods

Before activity evaluation, essential oil chemistry was analysed and confirmed by GC-FID and GC/MS, respectively. The *in vitro* antioxidant capacity of the essential oils was evaluated by using the DPPH• and ABTS• methods. The antibacterial potential was determined using the *in vitro* broth microdilution assay against methicillin resistant *Staphylococcus aureus, Candida albicans,* and *Streptococcus mutans*. In addition, antioxidant and antimicrobial activities of all essential oils were investigated bioautographically. The most active essential oils were combined with checkerboard assay.

Results

When compared *Anethum graveolens* essential oil was determined as the most effective essential oil using the DPPH radical on bioautographic assay. In the broth microdilution assay, the MIC value of *C. cyminum* essential oil prevented the growth of resistant microorganism at a concentration of 0.5 mg/mL. *C. cyminum* essential oil was effective at a very low concentration (0.98 µg/mL) against *S. mutans.*

Conclusion

C. cyminum and *Anethum graveolens* essentials oil showed strong effects against the tested human pathogenic microorganisms as combination.

ACKNOWLEDGMENTS

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Composition of wasabi flowers (*Eutrema japonicum* (Miq.) Koidz.) distillation products

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Keywords: wasabi, essential oil, hydrolate, volatiles, distillation

Objective

Eutrema japonicum (Miq.) Koidz. (syn. *Wasabia japonica* (Miq.) Matsum.) it is so-called wasabi or Japanise horseradish. It is a peranial plant which belongs to the Brassicaceae family. *E. japonicum* naturally grows on shaded, wet banks of cold moutains springs and streams. The paste made from wasabi rhizomes has been used for a long time in Japanes traditional food such as sushi and shashimi as pungent spice. Due ti this reason *E. japonicum* is cultivated on a large scale in Japan, South Korea, New Zeland and Taiwan. Literature studies have shown that *E. japonicum* leaves, rhizome and flowers extracts contains many different metabolites such as allyl isotiocyanate derivatives, flavonoids, phenylpropanoids and carotenoids. Nevertheless in scientific literature there is only few data about volatiles comounds isolated from wasabi flowers. That is why we chose *E. japonicum* as an interesting research material.

Methods

The *E. japonicum* flowers were collected from wasabi farm in central Poland. The plant material was harvested during flowering period in January and February 2021.
Dried wasabi flowers was shredded and subjected to Clevenger type apparatus. Then immerse in water plant material was distilled for four hours for essential oil production. The distillation was repeated three times to confirm the results.Teh essential oil composition was analysed using GC-FID-MS.

Results

From 300g plant material 1.7 mL of essential oil was collected. This product reveald pungent unpleasant aroma. According to GC-FID-MS analysis 97.2% of total oil was identyfied. Nitriles was the main group of constituents of in this product e.g. but-2-enenitryle (75.0%), 7-methylthioheptanonitrile (5.1%) and pent-3-enonitrile (1.0%). Isothiocyanates such as allyl isotiocyanate (5.7%), isopropyl isothiocyanate, sec-butyl isothiocyanate, hexen-5-yl isothiocyanate were also identifed. The presence of these compounds was also been confirmed in the literature for wasabi leaf extract. Trace amounts of terpenes were also identified in the analyzed oil.

Conclusions

Wasabi flower oil has a specific composition different from other essential oils, according to our previous experiments we suspect its application as bio-herbicide what will be investigated in our further investigations.

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Essential oils from cones of coniferous trees – composition, biological properties and potential application in nanoemulsions

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Keywords: essential oil, cone, tree, *Abies*, *Spruce*

Objective

Trees, including conifers, constitute not only a renewable source of valuable wood but also of a variety of natural products, semi-products and chemical substances used in many branches of industry. Hence, a priority of the work was the determination of cone volatiles with their possible use as potential components of cosmetics and fragrances, while increasing use of so-called biomass (cones) generated during regular felling. The reseach has to expand the modest knowledge on content and composition of cone essential oils (EOs) of many species od coniferous trees. This work is based on over ten years of research based on the determination of composition of cone essential oils of fir species such as: *Abies alba, A. koreana, A.cephalonica, A. arnoldiana, A. nordmanniana, A. concolor,* one specie of *Tsuga canadensis* as well of spruce species: *Picea abies, P. pungenes* and *Picea orientalis*. Selected essential oils were assessed in terms of their biological activity both cytotoxicity (normal skin cells and melanoma cell lines) and antimicrobial properties. The EOs from different tree's parts of some above genus are used commercially in the cosmetic and fragrance industry, especially the oil from fir cetin. In our work for the first time selected cone essential oils of interesting, forest-like, resinous with citrus note scent were used as fragrance agents in nanoemulsions.

Methods

Methods used for isolation essential oils (EOs), determination of EOs composition, determination of EOs cytotoxicity as well antimicrobial properties have been described in literature [1].

Results

Tested essential oils were exhibited moderated antimicrobial properties. Determination of cytotoxicity of the cone essential oils was assessed in normal skin cells (fibroblasts, keratinocytes) and in melanoma cell lines. Cell viability was not affected by tested cone EOs in all cell lines. Thymol used as a reference agent was significantly more cytotoxic than tested oils (IC50 values in range 50-65 μg/mL) indicating safety of essential oils. Moreover, incubated with essential oils in a concentration up to 50 μg/mL was not impaired whereas it was reduced by 30% after exposition to 50 μg/mL of thymol. Tested essential oils did not interfere with one of the most fundamental function of fibroblasts in the skin - collagen biosynthesis in fibroblast. Cones of firs are good source of essential oil while cones from spruces and hemlock contained small amount of volatile terpenes. All EOs were mainly composed of monoterpenes and their oxygenated derivatives.

Conclusions

Bioassay screening of selected essential oils showed that cone EOs were safe for human skin and can be use as potential flavour agent in nanoemulsions, however only for EO from cones of *Abies nordmanniana* and *Picea pungens* which contained slightly higher amount of volatile alergenes, these volatiles are part of the Cosmetic Regulation with labelling (INCI) and concentration restrictions.

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Importance of chemical descriptors. Portuguese fennel and oregano

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Keywords: Chemical descriptors, medicinal and aromatic plants, essential oils, fennel, oregano

Objective

Sharing information on countries plant resources is important to academics, stakeholders (farmers, producers, retailers, etc.) and policy makers. To share information, it is necessary to collect, record and describe data uniformly, to be comparable. There are nowadays six types of descriptors [1,2] that mostly gather morpho-agronomic characters (such as plant's height, flowering patterns, among others), but also genetic marker data and traditional knowledge. Given the economic importance of medicinal and aromatic plants, and some of their products, such as essential oils, it is becoming evident the relevance of gathering information on countries plant resources chemical variability to complement the existing descriptors. The goal of this work was to evaluate the chemical variability of fennel and oregano maintained, respectively, at Banco Português de Germoplasma Vegetal (BPGV) and at Escola Superior Agrária de Elvas (ESAE) experimental fields.

Methods

The essential oils (EOs) and hydrolates isolated from nineteen plant samples (accessions) of fennel fruits and fourteen of oregano flowering aerial parts, were analyzed by Gas Chromatography (GC) and GC coupled to Mass Spectrometry (GC-MS). EOs composition was used to determine the relationship between samples as in [3].

Results

Cluster analysis showed high correlation between fennel samples, which were dominated by estragole (= methyl chavicol, 23-76%), *trans*-anethole (1-40%) and fenchone (14-30%) in cluster 1, and *trans*-anethole (44-56%), fenchone (21-34%) followed by estragole (3-23%) in cluster 2. The main volatiles of fennel fruits included the phenylpropanoids estragole (3-76%) and *trans*-anethole (1-56%). EO cluster analysis evidenced low correlation between the two oregano samples clusters, with the dominance of monoterpene hydrocarbons in cluster 1 (10-76%), particularly γ-terpinene (2-32%) and *p*-cymene (5-27%). In cluster 2 oxygen-containing monoterpenes were the main volatiles (45-91%), namely linalool (0-84%) and carvacrol (0-52%). Thymol was dominant in cluster 1 (11-50%).

Conclusions

The obtained results highlighted the occurrence of chemotypes and the importance of complementing current descriptors with chemical data.

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Effects of immortelle essential oil and its side products on plant germination

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Keywords: Immortelle, Helichrysum, essential oil, hydrosol, decoction, side-products, germination

Objective

This study analyzed the chemical profiles of immortelle (*Helichrysum italicum* (Roth.) G. Don) essential oil, hydrosol, and decoction obtained by industrial distillation from aerial parts of plants from Montenegro and germination inhibitory effects.

Methods

The chemical profiles of oils and hydrosol were determined by gas chromatography, while the profiling of decoction was approached by liquid-mass chromatography. The germination inhibitory effects were tested on white mustard, spring wheat, and great millet. Content of chlorophyll *a*, chlorophyll *b*, carotenoids, sugars, phenolic compounds, and proline was assessed spectrophotometrically.

Results

The major components of the essential oil were α-pinene, γ-curcumene and *ar*-curcumene, while the most dominant components of the hydrosol were italidione I, italidione II, and allyl heptanoate. The most abundant polyphenols in decoction were three caffeoylquinic acid derivatives, acetosyringone, and arzanol. The essential oil did not inhibit the germination of white mustard and spring wheat while the 100% decoction caused a significant inhibition of seedlings' growth. Quantitative changes in chlorophyll *a*, sugars, phenolic compounds, and proline were observed following the seedlings' essential oil and decoction treatments.

Conclusions

An inhibitory effect of immortelle decoction places it among the potentially useful waste products that could find application in sustainable agriculture as a botanical pesticide.

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Chemical composition of Essential oils from different parts of *Echinophora orientalis* Hedge & Lamond in Turkey

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Keywords: *Echinophora orientalis,* Apiaceae, essential oil

Objective

Six species of the genus *Echinophora* (Apiaceae), including three endemics, are found in Flora of Turkey. *E. orientalis* Hedge & Lamond has been reported to be added to "helva," a Turkish sweet, to impart fragrance and tenderness. *E. orientalis* called Narrow Leaves Prickly parsnip and East Prickly parsnip in Iran. The antioxidant properties of essential oil of leaves, roots, and grains of this species have been reviewed, where the highest antioxidant activity is related to the root [1,3].

Methods

In this study, the stem, flowers, aerial parts and root of *Echinophora orientalis* were collected in Van, Turkey. The plant material was identified by the Prof. Dr. Osman Tugay (KNYA17.742). The essential oils (EOs) of *E. orientalis* different parts were obtained by hydrodistillation using a Clevenger type apparatus for 3h. Essential oils were analysed both by GC-FID and GC-MS, simultaneously.

Results

The GC-FID and GC-MS analysis showed that major components of aerial parts, flowers, and steam essential oils were myrcene (50.2, 56.2, 48.7%); α-phellandrene (9.9, 11.9, 13.3%), and p-cymene (18.6, 7.7, 13.5%), respectively. Myristicin (42.5%), terpinolene (38.1%) and myrcene (5.8%) were found as the main constituent of the EO of root.

Conclusion

To the best of our knowledge, no chemical compositions of the essential oils of *E. orientalis* flowers and stem have been reported in the literature.

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Chemical composition of *Ruta chalepensis* L. Essential oil from Turkey

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Keywords: *Ruta chalepensis*, rutaceae, essential oil

Objective

The genus *Ruta*, known as common rue, belongs to the Rutaceae family, which features many shrubby plants that are native to the Mediterranean region and are represented by 40 species in the world [1]. Out of these 40, only two species *Ruta chalepensis* L. and *Ruta montana* L., are widespread in Turkey [2]. *R. chalepensis* is commonly called 'kokar sedefotu' (psoriasis herb) in Turkey and is used in decoction as emmenagogue and analgesic [3].

Method

In this study, the aerial parts of *Ruta chalepensis* was collected in Alanya-Antalya, Turkey, in 2021. The plant material was identified by the M.M. Hürkul (Herbarium number: AEF30941). The essential oils (EO) of *R. chalepensis* aerial parts was obtained by hydrodistillation using a Clevenger type apparatus for 3h. Essential oils were analysed both by GC-FID and GC-MS, simultaneously.

Results

Hydrodistillation of the aerial parts of *R. chalepensis* yielded 0.027 % of yellowish oil with strong odor. Twenty-three compounds from the essential oil were identified using GC and GC-MS which represented 98.6% of the oil of *R. chalepensis*. The major components of EO were 2-undecanone (75.3%), and 2-nonanone (12.6%).

Conclusion

The chemical composition of *R. chalepensis* essential oil from different parts of the world (Turkey, North India, Algeria, Tunisian, and Iran) have been reported by many researchers. The major compounds of essential oils were reported as 2-undecanone and 2-nonanone [4]. Our study showed similar results to the literature.

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A series of esters of diastereomeric menthols: comprehensive mass spectral libraries and gas chromatographic data

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Keywords: diastereomeric menthols, menthyl esters, EI Mass Spectral Libraries, GC RI Libraries, MS and GC data collections

Objective

Gas chromatography–mass spectrometry (GC–MS) is commonly used and accepted for the unambiguous identification of compounds based on the use of mass spectral matching in combination with chromatographic retention data and co-injection with pure reference compounds.
The scarcity of references and literature dealing with the mass spectral and GC-retention data implies that some of these compounds remain most likely unidentified and/or even misidentified, particularly when they are present in mixtures of dozens and often hundreds of volatiles, such as essential oils and food aroma extracts. In many cases, the identification of 'known unknowns' remains a complex problem, particularly in the analysis of compounds with significant inter-laboratory differences in RI data or in cases when no literature RI values are available. There are many cases where even the co-injection with pure reference compounds does not allow successful identification, as will be shown in this work [1].
In this context, the aim of this study was the creation of suitable mass spectral and gas chromatographic data libraries for a series of esters of diastereomeric menthols – menthol, isomenthol, neomenthol, and neoisomenthol – compounds with olfactory properties that have been used as flavoring agents in a range of food products, cosmetics, tobacco, and perfumery, as well as in pharmaceutical and other branches of industry.

Methods

A series of esters of diastereomeric menthols was obtained by synthesis using the Steglich approach and analyzed by GC–MS using both non-polar and polar capillary columns.

Results

RI libraries of about 150 compounds – a series of esters of menthol, isomenthol, neomenthol, and neoisomenthol with selected acids – were created utilizing both non-polar (HP-5MS) and polar (HP-Innowax) capillary columns [1]. Also, MS libraries containing electron ionization MS recorded on single quadrupole as well as on quadrupole ion-trap mass detectors, that can be used for automated profiling, together with the RIs on non-polar and polar columns were compiled [1]. The results point out to frequent misidentification of neoisomenthyl acetate as isomenthyl acetate in the literature and the means how to resolve this issue was suggested.

Conclusions

A comprehensive data collection of mass spectral and gas chromatographic data of a series of esters of diastereomeric menthols, in total 150 chemical entities, was created. Also, MS libraries containing electron ionization MS recorded on single quadrupole as well as on quadrupole ion-trap mass detectors together with the RIs on non-polar and polar columns were compiled. The outcomes of this study provide chemists and food technologists a useful tool in the field of food analysis of compounds with important food aroma properties.

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Chemical Investigation on *Salvia officinalis* L. Affected by Multiple Drying Techniques - The Comprehensive Analytical Approach

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Keywords: sage, odour-active compounds, dehydration, quality, essential oils, low molecular acids, higher terpenoids

Objective

In light of large scale production of *Salvia officinalis* L. and its complex storage and delivery chain, the efficient preservation process is required. At this moment, the most popular preservation method is drying, therefore a comprehensive experiment to evaluate the influence of multiple drying techniques on sage was conducted. Convective drying at 40, 50 and 60 °C, vacuum-microwave drying with powers 240, 360 and 480 W and combined drying consisting of convective pre-drying at 50 °C followed by vacuum-microwave finish-drying with power 360 W were applied. To evaluate the effect of particular procedures chemical analyses were performed, namely evaluation of changes in bioactive volatile constituents, odour-active compounds and various groups of non-volatile bioactive constituents of sage. The obtained results shown, that to receive the product with expected quality, it is necessary to identify the designation of the product before treatment, since particular groups of phytochemicals react differently during drying.

Methods

Sage leaves were subjected to three different drying protocols, namely, convective drying (CD), vacuum-microwave drying (VMD) and combined drying consisting of convective pre-drying and vacuum-microwave finish-drying (CPD-VMFD). Hydrodistillation was then performed to extract essential oils, the prepared samples were analysed using GC-MS (Shimadzu GCMS QP 2020, Kyoto, Japan) equipped with a Zebron ZB-5 MSi capillary column (30 m×0.25 mm×0.25 147 μm; Phenomenex, Torrance, CA, USA). Low molecular acids (by GC-MS), higher terpenoids (by GC-MS) and rosemary acid (by LC-MS) content were also analysed.

Results

Depending on the analyzed group of compounds, the most optimal drying methods were: for odour-active compounds (CD 60 °C and VMD 240W), for low-molecular acids (CD50/VMD360W), for higher terpenoids (CD 50 °C and VMD 480W), for volatile bioactive compounds (CD 40 °C), for rosmarinic acid (CD 40 °C), for total essential oil (CD 50 °C and VMD 480 W).

Conclusions

The comprehensive experiment, evaluating various drying techniques in light of multiple chemical analyses, was performed due to illustrate the influence of particular treatments on the quality of *Salvia officinalis* L. The obtained results allowed to predict, which treatment would be optimal for particular groups of sage phytochemicals. As it was shown in our research, the issue of choosing appropriate drying technique and its parameters is a complex task. It would be extremely hard to identify one optimal method due to receive the highest retention of all valuable phytochemicals, thus, the drying method and its selection has to be dependent on the designation of the dried *S. officinalis* product. The other aspect is the economic profitability of the chosen drying procedure, what was not considered in this study, however will have a significant influence on this choice.

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Evaluation of Several *Lavandula* species for Antimicrobial and Antioxidant Activity with TLC Bioautography method

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Keywords: Lamiaceae, Linalool, Essenital oil, Bioautography, Antimictobial

Objective

The aim of this study was to identify the chemical composition and evaluate the antioxidant and antimicrobial activity of *Lavandula angustifolia* Mill., *Lavandula stoechas* L., and *Lavandula* × *heterophylla* Viv. against different human pathogenic microorganisms.

Methods

The antioxidant and free radical scavenging activities of *L. angustifolia*, *L. stoechas*, *L. heterophylla* and their charasterictic and main compound linalool was carried out by TLC-bioautography method based on the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays to compare linalool and oils main active constituents. The antimicrobial activity of the essential oils samples and linalool were tested using the *in vitro* broth microdilution assay towards two different microorganisms. Methicillin-resistant *Staphylococcus aureus* and *Streptococcus mutans* were used for the study.

Results

In previous studies with same essential oil samples, chemical analysis of *L. angustifolia* essential oil showed four main components including linalool (22.4%), linalyl acetate (19.2%), camphor (17.9%), 1,8-cineole (12.3%). *L. stoechas* oil contained predominantly camphor (54.7%) and bornyl acetate (21.41%). The major components of *L. heterophylla* essential oils were linalool (30.6%), linalyl acetate (19.6%), camphor (15%), 1,8-cineole (11.3%). They were found to be active against both the microbes used for the activity. The minimum inhibiting concentration (MIC) of *L. angustifolia*, *L. stoechas*, *L. heterophylla* essential oils against methicillin-resistant *S. mutans* and *S. aureus* was calculated as 0.031, 0,015, 0.015 and 1, 0.5, 0.5 mg/mL respectively. The essential oils and linalool were found to be effective both against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical on bioautographic assay.

Conclusions

The overall, *L. angustifolia, L. stoechas*, and *L. heterophylla* essential oils studied here exhibited potent free radical scavenging and antimicrobial activities in a series of *in-vitro* tests. The bioautography screening of antioxidant compounds led to the identification of linalool as the major antioxidant constituent of the oil. In addition, *L. angustifolia, L. stoechas*, and *L. heterophylla* essential oils showed strong effects against the tested human pathogenic microorganisms on bioautography method with the microdilution assay.

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Antioxidant and Antimicrobial Activities of Four *Origanum* Species with Bioautography Assay

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Keywords: *Origanum*, antioxidant, antimicrobial, bioautography, carvacrol

Objective

Origanum, a genus of important medicinal and aromatic plants, belongs to the family Lamiaceae which includes various species with characteristic odor and flavor of flowers and leaves. The genus *Origanum* naturally occurs in the northern hemisphere and mostly it is distributed in the Mediterranean region. There are 28 species (32 taxa) in Turkey. [1,2]. In this study, four different *Origanum* essential oils (*O. majorana, O. minutiflorum, O. vulgare,* and *O. onites)* were investigated according to their antimicrobial and antioxidant activities with bioautographic assays, and their chemical constituents evaluated.

Methods

For the experiment, analytically approved essential oils were bought from Turkey. Antioxidant activities of the essential oils were evaluated with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic) acid (ABTS) bioautography methods. DPPH solutions were displayed in the form of yellowish fluorescent bands with purple background, and ABTS solutions were displayed in the form of colorless or pink spots with a green background, which were easy to be identified and were of high sensitivity [3,4]. The antibacterial potential was determined using the *in vitro* broth microdilution assay against methicillin-resistant *Staphylococcus aureus* and *Streptococcus mutans*. According to our knowledge TLC–fingerprinting of different *Origanum* species was studied before [5] however, this is the first time that it researches four different *Origanum* species from Turkish flora and compares the activity-chemotype relationship between them.

Results

According to the previous GC-MS study with the same essential oil samples, *O. majorana, O. minutiflorum, O. vulgare*, and *O. onites* essential oils showed a relatively high carvacrol percentage and linalool, γ-terpinene, p-cymene was found in different percentages in the analyzed essential oils [6,7]. The fingerprinting of essential oils obtained from four *Origanum* specimens was studied by thin-layer chromatography. *Origanum* essential oils and their major constituent carvacrol were found to be effective against both ABTS and DPPH radicals. In the broth microdilution experiment, the MIC value of *O. majorana*, *O. minutiflorum*, *O. vulgare*, and *O. onites* essential oils against methicillin-resistant *S. aureus* microorganism was calculated as 62, 62, 31, and 31 µg/mL respectively. Against *S. mutans*, *O. majorana*, *O. minutiflorum*, *O. vulgare*, and *O. onites* essential oils have a MIC value of 3, 3, 0.5, and 1 µg/mL respectively.

Conclusions

TLC–fingerprinting of four different *Origanum* species was performed for the first time as a comparitevely study and enabled the discrimination of chemotypes. TLC–bioautography showed that carvacrol is the main common antioxidant compound in tested *Origanum* sp. essential oils. Moreover, some spots didn't determine under the TLC but showed significant antioxidant activity. In conclusion, *Origanum* essential oils showed potential against the tested human pathogenic microorganisms.

Analysis of lipid profiles of mycobacteria stressed with cinnamaldehyde from cinnamon essential oil

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Keywords: cinnamaldehyde, Mtb, metabolomics, natural products

Objective

Cinnamaldehyde is natural organic compound found in large quantities in essential oil obtained from bark of cinnamon trees (*Cinnamomum verum* J.Presl) and other species of the genus Cinnamomum like *C. cassia* (L.) J. Presl and *C. camphora* (L.) J. Presl. Essential oil of cinnamon bark contain up to 90% cinnamaldehyde [1], which posseses antimycobacterial activity proved for laboratory strains (H37Rv and H37Ra) [1-2] and for clinical isolates [3]. What is more, cinnamaldehyde was shown to threaten the mycobacterial plasma membrane integrity and to activate bacterial stress response system [1]. To get more insight into mechanism of action of cinnamaldehyde against mycobacteria (*Mycobacterium tuberculosis* H37Ra) we determined differences in lipid profiles of bacteria stressed with this compound in comparison of control culture.

Methods

Bacteria were exposed to the action of cinnamaldehyde for 24h. Then biomass was harvested, lipids extracted and subjected to untargeted LC-MS analysis [4]. Features were defined as a three-dimensional value of m/z, retention time (RT) and intensity (cloud plots). Then, features with equivalent mass and retention time in test and control samples were aligned what enabled pair-wise comparison of MS signal intensity to monitor the metabolites that were either increased/decreased or present/absent.

Results

Lipophilic extracts subjected to lipids analysis showed that majority of detected compounds belonged to nonpolar glycerolipids (24%) and polar glycerophospholipids (57%). Less abundant classes of compounds were fatty acyls, saccharolipids, polyketides and prenol lipids. The comparison of test samples to control showed predominant upregulation in almost all classes of described lipids.

Conclusions

The current study revealed that bacteria exposed to cinnamaldehyde reorganized outer membrane as a physical barrier against stress factor. They lowered cell wall permeability and inner membrane fluidity and redirected carbon flow to store energy in triacylglycerols. Based on our findings cinnamon essential oil can be useful as an adjuvant for the eradication of mycobacteria. This is a new application of this natural product extracted from cinnamon bark commonly used in the kitchen.

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Fungicidal Effects of Essential Oils in Low Oxygen Environments: An innovative approach for Heritage Conservation

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Keywords: heritage conservation, fungicidal properties, low oxygen environment, encapsulated essential oils

Overview

A common problem among art and museum collections worldwide is the deterioration of different organic materials (such as fur, skin, stuffed animals filling) by different organisms (mainly insects and fungi) which thrive on them, causing structural and aesthetical damage [1]. Aggressive disinfection treatments are not suited for most heritage items since they often bleach, degrade or otherwise affect their integrity. An alternative is the use of Low Oxygen Environments (LOEs) where oxygen is replaced with another gas (nitrogen) in a sealed container. This is efficient against insects but has limited effects on fungal organisms, especially their spores. Many essential oils (EOs) display fungicidal properties and have been applied to cultural heritage conservation. Nevertheless, their effect on spores is also limited. This project aims to develop a mitigation strategy that is eco-friendly, cost-effective and applicable to a large number of items simultaneously, by enriching LOEs with volatile compounds from EOs with known fungicidal effects. The synergistic effects of simultaneous oxygen deprivation and biochemical effects caused by EOs' organic compounds should increase the effectiveness of the disinfection of active fungi, spores and hyphae development. This work will start with sampling museum items and the isolation and identification of different fungal organisms present. Then, degradation essays will be performed *in vitro* to assess the ability of obtained isolates to degrade materials from which they were retrieved, separating casual and ubiquitous species from relevant, potentially damaging ones. In parallel, a careful selection of EOs will be made (e.g. *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L. and *Thymus vulgaris* L.) attending to their known fungicidal effects (against *Aspergillus niger*, *Cladosporium* spp., *Penicillium* spp) [2]. These EOs will be isolated by hydrodistillation following chemical characterization. They will then be encapsulated into suitable nanomatrices for application in-line within the LOEs gas inlet to maximize their vaporization into the artificial atmosphere. After the selection of organisms and EOs encapsulation strategies to be tested, *in vitro* essays will be performed to assess the fungicidal effects of the EOs under low oxygen conditions, especially concerning fungal growth, conidial development and spore inactivation. These essays will be followed by in situ tests using actual contaminated items from the museum, finishing our proof of concept.

Conclusions

If application is successful, and good results are attained, we will proceed with the development of adequate guidelines for its application by other institutions since many of them already have an anoxy equipment in-house.

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Inhalation of Lavender aroma oil reduces frequency of urination through reduced serum adrenaline and noradrenaline levels in urethane-anesthetized rats

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Keywords: Lavender, nocturia, urinary frequency, monoamines, aroma oil

Objective

Lavender oil has been long used as a relaxant especially for those with sleeping disorders. Since nocturia is closely associated with sleeping disorders, we examined how inhaling lavender scent would affect the frequency of urination in rats under light urethane anesthesia, as well as the monoamine concentrations in the serum.

Methods

Twenty-four rats were anesthetized with 0.6 g/kg urethane injection in the subcutaneal and intraperitoneal areas to induce a sleep-like state. Cystometry was performed via transurethral catheter before, during and after inhalation of lavender essential oil (n=8), or the Peace & Calming Blend essential oil (n=8), for 120 min. The control group (n=8) underwent cytometry but did not undergo inhalation treatment. After the 120 min of treatment, blood was collected from the three groups and serum monoamine levels were measured

Results

In the lavender inhaled group, the intervals between the bladder contractions as well as the urinary volume were significantly increased. In addition, the bladder capacity, which is derived from the urinary volume and the residual volume, increased in the lavender treated group. Other parameters did not differ significantly to that of the control group. As for the blend oil (Peace & Calming) treated group, there were no significantly altered parameters as compared to the control group. The monoamine analysis shows that serum adrenaline and noradrenaline levels (but not serotonin) significantly decreased in the lavender treated group compared to the control group. Peace & Calming treated group did not show any significant changes to the parameters as compared to the control group.

Conclusions

It was observed that the lavender scent may reduce the frequency of by regulating the sympathetic nervous system through reduced serum adrenaline and noradrenaline levels.

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pH-responsive Citral Nanocapsules with Tannic Acid-Fe^{III} Coordination Complexes

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Keywords: flavour, tannic acid-Fe^{III} coordination complexes, pH-responsive release, antioxidant, antibacterial

Objective

Flavour, especially for aroma compounds existed in citrus essential oil, is prone to volatize and oxidize. This study aimed to contruct a pH-responsive delivery system for citral encapsulation. Citral encapsulated tannic acid-Fe^{III} nanocapsules (citral@TA-Fe^{III}) were prepared to control the release behaviour, improve the chemical stalibity, maintain the odor attribute, and enhance the antibacterial effects of citral.

Methods

Citral@TA-Fe^{III} nanocapsules were prepared on citral emulsion droplets through the coordination interaction of TA and Fe^{III}. The morphologies and loading amount of nanocapsules were determined by transmission electron microscopy (TEM) and thermogravimetric analysis (TGA). The release behaviour, chemical stability, odor attribute, of citral were investigated by ultraviolet-visible (UV-vis) absorption spectra, gas chromatography (GC), and sensory evaluation, respectively. The antibacterial effects of nanocapsules were calculated by plated counting method.

Results

Citral@TA-Fe^{III} nanocapsules were obtained with core-shell structures with the average diameter of about 444 nm. The loading amount of citral in nanocapsules was 12.79%. The release behaviour of citral was pH-responsive which reached 38.2% at pH 7.0, 52.2% at pH 5.0, 86.6% at pH 3.0 after 24 h. The total phenolic content of capsules was 108.68 mg TAE g^{-1}, indicating the phenolic groups of TA was maintained in capsules. Capsules showed excellent antioxidant activities with the DPPH radical scavenging rates of 53.54%. After storage for 14 days at 37 ^{o}C, the citral retention rate of capsules was significantly higher than that of emulsion, the lemon-like odor was well preserved and the gasoline-like and bitter almond-like odor were retarded. Capsules showed strong antibacterial effects against *S. aureus* and *E. coli*.

Conclusions

In this paper, flavour capsules with pH-responsive, antioxidant, and antibacterial shells were presented. Citral as a model flavour compound could release in a controllable way by changing the environment pH. Compared with emulsion, the chemical stability of citral was significantly improved, fresh lemon odor was well maintained, and off-odor was effectively inhibited after storage. The design of citral@TA-Fe^{III} nanocapsules could unlock a simple and straight encapsulation method of flavour and fragrances and widen their multifunctional application.

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Litsea cubeba essential oil pickering emulsion stabilized with epigallocatechin gallate loaded mesoporous silica nanoparticles

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Keywords: *Litsea cubeba* essential oil, mesoporous silica nanosphere, Pickering emulsion, protective, oxidation, stability

Objective

The oxidation of *Litsea cubeba* essential oil in food emulsions remains challenging to keep food quality and shelf-life. This study aimed to achieve a dual stabilization to both oil phase and antioxidant in Pickering emulsions. *Litsea cubeba* (*Litsea cubeba* (L.) Pers.) essential oil (WD) from China Pickering emulsions were prepared with epigallocatechin gallate (EGCG) loaded mesoporous silica nanopsheres (MSN) as emulsifier to improve the physical stability, chemical stability and olfactory attribute of Pickering emulsions.

Methods

MSN were prepared via sol-gel method with CTAB as template and cyclohexane as pore swelling agent. Pickering emulsions were prepared by mixing *Litsea cubeba* essential oil with EGCG@MSN water dispersion under ultrasonic treatment. The physical stability, chemical stability, and olfactory attribute of Pickering emulsions were obtained by optical microscopic images, gas chromatography-mass spectrometry-olfactometry (GC-MS-O).

Results

MSN were obtained with uniform nanosphere structures with the average diameter of about 80 nm. The specific surface area, pore volume and average pore size were 557.47 m^2 g^{-1}, 0.93 cm^3 g^{-1} and 2.62 nm, respectively. When EGCG was 0.8 g g^{-1}, EGCG loading amount was 276.23 mg g^{-1} and the contact angle of EGCG@MSN was 79±1°. The average droplet size of Pickering emulsions was 1.35±0.02 μm which was stable for 4 weeks. Temperature and pH value had less impact to any phase separation. Cis-limonene oxide, cis-linalool oxide, and 3-methyl-2-butenal are the oxidation products of *Litsea cubeba* essential oil. The contents of these compounds were significantly decreased after storage compared with emulsions stabilized by Tween 80. The floral and fruit notes of Pickering emulsions after storage were maintained and the pungent and fatty notes were inhibited.

Conclusions

In this paper, a dual stabilization strategy of Pickering emulsion, to stabilize the encapsulated *Litsea cubeba* essential oil phase and antioxidant loaded on emulsifier was presented. EGCG loaded MSN physically and chemically stabilize the emulsion. The emulsions with various amount of emulsifier and oil under various pH and temperature were uniformly stable with at least one-month observation. The odors and volatile components of *Litsea cubeba* essential oil in emulsion were well preserved, and much better than conventional emulsion. This strategy could inspire new designs for functional Pickering emulsions.

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Clinopodium nepeta L. Kuntze Aerial Parts Essential Oil Antiproliferative Activity

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Keywords: *Clinopodium nepeta* L. Kuntze, essential oil, hydrodistillation, antiproliferative activity, MTT test

Objective

Since ancient times, plants, as well as their essential oils or extracts, have been used for various purposes in ethnomedicine. Natural-based products have become popular for their simple and cheap preparation due to their origin, variety and availability. Compared to pharmaceutical drugs they are considered safer and exhibit beneficial therapeutic effects [1]. The objective of this study was to determine antiproliferative activity of four hydrodistilled essential oils of *Clinopodium nepeta* L. Kuntze (sub-Medıterranean part of Bosnia and Herzegovina) against androgen-sensitive prostate cancer cells (PC-3) and androgen-insensitive prostate cancer cells (Du-145).

Methods

Clinopodium nepeta L. Kuntze (family Lamiaceae) was classified in different genera (*Calamintha*, *Satureja*, *Thymus*), but eventually into genus *Clinopodium* which was accepted by Euro+Med PlantBase [2]. Samples of *C. nepeta* aerial parts (flowers and leaves) were collected during the flowering period in August 2021. in southern areas of Bosnia and Herzegovina. Hydrodistillation was performed with a modified Clevenger type apparatus for 2 h from 50 g of shade-dried plant material. Obtained essential oil (EO) was dried using anhydrous sodium sulphate and kept at 4 ºC in proper dark glass containers, until the analysis. The MTT cytotoxicity assay was used to investigate cellular metabolic activity as an indicator of cell viability and expressed as IC_{50} value.

Results

The results showed that the tested EOs have high toxicity against the *in vitro* tested malignant cells [3]. The highest cytotoxicity showed *C. nepeta* EO against androgen-insensitive prostate cancer cells. The best results were those for EOs of *C. nepeta* from Počitelj and Lokve with IC_{50} values ranging from 3.06±0.08 to 5.06±0.65 μg mL^{-1} for both investigated malignant cells. EO of *C. nepeta* from Blagaj showed potentially high toxicity against PC-3 and Du145 cells with IC_{50} from 13.27±0.24 to 17.42±0.81 μg mL^{-1}[3]. These results are in correlation with the traditional use of *C. nepeta* in Bosnia and Herzegovina and its beneficial effect on the treatment of various diseases. In relation to the sensitivity of malignant cell lines to the cytotoxic effect of the EO, it should be noted that Du145 cells were more sensitive compared to PC-3 cells.

Conclusions

It is obvious that *C. nepeta* EO have significant cytotoxic potential against both tested cancer cell lines. Furthermore, due to absence of research results on *C. nepeta* EOs antiproliferative activity, this paper has presented preliminary data that is particularly interesting given the proven medicinal properties of this plant and their usage in phytotherapy and traditional medicine.

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Litsea cubeba essential oil pickering emulsion stabilized with epigallocatechin gallate loaded mesoporous silica nanoparticles

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Using biostimulants as a mitigating strategy for hail-damaged rose geranium

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Keywords: brassinosteroids, cytokinin, essential oil, gibberellic acid, hail damage simulation

Objective

Hailstorms cause significant yield and financial losses to the agricultural sector. There are reports that hail can cause severe damage to rose geranium (*Pelargonium graveolens* spp.) plants. There are currently no mechanisms in place to alleviate hail-damaged essential oil plants, such as rose geranium. This study aims to develop a strategy for hail damage, using agricultural biostimulants as an alleviating remedy for hail-damaged rose geranium.

Methods

The first experiment was conducted using combined agricultural biostimulants (cytokinin [CK] + gibberellic acid [GA]). Treatments comprised of three simulated hail damage levels: zero% defoliation (intact plants); 50%; and 100% defoliation, as well as two levels of biostimulants: CK 0.32 mg/L + GA 150 mg/L (low); CK 0.64 mg/L + GA 300 mg/L (high); and distilled water as a control. The second experiment was run simultaneously, using four experimental treatments of abscisic acid (ABA) and methyl jasmonate (MeJA): 75 µM ABA; 150 µM ABA; 10 mM MeJA; 20 mM MeJA; and distilled water as a control. In this experiment, the treatment was applied daily for either seven or 14 days, and the hail damage simulation was 100% defoliation only. The third experiment was conducted to determine the effects of agricultural biostimulants containing GA; brassinosteroids (BRs); and trace CKs on the recovery of three levels (low, medium, and high), and a control of simulated hail-damaged rose geranium plants (as before). All these experiments had a factorial treatment design, laid out in a randomised complete block design with three replications.

Results

From these studies, it was therefore recommended to consider applying a low to medium concentration of GA, BRs and traces of CKs to improve, and recover the growth of the herbage and essential oil quality (C:G ratio <3) of rose geranium plants with 100% foliage loss from hail damage. Overall, the highest mean biomass was recorded when the highest concentration of biostimulants were applied. Rose geranium plants that suffered 50% simulated hail damage mostly had higher mean essential oil yield compared to plants that suffered 100% simulated hail damage and treated with medium biostimulants. High-quality essential oil, with a C:G ratio below three, was recorded on all defoliated plants compared to the intact plants.

Conclusions

Rose geranium plants are grown for essential oil production; therefore, the aerial herbage material is a crucial component of the crop. It has been shown from these studies that hail can causes significant damage to these plants, reducing this valuable material, and directly affecting the essential oil yield and quality. This study contributed to the development of a mitigation strategy for hail-damaged rose geranium plants using agricultural biostimulants.

Comparative Evaluation of Chemical Composition and Antimicrobial Activity of Calendula *(Calendula officinalis* L.*)* Essential Oil and Absolute from Egypt

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Keywords: *Calendula officinalis*, essential oil, antimicrobial activity, chemical composition**.**

Objective

Calendula officinalis (fam. *Compositae*) plant is native to Central, Eastern, and Southern Europe. The main commercial sources are from Eastern Europe and Egypt [1]. The numerous pharmacological applications of Calendula cover anti-inflammatory, astringent, anti-hemorrhagic, cutaneous healing, antioxidant, anti-carcinogenic, immunological, and anti-infectious amongst other properties recognized by the EMA and the WHO [2]. Products derived from Calendula are foremost dedicated to herbalism (*e.g.* dried blossoms/ petals) and then to the production of "calendula oil" - a calendula macerate in fixed (vegetal) oils. The aromatic sector of *C. officinalis* comes last which is why it arguably offers the greatest growth potential. Examples of what such aromatics could be are concretes (solvent extracted), absolutes from concretes, diverse extracts (*e.g.* CO_2 SC extraction, other solvents). Never has an essential oil (EO) of Calendula by steam distillation seemed viable to this day. A. Fakhry & Co. was lately able to produce commercial quantities of Calendula EO filling that gap. To the best of our knowledge, this work is the first systematic study of the chemical composition and antimicrobial activity (against 11 bacteria and 2 fungi) of Calendula EO and absolute produced on an industrial scale.

Methods

Both Calendula EO and absolute were obtained from fresh flowers, sourced from the Fayoum and Beni Suef provinces in Egypt. The chemical composition of the oil and the absolute were evaluated by GC-MS on a GC-QP2020 system (Shimadzu, Kyoto, Japan). MIC values were determined in 96-well plates using the Alamarblue® viability assay protocol in quadruplicate.

Results

1 ton of fresh blossoms produced on average 0.2 kg and 1.5 kg of calendula essential oil and calendula absolute, respectively. Major constituents of Calendula EO are sesquiterpene hydrocarbons and their oxygenated derivatives: cadin-4-en-10-ol (27%), δ-cadinene (15%), epi-α-muurol (12%), γ-cadinene (5%), cubeban-11-ol (4%), α-muurolene (4%), viridiflorene (3.6%), copaborneol (2%). In Calendula absolute, the major constituents are fatty acids and their esters, sesquiterpene hydrocarbons and their oxygenated derivatives. Both Calendula EO and absolute show a wide antimicrobial activity against the tested bacteria and fungi. It was shown that Calendula EO is most active against *Pseudomonas aeruginosa* (ATCC 15442) with MIC 6,25 µg/ml, and the absolute against *Kocuria rhizophila* (ATCC 9341) with MIC 6.25 µg/ml.

Conclusions

Calendula EO presents the advantages of being 100% composed of itself (*i.e.* no additives, collateral support macerate material, etc.), obtained without the use of petroleum-based solvents but instead by steam distillation, standardizable under ISO/TC-54 standards. Further research is required to confirm its potential as a natural preservative, and as an aromatherapeutic material.

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Variation in Chemical Composition and Antibacterial Activities of Essential Oils from Two *Ocimum* Species

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Keywords: *Ocimum sanctum* L., *Ocimum gratissimum* L., essential oil, eugenol, caryophyllene, antibacterial

Objective

This study revealed variation in essential oil compositions from the different parts of two different *Ocimum* species and antibacterial effects of respective oils.

Methods

The flowers, leaves, stems and roots of *O. sanctum* and *O. gratissimum* were collected from Kushtia region, Bangladesh. The oils derived by hydro-distillation from the different parts were dried over anhydrous sodium sulphate (Na2SO4) and analysed by GC-MS (Model QP 2010, Shimadzu, Japan) furbished with a ZB-1 MS fused silica capillary column (30 m × 0.25 i.d., film thickness 0.25 µm). For antibacterial study of essential oil, five bacteria were used including three Gram-positive bacteria, methicillin-resistant *Staphylococcus aureus* (clinical isolate), *Klebsiella pneumoniae* ATCC 700603, and *Enterococcus faecalis* ATCC 291212 as well as two Gram-negative bacteria, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 35218 and all these bacteria were obtained from Department of Microbilogy, Islamic University, Kushtia, Bangladesh. The antibacterial test was carried out by disc diffusion assay as described by Chandrasekaran and Venkatesalu [1].

Results

Sixty seven components were identified from these species and eugenol (1.00-27.66%), methyl eugenol (0.78-28.52%), β-elemene (5.10-20.98%), caryophyllene (0.94-43.18%), caryophyllene oxide (1.1-10.81%), palmitic acid methyl ester (3.20-21.38%), oleic acid methyl ester (2.91-40.7%) and linoleic acid methyl ester (1.55-74.71%) were the most abundant components of the essential oils, but the percentage of most of the compounds in different species and different parts varied greatly. The essential oils contain monoterpenes, sesquiterpenes and aldehydes/ketonic derivatives in flower and leaf parts of *O. sanctum* and in the leaf part of *O. gratissimum*. The essential oils of these *Ocimum* species showed good antibacterial activities against the bacteria, methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli* with inhibition zones ranging from 10-31 mm.

Conclusions

The essential oils from flower and leaf part of *O. sanctum* and the leaf part of *O. gratissimum* contain rich active components e.g. monoterpenes, sesquiterpenes, aldehydes/ketonic compounds (especially eugenol). So, these essential oils were found to have higher inhibitory effect on the tested bacteria compared to the other oils of this study. Our study will help pharmaceutical, agro-chemical, and herbal medicinal industry to have a clear comparison on chemical compositions of different parts of *Ocimum* species and identify plant parts for extraction of most active essential oils.

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PP-A44

Phytochemical Composition of *Laurus nobilis* L. leaves, fruits and seeds essential oils from Mostar, Bosnia and Herzegovina

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Keywords: *Laurus nobilis* L., essential oil, hydrodistillation, GC/MS, chemical composition

Objective

Laurus nobilis L. belongs to the family Lauraceae, which comprises numerous aromatic and medicinal plants. It is commonly known as bay, sweet bay and true laurel and it is widely distributed in 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 [1]. 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 [2]. *Laurus nobilis* L. fruits EO are generally utilized for the production of perfumed soaps, antidandruff hair lotions and candle manufacture because of their chemical composition [2]. Leaves EO has proven antibacterial and antimicrobial properties [3].

Methods

Laurus nobilis L. was collected from Mostar, Bosnia and Herzegovina. In the present study, extraction of bay leaf, fruit and seed essential oils was obtained by hydrodistillation in Clevenger-type apparatus. Obtained EOs were dried over anhydrous Na_2SO_4 and stored in sealed dark vials until analysis. Phytochemical composition of EO volatile compounds was analysed by gas chromatography coupled with mass spectrometry (GC/MS).

Results

A total of 13 constituents were identified in leaf, 32 in fruit and 39 in seed *L. nobilis* EOs. In all three plant parts the major component was 1,8-cineole (leaves 50.66%, fruits 29.28% and seeds 28.06%). Other predominant components were linalool, bicyclic monoterpenes sabinene, α-pinene and β-pinene followed by monocyclic monoterpene α-terpenyl acetate. Components identified in the fruit and seed but not found in the leaf were (E)-β-ocymene, camphene, β-elemene, bornyl acetate and *trans*-caryophyllene.

Conclusions

In conclusion, our study has shown differences in numerous of identified compounds in essential oils obtained from *Laurus nobilis* L. different plant parts. Although there is not enough evidence about the biological role of 1,8-cineole, several studies have suggested that cineoles, particularly 1,8-cineole have inhibitory effect in germination antitumor activity on certain types of cancer cells by inducing apoptosis. Therefore, we will focus our further research on examining the antiproliferative activity of bay EOs on different human cancer cell lines.

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POSTER SESSION - B

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Subcritical extracts from main oil bearing roses - a comparative chemical profiling

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Keywords: subcritical freon extraction, gas chromatography-mass spectrometry, oil bearing roses, 2-phenyl ethyl alcohol, terpenes

Objective

The chemical composition of the rose extracts, similarly to the rose essential oil, should be strongly affected by the botanical and geographical origin of the raw plant material, environmental conditions, the production method (the extraction selectivity of the liquefied gases, for example) and storage. Therefore, the aim of the current study is to perform a comprehensive chemical profiling of 1,1,1,2-tetrafluoroethane (freon R134a) subcritical extracts from the main commercially grown oil bearing rose species, namely: *R. damascena* Mill. *R. gallica* var. officinalis Thory., *R. centifolia* L. and *R. alba* L., by means of gas chromatography and to compare their chemical compositions.

Methods

Extraction of the raw material (fresh rose flowers) was performed using 1,1,1,2-tetrafluoroethane in a pilot apparatus. Gas chromatography-mass spectrometry (GC/MS) and gas chromatography with flame ionisation detection (GC-FID) was used for the chemical profiling. The identification of the compounds was performed using commercial mass spectral libraries and retention times/indices.

Results

One hundred and three individual compounds were identified by GC/MS and their quantitative content was determined by GC-FID, representing 89.8 %, 92.5 %, 89.7 % and 93.7 % of the total content of *Rosa gallica* L., *Rosa damascena* Mill., *Rosa alba* L. and *Rosa centifolia* L. extracts, respectively. The compounds found in the extracts are representatives of the following main chemical classes: mono-, sesqui- and triterpenoids, phenylethanoids and phenylpropanoids, and aliphatic hydrocarbons. Fatty acids, esters and waxes were found, as well. The study revealed that 2-phenylethanol is the most abundant component, ranging 9.0 – 60.9 %, followed by nonadecane+nonadecene with 5.1 – 18.0 %, geraniol (2.9 - 14.4 %), heneicosane (3.1 - 11.8 %), tricosane (0.1 - 8.6 %), nerol (1.3 - 6.1 %) and citronellol (1.7 – 5.3 %). The extracts demonstrate specific chemical profile, depending on the botanical species – phenylethanoids and phenyl propanoids are the main group for *R.damascena*, aliphatic hydrocarbons - for *R. alba* and *R.centifolia*, while both are found in almost equal amounts in *R.gallica*. Terpenoid compounds content shows relatively broad variations: monoterpenes – 11.9 – 25.5 %, with maximum in *R.centifolia*; sesquiterpenes – 0.6 – 7.0 %, with maximum in *R.gallica* and triterpenes - 0.4 - 3.7 %, with maximum in *R.gallica* extract.

Conclusions

The chemical profile of subcritical (1,1,1,2-tetrafluoroethane) extracts from main oil bearing rose species, dominated by 2-phenylethyl alcohol in the range of 9.0 -59.3% brings them closer to the rose absolute and could be successfully used in cosmetics and aromatherapy.

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A comparative chemical profiling of Damask and Kushui rose essential oils, produsedin China

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Keywords: *R. damascena* Mill., Kushui rose, oil-bearing roses, gas chromatography-mass spectrometry, chemical profiling

Objective

China has the largest cultivation area in the world for oil-bearing roses, however, compared with Bulgaria and Turkey, China lags behind in essential oil production, regarding both amount and quality of the rose oil. The main species with industrial application in China are Kushui type rose, multi-petalled roses and Damask rose. The chemical composition of the rose essential oil is strongly affected by the botanical and geographical origin of the raw plant material, environmental conditions, the production method, etc. Therefore, the chemical composition and aroma profile of Chinese rose essential oils - four Damsk rose (*R. damascena* Mill.) essential oil samples from Uyghur, Xiujiang and a sample of essential oil from famous Kushui rose (*R.sertata* x *R. rugosa*), Gansu province, were studied by means of gas chromatography-mass spectrometry (GC/MS) and gas chromatography with flame-ionization detection (GC-FID).

Methods

The studied rose oil samples are industrial type essential oils, derived in 2017 (Kushui rose) and 2019 (Damask rose). Gas chromatography-mass spectrometry (GC/MS) and gas chromatography with flame ionisation detection (GC-FID) was applied for the chemical profiling. The identification of the compounds was performed using commercial mass spectral libraries and retention times/indices.

Results

Rose oil is a very complex mixture containing compounds with high structural diversity. GC/MS and GC-FID analysis of the aroma constituents present in the Chinese rose essential oils reveals, that *R. damascena* essential oil samples are, in general, not consistent with the International standard ISO 9842:2003 for the rose oil, and the chemical profile of Kushui rose oil differs most significantly. The most abundant class of aroma compounds presented in the Chinese rose oil are monoterpene alcohols: β-citronellol+nerol (42.66-45.35% in Damask rose and 37.75% in Kushui rose), geraniol (8.37 %), followed by linalool (2.37-1.49%). Phenylethyl alcohol, which is responsible for the characteristic rose-like odor of Rosaceae plants was found in relatively high amount in Damask rose oils (2.30%) and only in trace amount in Kushui rose oil – 0.05%. Eugenol and its methyl ether – methyl eugenol were found in much higher concentration in Kushui rose – 3.53 and 0.63%, respectively, than in Damask rose oils. The same was observed for farnesol (2.99 % in Kushui rose and 0.615 in Damask rose).

Conclusions

The comparative study of the chemical composition reveals higher amounts of characteristic aroma constituents in the essential oils from Damask rose, than in Kushui rose. In addition, the chemical profile of Chinese *R. damascena* rose oil samples differs significantly from Bulgarian *R. damascena* rose oil (ISO 9842:2003).

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Bio-insecticidal efficacy of the wild parsnip essential oil against the yellow mealworm

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Keywords: *Pastinaca sativa*, essential oil, *Tenebrio molitor*, contact toxicity

Objective

Yellow mealworm (*Tenebrio molitor* L., Coleoptera: Tenebrionidae) belongs to the most common as well as the most harmful grain pests. Larvae of yellow mealworm damagedifferent fractured grains of maize, wheat orsoybean, their metabolism products cause grainscontamination [1]. The study objective of the study was to evaluate bio-insecticidal efficacy of the wild parsnip (*Pastinaca sativa* L., Apiales: Apiaceae) essential oil against the yellow mealworm larvae, pupae and adults. Wild parsnip is an autochthonous plant species within the area of Slovakia and its wild form is found to be a natural component of the grassy biotopes. On the other side, species is found to be invasive within the area of North America, with proven consequences for the human and animal health [2].

Methods

Pure, 100% essential oil from the wild parsnip roots was commercially available (Luminescents Essential Oils, origin: India). Bio-insecticidal efficacy was tested in the contact toxicity test, when larvae, pupae and adults were exposed to six EO concentrations - 5%, 12.5%, 25%, 50%, 75% and 100%, responded to 0.05, 0.12, 0.25, 0.6, 0.74 and 0.98 mg/µL. As a solvent and control acetone was used. The 1 µL of the prepared solutions + 2µL and 3µL (1.96 and 2.94 mg/µL) of the pure 100% undiluted EO respectively was applied to the thorax of larvae, pupae and adults Mortality was determined in % based on data after 48 hours exposure [3].

Results

The dose dependence was observed, as the mortality increased continuously with the EO concentration. The highest average mortality was observed by the adults (25%), followed by pupae (24%) and larvae (18.75%). Lethal doses were as follows: LD50 1.39 mg/µL/LD90 4.39 mg/µL for adults, LD50 1.55mg/µL/LD90 8.03mg/µL for pupae and LD50 1.98 mg/µL/LD90 5.73 mg/µL for larvae. Larvae seems to be the most resistant to the wild parsnip EO contact toxicity.

Conclusions

In aim to replace dangerous and toxicfumigants and insecticides essential oils seem to bepossible tools for crops and stored productsprotection against insect pest, as the human andecological safely alternative in agriculture andintegrated pest management.

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Larvicidal activity of the three essential oils against drain fly (*Clogmia albipunctata*)

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Keywords: *Psychodidae*, essential oil, eucalyptus, lavender, lemongrass, larvicidal activity

Objective

Drain fly (*Clogmia albipunctata*, Diptera: Psychodidae: Psychodinae) is a very widespread species, that occurs in tropical and temperate climates all over the world. It is most commonly found in anthropogenic habitats such as bathrooms, kitchens and sewers. It is a non-hematophagous species, however, drain fly poses human health risk associated mainly with the mechanical transmission of various pathogens and is one of the insects that cause accidental myiasis [1,2,3]. (Rupprecht et al. 2020, Zittra et al., 2020, Oboňa et al., 2021). On the other side, various essential oils is used for the household cleaning as an ecological alternative to conventional cleaning products. In our study we tested, if one of the most used EOs i.e. *Eucalyptus globulus* L., *Cymbopogon flexuosus* L. and *Lavandula angustifolia* L. can somehow limit the survivorship of the drain flies within households. To our very best knowledge, this is the first time, when such a testing with *Clogmia albipunctata* larvae was realized.

Methods

Third instar larvae of drain fly from our own breeding were exposed to 2.85, 5.7 and 8.55 µl of eucalyptus, lemongrass and lavender EO, dissolved in 1 ml of 2% DMSO, corresponding to 2.7, 5.5 and 8.2 mg/mL; 2.25, 4.9 and 7.27 mg/mL; 2.42, 4.85 and 7.27 mg/mL respectively [4]. Essential oils were comercially available. For the bioassay test, we used the dipping method with several modifications. Larvae mortality was recorded after 25, 45 and 60 minutes.

Results

Mortality was dose and time depended, as the number of death larvae increased with the EO concentration as well as time of exposition. The highest average lavicidal mortality was connected with lemongrass, followed by eucalyptus and lavender EO. Lethal doses were as follows: eukalyptus EO LD50 1.89mg/mL/LD 90 4.58 mg/mL, lemograss EO LD 50 1.51 mg/mL/LD90 12.97 mg/mL, lavender EO 4.22 mg/mL/LD90 14.18 mg/mL.

Conclusions

Our results indicate, that essential oils used for the household cleaning have some potential to limit survivorship of the drain fly larvae.

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Giant Hogweed essential oil larvicidal activity against Asian bush mosquito

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Keywords: *Heracleum mantegazzianum*, essential oil, *Aedes japonicus*, larvicidal activity

Objective

Giant Hogweed (*Heracleum mantegazzianum* Sommier et Levier, Apiales: Apiaceae) represents one of the most dangerous invasive neophyte in Slovakia and throughout the Central Europe. The species was confirmed to produce various spectrum of the biologically active compounds [1]. On the other side, the Asian bush mosquito *Aedes japonicus* Theobald, 1901 (Diptera: Culicidae) is one of the most expansive mosquito species in the world [2]. Nevertheless, the way of its biological control based on the plant active compounds is just very less studied. The objective of our study was to test essential oil from the Giant Hogweed seeds for its larvicidal activity against the larvae of Asian bush mosquito. To our very best knowledge, this is the first time, when such a testing was provided.

Methods

Fully matured maternal plants of Giant Hogweed were collected within the cadastre of Lekárovce village, Eastern Slovakia. Essential oil from the dried seeds of *H. mantegazzianum* was hydro-distilled and composition was analyzed using GC-MS-FID. In the larvicidal bioassay, the fourth-instars larvae of Asian bush mosquito, collected from the rainwater barrels at a private garden, were exposed to 0.035, 0.060, 0.085, 0.110 and 0.135 mg/mL of essential oil, diluted in the 2% aqueous DMSO [3]. A mortality count was conducted 24 h after the experiment was performed.

Results

Essential oil was dominated by Octyl acetate with the representation >58%. Essential oil showed a larvicidal effect against *Ae. japonicus* with the average mortality 58.8% and the LC50 = 0.058 mg/mL and LC 90 = 0.158 mg/mL. In comparison to previous study [4] when LC50 = 0.017 mg/mL and LC 90 = 47 mg/mL were obtained with the colve EO, essential oil from *H. mantegazzianum* seeds shoved just about three times lover larvicidal activity against *Ae. japonicus* in our study.

Conclusions

The results of our study indicate, that the essential oil from Giant Hogweed may be potentially explored as “eco-friendly” plant bio based mosquitocide against Asian bush mosquito.

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Composition of the essential oil from different plant parts of *Salvia verticillata* growing wild in Lower Austria

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Keywords: *Salvia verticillata*, essential oil, germacrene D, β-caryophyllene, bicyclogermacrene

Objective

Salvia verticillata L., whorled clary, (Lamiaceae) is growing in Austria mainly in the Pannonian region in semi-dry and dry meadows. The plant is distributed from Central Europe to Iran. Published literature reports *Salvia verticillata* essential oil compositions from the Eastern Mediterranean region and Near to Middle East regions. Data from Central Europe are scarce. So, the objective of this work was to evaluate the chemical composition this plant from Austrian locations and to differentiate between the plant parts.

Methods

The plants were collected while fully flowering in July and August 2021 on three locations in Lower Austria, south of Vienna, separated into the parts stems, leaves and inflorescences. After drying in the ambient air, the essential oils were isolated in a Clevenger type distillation unit. The oil were taken up in hexane and compound identification and composition was performed by GC-MS and GC-FID.

Results

Inflorescences had about 0.2 % essential oil, leaves 0.1 % and stems less than 0.01%. The essential oils were composed mainly of sesquiterpene hydrocarbons. On average, in all three plant parts the following compounds were identified in decreasing order: germacrene D > β-caryophyllene > bicyclogermacrene > α-humulene > spathulenol > β-bourbonene as presented in Table 1.

Table 1: Mean essential oil composition (%) of the *Salvia verticillata* plant parts

Compound	Stems	Leaves	Inflorescences
Germacrene D	46.8	49.1	18.4
β-Caryophyllene	13.6	8.2	16.3
Bicyclogermacrene	8.7	6.0	16.1
α-Humulene	5.7	3.4	7.5
Spathulenol	5.1	3.5	6.4
β-Bourbonene	4.1	2.3	1.3

Conclusions

Salvia verticillata is a plant low in essential oils. The six compounds mentioned above accounted for roughly 66 to 84 % of the essential oil. A comparable distribution has been found in essential oils from Italy [1] and Serbia [2], but there are reports from Greece, Turkey and Iran that mention a higher proportion of monoterpenes than in the actual results or even a completely different oil composition.

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Industrially important hemp: essential oil and their bioassays

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Keywords: hemp, antioxidant, anti-inflammation, skin whitening, antimicrobial

Objective

Cannabis sativa (L.) commonly known as hemp is majorly cultivated for seed and fiber production; moreover, it has wide application in the food and beverage industry as a flavoring agent, in the cosmetic industry as well in the pharmacological industry. Owing to the immense emerging industrial application the present study was undertaken to investigate the composition of essential oil of *Cannabis sativa* (L.) and their various bioactivities related to oxidation, inflammation, skin whitening, neurodegenerative disorders as well as antimicrobial potential.

Methods

The planting material of *Cannabis sativa* was collected from Manipur, India. The essential oil was extracted by hydrodistillation technique using Clevenger apparatus from the leaves and analyzed using gas chromatography-mass spectroscopy. For the estimation of antioxidant potential the DPPH assay was performed by a slight modification of the method given by Noumi et al. (2011). Furthermore, the antioxidant activity was further confirmed ABTS assay which was carried out following the standard protocol of Re et al. (1999). The anti-inflammatory activity of hemp essential oil was determined by protein denaturation assay as per protocol of Sangita et al. (2012). For further confirmation of anti-inflammatory activity, a slightly modified method of protease inhibitor assay was performed according to Kunitz, (1947). Acetylcholinesterase (AChE) activity of hemp essential oil was analyzed following the protocol of Ellman et al., (1961). The tyrosinase inhibitory activity was assayed using a modified dop-achrome method (Sarikurkcu et al., 2018) to screen the skin whitening property of hemp essential oil. The antimicrobial activity was estimated using agar disc diffusion method and minimal inhibitory concentration assay. For the analysis the gram-positive bacteria used were *Staphylococcus aureus* (ATCC-11632), *Streptococcus mutans* (ATCC-25175), *Bacillus subtilis* (ATCC-11774). While *Salmonella typhimurium* (ATCC-13311) and *Salmonella enteric* (ATCC-35664) were used as gram-negative bacteria. In addition to that five fungal strains namely, *Aspergillus niger* (ATCC-16885), *Fusarium keratoplasticum* (ATCC-36031), *Aspergillus oryzae* (ATCC-10124), *Saccharomyces cerevisiae* (ATCC-9763), and *Aspergillus fumigatus*, (ATCC-204305) respectively were employed for evaluation of the antifungal activity.

Results

GC/MS analysis revealed that the hemp essential comprises of epicurzerenone (19.88%) as the major compound followed by caryophyllene (15.64%) and camphor (12.8%). The other compounds were present as minor and trace compounds. The essential oil exhibited high antioxidant activity with 14.61 µg/mL as the IC_{50} value. Similarly, for the anti-inflammatory assays 12.55 µg/mL was the IC_{50} value in albumin denaturation; 19.20 µg/mL in protease inhibitor exhibitng the anti-inflammatory potential oil the essential oil. The essential oil showed moderate anti-tyrosinase activity. The essential oil exhibited IC_{50} 18.27 µg/mL in anticholinesterase activities revealing potential cholinesterase inhibiting activity. However, the hemp essential oil did not show any antimicrobial properties against any of the microbial strains.

Conclusions

The present investigation gives the foundation that hemp essential oil has the potential for future formulation and development as an antioxidant, anti-inflammatory, and cholinesterase inhibiting source. Hence, the present investigation has potential in the area of pharmaceuticals.

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Essential oil composition of eight *Salvia* taxa native to Greece

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Keywords: *Salvia*, essential oils, Greece, GC/MS

Objective

Salvia L. (sage) is the largest genus of the Lamiaceae family, which is native to the Mediterranean area and includes over 900 species worldwide [1]. The aim of this study was to determine the essential oil composition of eight *Salvia* taxa growing in Greece. The essential oils from *Salvia amplexicaulis* Lam., *S. aethiopis* L. and *S. sclarea* L. from the region of Serres (North-East Greece), *S. tomentosa* Mill. and *S. argentea* L. from Kozani (North-West Greece), *S. verticillata* L. subsp. *verticillata* and *S. officinalis* L. from Ioannina (North-West Greece) and *S. fruticosa* Mill. from the island of Symi (South-East Greece) were analyzed by means of GC/MS. Overall, the objective of this research was to investigate the variability in the essential oil profiles of *Salvia* taxa collected from different locations, willing to define the quality profile of these oils.

Methods

The aerial parts of the wild plants were collected during the summer of 2021. The essential oils were obtained by hydrodistillation using a Clevenger type apparatus and were analyzed by GC/MS method. GC/MS analyses were performed on a Hewlett-Packard 7820A-5977B MSD system, operating in EI mode (70eV) equipped with an HP-5 MS capillary column (30m x 0.25mm I.D., film thickness: 0.25μm) and a split-splitless injector. The temperature program was from 60°C to 300°C at a rate of 3°C/min. Helium was used as a carrier gas at a flow rate of 1.4 mL/min. The injector volume was 1μL.

Results

The major component found in the essential oil of *S. amplexicaulis* and *S. ar*gentea was germacrene D, while *S. aethiopis* and *S. verticillata* subsp. *verticillata* contained β-caryophyllene as the dominant constituent. In contrast, the major compound of the essential oils of *S. tomentosa*, *S. officinalis* and *S. fruticosa* was 1,8-cineole, while *S. sclarea* was rich in linalool acetate. The chemical compounds, distributions, and concentrations of the investigated essential oils varied according to the different taxa. Differences between the essential oils obtained by the eight native taxa in relation to the different geographical locations were discussed, as well as compared with the results of previous investigations of the same taxa in Greece [2-4].

Conclusions

Our results reflect the variability of the essential oil composition between the eight different *Salvia* taxa collected from Greece.

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The effectiveness of microneedling therapy with and without local lidocaine anesthesia in improvement of skin conditions

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Keywords: microneedle mesotherapy, skin treatments, cosmetology, lidocaine

Objective

The aim of the study was to evaluate the effectiveness of microneedle mesotherapy performed with and without lidocaine anesthesia, by measuring the physiological parameters of the skin (hydration, transepidermal water loss, elasticity, level of epidermal exfoliation, skin profilometry) using two specialized devices MPA from Courage + Khazaka Electronic GmbH and Nati Skin Analizer Beauty of Science.

Methods

The skin conditions were assessed using biophysical methods. Two different devices: Courage + Khazaka MPA and Nati Skin Analizer Beauty of Science were used in the study. The analysis of skin hydration and transepidermal water loss was carried out using a Corneometer and Tevameter. The level of skin greasy was determined with the Sebumeter, while skin elasticity was determined with the Reviscometer and skin roughness measurements (profilometry) were done using the Skin-Visiometer.

Results

Based on preliminary results, a greater improvement in skin conditions was observed in microneedle mesotherapy without the use lidocaine anesthesia.

Conclusions

There is limited number of available reports on the effect of local lidocaine anaesthesia in microneedling therapy. The results of this study will validate and provide a scientific guideline on the use of local anaesthesia in microneedling therapy by the comparative analysis of the skin condition before and after procedure with and without the application of anaesthesia.

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Essential oils from some species and cultivars of the *Mentheae* tribe: chromatographic profiles and antimicrobial properties

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Keywords: *Thymus* sp., *Salvia sclarea*, *Monarda didyma*, essential oil, GC/MS, antimicrobial activity

Objective

The aim of the study was the analysis of chemical composition and antimicrobial properties of essential oils (EO) obtained from 5 representatives of the *Mentheae* Dumort. tribe (*Lamiaceae* Martinov family) cultivated in Ukraine: *Salvia sclarea* L., *Thymus serpyllum* L., *Thymus vulgaris* L. (cultivar 'Jalos'), *Thymus pulegoides* L. (cultivar '2/6-07'), and *Monarda didyma* L. (cultivar 'Cambridge Scarlet'). The appearance of the new cultivars and chemotypes among species of this tribe [1, 2] encourages searching for representatives possessing significant antimicrobial effects [3].

Methods

The gas chromatography-mass spectrometry (GC/MS) method was applied for the component analysis of EO which were isolated from the herbs of plants by hydrodistillation. The antimicrobial properties of the EO were determined using agar diffusion test against typical strains of *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), and *Candida albicans* (ATCC 885-653) as well as their clinical strains.

Results

It was found the domination of such compounds in the EO of studied species: linalyl acetate (45.51%) and linalool (38.98%) in *Salvia sclarea*, α-citral (27.10%) and *β*-citral (17.11%) in *Thymus pulegioides*, thymol (47.33%), o-cymene (14.34%) and γ-terpinene (11.57%) in *Thymus vulgaris*, thymol (52.26%) and isothymol methyl ether (11.28%) in *Thymus serpyllum*, and thymol (47.54%) as well as eucalyptol (17.82%) in *Monarda didyma*. The most considerable antimicrobial influences of tested EO were against typical and clinical strains of *Candida albicans* and *Staphylococcus aureus*. It should be mentioned that clinical isolates of pathogenic microorganisms used in the study were insensitive to several antibiotics. The EO of *Thymus vulgaris* and *Thymus serpyllum* displayed a wide spectrum of action and the highest antibacterial effects that could be attributed to the significant content of aromatic monoterpenoids in them.

Conclusions

The chemotype peculiarities and antimicrobial properties of EO isolated from the studied species and cultivars were investigated. The most tested EO could be recommended for use as the active pharmaceutical substances with antimicrobial activity or excipients for conservation of food and cosmetics.

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Antibacterial activity of "Thieves Oil" as a natural biocide with potential application for enriching shoe leather

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Keywords: essential oils, "Thieves Oil", antibacterial activity, natural biocide

Objective

The aim of the analyses was to verify the antibacterial properties of an oil available on the Polish market under the trade name "Thieves Oil" in the context of the possibility of using it as a natural biocide for imparting hygienic properties to leather intended for inside parts of footwear. "Thieves Oil" is an olfactorily pleasing composition of 6 natural essential oils, i.e. *Eugenia caryophyllus* bud oil, *Citrus limon* peel oil, *Cinnamomum zeylanicum* leaf and bark oil, *Eucalyptus globulus* leaf oil and *Rosmarinus officinalis* leaf oil. Most of those mentioned are associated with strong antimicrobial activity. In the authors' earlier studies it was shown that the application of essential oils from *Origanum vulgare, Cinnamomum zeylanicum*, and *Thymus vulgaris* during bath finishing and/or by spraying can provide cowhide lining leather with resistance and activity against strains of bacteria and fungi that are pathogenic and cause biodeterioration of leather.

Methods

Tests for antimicrobial activity of "Thieves Oil" were carried out against reference strains of *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538) using the diffusion disc method and the diffusion well method. With regard to the former, tissue paper discs (Ø 6 mm) were placed on culture media inoculated with the bacterial suspension, and 10 µl of oil was applied to the discs at the appropriate dilution (dilution in Mueller-Hinton broth with added emulsifier Tween 20 in the concentration range 1-50%). In the diffusion well method, 50 µl of oil was introduced into the wells (Ø 6 mm) at the appropriate dilution. In both methods, cultures were incubated for 24h at 36±1°C. The size of the zones of bacterial growth inhibition from the edge of the disc/well to the visible growth limit were measured after incubation, in accordance with the guidelines of PN-EN ISO 20645:2006 recommended for testing the properties of light industry products.

Results

The results of the tests of antibacterial activity of "Thieves Oil" are presented in Table 1.

Table 1. Antibacterial activity of "Thieves Oil"

Microorganisms	Oil concentration [%]							
	1	5	10	50	1	5	10	50
	Inhibition zone [mm]							
	Diffusion disc method				Diffusion well method			
Escherichia coli	-	2*	2*	4**	-	0-1	1	2-3
Staphulococcus aureus	-	1**	3**	5**	-	0	2	3-4

"-" no zone; *irregular zone; **oil spillage outside the disc, microcolonies in the zone.

Source: Author's own research.

Conclusions

Based on the test results obtained with the diffusion disc method and the diffusion well method, it was concluded that "Thieves Oil" at a concentration of 1% does not show an antibacterial effect against the strains tested, and at a concentration of 5% this effect is limited. The effect obtained for concentrations of 10% and 50%, according to the methodology of PN-EN ISO 20645:2006, was considered satisfactory, indicating the possibility of practical application of the oil for leather enriching.

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Essential oils' components for the preparation of natural eutectic solvents

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Keywords: natural eutectic solvents, *Cannabis sativa* L., cannabinoids, flavonoids, UHPLC-PDA

Objective

Essential oils (EOs) represent an outstanding natural source of active compounds that are exploited in different fields for their healing properties (e.g antibacterial, antiviral, antiinflammatory...) and pleasant aroma. The quality and activity of EOs depend strictly on their chemical composition, and several classes of volatile substances were isolated, including hydrocarbons, lactones, phenols, esters and aldehydes [1]. Deep eutectic solvents (DESs) have become popular in recent years, thanks to their low toxicity, easy preparation, a wide spectrum of applications and low cost of raw materials. Among them, natural compounds have been widely used for the preparation of eutectic mixtures, to increase their sustainability [2]. In this sense, menthol and similar terpenoids that can be isolated from EOs arise as good candidates to prepare natural DESs with hydrophobic characteristics. In this work, a case study involving six compounds (carvacrol, eugenol, linalool, menthol, terpinen-4-ol and thymol) naturally occurring in EOs and used to prepare natural eutectic solvents (ESs) for the extraction of phytochemicals from *Cannabis sativa* L. aerial parts, is presented.

Methods

The six compounds were mixed in 1:1 molar ratio and heated at 40° C for 30 minutes, under magnetic stirring to form ten different ESs that were characterized by FTIR, ^{1}H NMR spectroscopy and in terms of hydrophobicity. These solvents were used as greener alternatives to traditional organic solvents (e.g methanol, acetone, hexane...) in a fast and user-friendly microextraction approach. After a careful optimization, the extraction method required 100 mg of the hemp plant, 2 mL of water as co-solvent and 100 µL of ES. This latter was dispersed in the sample by a vortex step followed by 10 minutes of ultrasound to help the release of the target compounds (flavonoids and cannabinoid acids) from the plant. The ES-rich phase was then separated by centrifugation and diluted in 500 µL of methanol 70% before the analysis in the UHPLC-PDA system.

Results

The method was validated and the performance of extraction of the natural ESs was compared with a conventional solid-liquid extraction with methanol. Thanks to the hydrophobic features of the ESs tested in this study, cannabinoid compounds demonstrated a higher enrichment compared to the methanolic extraction which, on the contrary, was more effective in the isolation of the more polar flavonoid glycosides.

Conclusions

The exploitation of natural resources in several fields has become fundamental in recent years, in view of a sustainable growth and development. This study demonstrated that EOs can be used as a valuable source of phytochemicals for the preparation of greener solvents for several applications, including more sustainable extraction methodologies, in accordance with the principle of Green (Analytical) Chemistry.

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Automation of data entry in electronic sheets for predicted response factor calculation in essential oil analysis

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Keywords: essential oils, electronic sheets, predicted response factors, gas chromatography.

Overview

Essential oils are complex mixtures and the analysis of mass spectra and retention indices, even with automated software, is time demanding. For routine operation, the proprietary software embedded in the GC-FID and GC-MS equipment do the job. In research laboratories, particularly those dealing with the analysis of natural products, many inputs cannot be set before analysis, since they are dependent of the chemical identity of the compounds present. Even in post-run data analysis, many times the parameter input, such as the response factors, must be entered line by line.

To overcome this laborious and time-consuming task, sets of electronic sheets using pre-existent functions in Microsoft Excel® can be built to automatize data entry and processing. These sets include sheets for the calculation of dilutions, internal standard normalization, linear retention indices, response factors (RFs), relative and absolute quantitation [1]. At our lab, the application of these sheets reduced data transcription errors and labor hours (for 1200 samples, in triplicate, by 500 h/year).

When accurate quantitative data is necessary, the use of response factors (RFs) are mandatory. As for EO not all standards are commercially available, RFs may be determined by injection of several standards (3 to 5) for each class of compounds to be quantified, such as monoterpenes, monoterpenic alcohols and ketones, sesquiterpenes and sesquiterpenic alcohols, arylpropanoids etc., and the average values applied [2]. Alternatively, predicted RFs can be calculated using the molecular formulas of the compounds, as demonstrated for flame ionization detectors by Laumer et al. [3]. Posteriorly, an IOFI working group developed an electronic sheet for the calculation of the predicted RFs [4].

Herein we present a modification to improve a little further the efficiency of ours previous set [1], by adding a function to automatize data entry in the sheet for predicted RF calculation. A databank sheet was assembled with the name of the compounds usually found in essential oils and the corresponding atom distribution (C, H, O…) of the molecular formulas. A search algorithm (function "VLOOKUP") links the identification column to the databank and the predicted RF calculation sheet. Once a compound is identified and its name typed in the "identification" column, the algorithm recovers the correspondent molecular formula from the databank and returns the data to the predicted RF calculation sheet, cutting off the need of manual data entry. The databank sheet is editable and new compounds can be added anytime.

Conclusions

The automation of data entry for the predicted RF calculation sheet improved the laboratory routine by reducing the amount of time needed for data processing as well as the occurrence of typing errors.

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Composition of the essential oils of the flowers and leaves of the fireweed (*Chamerion angustifolium* (L.) Holub) growing in the wild in Lithuania

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Keywords: Fireweed, essential oil, GC-MS, hexadecanoic acid, linalool

Objective

Fireweed (*Chamerion angustifolium* (L.) Holub or *Epilobium angustifolium* L.) is one of the most popular specie in genus of Epilobium. For a long time it has been used as a medical plant due to its inflammatory, antimicrobial or antioxidant properties [1]. Despite the widespread use of the plant in traditional medicine, most of the scientific work has been devoted to the analysis of its non-volatile organic compounds and their bioactivity, especially to the ability of scavenging free radicals. Literature data about volatile organic compounds emitted by fireweed plant are very scares. Mostly they were collected and analysed using SPME-GC-MS methods. This was the main trigger for our research on evaluation of the yield and chemical composition of essential oils obtained from inflorescence and leafs of fireweeds collected in different habitats in Lithuania.

Methods

Plants of fireweed growing in five natural habitats were collected during flowering stage. Inflorescences and leaves were separated and dried at room temperature indoors out of direct sunlight. The essential oils were obtained by hydrodistilation using Clavenger type apparatus and their chemical compositions were analysed employing a gas chromatography-mass spectrometry system GC-2010Plus/GCMS-QP2010 Ultra (Shimadzu, Japan). Sepration of volatile organic compounds was done on non-polar column Rxi-5MS (33m-0.25mm-0.25µm) (Restek, USA). Identification of compounds was completed comparing mass spectra with those available in data bases (NIST, Willey, Flavour & Fragrance).

Results

The yield of the essential oil depended mostly on the part of the plant. As the quantity of leaves was twice and more that of flowers collected from the same plant, in the same habitat, the quantities of essential oil differed significantly (for flowers from 0.0 to 0.05 % (v/w) and for leaves from 0.0 to 0.8 % (v/w)). The composition of essential oils of inflorescence and leaves do not vary significantly, is dominated by high molecular weight compounds, long-chain hydrocarbons, their esters and alcohols. Quantitative differences were found between essential oils extracted from raw material from different habitats. Hexadecanoic acid, tetradecanoic acid and linalool were found to be most characteristic compounds of fireweed flowers or leaves essential oils comprising respectfully up to 24 %, 11 % and 16 % of all identified compounds.

Conclusions

In terms of essential oil yield, it has to be said that from such a large biomass, it is very poor, and the fireweed can hardly be a valuable raw material for essential oil extraction. Major compounds of flower and leaves essential oils were found to be hexadecanoic acid, teradecanoic acid and linalool.

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Analysis of the lavender (*Lavandula angustifolia*) essential oil market in Lithuania in 2021

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Keywords: Lavender, essential oil, GC-MS, adulteration, market

Objective

Lavender (*Lavandula angustofolia*) essential oil (EO) is one of the most well-known, widely used, highly olfactometrically recognisable essential oils in the world. At the same time, it is one of the most frequently adulterated EOs. Falsified essential oils are not only those which are completely devoid of the pure product and mimic the composition with synthetic compounds, but also those which, for economic or organoleptic reasons, are diluted with cheaper essential oils of a different part of the same plant, of a different subspecies or of a similar composition. Lavender essential oil is well-known to Lithuanian buyers and is the number one purchased and used oil. Since the price differences on the market are really high, the question was whether you can expect to get good quality authentic lavender EO for a low price. The aim of this study was to analyse the composition and quality of lavender essential oils available in the Lithuanian market by means of a simple chromatographic analysis.

Methods

All forty samples purchased were coded by numbers from 1 to 40 prior to analysis. 10 µl of each sample was diluted to a volume of 1 ml with a mixture of pentane and diethyl ether (1:1). Analysis was performed using a gas chromatography-mass spectrometry system GC-2010Plus/GCMS-QP2010 Ultra (Shimadzu, Japan). Sepration of volatile organic compounds was done on non-polar column Rxi-5MS (33m-0.25mm-0.25µm) (Restek, USA). Identification of compounds was completed comparing mass spectra with those available in data bases (NIST, Willey, Flavour & Fragrance).

Results

After GC-MS analysis all investigated samples were divided into tree main groups: those meeting the authenticity criteria (according to guidances of ISO and EP) [1]; those possibly mixed with other essential oils and those containing synthetic impurities. Only 49 % of the samples fell into the first group, while 31 % were presumed to be mixed with lavandin and/or spike lavender essential oils due to the high levels of 1,8-cineole and camphor found in the samples and unfortunatly 20 % contained syntetic substances: dihydrolinalool and dihydrolinalool acetate (that suggesting the addition of synthetic linalool and linalyl acetate), di-1,2-propylene glycol, glycerol diacetate, compounds used as solvents and odour stabilisers, and 3,5,5-trimethylhexylacetate, whose smell is described as powdery, lavender-like and soapy.

Conclusions

51% of the true lavender (*L. angustifolia*) oils purchased on the Lithuanian market and tested did not meet the authenticity criteria; aynthetic substances were found in as many as 20% of the oils; among the lavender EO purchased in pharmacies, none met the authenticity criteria.

ACKNOWLEDGMENTS

All samples were purchased by JSC Kvapu namai and analysed in their laboratory.

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Effect of *Cistus ladanifer* L. pre-treatment on essential oil yield

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Keywords: *C. ladanifer*, rockrose, steam distillation, round bales, shredding, essential oil

Objective

This study aimed to compare effect of two forms of feedstock biomass (whole bales and shredded bales) on essential oil (EO) yield during steam distillation process of rockrose (*Cistus ladanifer* L.).

Methods

Cistus ladanifer L. (*Cistaceae*) was mechanically collected (which is not a common practise) from two locations - Bustares and Hiendelaencina (Guadalajara, Castilla-La Mancha, Spain) in October 2021. Plants' age was approx. 6 years and 12 years, respectively. Using a harvester-baler (Biobaler WG55) driven by a 154-kW tractor (Valtra T194D), aerial biomass was cut at an approx. height of 15 cm above the ground and compressed into cylindrical bales. The bales were stored 4 months indoors (which did not affect the EO yield [1]). Average bale weight and moisture content (w.b.) were 177.3 kg and 16.4% for 6-years plants and 227.3 kg and 15.9 % for 12-years plants, respectively. Half of the bales were crushed to a size of 20 mm by means of a shredder (90 kW, slow rotating single-shaft type) just before distillation. The whole and shredded bales were distilled in a steam distillation pilot plant composed by a 1.8 m^3 stainless steel still, an electric boiler (ETE) to produce the steam (0.5 bar, 30 kg h^{-1}), a cooling system for steam condensation and a glass Florentine flask to separate hydrolate and EO by density. Batch distillations were performed with three repetitions per sample and a distillation duration of 6 hours. The obtained EOs were dried over anhydrous sodium sulphate and filtrated. The EO yield was calculated as a % (weight/weight) on a biomass dry basis (d.b.).

Results

The results showed (Table 1) that all repetitions exhibited higher EO yield from the distillation of whole bales. It could be caused by a better heat and steam transfer among the branches forming the bale. In the case of 6 years plants, the average oil yield of whole bales was approx. double that of shredded ones. The amount of EO extracted from non-treated bales of older plants increased by 37% over the mechanically pre-treated bales.

Table 1. EO yield (%, d.b.) of C. ladanifer – comparison of whole bales vs. shredded bales

Plant age	Whole bales	Shredded bales	Plant age	Whole bales	Shredded bales
	0.126	0.042		0.046	0.028
6	0.129	0.073	12	0.033	0.025
	0.122	0.067		0.033	0.029
Aver±St.dev.	0.126±0.004	0.061±0.016	Aver±St.dev.	0.037±0.008	0.027±0.002

Conclusions

In this study, *C. ladanifer* produced higher oil yield when distilled directly from harvested bales. This factor can lead to minimization of energy consumption and optimization of operating conditions of rockrose EO production.

ACKNOWLEDGMENTS

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Annual variation of yield and main composition of rockrose (*Cistus ladanifer* L.) essential oil

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Keywords: *C. ladanifer*, essential oil, composition, rockrose, steam distillation, yield

Objective

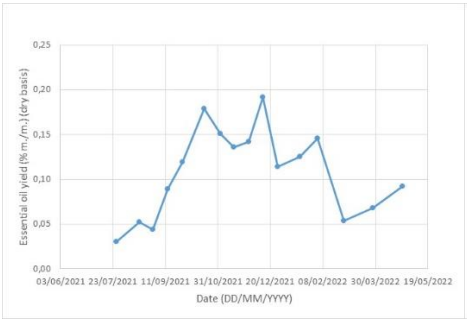
The objective of this work is to define the most appropriate harvesting season of rockrose (*Cistus ladanifer* L.) collected in central Spain regarding its essential oil yield and main composition.

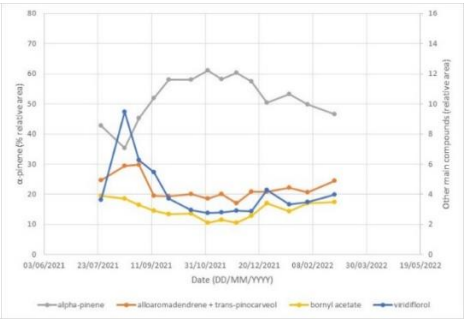
Methods

Cistus ladanifer L. samples were manually collected in a shrubland regenerated after a wild fire in Bustares (Guadalajara, central Spain) happened in 2014. Monthly or fortnightly samples of 80 kg were collected, being cut at an approximate height of 15 cm aboveground. The biomass material was shredded to a size of 20 mm by means of a shredder (90 kW, slow rotating single-shaft type). Afterwards, three batches of 15 kg were distilled during 1 hour in a steam distillation pilot plant composed by a 50 L stainless steel still, an electric boiler to produce the steam (0.5 bar, 13 kg h^{-1}), a cooling system for steam condensation and a glass Florentine flask to separate hydrolate and essential oil by density. Finally, the three samples of essential oil obtained were blended and analysed with GC (FID)-MS, using the relative area percentage. The used apparatus were Agilent HP 8890/5977 (GC-MS) and Agilent HP 8890 (GC-FID), both equipped with DB-WAX UI fused silica columns (60 m × 0.25 mm inner diameter, film thickness 0.5 µm) and retention time locking.

Results

In the following figures, the essential oil yield, expressed in % (mass/mass, dry basis), and its composition (compounds >5%, in % relative area) are shown. As can be seen, the essential oil yield was higher during the autumn and the beginning of winter. Concerning the composition, the main compounds were α-pinene, alloaromadendrene quantified together with trans-pinocarveol, bornyl acetate and viridiflorol. The highest α-pinene content was registered in the months of maximum yield, while the highest contents of alloaromadendrene, trans-pinocarveol, bornyl acetate and viridiflorol were registered during the summer.





Conclusions

The maximum essential oil yield of *Cistus ladanifer* L. collected in central Spain from summer 2021 to spring 2022 was obtained between September and February, registering an increase of α-pinene and a decrease in the rest of the main compounds (alloaromadendrene + trans-pinocarveol, bornyl acetate and viridiflorol) in these months.

ACKNOWLEDGMENTS

This work has been funded by the Spanish Ministry of Science and Innovation (MCIN) through the Project PID2020-114467RR-C32.

Composition and anti-MRSA activity of selected brewing quality varieties of *Humulus lupulus* L. cultivated in Poland

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Keywords: *Humulus lupulus*, MRSA, extracts, essential oils, GC/MS, LC/MS

Objective

Hop plant (*Humulus lupulus* L., Cannabaceae) has a long history in traditional medicine, and is well-known as bittering agent in the brewing industry. Hops in beer provide rich aroma and flavour depending on hop variety and its phytochemical composition. There are more than 200 different varieties of hops grown worldwide. The content and composition of the violatiles present in hop varies greately depending on variety. Researches show that hop derivatives are effective in inflammatory disorders, osteoporosis, antiproliferative inhibition and cancer cells apoptosis induction, sedation, bactericidal and fungial agents [1,2]. The aim of this study was to investigate the chemical composition of 11 brewing quality varieties of *Humulus lupulus* L. and to determine anti-MRSA activity of essential oils (EOs) and methanolic extracts (MEx) obtained from these varieties. All varieties were cultivated in Poland and they were: 'Iunga', 'Marynka', 'Sybilla', 'Oktawia', 'Lubelski', 'Tomyski', 'Magnum', 'Perle', 'Tradition', 'Cascade' and 'Chinook'.

Methods

Hop pellets type 90 of all mentioned verieties were stored in vacuum sealed packages in freezer prior to the examination. EOs were obtained by hydrodistillation in Deryng-type apparatus and content of each was measured. GC/MS analysis was performed and composition of each EO was compared. MEx were obtained by ultrasound assisted extraction of hop pellets with methanol (3 x 5:1 solvent/plant material ratio). Each extract were vacuum-distilled in low temperature. Each extract was analyzed by use of LC/MS and GC/MS technique and compositions were compared. Thin layer chromatography-direct bioautography (TLC-DB) assay was performed for both EOs and MEx on ATCC 43300 MRSA (methicillin-resistant *Staphylococcus aureus*) strain.

Results

GC/MS analysis showed differences in composition of essential oils obtained from different hop varieties. In each EO sample main compound was either β-myrcene ('Marynka', 'Magnum', 'Cascade' and 'Chinook') or humulene ('Iunga', 'Oktawia', 'Lubelski', 'Tomyski', 'Perle' and 'Tradition'). TLC-DB showed that all examined EOs are active against MRSA. Highest activity was revealed for these EOs where oxygenated compounds were most abundant; 'Oktawia', 'Lubelski' and 'Perle'. GC/MS analysis of the MEx showed that most abundant compounds were β-acids and β-caryophyllene, it also indicated that small amounts of other EO compounds were present although further investigation proved that most of the β-myrcene was evaporated with methanol during distillation. LC/MS analysis of MEx proved the presence of typical for *Humulus lupulus* compounds such as; α-acids (humulone and cohumulone), β-acids (lupulone, postlupulone and prelupulone) and prenylflavonoids (xanthohumol and 6-prenylnaringenin) but there were significant differences in the concentration. TLC-DB performed for MEx showed significant activity of spots identified as α-acids, although spots identified as β-acids were also active. Most visible MRSA growth inhibition was spotted for 'Iunga', 'Magnum' and 'Perle' where both 'Iunga' and 'Magnum' had the highest α-acid content declared by the producer.

Conclusion

This research proved that both essential oils and methanolic extracts of brewing quality *Humulus lupulus* (L.) are effective against MRSA.

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Essential oil of flowers of sweet mock orange (*Philadelphus coronarius* L.) and its potential antiepileptic activity

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Keywords: *Philadelphus coronarius*, essential oil, zebrafish, epilepsy, gas chromatography

Objective

Philadelphus coronarius L. also known as sweet mock orange is a shrub belongings to Hydrangaceae family, it is popular ornamenting plant, cultivated in gardens, which white blossoms are strongly scented. The aim of this study was to obtain the essential oil from flowers of the *Philadelphus coronarius* L., that were collected in Poland form private garden, and then GC/MS analysis was performed to identify the composition of oil, followed by biological assay to evaluate antiepileptic activity on PTZ zebrafish model.

Methods

Essential oil (EO) was obtained from flowers of *Philadelphus coronarius* L. by hydrodestillation in Deryng apparatus, however due to very small yield of oil hydrolate was collected and then extracted with diethyl ether and further ether was evaporated.

To identify the compounds of essential oil we used gas chromatography with mass spectrometer (GC/MS).

Biological assay was performed on zebrafish epilepsy model using PTZ that is modulator of $GABA_A$ receptor that induce seizures in animal models. The larvae of wild-type strain of zebrafish (Danio rerio) was incubated with different, non-toxic concentrations of *Philadelphus coronarius* essential oil, after overnight incubation the activity of larvae before and after addition of PTZ was measured using ZebraBox tracking device (ViewPoint, France).

Results

The results of GC/MS analysis indicated the presence of multiple long chained hydrocarbons and oxygenated hydrocarbons (e.g. Z-jasmone).

The EO shows antiseizure protection in most of tested concentrations (up to 55.5% reduction of seizures compared to larvae treated only with PTZ).

Conclusions

The exact mechanism of antiseizure activity of *Philadelphus coronarius* L. essential oil is unknown. The activity might be due to modulation of $GABA_A$ receptor, however presence of long-chained hydrocarbons might decrease absorption of seizure inductor (PTZ), herein further research has to be performed.

Anticancer potential of volatile compounds from endophytic microorganisms of the *Marchantia polymorpha* L.

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Keywords: liverworts, endophytes, diketopiperazine, sesquiterpenoids, anticancer activity, *Marchantia polymorpha*

Objective

Liverwort endophytes could be a source of new biologically active substances, especially when these spore-forming plants are known to produce compounds that are not found in other living organisms [1]. Despite the significant development of plant endophytes research, there are only a few studies describing liverwort endophytic microorganisms and their metabolites. In the presented study, the analysis of the volatile compounds obtained from thallose liverwort species, *Marchantia polymorpha* L. and its endophytes was carried out. For this purpose, non-polar extracts of plant material and symbiotic microorganisms were obtained. The objective of this work was to determine chemical composition of organic extracts and to evaluate their cytotoxicity on a panel cancer and normall cell lines.

Methods

The thalli of *Marchantia polymorpha* L. were collected in November 2020 from its natural state in the Lublin region (Cieleśnica, Biała Podlaska district) by A.L. The isolation and cultivation of endophytes was carried out with the use of sterile materials under aseptic conditions. After the incubation period of 7 days, the medium was harvested with endophytes and subjected to the extraction process. The obtained hexane and ethyl acetate extracts were concentrated and subjected for chromatographic analysis. The GC-MS analysis of the obtained extracts was carried out with the use of a Shimadzu GC-2010 Plus gas chromatograph, coupled with a QP 2010 Ultra mass detector. The cytotoxicity testing was carried out using microculture tetrazolium assay (MTT). The study was performed on a panel of cancer (FaDu, HeLa, and SCC-25) cell lines and normal (VERO).

Results

The most characteristic volatile metabolites of diethyl ether extract obtained from the thalli of *Marchantia polymorpha* are two sesquiterpene alcohols belonging to cuparane type, cyclopropanecuparenol and its diastereoisomer, *epi*-cyclopropanecuparenol. Compounds with the structure of diketopiperazine in the endophyte extract were identified. The cytotoxicity of ethyl acetate extracts from endophytic microorganisms revealed significant anticancer potential towards hypopharyngeal squamous cell carcinoma and cervical adenocarcinoma.

Conclusions

The results presented in this paper prove how important the source of metabolites are endophytic microorganisms. It is also very important that there are many plant species for which endophytes and the compounds produced by these microbes have not yet been analyzed. The first phytochemical analysis of metabolites present in the endophytes of the liverwort *Marchantia polymorpha* allowed the identification of interesting compounds. Due to the completely different type of the identified metabolites found in the endophytes of the *M. polymorpha* compared to those identified in the liverwort, further research is necessary.

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The use of the Soxhlet apparatus for obtaining geranium extracts

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Keywords: geranium extracts, extraction Soxhlet apparatus

Objective

The extract obtained from the leaves of geranium (*Pelargonium graveolens*) is used in perfume and cosmetic industries, and also in aromatherapy. This extract found applictaions in reducing pain due to post-herpetic neuralgia as well as treating dysentery, hemorrhoids, inflammation, and cancer [1]. Usually, geranium oil is obtained by a hydrodistillation. The aim of our work was to obtain extract from geranium leaves using the Soxhlet apparatus. The extract of this plant was obtained in two stages. Such a procedure was aimed at obtaining a higher concentration of geraniol in the final extract.

Methods

Geranium leaves from plants grown in our laboratory were used in the research. All the leaves were freshly plucked and healthy (Figure 1a). Ethanol with the purity of 99.8% was used for the extraction. Extraction was performed using a Soxhlet apparatus and with help of two steps method. First, about 16 g of fresh leaves were weighed and placed in the Soxhlet apparatus, then about 86 g of ethanol were poured into the flask (Figure 1b). After 4 hours, the next portions of leaves were weighed and the extract obtained from the first stage served as the extraction agent in the second stage (Figure 1c). In the second stage, about 16g leaves were planted in the Soxhlet apparatus. In the second stage, the extraction was carried out for 4 h (Figure 1d).

a) b) c) d)

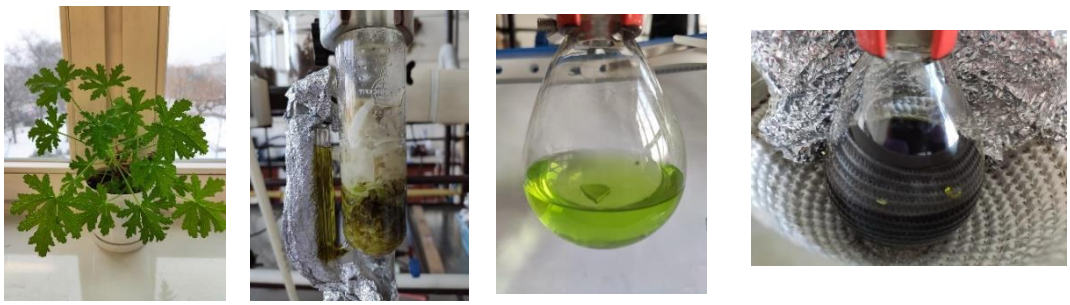

Figure 1. Geranium leaves used in the extraction process (a), the Soxhlet apparatus filled with leaves at the end of the first stage (b), and the extracts obtained after the 1st (c) and 2nd stage of extraction (d)

Results

Thanks to the use of a 2-stage extraction with ethanol in the Soxhlet apparatus, it was possible to obtain an ethanol extract, which contained as the main ingredients: cis-geraniol (29.53%), b-citronellol (17.41%), menthon (12%), geranyl tiglate (5.67%), eudesmal (16.91%) and arostolene (10%). This extract contains a significantly higher amount of geraniol in relation to the amount described in the literature [1] - 6%.

Conclusions

Thanks to the use of this method and obtaining an increased content of geraniol in the extract, these extracts can become a source of natural geraniol for organic syntheses in the future.

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Methanol extraction of needles and cones of Scots pine using Soxhlet apparatus

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Keywords: plant materials, pine cones, pine needles, Soxhlet apparatus

Objective

Extracts derived from various parts of coniferous trees, such as needle, bark, stem, wood, cone, nut and leaves are widely used as medicinal subtances. These compounds are used as antiseptic, anticancer, antirheumatic, anti-inflammatory and antitoxic agents. The medicinal properties of extract from coniferous trees allow the use of these compounds in the treatment of various diseases, including respiratory diseases [1]. The aim of our work was to obtain the extracts from the needles and undeveloped cones of Scots pine using the Soxhlet apparatus and to qualitatively analyze the components present in the extracts by GC-MS.

Methods

The raw materials for the study were dried needles and undeveloped cones of Scots pine (Gwizdów, Poland) (Figure 1). First, 20,128 g of Scots pine needles and 20,042 g of undeveloped Scots pine cones were weighed. The raw materials were then crushed in a mortar and placed in a Soxhlet apparatus. Later, about 200 ml of methyl alcohol was introduced into a 500 ml round-bottom flask. The process of extraction of needles and undeveloped pine cones was carried out for 4 hours. After several cycles of the Soxhlet apparatus, the valuable organic compounds present in the needles and undeveloped cones of Scots pine were found in the methanol extract.

a)  b) 

Figure 1. Raw materials used in the extraction process: a) pine needles, b) undeveloped pine cones

Results

It was shown that the main components of the extract from the needles of Scots pine were the following compounds: alpha-pinene (20.39%), camphene (2.29%), 3-carene (4.40%), borneol (5.16%), beta-cadinene (7.54%), tau-cadinol (3.06%) and cubenol (7.20%), while the methanol extract of undeveloped pine cones contained the following main constituents: alpha-pinene (1.43%), 3-carene (11.56%),verbenone (2.30%) and 3-O-methyl-d-glucose (5.83%).

Conclusions

The needles and undeveloped cones of Scots pine are a source of valuable organic compounds. Extraction of needles and undeveloped pine cones using a Soxhlet apparatus and methyl alcohol as a solvent proved to be an effective method for extracting organic compounds. The extract from common pine needles contained a significantly higher amount of alpha-pinene and a lower amount of 3-carene compared to the amount present in the extract from undeveloped pine cones.

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Analysis of Essential Oil (Phytoncide) Components of Trees for Development of Platform for Smart Forest Utilization

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Keywords: Phytoncide, Pine tree, Hinoki Cypress tree, pinene, smart forest utilization platform

Objective

The aim of this study is to analyze the composition of essential oil of trees (Pythoncide) in the atmosphere so that the platform device can generate the same scent as the forest in the process of developing a smart forest utilization platform. Phytoncide is defined as essential oil extracted from trees, and the antibacterial and sedative effects of phytoncide have been reported. Due to the recent development of the 4th Industrial Revolution element technology and the Covid-19 situation, the demand for smart forest utilization platforms that allow people to experience forests indoors is increasing, and these platforms need to include attachments that can generate the same scent as forests.

Method

We analyzed the composition of phytoncide in the atmosphere by collecting the atmosphere of Pine trees (*Pinus densiflora*) and Hinoki Cypress trees (*Chamaecyparis obtusa*) forests in South Korea which is a temperate climate region. The concentration of phytoncide was measured at a 30-minute cycle before and after sunrise, peak, and sunset.

Results

Although some differences were found in the composition of phytoncide by forest type, the proportion of "pinene" substances was steadily high. It was observed that the ratio of phytoncide concentration and substance varies depending on the temperature, humidity, wind speed, and amount of light in the atmosphere.

Conclusions

This study was conducted to understand the composition of essential oil from trees (*Phytoncide*) components in the forest atmosphere in order to include an attraction that can generate the same scent as the forest in a smart forest utilization platform that allows visitors to experience the forest indoors. As a result, it was possible to get basic data that could reproduce the concentration of essential oil of the same tree as the natural forest in the indoor air. These results are expected to be effectively used to design an environment similar to nature indoors.

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Association of *Conobea scoparioides* Cham. & Schltdl essential oil with parabens

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Keywords: preservatives, essential oils, *Conobea scoparioides*, antimicrobial activity, paraben association

Objective

The present work aimed to evaluate the chemical composition of the essential oil of *Conobea scoparioides* Cham. & Schltdl., its antimicrobial potential, and its association effect of with parabens for a future application as a preservative system in cosmetics.

Methods

Essential oil: *Conobea scoparioides* Cham. & Schltdl, essential oil was a commercial sample obtained from International Flavors & Fragrances (IFF) (Santana do Parnaíba, São Paulo, Brazil).
Chemical analysis: The oil was analyzed by GC and GC/MS. GC using a method previously described [1].
Antimicrobial Assay: The activity determined by a microdilution method against *Aspergillus brasiliensis* (ATCC 6404), *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027). *Burkholderia cepacia* (ATCC 25416) and *Staphylococcus aureus* (ATCC 6538), after reading the absorbance at λ=630 nm, after 24 -48 h of incubation [1].
Association effect: The variables selected for this study were the predetermined MIC's of methylparaben and propylparaben along with the essential oil, using a centroid simplex network with additional points of the three components mixture, composing ten different test points [2].

Results

The major oil compounds were thymol methyl ether (39.2%), thymol (33.8%) and α-phellandrene (15.9%). The antimicrobial activity of essential oil was accessed through the minimum inhibitory concentration (MIC), whose results in μg/mL were *S. aureus* 650.7, *E. coli* 721.5, *P. aeruginosa* 1748.0, *B. cepacia* 833,0, *C. albicans* 521.4, and *A. brasiliensis* 300,0. The association of methylparaben, propylparaben, and essential oil, against the same microorganisms, resulting in an optimum concentration (μg/mL) of 1120 for methylparaben, 350 for propylparaben and 675 for the essential oil. The essential oil of *C. scoparioides* showed an important antimicrobial potential both alone and in association with synthetic preservatives.

Conclusions

C. scoparioides essential oil presented thymol as one of the main compounds that already showed antimicrobial activity. The antimicrobial potential of the essential oil alone and associated with parabens, suggested that this oil can be used to compose a preservative system for cosmetic formulations containing lower amounts of synthetic compounds.

ACKNOWLEDGMENTS

CAPES for the MSc. Scholarship and International Flavors & Fragrances (IFF) for providing the essential oil.

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Effect of nanoemulsions with caraway oil on seedlings growth of maize and barnyard grass

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Keywords: phytotoxicity, herbicidal effect, thermal heat, Raman spectroscopy

Objective

Essential oils could serve as an important source of active compounds for crop protection with the help of nanotechnological methods. The previous experiments showed that caraway oil significantly inhibited the germination and growth of different weed species [1,2]. This study aimed to determine the herbicidal potential of European origin caraway (*Carum carvi* L.) seed essential oil, based on the physiological response of seedlings of barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.) and maize (*Zea mays* L.).

Methods

The nanoemulsions (Ns) consisted of caraway oil, Eco Tween 80, and deionized water prepared by ultrasonification. The oil and surfactant proportion in the Ns was 1:1, and concentration was 1.0-5.0%. The metabolic activity of seedlings was measured using an isothermal calorimeter TAM III. Seedlings of maize or barnyard grass were placed in the calorimetric ampoules and the thermal power curves were recorded for 24 h. The Raman spectra of lyophilized seedlings were recorded using a Nicolet NXR 9650 FT-Raman Spectrometer equipped with an Nd:YAG laser and a InGaAs detector, in the range of 400 to 2000 cm^{-1}. A hierarchical cluster analysis was performed and the spectral distances were calculated using Ward's algorithm.

Results

Seedlings growing in the presence of NS displayed an overall inhibition of metabolism (Figure 1). Cluster analysis showed differences in chemical composition (carbohydrates) between Ns-treated seedlings at different concentrations compared to the control and ADJ-treated seedlings.

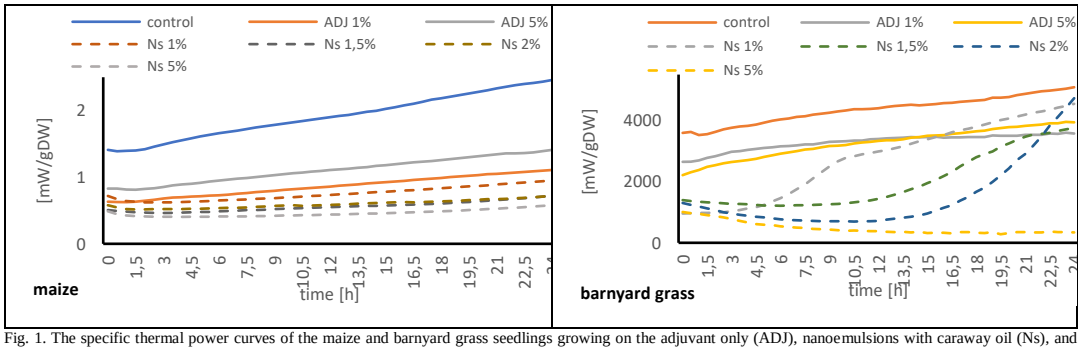

Fig. 1. The specific thermal power curves of the maize and barnyard grass seedlings growing on the adjuvant only (ADJ), nanoemulsions with caraway oil (Ns), and water (C).

Conclusions

Caraway nanoemulsion with Eco-Polysorbate 80 strongly inhibits maize and barnyard grass seedlings. It should be further optimized and analyzed in controlled and field conditions.

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PP-B26

Production Fields of Peppermint at University of Presov, Slovakia, detailing to Analyses of its Essential Oils

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Keywords: composition, essential oil, Eastern Slovakia, fields, GC/FID, peppermint

Objective

Increasing demands for both crude herbs and natural products based on herbs, the risk of excessive collection from the wild, and more protected areas have led to a growth in small- and large- scale cultivation. The university field with an area of 0.46 hectares at University of Presov in Presov, the East Slovakia, are concentrated to all aspects of their biodiversity, breeding and cultivation. As a result of the breeding work of scientists at the University of Presov in Presov, Slovakia, was new peppermint variety "Kristinka", confirmed by a decision at the level of the CPVO in Angers, France in 2017 [1]. The objective of this study was the qualitative and quantitative analysis of the essential oil composition in peppermint grown on the university field over last four consecutive years.

Methods

Essential oil from peppermint plants was isolated from dried drugs by repeated hydro-distillation (from 6 to 8 times depending on sample size) and the weight of oil was determined gravimetrically [3]. Composition of peppermint essential oils was determined by capillary GC-MSD system on a Varian 450-GC instrument together with a Varian 220-MS with Split-Splitless injection port, MSD detector.

Results

According to the results shown the essential oil content in peppermint leaves grown on the university field reached values between 3.00 ± 0.15 to 3.40 ± 0.25 %. Essential oil extracted from peppermint variety "Kristinka", was recorded to have 20 main natural components. It was found that menthol was the major constituent (from 68.00% ± 2.00 to 74.00 % ± 2.66) followed by menthone (from 8.30% ± 0.5 to 17.00 % ± 1.00), limonene (from 3.0 % ± 0.5 to 3.5 % ± 0.5), iso-menthone (from 1.7 % ± 0.1 to 2.5 % ± 0.2), methyl acetate (from 1.0 % ± 0.5 to 3.5 % ± 0.5), piperitone (> 0.5 %), menthofurane (> 0.5 %), cineole (> 0.3 %), pulegone (> 0.3 %), isopulegol (> 0.5 %), carvone (> 0.5 %). In regard to menthol ($C_{10}H_{16}$), it is a bicyclic monoterpene chemical compound. Peppermint has the high menthol content, and is often used as flavouring in tea, ice cream, confectionery, chewing gum, and toothpaste. The oil also contains menthone and menthyl esters. It is the oldest and most popular flavour of mint-flavoured confectionery [2].

Conclusions

The introduction of medicinal plants as a part of the agro-technology development on the university field gives an opportunity to cultivate medicinal and aromatic plants with interesting characteristics in Eastern Slovakia. The peppermint plant material was after 5 years of testing for varietal diversity, balance and stability showed remarkable results. This is used in the production of seedlings for large-scale cultivation in Slovakia, but also abroad.

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Nanoformulation of a biopesticide based on combinations of essential oils of *Syzygium aromaticum* (cloves) and *Zingiber officinale* (roots) harvested in Cameroon

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Keywords : Nanoemulsion, Essential oils, Formulation, Optimization, Biopesticide

Objective

Aromatic plants are a gigantic pool of bioactive molecules. Essential oils (EO) extracted from different parts of plants are more or less endowed with antimicrobial properties dependent on their chemical compositions. Indeed, many researchers have demonstrated that, the EO of the flower buds of *Syzygium aromaticum* (SA) and the rhizomes of *Zingiber officinale* (ZO) have a strong inhibitory power on the growth of plant pathogens and therefore could be used in preservation in foodstuffs **[1]**. However, the use of raw EO is not optimal because they have poor chemical stability, low water solubility and high photosensitivity **[2]**. To remedy these limitations, new formulation methods are being developed, including nanoemulsions. The objective of the formulation is to optimize the use of EO, reduce the amount of plant matter for the extraction of EO and possibly potentiate the bioactivity of EO.

Methods

The EOs were extracted by hydrodistillation and the analysis of their chemical composition was done by Gas Chromatography coupled with Mass Spectrometry. The biopesticide was formulated by nanoemulsion using the pressure-based homogenization technique. By mixing surfactant xg (tween 80), yg (v/v) cotensioactive (Mono Propylene Glycol + Glycerol), he combination zg (SA/ZO: 7/3) and distilled water were mixed with stirring for 05 hours. The particle size was determined by laser particle size. The centrifugation, washability, staining and rheological tests made it possible to study the physicochemical stability of the emulsion.

Results

The emulsion obtained is white, homogeneous, milky in appearance and leaves a bluish reflection on the wall of the bottle. The centrifuged emulsion at 2000 and 3000 rpm successively remained homogeneous and uniphasic. The washability test reveals a direct emulsion (Oil in water) because there is a perfect dilution of the water in the emulsion, without any other visible modification. Also, it was noticed a concentric distribution and uniform dispersion of the dye throughout the emulsion volume. Laser particle size indicates a particle size of 132 nanometers, an obscuration rate of 5.11% and a SPAM of 1.330 units/mL. No rheological phenomena have been observed at more than 65 days.

Conclusions

HE-based nanoemulsions would therefore protect bioactive molecules from EO, reduce their volatility and optimize their use in plant protection.

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Chemical and Biological Investigation of *Cupressus torulosa* Needles Essential Oil

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Keywords: Cupressus torulosa, essential oil, GC/MS, GC/FID, anti-inflammatory activity

Objective

Cupressus torulosa D. Don, widely distributed in the north western Himalayan region of India, is an aromatic tree with various traditional uses of its aerial parts. This study aimed to determine the chemical composition of the essential oil isolated from needles of *C. torulosa* (CTEO) from India and scientifically validate the traditional claim of the use of the needles in treatment of inflammation disorders employing *in vitro* and *in vivo* assays.

Methods

Fresh needles (2 Kg.) of *C. torulosa* were collected from the Botanical Garden of the Forest Research Institute, Dehradun, India. The needles (600 g, in triplicate) were hydro distilled using Clevenger-type apparatus. CTEO was separated from the hydro distillate using dichloromethane, dried over anhydrous Na_2SO_4 and yield was determined on a moisture-free basis. Composition of the CTEO was determined using GC-FID and GC-MS on a DB-5MS column (30 m x 0.25 mm x 0.25 µm). GC oven temperature was set to rise from 40°C (4 min hold) to 220°C (15 min hold) at 4°C/min. Compounds were identified by comparison of their KRIs, determined using a mixture of *n*-alkanes (C_6-C_{24}) under identical experimental conditions, and mass spectra with NIST17 and F&F library. Quantification of the compounds was done according to the IOFI-recommended practice using methyl octanoate as an internal reference. *In vitro* anti-inflammatory activity of CTEO was evaluated using egg albumin denaturation and protease inhibitory assays. Acute toxicity of CTEO on female mice was evaluated following the OECD guideline 423. *In vivo* activity of the CTEO was performed using xylene-induced ear edema model on Wistar rats of either sex divided into four groups (six in each group). Group 1 received CTEO 20 mg/kg b.w, Group 2 received CTEO 40 mg/kg b.w, Group 3 received standard sodium diclofenac 10 mg/kg b.w and group 4 served as negative control. After 30 mins of dose administration, xylene was smeared on the surface of the right ear and the left ear was used as control. Ear edema was measured using a vernier caliper.

Results

A greenish-yellow CTEO with a yield of 0.6% was obtained. Twenty-three compounds amounting to 91.63% of the oil, with terpinen-4-ol (31.42%), sabinene (8.79%), and limonene (7.56%) as major components, were identified. CTEO demonstrated promising *in vitro* anti-inflammatory activity (IC_{50} 27.32 µg/mL and 126.89 µg/mL compared to standard sodium diclofenac (IC_{50}15.03 µg/mL and 69.42 µg/mL) in egg albumin denaturation and protease inhibitory assays, respectively. CTEO was found to be non-toxic at 300 mg/kg b.w. with no signs of abnormal locomotion, seizures and writhing. In xylene-induced ear edema model, CTEO exhibited significant increase in inhibition of edema till 1h (43.15% and 71.07% inhibition after 0.5h and 77.14% and 80.71% inhibition at 1h at dose of 20 mg/kg b.w. and 40 mg/kg b.w, respectively) compared to standard sodium diclofenac (47.72% and 45% inhibition after 0.5h and 1h, respectively at dose of 10 mg/kg b.w.). Terpinen-4-ol, being a strong anti-inflammatory agent, may be responsible for the significant activity of CTEO along with the synergistic contribution from other major and minor components of the oil.

Conclusion

Terpinen-4-ol (31.42%) rich CTEO exhibited promising *in vitro* as well as *in vivo* anti-inflammatory activity thus supporting the traditionally accepted medicinal use of the needles. CTEO appears to be a potent natural agent that can be developed into anti-inflammatory formulation(s) with further explorations.

ACKNOWLEDGMENTS

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PP-B29

Volatile Composition, Toxicity, Analgesic, and Anti-Inflammatory Activities of *Mucuna pruriens*

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Keywords: *Mucuna pruriens utilis*, essential oil, (*E*)-2-hexenal, anti-inflammatory activity, anti-nociceptive activity

Objective

The objective of this work was to evaluate the chemical composition of *Mucuna pruriens* collected from Imota Ikorodu Forest Reserve, Lagos State, Nigeria. This study aimed to isolate, characterize; evaluate the anti-nociceptive and anti-inflammatory properties of the essential oils of the leaves of *Mucuna pruriens* collected. The volatile constituents, toxicity, antinociception, and anti-inflammatory activities of the essential oil obtained from the leaf of *Mucuna pruriens* collected were reported.Several studies have reported the pharmacological evaluations of *Mucuna pruriens*, however, this present study is aimed at reporting for the first time, the chemical constituents, antinociceptive, and anti-inflammatory activities of the volatile oil of *M. pruriens* leaves growing in Nigeria.

Methods

Hydrodistillation was carried out with a distillation unit designed according to the specification. The oil was distilled over water and collected in hexane at normal pressure at about 100°C. The essential oil was analyzed comprehensively utilizing gas chromatography (GC)-flame ionization detector and GC coupled with mass spectrometry (MS) using the HP-5 column. Wistar rats of about 150-200 g of both sexes were provided by the Biochemistry Department animal unit of Lagos state University, Ojo-Lagos, Nigeria and accommodated within the animal facility for the animal study. The antinociceptive and anti-inflammatory assays were analyzed by a hot plate, formalin, and carrageenan-induced edema assays, respectively. Carrageenan of analytical grade was obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA; Batch Number: SLBR0530V). Piroxicam (May and Baker; Batch Number: MT2056) and ibuprofen injection (Dizpharm, Nigeria Ltd.,Batch Number: 180606) were purchased from Juta Pharmacy, Lagos, Nigeria.

Results

The essential oil was obtained in a yield of 0.2% (v/w) calculated on a dry weight basis. A total of 36 compounds representing 94.8% of the oil contents were identified. The oil contained a high content of (*E*)-2-hexenal (19.0%), linalool (8.9%), 1-hexanol (6.6%), and *trans*-dehydroxylinalool oxide (5.2%). The analgesic property of the essential oil was slightly significant ($P < 0.5$) only at the third hour for the 400 mg/kg while other doses are less active. The rate of inhibition was moderate (24.1%-54%) during the analgesic phase of the formalin assay. The rate of inhibition at the anti-inflammatory phases of both formalin and carrageenan were significantly high (100%) and $P < 0.001$ for all the doses during the reaction duration.

Conclusion

The potential proinflammatory mechanism might be due to effects on several proinflammatory mediators, including, histamine, serotonin, and bradykinin, and the ability of the essential oils to act as centrally mediated opioid analgesic. *Mucuna pruriens* essential oils displayed a high anti-inflammation potential and can be used as a potential centrally mediated opioid antagonist against analgesia.

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The chemical constituents, anti-nociceptive and anti-inflammatory effects of essential oil from the leaves of *Gmelina arborea* Roxb. e x. Sm.

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Keywords: *Gmelina arborea*, essential oil, anti-nociceptive, Dendrolasin

Objective

Gmelina arborea (Lamiaceae) is a plant with medicinal properties against bilious fever, gonorrhea, and cough. This study was designed to determine the chemical constituents and to evaluate the anti-pain properties of the essential oil of the leaves of *Gmelina arborea*.

Methods

The essential oil was isolated using the hydrodistillation method in an all-glass Clevenger-type apparatus and characterized by Gas Chromatography (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) and evaluated for its anti-inflammatory and anti-nociceptive properties using carrageenan-induced, formalin, and hot plate model as reported previously [1,2].

Results

The essential oil was obtained in a yield of 0.80% (w/w) calculated dry weight. A total of 25 components were identified, accounting for 94.75% of the total oil characterized by GC and GC-MS. The main contents are methyl isoeugenol (28.24%), (*E*)-α-ionone (14.18%), *E*-geranyl acetone (7.32%) and Dendrolasin (7.09%). The essential oil at 100-400 mg/kg dose significantly ($p<0.5$-0.001) reduced inflammation. The 100 mg/kg hot plate model showed high inhibition ($p<0.001$) at all hours; the formalin assay revealed a very low inhibition rate at the inflammatory and neurogenic phases. The potential mechanism could be due to the inhibition of pro-inflammatory mediators and the ability of the essential oil to act as centrally mediated opioid analgesics. The study agreed with earlier studies on the inflammatory activities of *G. arborea.*

Conclusions

This study found out that the leaves of *G. arborea* produce a low yield of essential oils. The oil is predominantly dominated by phenylpropanoids and apocarotenoids compounds, comprising of methyl isoeugenol, (*E*)-α-ionone, *E*-geranyl acetone, and Dendrolasin. In addition, the essential oil of *G. arborea* are non-toxic, inhibiting nociceptive receptors and reducing edema caused by inflammation. The extent of anti-inflammation activity reported was independent of the dose. Monoterpenoids and phenylpropanoids compounds reported in this study contribute significantly to the antinociceptive and anti-inflammatory properties of the essential oil. The mechanism of action is predicated on the high penetrating and binding effect of the constituents on pain receptors. This study has partially revealed the ethnobotanical use of the extracts of *G. arborea* for wound and sore treatment. Further molecular studies to show the mechanism of inhibition are ongoing.

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PP-B31

Antimicrobial Activity and Constituents of *Tsoongiodendron odorum* Chum and *Manglietia chevalieri* Dandy Essential Oils from Vietnam

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Keywords: *Tsoongiodendron odorum*, *Manglietia chevalieri*, essential oil, antimicrobial activity

Objective

This paper is a contiuation of an extensive research whereby the chemical constituents and biological activities of essential oils from Vietnamese plants are evalted and reported [1-3]. This study determine the chemical composition and antimicrobial activity of essential oils from the leaves of *Tsoongiodendron odorum* Chum and *Manglietia chevalieri* Dandy (Myrtaceae) collected in Vietnam for the first time.

Methods

The leaves of *T. odorum* and *M. chevalieri* harvested from Pù Hoạt Natural Reserve (GPS 19°42′18″N, 104°49′42″E), Vietnam, at elevation of 337 m. The plant samples were collected in April 2020. The constituents of the essential oils were determined using gas chromattography (GC) on Agilent Technologies HP 7890N Plus Chromatograph (HP-5 MS column, 30 m x 0.25 mm, film thickness 0.25 μm) and gas chromatography-mass spectrometry (GC/MS, mass spectrometer HP 5973 MSD) techniques. The identification of constituents from the individual GC/MS spectra of leaves and stems of *S. henryi* subsp. *hoatii* was performed based on retention indices (RI) determined with reference to a homologous series of *n*-alkanes, under identical experimental conditions. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition. The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay.

Results

The constituents occurring in higher amount in the leaf essential oil of *T. odorum* were β-pinene (20.4%), β-caryophyllene (6.4%) and α-humulene (5.6%). On the other hand, *cis*-β-elemene (12.7%), β-caryophyllene (8.4%), β-slinene (7.4%), α-selinene (6.8%), and (*E*,*E*)-α-farnesene (5.4%) constitute the bulk of essential oil of *M. chevalieri*. Of the two essential oils, the leaf oil of *T. odorum* displayed promising antibacterial activity against *Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, and *Bacillus cereus* ATCC14579; and anti-candidal action towards *Candida albicans* ATCC10231, with minimum inhibitory concentration (MIC) value of 32.0 μg/mL. The corresponding IC_{50} values were 19.56, 20.43, 22.45 and 20.19 μg/mL, respectively. The chemical constituents and antimicrobial activity of *T. odorum* and *M. chevalieri* essential oils are being reported for the first time.

Conclusions

The chemical constituents and antimicrobial activity of *T. odorum* and *M. chevalieri* essential oils may provide clues to the potentials of the plants in amelioration of microbial infections.

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Antimicrobial Activity and Constituents of *Schisandra henryi* subsp. *hoatii* Leaf and Stem Essential Oils from Vietnam

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Keywords: *Schisandra henryi* subsp. *hoatii*, Myrtaceae, essential oil, terpenes, antimicrobial activity

Objective

The main objective of the present work was to evaluate the chemical composition and antimicrobial activity of essential oils the leaves and stems of *Schisandra henryi* subsp. *hoatii* N.S.Ly & X.T.Nguyen,. from Vietnam. This is part of an extensive research aimed at the characterization of the chemical constituents and biological activities of unexploited Vietnamese flora.

Methods

2.0 kg of fresh sample of each plant parts were subjected to separate hydrodistillation using a Clevenger-type apparatus as described previously [1-3]. The essential oils were collected in separately in sealed sample bottles before analysis. The constituents of the essential oils were determined using gas chromattography (GC) on Agilent Technologies HP 7890N Plus Chromatograph (HP-5 MS column, 30 m x 0.25 mm, film thickness 0.25 μm) and gas chromatography-mass spectrometry (GC/MS, mass spectrometer HP 5973 MSD) techniques. The identification of constituents from the individual GC/MS spectra of leaves and stems of *S. henryi* subsp. *hoatii* was performed based on retention indices (RI) determined with reference to a homologous series of *n*-alkanes, under identical experimental conditions. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition. The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay.

Results

The yields of the essential oils were 0.28% and 0.30% (w/w), respectively. The major compounds of the leaf essential oil were (*E*)-nerolidol (20.7%), caryophyllene oxide (11.2%) and β-caryophyllene (8.6%), while β-caryophyllene (21.2%), sabinene (8.5%) and α-pinene (7.4%) were the main constituents of the stem essential oil. The essential oils showed antimicrobial activity towards *Enterococcus faecalis* ATCC299212 with minimum inhibitory concentration (MIC) value of 128 μg/mL. The stem oil displayed activity against *Bacillus cereus* ATCC14579 with MIC value of 64.0 μg/mL and *Staphylococcus aureus* ATCC25923 with MIC value of 128.0 μg/mL. The essential oil did not displayed anti-candidal activity.

Conclusions

The results in the present paper are being described for the first time. The studied essential oils of *S. henryi* subsp. *hoatii* may further be exploied for their antimicrobial potential.

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Antifungal activity of the selected essential oils and their interactions with nystatin against *Candida* spp.

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Keywords: antifungal activity, superficial infections, essential oil, nystatin, synergism

Objective

Recently, the occurrence of superficial infections caused by fungi belonging to *Candida* spp., has increased significantly, especially in immunocompromised patients. Additionally, the considerable number and intensity of side effects, mainly related to the usage of high antifungals concentrations, the constantly increasing drug resistance, makes fungal infections a serious clinical problem. Therefore, it is necessary to search for alternative therapies or other antimycotics, e.g. natural compounds such as essential oils (EOs). In these studies, we decided to verify the anticandidal activity of five selected EOs and their effect of combination with nystatin.

Methods

To evaluate antifungal activity of some EOs: thyme, lawender, clove, oregano and sage oils (dr Beta, Poland) against strains of *Candida* spp.: *C. albicans, C. glabrata, C. parapsilosis* and *C. krusei* (from American Type Culture Collection – ATCC) the broth microdilution method was used according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [1] and Clinical and Laboratory Standards Institute (CLSI) guidelines [2]. The MIC (Minimal Inhibitory Concentration) of the EOs and nystatin (Sigma-Aldrich Chemicals, USA) was examined using the two-fold dilutions in a liquid medium in 96-well polystyrene plates. The MFC (Minimal Fungicidal Concentration), defined as the lowest concentration of EO required to kill fungi, was determined by transferring the culture samples from the wells onto Sabouraud agar after MIC determination. To verify the interactions of EOs and nystatin, the fractional inhibitory concentration (FIC) was assessed by checkerboard technique and then ΣFIC values were calculated and interpreted [3].

Results

Based on our results, all studied EOs showed antifungal activity against *Candida* spp. strains with MIC = 0.5 – 8 mg/mL and MFC = 0.5 – 16 mg/mL. It seems that the clove and oregano EOs indicated the highest antifungal effect (MIC = 0.5 – 2 mg/mL). Similar MIC values were found for thyme and sage EOs (MIC = 1 – 4 mg/mL). In turn, lavender EO was slightly less effective (MIC = 2 – 8 mg/mL). The values of MFC of the studied oils were the same or 2-4-fold higher than MIC values. We observed a beneficial, synergistic interaction between thyme EO and nystatin towards yeasts (ΣFIC = 0.5). Moreover, additive effect (ΣFIC = 0.75) was showed for lavender and clove EOs in combination with nystatin. In the case of oregano and sage EOs no interactions was found.

Conclusions

These results showed the susceptibility of *Candida* spp. strains to the studied EOs *in vitro*, especially to clove and oregano oils. It should be noted synergistic action of nystatin with thyme EO and additive effect with lavender and clove EOs. Therefore, the studied EOs seems to be promising antifungal agents. In addition, combining them with antimycotics may increase their effectiveness and find wide application in the treatment of surface candidiasis.

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Mints producing thymol? A comprehensive study of five Hungarian *Mentha longifolia* (L.) L. chemotypes

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Keywords: *Mentha longifolia* (L.) L.; essential oil; thymol; carvacrol; chemotypes

Objective

Mentha longifolia (L.) L. is considered to be the most widespread wild-growing mint taxon of the Earth [1]. We studied the essential oil (EO) yield and chemical diversity of five Hungarian accessions. These were involved to a cultivation experiment as a part of the first phytochemical evaluation of the species in Europe. The goal is to establish the cultivation of this species as a source of flavoring and preservative agents.

Methods

Accessions annotated as HOR-1, HOR-2, EGR3, DOM and KBT were selected from 36 wild-growing populations. Cultivation sites were Eger (N 47.906834°; E 20.388889°) and Budapest (N 47.398820, E 19.149270). Both sites were sampled in full bloom in 2019 and 2020. Air-dried samples were processed in a Clevenger apparatus according Ph. Hg. VII. Components were identified via GC-MS using standards, retention indices and spectral libraries.

Results

EO yield (1–2 mL/100 g dry weight) was dependent on accession and year. HOR-1 is assumed to be a new chemotype in the species, dominated by carvacrol (19.28–20.56%), thymol (13.36–13.90%), carvacryl acetate (8.81–10.40%), *para*-cymene (7.24–8.01%) 1,8-cineole (14.87–17.45%). Concentration of these showed minor fluctuations. However, a turn of composition was observed in EO of HOR-2, as one batch was based on thymol (19.79%) and 1,8-cineole (14.93%), while the others were rich in dihydrocarvones (up to 69%). EO of EGR, DOM and KBT based on limonene-3-oxo metabolites and sesquiterpenes which are typical for the species [2].

Conclusions

The γ-terpinene pathway in the genus *Mentha* is less known and may be rare. We demonstrated that chemotypes of horsemint might also differ in phenotypic appearance of chemical traits influenced by habitat or development. Our results led to the conclusion that any statement on chemotype needs detailed examinations.

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The perception of Naturology students from inhaling the pink peper essential oil (*Schinus terebinthifolius* Raddi)

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Keywords: *Schinus terebinthifolius* Raddi, pink pepper essential oil, psychological, perception, inhalation.

Objective

The pink pepper's essential oil (EOpp), extracted from the ripe fruit of *Schinus terebinthifolius* Raddi, is composed mainly of monoterpenes, and it is used to treat diseases of the respiratory system, ringworm and candidiasis. Studies have demonstrated anticancer (cytotoxic), antioxidant, insecticidal, cicatrizing, fungicidal, bactericidal and antimicrobial effects, mainly confirmed by in vitro research. Pre-clinical tests with EOpp demonstrate no toxicity. [1-3]

It is known that there are several bibliographic sources that describe psycho-emotional effects, as well as symbolic aspects related to the use of essential oils (EOs). However, there is a lack of current scientific studies that address these effects described in the literature, resulting from the stimulation of the limbic system triggered by the inhalation route, as well as how much it can interfere in the effectiveness of treatment with the use of EOs. Due to the lack of clinical studies on aromatherapy that elucidate its effects mainly related to psychological aspects, and the scarcity of information related to the psycho-emotional and symbolic aspects of Eopp from Brazil, this research aimed to describe the perceptions and psychological associations of the University of Southern Santa Catarina (Universidade do Sul de Santa Catarina – UNISUL) Naturology students, when inhaling the Eopp.[3]

Methods

This is a qualitative, descriptive, exploratory and field research. This research was conducted with 30 participants in individual in-depth interviews. For the application of the research, a glass bottle containing 10.1 ml of the EOpp from Laszlo brand was used. [3]

Results

The inhalation of EOpp was related to the perception of physical sensations such as relaxation, sedation and improvement of breathing, as well as discomfort in the respiratory system, such as nose stinging; childhood memories, and other past recollections, feelings of comfort, welcome, warmth, tranquillity, calm, well-being and peace; and improvement of self-perception, sense of presence and decreased thoughts. [3]

Conclusions

It was possible to conclude that most of the feelings and emotions expressed by the interviewed participants were more directly related to the physical sensations and memories that emerged with the inhalation of EOpp, than with EOpp itself. Therefore, it seems that it depends a lot on the individuality of each person, on the physiological response and on which memory emerged with the inhalation of EOpp, to obtain emotional or sentimental responses. It is emphasized the importance of the aromatherapist to be careful with the EO used on patients, because they can directly or indirectly stimulate memories, emotions or sensations with connotations both positive and negative for the individual. [3]

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Citrus limon waste in South Africa: Medicinal, Sustainable Agriculture and Bio-resources Potentials

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Keywords: *Citrus limon*, essential oils, antioxidant, citrus feed, *Sitophilus zeamais*

Objective

Lemon fruits are often purchase for the juice content which is regarded as a good source of vitamin C, while the fruit peels and the tree leaf are regarded as waste. This study investigated the chemical profile of the essential oils of *Citrus limon* waste, antioxidant bioassay to unveil the hidden biological potential of these waste for the management of some illnesses and its use as use in postharvest preservation against *Sitophilus zeamais* in maize. Simulation of lemon peel for sustainable agriculture and socio-economic benefits was evaluation.

Methods

Essential oils were isolated from the leaf and peel using hydrodistillation method and the constitutes were identified by GCMS. Antioxidant assay was done using DPPH and Ferric reducing power methods. Contact toxicity, fumigation and repellence assay was carried using known methods.

Results

The chemical composition and antioxidant activity divulges that Lisbon lemon leaf and peel oils are of medicinal value and should not be treated as waste. Sustainable agriculture and socio-economic benefits of lemon peel waste reveal the waste as alternative source of insecticide and maize ingredient in livestock feed production.

Conclusion

It was observed that fruit peels had higher oil (w/w) yields than leaf from both fresh and dried materials. Limonene a major citrus constituent was present in all essential oil with percentage composition ranging from (12.0-52.5%). The presence of limonene in all indicates that South African *Citrus limon* essential oils (leaf and peel) can be classified as medicinal essential oil and hence of great value instead of it being treated as a waste.

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Effects of different organic fertilizers and plant densities on chemical componds of essential oil in *Satureja mutica* Fisch. & C. A. Mey. under rain fed conditions

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Keywords: Carvacrol, GC, GC/MS, *Satureja mutica*, Thymol

Objective

In order to investigate the effect of Plant density and feeding with organic fertilizers on the quantitative and qualitative yield of essential oils in *Satureja mutica* Fisch. & C. A. Mey. under rainfed conditions.

Methods

an experiment in the form of split plots based on randomized complete blocks with three replications in 3 crop years (2018-2020) was carried out in the Research and Education Center of Agriculture and Natural Resources of Kermanshah province. The main factor included 3 fertilizer treatments and the sub-factor was 3 densities (2.66, 4 and 8 plants per square meter) in rows with a distance of 50 cm and with 3 planting distances (25, 50 and 75 cm). the EO extracted by water distillation and Clevenger methods (British Pharmacopoeia, 1993). the EO content and EO yield calculated and finally EO chemical compounds were studied by a gas chromatograph with specifications (Ultra Fast Model) Thermo-UFM and with Chrom-Card A/D data processor, cap-column Ph-5 (non-polar) made by thermo company, Italy and a Varian 3400 GC/MS device connected to mass spectrometer (Saturn II), with ion telephoto system and ionization energy of 70 electron volts. It has a DB-5 column which is a semi-polar column (length 30 m, inner diameter 0.25 mm and thickness of static phase layer equal to 0.25 microns). Data were Analysed by SPSS (ver.16) software.

Results

The nine chemical compounds include thymol, charvacrol, γ-terpinene, ρ-cymene, α-terpinene, α-pinene, β-pinene, camphen, and trans-caryophyllene were identified. The EO percent and chemical compounds did not show any difference between years. There was a significant difference between fertilizer treatments for EO percent, EO yield, thymol, carvacrol and other chemical compounds at the level of 1%. There was a significant difference between planting densities only for EO yield. Based on Perincipal Component Analysis, thymol had a very positive relationship with the treatment of rotten cow manure and the highest amount was observed in this treatment but carvacrol has a positive relationship with enriched straw treatment and its trend is negative and contrast with thymol.

Conclusions

Organic fertilizers such as rotten cow manure and Enriched straw were affect the quality and quantity of Eo in *S. Mutica* and inhanced the EO content, Thymol and Carvacrol. Plant dencity affect EO yield but no EO chemical componds.

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Chemical compounds of EO in creeping savory under rainfed conditions, organic fertilizers and plant density

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Keywords: Carvacrol, GC, GC/MS, *Satureja spicigera*, Thymol

Objective

Satureja spicigera is an Iranian native medicinal species that growing on rock walls of Iran. This study was conducted for the possibility of rainfed cultivation and to achieve to proper plant density and organic fertilizers for enhanced EO quantity and quality in *s. spicigera*.

Methods

An experiment was conducted by a split plot design based on RCBD with three organic fertilizers treatment and three planting densities in Mehregan research station of Kermanshah (Iran) during 2017-2018 under rainfed condition. the EO extracted by water distillation and Clevenger methods (British Pharmacopoeia, 1993). the EO content and EO yield calculated and finally EO chemical compounds were studied by a gas chromatograph with specifications (Ultra Fast Model) Thermo-UFM and with Chrom-Card A/D data processor, cap-column Ph-5 (non-polar) made by thermo company, Italy and a Varian 3400 GC/MS device connected to mass spectrometer (Saturn II), with ion telephoto system and ionization energy of 70 electron volts. It has a DB-5 column which is a semi-polar column (length 30 m, inner diameter 0.25 mm and thickness of static phase layer equal to 0.25 microns). Data were Analysed by SPSS (ver.16) software.

Results

The results showed that the highest EO content was obtained in the treatment of rotten cow manure/high density (3.75%) and then in enriched straw /low density (3.73%). The lowest EO content was observed in the farm soil/high plant density treatment (2.78%). The highest EO yield was obtained in enriched straw/high density (72.59 kg ha^{-1}) and the lowest (16.76 kg ha^{-1}) in the farm soil/low density. A total of 13 compounds were identified in the EO, which contains more than 97% of the EO compounds. The main compounds of essential oil were thymol (29.13%), carvacrol (23.84%), ρ-cymene (21.39%) and γ-terpinene (15.74%). The other identified compounds were Methyl ether carvacrol, methyl ether thymol, myrcene, terpinolene, terpinene-4-ol, α-pinene, α-thujoune, β-bisabolene and trans-caryophyllene. The fertilizer treatments had significant effect on some EO chemical compounds. There were significant differences between fertilizer treatments for myrcene, γ-terpinene, terpinene-4-ol, methyl ether thymol and trans-caryophyllene ($p< 0.01$) and for α-pinene ($p< 0.05$). Also, there were significant differences between different planting densities for α-pinene, terpinolene, terpinene-4-ol and thymol ($p< 0.01$) and for α-thujone, methyl ether thymol and carvacrol ($p< 0.05$). The year/fertilizer interaction was significant for γ-terpinene and trans-caryophyllene ($p< 0.01$). The year/density interaction was significant only for the α-pinene ($p< 0.05$). Fertilizer/density interaction was significant ($p< 0.01$) for α-thujoune, γ-terpinene, terpinolene, trans-caryophyllene, terpinene-4-ol, methyl ether thymol, and for myrcene and thymol ($p< 0.05$). Year/fertilizer/density interactions were significant only for the α-pinene ($p< 0.01$).

Conclusions

The highest amount of thymol (33.68%) was obtained in rotten cow manure/low density treatment and the highest percent of carvacrol (26.55%) in farm soil/high density treatment. The use of organic fertilizers in *S. Specigera* cultivated under rainfed condition increased the EO content, EO yield and thymol content, but decreased carvacrol production.

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The effect of water stress on essential oil content and yield of peppermint

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Key words: Mint, Essential oil, Menthol

Objective

Peppermint (*Mentha* × *piperita*) is one of the most important aromatic and medicinal plants of the Lamiaceae family. Although plants can accumulate biosynthetic secondary metabolites which increase their medicinal value, these secondary metabolites are affected by environmental factors. The availability of water for plants has a major impact on physiological processes, biosynthesis of secondary metabolites, enzyme activity, and quantity and quality of secondary metabolites.

Methods

To investigate the effects of different levels of water stress on the phytochemical composition of peppermint, a randomized complete block design experiment was conducted in 2020 and 2022. Irrigation levels include control (irrigation after depleting 25% of field capacity (FC)), moderate stress (irrigation after depleting 50% of FC) and severe stress (irrigation after discharging 75% FC). In this study, the essential oil of Peppermint was extracted using the Clevenger apparatus and assessed using gas chromatography-mass spectrometry (GC-MS).

Results

In two years, essential oil yield decreased as water stress intensity increased, while essential oil content increased. The highest amounts of peppermint essential oil were Sabinene, β-Pinene, 1,8-Cineole, Linalool, Mentone, Menthofuran, Menthol, trans-Caryophyllene, Germacrene-D. This study revealed that water stress increased the content of Mentone, Menthol, 1,8-Cineole, Linalool, Menthofuran, Menthol, Menthyl acetate, 1,8-Cineole, and trans-Caryophyllene.

Conclusions

In conclusion, water stress has a significant effect on the yield and content of essential oil as well as the amount of constituents of peppermint essential oil and can be optimized by the environmental conditions of peppermint cultivation, especially in arid and semi-arid regions, resulting in increased levels of important and functional compounds.

Hemisynthesis of limonaketone a barely available monoterpene in *Cedrus atlantica* essential oil: Viable route and MEDT study

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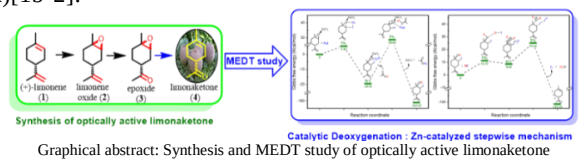
Keywords: *Cedrus atlantica*, limonaketone, limonene, deoxygenation, MEDT study.

Objective

Intense interest was given to derived carbonyls from monoterpenes, a class of naturally occurring compounds in plants which possess a range of pharmacological properties. In contrast, only few studies are reported in literature for the isolation, synthesis, and reactivity of limonaketone [1]. Herein, we report a novel, easy and efficient pathway as well as density functional theory (DFT) mechanistic study for the synthesis of optically active limonaketone (*Graphical abstract*), a high added value monoterpene, starting from limonene, natural and low-cost feedstock [2]. The strategy was developed in an excellent 3-step synthesis of limonaketone, potentially important and barely available in the Morrocan *Cedrus atlantica* essential oil (0.1% yield)[1b-2].



Graphical abstract: Synthesis and MEDT study of optically active limonaketone

Methods

The hemisynthesis of limonaketone (**4**) was carried out as follow: (i) Aerobic epoxidation of limonene (**1**) catalyzed by a ruthenium complex; (ii) Ozonolysis of limonene oxide (**2**) to obtain the characteristic ketone function; (iii) Deoxygenation of epoxide (**3**) catalyzed by Zn powder. All the prepared compounds were characterized by NMR and GC–MS analyses. The Molecular Electron Density Theory (MEDT) study of the Zn-deoxygenation step was performed using DFT calculations at the M06–2X/6–311G(d,p) (LANL2DZ for Zn) level.

Results

The optically active limonaketone was prepared in 3 steps with a total yield of 84%. Moreover, the yield of each step was (i) 91%, (ii) 92%, and (iii) 100% (after an optimisation study of Zn-deoxygenation). The observed chemoselectivity in the Zn-deoxygenation step as well as its corresponding mechanistic pathway were explained by MEDT study. Furthermore, the mechanistic aspect of each reagent or additive in the Zn-deoxygenation was investigated. Accordingly, the deoxygenation reaction of epoxide (**3**) follows a stepwise mechanism with an easy reaction in terms of energetic, which is in good agreement with the experimental observations.

Conclusions

Novel, easy, and efficient hemisynthesis of optically active limonaketone was carried out starting from limonene. The reactions afford an optically pure limonaketone with an overall yield of 84%. The obtained chemoselectivity was explained by means of MEDT study as well as local reactivity indexes obtained from the Parr functions.

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Effect of chitosan coating enriched with cinnamon essential oil on apple fruit quality during storage

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Keywords: Apple, Chitosan, Cinnamon essential oil, Coating, Storage

Objective

The aim of this study was to evaluate the effect of Chitosan-Cinnamon essential oil combination, as a natural packaging material, on apple fruits quality during cold storage. The essential oil was obtained in Tunisia by steam distillation of bark cinnamon (*Cinnamomum verum* J. Presl, 1825).

Methods

The Chitosan was obtained from shrimp exoskeletons and Cinnamon essential oil was added to form a liquid packaging material. The apple fruits were soaked in Chitosan and Chitosan-Cinnamon essential oil for 1 min and left to dry, then stored at 4 C° for 0, 5, 10, 20 and 30 days. Several quality parameters (% of weight loss, pH, titratable acidity), chemical composition (phenols and flavonoids contents), antiradical capacity (DPPH) and sensory analysis were evaluated. Chemical composition of Cinnamon essential oil was evaluated by GC-MS analysis.

Results

The obtained results demonstrated that apples treated with Chitosan-Cinnamon essential oil have the lowest weight loss, however the highest one was registered for untreated apples. Titratable acidity decreased over the time across all of the samples while the pH values were relatively stable during the experiment and no difference was seen between untreated and treated fruits during of storage. The phenols content, flavonoids content and antiradical capacity of treated apples with Chitosan and Chitosan-Cinnamon essential oil were higher than the control fruits. Regarding the sensory analysis, the use of the Chitosan-Cinnamon essential oil found to preserve the odor, color and the overall acceptability of apple fruits. In summary, our research showed that the Chitosan and Chitosan-Cinnamon essential oil used as a natural packaging material allowed the reduction of weight loss, the increase of phenols and flavonoids contents, the enhancement of antiradical ability and the preservation of sensory quality during the cold storage of apple fruits. This powerful activity is related to the richness of cinnamon essential oil on *trans*-Cinnamaldehyde.

Conclusions

These results suggest that Chitosan-Cinnamon essential oil could be an attractive, promising and a natural packaging alternative to be used for maintaining apple fruits quality and extending their postharvest life.

ACKNOWLEDGMENTS

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PP-B42

Antibacterial effects of clove and fennel essential oils and their main compounds: a comparative study

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Keywords: Monoterpenes, Clove, Seed of Fennel, Essential oil, Antibacterial activity

Objective

Phenylpropanoids have been known for centuries as the fragrant components of the essential oils (Eos) obtained from several parts of the plants. These compounds generated considerable biological interest such as antibacterial activity [1]. This study aimed to evaluate the chemical composition of essential oils of two plants, Clove (*Syzygium aromaticum* (L.) Merr. & Perry) from Madagascar and Fennel seeds (*Foeniculum vulgare* Mill.) from Morocco, isolate their main chemical compound, and to evaluate the antibacterial activities of Eos and the isolated compounds against three bacterial strains *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Methods

The essential oils were extracted using hydrodistillation type Clevenger and analysed by Gaz Chromatography-Mass Spectrometry (GC/MS) to determine their chemical composition. The major compound of Clove oil was isolated according to the method described by Mimpin Ginting [2]. The fennel seeds oil was purified by silica gel chromatography to obtain its main product. The structures of isolated products were identified and confirmed by GC/MS, Infrared spectroscopy IR, ^{1}H, and ^{13}C NMR. The agar disc diffusion method was used to evaluate the antibacterial potentiality of the two Eos and the isolated compounds against three strains *E. coli*, *S. aureus* and *P. aeruginosa*.

Results

The two Eos were obtained with a good yield, 12 % of *Syzygium aromaticum* and 3.2 % of *Foeniculum vulgare*. We noted that the Eos are chemotype presenting their majors' compounds with 68 % of eugenol and 93 % of estragol in Clove and fennel seeds respectively [3]. The antibacterial activity showed that the isolated compounds, eugenol and estragol presented a high efficiency against the tested strains compared to their Eos, while the eugenol give the best result.

Conclusions

The GC-MS study revealed that the Eos were chemotype presenting two phenylpropanoids, eugenol and estragol as the major compounds in clove and fennel seeds respectively. The antibacterial activity of the Eos, eugenol and estragol against *E. coli*, *S. aureus* and *P. Aeruginosa* showed that a synergistic effect reduces the efficiency of the major compound.

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Exogenous application of silicon nanoparticles encourage essential oil production and functional activities of menthol mint (*Mentha arvensis* L.) under chromium toxicity

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Keywords: Essential oil, menthol, *Mentha arvensis*, nanoparticles, heavy metals.

Objective

"The purpose of my research is to provide the information that how essential oil (EO) composition of *Mentha arvensis* L. (Lamiaceae) leaves from India, which were already in chromium stress, changes when supplemented with Silicon nanoparticles". Menthol mint (*Mentha arvensis* L.) is a plant of substantial medicinal value in both traditional and modern medicine, owing to its EO. In view of beneficial aspects of Silicon Nanoparticles (SiNPs), the current study was conducted to evaluate the influence of SiNPs on physiological and biochemical as well as EOs production of the plant under chromium (Cr) toxicity. A simple randomised pot experiment was designed in Aligarh Muslim University, India, to explore the effect of various foliar concentrations of SiNPs (40, 80, 120, 160 & 200 mg L^{-1}) on the overall performance of the plant under Cr stress (60 mg kg^{-1} of soil). The present study indicates that Cr stress reduced yield, quality and quantity of EOs and its active constituents, particularly menthol. Consequently, exogenous application of SiNPs at the applied concentration (120 mg L^{-1}) exhibited a beneficial effect, was found to be the most effective in improving Cr tolerance by strengthen antioxidant dense metabolism and other functional activities of the plant which in turn increases the menthol content in plant.

Methods

Treated leaves were collected and finally chopped. the EO content of leaves of M. arvensis were estimated by distillation method for 3 hours using a Clevenger's apparatus [1]. The EO was dried over anhydrous sodium sulfate and preserved in sealed glass vials at 4 °C for the gas chromatography (GC) analysis. The active constituents of the EO were determined using GC apparatus [(Agilent) USA, 7890B] equipped with a capillary column HP5, flame ionization detector and an injector with Nitrogen as carrier gas. Identification of the active constituents, menthol, menthone and menthyl-acetate was based on retention time. It was quantified as the per cent content comparing their peaks with the peaks obtained from the reference standard reported in the literature [2].

Results

The essential oil content of *Mentha arvensis* significantly decreased with the application of Chromium. The decrease in the content of α-pinene, β-pinene, sabinene, β-myrcene, and menthyl acetate as compared with control, were observed with the application of 60 mg Cr kg^{-1} soil. Furthermore, the foliar application of SiNPs significantly enhanced the content and yield of EO. The maximum increase of menthol, menthone and menthyl-acetate yield in plants were observed by the supplementation of 120 mg L^{-1}. This increase in the content of EO by SiNPs, revealed by Gas chromatograms, possibly due to improved enzymatic activities of monoterpenes biosynthetic pathways.

Conclusion

Exogenously sourced elicitor (SiNPs) have been applied to the plants through foliage to enhanced yield of valuable secondary metabolites, content and yield of EO which are various of medicinal uses.

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Contribution to the domestication and valorization of *Artemisia herba alba*, a multipurpose species threatened by extinction.

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Keywords: *Artemisia herba alba*, multiplication and crop, Valuation, Essential Oil, Chemical Composition

Objective

In Tunisia, the white wormwood (*Artemisia herba alba*), which is distinguished by its vernacular name "Chih",is a herbaceous plant with woody and branched stems, belonging to the Artemisia genus and the Asteraceae family. This species is commonly used in traditional therapeutic practices for the treatment of several diseases (e.g., digestive tract illness and diabetes). *Artemisia herba alba* is a pastoral species that grows well in semi-arid and arid environments, and characterized by its high plasticity. It has long been known as one of the few species that tolerates drought, which is becoming longer and more recurrent around the circum- Mediterranean region. In Tunisia, its distribution range extends over a large part of the plains and lowlands characterized by fine-textured soils (silt and silty-clay) in the northwest and central west regions. Over time, the white wormwood has experienced a steep decline due to chronic anthropogenic disturbances (grazing and land clearing). Recently, climate change has exacerbated its decay, which may threaten the persistence of plants that survived challenging conditions if climate warming will continue in the coming decades. Hence the reintroduction of this species has become increasingly crucial in order to restore degraded lands. The present work aims at studying the vegetative propagation and cultivation of the species, and to verify whether its domestication affects its biochemical composition.

Methods

In this study, the plant material was sampled in the northwest region of Tunisia (Governorate of Kef). Two methods of cuttings were tested, namely herbaceous cuttings and hardwood cuttings. The two approaches were applied at three different dates of the plant life cycle, namely December, January and February respectively. Prior to transplanting, the cuttings were immersed into a rooting hormone solution at 50% concentration (Exuberone). After rooting, the seedlings produced were replanted in the planting site for the monitoring and evaluation of their growth and their chemical composition.

Results

Our results showed that the date of cuttings had a significant effect on the success rate of the cuttings. The December date gave the highest success rate, which reached around 40% versus 20% and 6% for January and February cutting dates respectively. With regard to the effect of the cutting methods (herbaceous and woody), the results revealed that the woody cuttings treated with a solution of Exuberone at a concentration of 50% were significantly more effective for the multiplication of this species (40% success rate) compared to 25% success rate obtained for herbaceous cuttings. Regarding the yield of essential oil (EO), based on the yield achieved on the dry matter from plants grown in natural environment, we found that the yield of EO obtained on the dry matter from cultivated plants was not strongly affected by culture. It was around 1,1 ml/100 g of DM for spontaneous white wormwood against 0,9 ml/ 100 g of DM for cultivated white wormwood. The characterization of EO by its injection with GC-MS, allowed us to assess that the chemical composition of the dry biomass of the white wormwood was affected by the culture and varied qualitatively and quantitatively from the plants grown in their natural environment. Thus, in dry biomass from the natural environment, EO was composed of five major molecules, in order of importance we found, camphor (55,38%), eucalyptol (16,24%), camphene (11,64%), Chrysanthenone (11,53%) and thyjone (3,31%). While in the cultivated one, the EO was composed in order of importance of camphor (55,96%), eucalyptol (18,42%), Chrysanthenone (13,78%), camphene (10,15%) and thymol (0,45%).

Conclusions

The woody cuttings in the month of December seem to be an effective approach for the multiplication of the white wormwood either for the restoration of the degraded lands of this species or for its cultivation to other purposes such as biochemical proprieties or/and fodder production.

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Effect of *Satureja hortensis* L. essential oil on the shelf-life of liquid whole eggs during storage

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Keywords: Summer savory; essential oil; GC/MS; liquid whole eggs; aromatisation; shelf life

Objective

The Algerian essential oil (EO) extracted by hydro-distillation from Satureja hortensis L. (S. hortensis) was characterised by gas chromatography–mass spectrometry (GC-MS). S. hortensis (summer savory) was chemotype carvacrol (54.2%) and γ-terpinene (21.1%). In its second part, the present study was conducted to evaluate in vitro, antioxidant activity of savory EO using the 2,2-di-phenyl-1-picrilhydrazyl (DPPH•) free radical scavenging assay. Finally, the EO was screened for biological activities in liquid whole eggs (LWE). The ability of savory EO to scavenge the free radical (DPPH·) was very high, exceeding 80%. The long-term oxidative and microbial stability of LWE was positively influenced by EO treatments, increasing the shelf life to more than eight (> 8) days under refrigeration. Therefore, the results obtained in this work confirm savory EO treatment as a promising technology to extend the commercial shelf life of liquid egg products, as well as to maintain the quality of LWE as ingredient in innovative food products.

Methods

The *S. hortensis* EO was obtained from dried aerial parts of plant by hydro-distillation. The Compounds were identified by Linear Retention Index (LRI) for analysis of gas chromatography– mass spectrometry data.

In vitro antioxidant activity was determined by DPPH radical-scavenging methods. To evaluate the potential biological capacity of savory EO in liquid whole eggs (LWE), the shelf life extension of EO treated LWE was undertaken.

Results

The storage of LWE demonstrated that treatment with summer savory EO resulted in an increase of retail shelf life. Results revealed that treated samples with 400 ppm savory EO showed two additional days of shelf life compared to control samples. Moreover, samples treated with LWE 800 and 1000 ppm savory EO did not suffer a notable deterioration, as indicated by the high retail shelf life period, which was extended three additional days compared to control samples.

Conclusions

The EO extracted from Algerian summer savory contained 92% bioactive monoterpenes, both hydrocarbons (35.6%) and oxygenated ones (56.4%). Results showed that the oxidative, microbial and sensory stability of the liquid whole egg was positively influenced by the treatment with summer savory EO, even increasing the shelf life to more than eight days with the treatments of LWE800 and LWE1000. According to the increasing trends of the chemical markers (TBARS), microbiological (TAP, LAB, Pseudomonas) and sensory (off-odour), the expected life of the batches LWE800 and LWE1000 could easily reach 10 days. Therefore, the results obtained confirm that the addition of summer savory EO is a promising technology to extend the commercial life of egg products, which could be applied at an industrial level.

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Composition of essential oil, antioxidant activity and phenol and flavonoid content of different parts of *Postia puberula* at post flowering stage

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Keywords: DPPH, β-carotene/linoleic acid, Cadinol, Caryophyllene

Objective

Postia puberula belonging to Asteraceae family, is a shrub plant and it's habitat is South of Iran. In this study, the essential oil composition, flavonoid and phenolic contents, and antioxidant activities of methanolic extract of stems, leaves and fruits in *Postia puberula* at the post-flowering stage were determined.

Methods

Essential oils were analyzed by using GC and GC/MS, The antioxidant activity were measured by 2, 2-diphenyl-1-picrylhydrazyl and β-carotene/linoleic acid assay, also phenol and flavonoid content were measured by gallic acid and quercetin as standard compounds.

Results

Results showed that cadinol (13.30%), hexenyl benzoate (12.06%) and caryophyllene oxide (11.96%) were the main compounds found in the leaves while; the stems were rich in cis-3-hexenyl benzoate (7.07%), hexadecanic acid (5.75%) and δ-cadinol(5.71%), also the major compound of fruits were nuciferol (8.98%), benzyl benzoate (7.39%) and hexenyl benzoate (6.55%). In both DPPH and β-carotene/linoleic acid tests the leaves have stronger antioxidant activity compared with other organs, also, the results demonstrated that the leaves have more flavonoid and phenolic contents than the others.

Conclusions

Organ type affects the constituents of essential oil, antioxidant activity, phenol and flavonoid content.

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Glycation products as aromatic compounds in food and fragrance products as alternative to essential oils

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Overview

Glycation is a non-enzymatic reaction between the reducing sugars or low molecular mass aldehydes (e.g., α-oxoaldehydes, hydroxyaldehydes) and the alkaline groups of proteins, lipids or nucleic acids, resulting in dozens of different compounds varying in structure and stability [1]. Depending on the context and type, these products are generally called Maillard Reaction Products (MRPs) or Advanced Glycation End Products (AGEs). While endogenous glycation products are very disadvantageous as they lead to serious metabolic problems in living organisms [1, 2], MRPs/AGEs may have a positive property - they give substances a specific, often very pleasant aroma [3]. Responsible for this are formed during glycation heterocyclic aroma compounds such as pyrazines, pyrroles and pyridines [4]. The formation of aromatic compounds during glycation take place in the food industry, where food is thermally processed to stimulate glycation and to obtain the desired changes in color, taste and aroma of food. Caramel-like aromas and other pleasant aromas can also be used in the perfume industry. Among the aromatic MPRs, the group of alkylpyrazines, oxazoles and oxazolines is distinguished, where these compounds are formed from the amino acid-non-specific pathway, when α-dicarbonyl (like methylglioxal or glyoxal) reacts with most types of amino acids forming α-aminocetone. Alkylpyrazines often have a very low odor threshold, so they are felt in very small amounts. An example of alkylpyrazines is 2-Methylpyrazine, 2,3-Dimethylpyrazine, 2,5-Dimethylpyrazine or 2,3,5,6-Tetramethylpyrazine, found e.g. in roasted seeds [5]. The next group are thiazoles, thiazolines, pyrrolines, and pyridines, formed in the amino acid-specific pathway, when in reaction amino acids such as cysteine and proline, α-aminocetone or α-dicarbonyl are involved [4]. It is worth highlighting 3-methylbutanal, which has malt aroma, and phenylacetaldehyde, with a characteristic honey-like smell. The last two compounds are derived from leucine and phenylalanine [4]. An interesting example is also 2,5-Dimethyl-4-hydroxy-3 (2H) -furanone (DMHF, known as furaneol), with an intense aroma, it was originally discovered as a key flavor component of strawberry [4]. Generally, 2-alkylthiazoles possess vegetable-like aroma [4]. There are many AGEs products, it seems that only a small part of them is identified. Work on distinguishing the libraries of glycayion products groups and describing their properties is still ongoing, requires highly specialized techniques (such as chromatographic methods coupled with mass spectroscopy) [2].

Conclusions

This work reviews the aromatic products that give the products the desired aroma. It should be noted that the isolation of AGEs from commonly available products is difficult and costly [2], so it is not easy to apply the knowledge of glycation products in the non-food industry. However, it is an interesting research direction involving chemistry, biotechnology and biomedical research, with the potential for using this knowledge in diagnostics and industry.

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Management of waste from the essential oil industry

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Keywords: biodiesel, burning, essential oils, recycling, waste

Overview

Essential oils are an increasingly used alternative to chemical products that can be toxic to both health and the planet. Apart from their aromatic qualities, these oils often have a healing value [1]. The production and use of essential oils, however, have their bottlenecks. The production of essential oils requires a huge amount of plants, which often come from crops where a significant amount of pesticides are used. Obtaining plants from the natural environment is often prohibited, as many of them are protected species. The disposal of expired essential oils and their packaging is also problematic. Most essential oils are extremely flammable, such as those derived from tea tree, clove, incense, eucalyptus, lavender, lemon, and peppermint. Their removal or cleaning of the spill requires additional precautions. Even small amounts of flammable oils are considered hazardous waste. Containers in which they are packaged before being recycled, and even trace amounts of a flammable liquid can pose a risk to workers in recycling plants. In addition, many essential oils are toxic to aquatic organisms and can have long-term effects on marine ecosystems. Most of them should not come into contact with water (they should never be thrown into the sewage system). So the question arises: how to manage the residual essential oils. When large quantities of out-of-date (rancid) essential oils are used, they are used to make biodiesel. Essential oils can be used for compression ignition of engines [2].

In addition, the very process of producing essential oils produces waste that is either composted or incinerated. However, it has been shown that plant residues from which essential oils are extracted can be a source of vitamins, antioxidants and other valuable substances. Therefore, projects are undertaken aimed at developing a technology of waste management from the production of essential oils [3]. It has been shown that these residues can be used in several different industries: food (to protect food products and extend their life), household chemicals (*e.g.* disinfecting liquids and cosmetics for cleaning floor surfaces, etc.), cosmetics, automotive (*e.g.* car fragrances), medicine (*e.g.* dietary supplements, throat preparations), gardening (*e.g.* plant protection chemicals), footwear (*e.g.* shoe insoles) [4].

Conclusions

Essential oils are a source of valuable compounds (vitamins, antioxidants) of natural origin. Plant residues after their production can be used, among others. in the food and cosmetics industry. The development of technologies for obtaining compounds from these residues will allow for the formation of less valuable waste, intended for composting or incineration.

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Isoprenoid-phospholipid conjugates as effective anticancer agents

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Keywords: isoprenoids, phospholipids, lipid carriers, anticancer activity

Objective

Isoprenoids are one of the most widely occurring group of natural compounds with documented useful properties. They exhibit strong fungicidal, antibacterial, antiviral, and anti-inflammatory activities. The results of many studies show also that they are able to inhibit effectively proliferation of cancer cell lines, causing inhibition of cell division and transition of cells into the phase of programmed cell death. The administered active doses, even in high concentrations do not cause such an effect in the case of healthy cells. Unfortunately, rapid metabolism of this group of compounds limits their practical application as chemoprevention substances, therefore, we proposed the novel hybrid molecules with the combination of isoprenoids with phospholipids and evaluated their antiproliferative potential towards selected cancer cell lines.

Methods

We synthesized four series of the terpene-phospholipids and their structures were confirmed based on the comprehensive spectroscopic data interpretation. Next the structured phospholipids were evaluated *in vitro* for their cytotoxic activity against several cancer cell lines.

Results

Terpene-phospholipids were synthesized in high yields (24-97%). Most of synthesized compounds showed a significantly higher antiproliferative effect on tested cell lines than free terpene acids. The phosphatidylcholines modified with monoterpene acid showed a significantly higher antiproliferative activity containing the isoprenoid units in the *sn*-1 position of phosphatidylcholine and palmitic acid in the *sn*-2 position. Among the most active phosphatidylcholine analogues conjugated with sesquiterpenoid acids, the most active was this containing 2,3-dihydro-3-vinylfarnesoic acid instead of fatty acids in both *sn*-1 and *sn*-2 positions. This derivative inhibited the proliferation of colon cancer cells at 13.6 μM.

Conclusions

Our results clearly showed that terpene-phospholipids demonstrate to be effective anticancer agents. The incorporation of isoprenoids acids into phosphatidylcholine significantly improves their selective cytotoxic activity which is strongly corelated with the structure of isoprene unit and its position in the structure of phosphatidylcholine.

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